CETP-deficiency manifests a unique plasma lipoprotein profile without other apparent symptoms. It is highly common in East Asia, while rare anywhere else. An environmental screening factor(s) should be conceived for this eccentric distribution, such as a region-specific infectious disease. Blood flukes use the host plasma lipoproteins as nutrient sources through the lipoprotein receptor-like systems and its Asian-specific species, Schistosoma (S) japonicum has been endemic in East Asia. The adults and eggs of S. japonicum take up cholesteryl ester (CE) from HDL for the egg embryonation to miracidia, a critical step of the hepatic pathogenesis of this parasite and this reaction was retarded with the HDL of CETP-deficiency. CD36-related protein (CD36RP) was cloned from the adults and the eggs of S. japonicum, with 1880-bp encoding 506 amino-acid residues exhibiting the CD36 domains and two transmembrane regions. Its extracellular domain selectively bound human HDL but neither LDL nor CETP-deficiency HDL, and the antibody against the extracellular domain suppressed the selective HDL-CE uptake and embryonation of the eggs. When infected with S. japonicum, wild-type mice developed less hepatic granulomatosis than CETP-transgenic mice by the ectopic egg embryonation. CD36RP is thus a candidate receptor of S. japonicum to facilitate uptake of HDL-CE necessary for egg embryonation. Abnormal HDL caused by CETP-deficiency retards this process and thereby protects the patients from development of hepatic lesions. S. japonicum infection is a strong candidate as a screening factor for CETP deficiency in East Asia.

There is no conflict of interest to declare for this work.

Subdivision Lipoprotein Metabolism

Presentation Preference Oral presentation

Additional information
Alternative treatment of atherosclerosis using nanoparticles and nanocomposites

Abstract nr. 2
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Cardiovascular Disease, LDL, Therapy

Atherosclerosis is a disease characterized by its occurrence in the arterial wall at susceptible sites in major conduit arteries. This disease is initiated by lipid retention, oxidation and modification which will eventually cause chronic inflammation. This process can result in stenosis or thrombosis. Atherosclerosis and its thrombotic complications are currently the chief cause of morbidity and mortality in the developed world. The traditional drug treatments for atherosclerosis are limited to the prevention of the formation (generation) of new atheroma plaques in the body. In this respect, the development of functional nanostructures, to provide the removal of free-LDL from the bloodstream, is absolutely necessary for those patients for whom traditional treatments (statins) fail to prevent the negative effects of atherosclerosis. We developed SPIONS (magnetic nanoparticles) functionalized with monoclonal antibodies that are able to interact with low density lipoprotein (LDL). That is, these SPIONS will act as molecular/magnetic traps for free LDL. The SPIONS-LDL complexes will be trapped using a magnetic field during the short extracorporeal circulation. The prepared samples have its biocompatibility and its capacity to interact with free-LDL investigated. The studies for the control of nanonavigation are progressing with good results for static and low speed flow. This magnetic separation-based filtration system will provide for the rapid removal of LDL, offering the patient an opportunity to improve his or her quality and duration of life.

[i] Organisation modiale de la santé – MTN Profils de pays, 2011

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Whole exome sequencing identifies novel causal variants in ABCA1 gene associated with familial hypoalfalipoproteinemia

Abstract nr. 3
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Co-author(s) - Yamagishi, Masakazu
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Genetics, HDL

**Background:** Plasma high-density lipoprotein cholesterol (HDL-C) is a quantitative, heritable risk factor for coronary heart disease. Several genes are known to cause extremely low HDL-C level in Mendelian manner, including APOA1, ABCA1, and LCAT. However, it is onerous to determine molecular diagnosis through conventional genetic analysis.

**Objectives:** This study aimed to identify the causal variants in a family with hypoalfalipoproteinemia of unknown pathogenesis through whole exome sequencing.

**Methods:** A family with autosomal recessive, familial hypoalfalipoproteinemia was identified. Despite the extremely low HDL-C level (HDL-C = 2 mg/dl), the proband did not exhibit any apparent abnormalities in any organs, including coronary arteries. Her parents exhibited almost normo-HDL cholesterolemia, suggesting autosomal recessive pattern of inheritance. Exome capture and sequencing were performed in this family members (the proband and her parents). Variants were filtered for quality of the exome sequencing, rarity, predicted functional significance, and segregation pattern.

**Results:** Among 305,202 variants found in this family, we found 21,708 nonsense, missense, or splice site variants, of which 5,192 were rare (minor allele frequency ≤0.01 or not reported) in 1000 Genome (Asian population). Filtering assuming recessive pattern of inheritance successfully narrowed down the candidate to the compound heterozygous mutations in ABCA1 gene (c.7173G>T or P2077H and c.6223C>T or S2046N).

**Conclusions:** Whole exome sequencing identified novel causal variants in ABCA1 gene associated with hypoalfalipoproteinemia. Those results provide new insights into the novel pharmacological target for ABCA1.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Predicting the Population at Risk of Atherothrombotic Disease (ATD)

Abstract nr. 4
Author Feeman, Jr , William, The Bowling Green Study, Bowling Green Ohio, United States of America
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis,Dyslipidemia,HDL,LDL

The cardinal risk factors for atherothrombotic disease (ATD) are dyslipidemia, cigarette smoking, and hypertension. Dyslipidemia is best defined in terms of LDL-cholesterol and HDL-cholesterol, and the author favors the Cholesterol Retention Fraction (CRF, or [LDL-HDL]/LDL). If only ATD of the heart is examined, then the population at risk of ATD can be defined in terms of CRF and LDL-cholesterol; however, if all forms of ATD are examined, then systolic blood pressure (SBP) must be added to the predictor.

There are 2841 patients in the author's general population database who have had full lipid profiles during the 1979-2003 time frame. If these people are arranged in a table stratifying the CRF values by their LDL-cholesterol values in a nested cohort manner, and if the incidence of ATD in each of these cohorts is examined, then three risk zones are evident. The highest risk zone includes all people whose CRF > 0.70 and LDL-cholesterol > 125 mg/dl (3.2 mmoles/L); 33% of these people sustained an ATD event. The medium risk zone includes all people whose CRF=0.60-0.69 and LDL-cholesterol=100-124 mg/dl (2.6-3.2 mmoles/L); 22% of these people sustained an ATD event. The low risk zone consists of all people whose CRF>0.59 and LDL-cholesterol<99 mg/dl (2.6 mmoles/L); 13% of these patients sustained an ATD event. If current cigarette smokers are excluded, the average age of ATD onset in the high risk zone is 66 years; in the medium risk zone, 72 years; and in the low risk zone, 75 years. These events are mainly ATD events of the coronary circulation.

When ATD events of the cerebral circulation are included, SBP must be included and the prediction of the population at risk of ATD evolves into a graph with CRF on the ordinate and SBP on the abscissa. The threshold line, above which lie the CRF-SBP plots of the vast majority of ATD patients have CRF-SBP loci of (0.74, 100) and (0.49, 140). The few ATD that occur in people with CRF-SBP plots below the threshold line do so in the aged and in the absence of current cigarette smoking, the prognosis is excellent.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
The Program on the Surgical Control of the Hyperlipidemias (POSCH) and the Lipid Regulatory Hypothesis

Abstract nr. 5
Author Feeman, Jr., William, The Bowling Green Study, Bowling Green Ohio, United States of America
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Dyslipidemia, HDL, LDL

The Lipid Regulatory Hypothesis of Esko Nikkila, MD, states that atherothrombotic disease (ATD) is best stabilized/regressed when LDL-cholesterol is lowered simultaneously with HDL-cholesterol being raised. The POSCH study involved a trial of dietary therapy, with half the patient population being randomized to undergo a partial ileal bypass. Lipid profiles were obtained at baseline and at one year; angiograms were done at baseline and at three years.

Changes in LDL-cholesterol and HDL-cholesterol were evaluated for their relationship to plaque outcomes. Since the Lipid Regulatory Hypothesis involves both LDL-cholesterol and HDL-cholesterol, a novel lipid predictor, the Cholesterol Retention Fraction (CRF, or \(\frac{LDL-HDL}{LDL}\)) was also used for evaluation.

Changes in LDL-cholesterol were stratified against changes in HDL-cholesterol, with the following results. Plaque stabilization/regression occurred if LDL-cholesterol levels fell so long as HDL-cholesterol levels did not fall too far or even if LDL-cholesterol levels provided that HDL-cholesterol levels rose sufficiently. Similarly, plaque progression occurred if LDL-cholesterol levels rose provided that HDL-cholesterol levels did not rise much, or if LDL-cholesterol levels fell provided HDL-cholesterol levels fell even more.

If the CRF rose, plaque progression occurred in all cases. If CRF values fell, plaque stabilization/regression occurred in all cases. Because the CRF predicted plaque outcome in all cases in POSCH, the CRF can serve as an indicator of plaque response to therapy.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Cardiovascular risk profile and metabolic syndrome in young police officers

Abstract nr. 10
Author Domagala, Teresa, Jagiellonian University School of Medicine, Krakow, Poland
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Co-author(s) - Kotula-Horowitz, Katarzyna
Co-author(s) - Dropinski, Jerzy
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Dyslipidemia, Lifestyle, Obesity

**Background:** There is an on-going debate about metabolic syndrome (MS) as a more viable predictor of cardiovascular disease (CVD) risk than individual risk factors. Stress also impacts prevalence of MS and coronary heart disease (CHD).

**Design:** The present study is focused on police officers whose occupation is deemed highly stressful.

**Methods:** 235 subjects mean aged 40.97 years were divided into two groups, with (46.38%) and without MS (53.62%). CV risk profile was assessed by interview, exercise ECG, measurement of endothelial function (flow-mediated dilation; FMD), carotid artery intima-media thickness (IMT) and select laboratory biochemical parameters. Coronary atherosclerosis was evaluated by computed tomography coronary angiography (CTCA). Levels of perceived stress were also assessed.

**Results:** MS was less associated with coronary artery atherosclerosis (OR=2.62, 95%CI 1.24-5.52) than with co-existence of classical CVD risk factors (OR=5.67, 95% CI 1.077-29.88; for 3 risk factors and OR = 9.0; 95% CI 1.24-66.23 for 6 risk factors), when compared to the subjects with 0-2 CVD risk factors. MS subjects had significantly higher IMT and no significantly lower FMD value. Perceived stress increased the chance of MS prevalence (OR 1.07; 95% CI 1.03-1.13; p=0.037), while in the multivariate regression model, stress impacted prevalence of coronary plaque (OR=1.05, 95% CI 1.001-1.010, p=0.04).

**Conclusions:** High prevalence of MS might be associated with exposure to job-specific hazards. Early comprehensive therapeutic intervention on CVD risk factors may potentially reduce overall risk of CV events in police officers.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
**Abstract**

**Physical activity and lung function in young police officers with metabolic syndrome**

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Topic: Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention

Keywords: Cardiovascular Disease, Inflammation, Lifestyle, Obesity

**Background:** Prevalence of metabolic syndrome (MS) continues to spread becoming a major clinical and public health problem, mainly due to its association with cardiovascular disease. Lack of regular physical activity (PA) and impaired lung function are associated with obesity, MS and CVD.

**Design:** The present cohort study aimed to investigate the level of PA and lung function in young police officers with multiple cardiovascular risk factors.

**Methods:** 235 studied subjects mean aged 40.97 years were divided into two groups, with (46.38%) and without MS (53.62%). Coronary atherosclerosis was evaluated by computed tomography coronary angiography. Ultrasensitive CRP (hs-CRP) in the serum and tissue necrotic factor α (TNF-α) in the plasma. PA (International PA Questionnaire) and standard spirometry were also assessed.

**Results:** MS subjects had a higher prevalence of coronary artery atherosclerosis, as compared to the non-MS subjects (p<0.0017). Significantly higher hs-CRP (p=0.0001), and non-significantly higher TNF-α plasma concentration in the MS subjects were encountered. In the MS subjects significantly lower leisure-time PA (p=0.0001) and non-significantly lower PA associated with transportation and total walking (p=0.08) were established. Logistic regression revealed leisure-time PA to reduce the chances for developing MS (OR=0.98; 95% CI 0.96-0.99, p=0.022). There were no differences with regard to moderate, vigorous and total PA. The MS subjects had significantly lower some lung function parameters than the non-MS ones.

**Conclusions:** Regular, leisure-time PA induced protective effects on coronary artery disease. Lower pulmonary function in the MS group may have resulted from obesity and systemic inflammation.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference: Electronic poster presentation

Additional information
Extreme Hypercholesterolemia Exacerbated by Breast Feeding: Infantile Cases of Sitosterolemia with Novel Mutations in ABCG5 and ABCG8 Gene

Abstract nr. 12
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Co-author(s) - Mabuchi , Hiroshi
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia,Familial Hypercholesterolemia,Genetics,Lipoproteins

Background: Sitosterolemia is an extremely rare inherited disease characterized by increased levels of plant sterols such as sitosterol, the cause of which is ATP-binding cassette (ABC) sub-family G member 5 or member 8 (ABCG5 or ABCG8) gene mutations.

Methods and Results: We tried to determine the molecular diagnosis for 6 Japanese infantile cases with severe hypercholesterolemia and systemic xanthomas without any mutations in LDL receptor (LDLR), proprotein convertase subtilisin/kexin type 9 (PCSK9), and LDL receptor adaptor protein 1 (LDLRAP1) genes, then evaluated their clinical features, especially the responsiveness to variety of therapies. We performed genetic analysis for ABCG5, and ABCG8 genes for those 6 cases, and identified 2 pairs of mutations in ABCG5 or ABCG8 gene in each case, including 3 novel mutations (c.130C>T and c.1813_1817delCTTTT in ABCG5, and c.1256_1257TC>AA in ABCG8) and 3 known mutations (c.1306G>A and c .1336C>T in ABCG5, and c.1285A>G in ABCG8).During their clinical courses, significant reduction of their cholesterol levels could be obtained through their weaning alone (from 540 ± 164 mg/dl to 147 ± 60 mg/dl, p < 0.05). Also, a substantial reduction of their sitosterol levels were observed using ezetimibe without any apparent side effects (from 90 ± 69 μg/ml to 52 ± 38 μg/ml, p < 0.05).

Conclusion: We have identified infantile Japanese sitosterolemic subjects with extreme hypercholesterolemia exacerbated by breast feeding. Their unique manner of response to weaning as well as to ezetimibe could provide us novel insights into the metabolic basis for cholesterol and plant sterols in human.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Effect of chronic hepatitis C infection on arterial stiffness is through systemic inflammation but not oxidative stress

Abstract nr. 13
Author Chou , Chang-Hua, Tainan SinLau Hospital, Tainan, Taiwan
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Co-author(s) - Chen , Ju-Yi
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Inflammation

Background
Our previous study has found both non-alcoholic fatty liver disease (NAFLD) and chronic hepatitis C virus (HCV) infection were associated with increased arterial stiffness. However, the possible mechanism has never been studied before.

Methods
We recruited 200 patients including 40 individuals were normal control (NC), 80 subjects were NAFLD, and 80 were HCV infection in this study. Arterial stiffness was assessed by Stiffness Index (SI) and Compliance Index (CI) derived from digital volume pulse by photoplethysmography. High-sensitive CRP (hsCRP) and TBARS (an oxidative stress marker) were measured in all subjects.

Results
HCV group had significantly higher SI (8.6 ± 2.2 m/s vs. 8.4 ± 2.4 m/s vs. 7.1 ± 1.5 m/s; p for trend = 0.001) and lower CI (3.06 ± 1.83 units vs. 3.82 ± 2.15 units vs. 4.93 ± 2.95 units; p for trend < 0.001) than NAFLD and NC. Using multi-variate linear regression analysis, we found that CI was independently correlated with HCV infection (beta = -0.212, p = 0.007). Furthermore, HCV had the highest hs-CRP (0.8 ± 0.8 ug/ml vs. 2.7 ± 2.5 ug/ml vs. 8.3 ± 7.7 ug/ml; p < 0.001) in all three groups. TBARS (17.9 ± 10.6 uM vs. 25.6 ± 11.4 uM vs. 18.7 ± 9.7 uM; p < 0.001) was significantly higher in NAFLD but not HCV group.

Conclusion
The underlying mechanism responsible for increased arterial stiffness in chronic HCV infection is different from NAFLD. It is possible due to systemic inflammation but not through increased oxidative stress.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Effects of Adiponectin on Vascular Strain of Carotid Artery Assessed by Speckle Tracking Echocardiography

Abstract nr. 15
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Imaging

Background: Adiponectin is a cytokine from adipose tissue associated with atherosclerosis. Vascular strain of carotid artery can be assessed by speckle tracking echocardiography and has been found in association with stroke. However, effects of adiponectin on carotid strain were never been studied before.

Methods: We recruited 89 consecutive elder patients (mean age 72.6 years, 31 men) from a community health survey program. Carotid B-mode image was acquired by using 10 MHz high resolution vascular probe equipped on an echocardiographic system. Cross sectional images of bilateral carotid artery 1 cm below carotid bulb were acquired and images were analysis offline. Circumferential strain (CS) and strain rate (CSR) of carotid artery were obtained by speckle tracking technique. We also measured carotid intima-medial thickness (IMT), and beta-index of carotid artery for local properties. The averages of bilateral measurements were used for analysis.

Results: Serum adiponectin was significantly correlated with carotid CS (r = 0.362, p = 0.001) and CSR (r = 0.313, p = 0.003) but not IMT and Beta-index. After multivariate analysis controlling age, blood pressure, body mass index, serum glucose level, adiponectin was still significantly correlated with CS (Beta = 0.313, p = 0.001) and CSR (Beta = 0.272, p = 0.010). Adiponectin was significantly correlated with procollage type I carboxyterminal propeptide, pro-matrix metalloproteinases I, and tissue inhibitor of metalloproteinases I.

Conclusions: Adiponectin was correlated with CS and CSR of carotid artery independently. This association was probably through the effects on matrix remodeling of carotid artery.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Mean Platelet Volume and Cardiovascular Outcomes in Acute Myocardial Infarction- a Prospective Cohort Study

Abstract nr. 16
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Pathogenesis, Thrombosis

Background: High levels of mean platelet volume (MPV) may be associated with adverse outcomes in patients with myocardial infarction (MI). We examined the association between MPV and the risk of death and adverse cardiovascular outcomes in patients with MI.

Methods: We studied consecutive patients with MI admitted to a tertiary care hospital during a period of 1 year. MPV was measured at admission and at third month. Patients were followed-up for one-year primary composite outcome of cardiovascular death, stroke, fatal or nonfatal MI and cardiac failure. Patients were classified according to tertile of baseline MPV.

Results: A total of 1206 MI patients, including 934 males (77.4%) and 272 females (22.6%) were studied. The mean age of the study population was 55.93 (SD 11.07) years. At one-year follow-up, 292 (28.57%) primary outcome occurred: cardiovascular mortality 78 (7.6%), fatal or nonfatal MI 153 (15.0%), stroke 30 (2.9%), and cardiac failure 128 (12.52%). Highest tertile MPV patients had higher primary outcome as compared with those with MPV in the lowest tertile (adjusted OR = 1.70; 95% CI: 1.18 to 2.45; p = 0.01). Total mortality was also more in high MPV group (adjusted OR 2.83; 95% CI: 1.49 to 5.35; p <0.001). There were no significant changes in mean MPV values at admission from those at third month interval [9.15, (SD 0.99) Vs 9.19 (SD 0.94); p=0.2].

Conclusions: Elevated MPV was associated with worse outcome in patients with acute MI. Elevated MPV in these patients may be due to inherently large platelets.
Everolimus-eluting Stents Reduce Monocyte Expression Of Toll-like Receptor 4: A Reason For Systemic Use of Everolimus?

Abstract nr. 17
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Intervention

Background: Toll-like receptors (TLR) are well known components of the innate immune system. Among them, TLR4 is related to the inflammatory responses after Percutaneous Coronary Intervention (PCI). Our purpose was to compare the monocytic expression of TLR4 following implantation of drug-eluting (DES) and bare stent (BMS).

Methods: The study was done in Shahid Madani Heart Hospital, Tabriz, Iran. Patients were divided into 2 groups: DES (n=95) and BMS (n=95). Blood collection was done before PCI, 2 hours and 4 hours after termination of PCI. Expression of TLR4 on monocytes was measured using flowcytometry (BD, US). Everolimus eluting stents were implanted for DES group (XIENCE, Abbott Vascular, US). Similar PCI protocols were performed.

Results: Figure A and B shows flowcytometry findings. No significant difference was seen in age, sex, use of medications, white blood cell count or the risk factors. A significant difference was present between DES and BMS before the intervention (P< 0.05). No significant difference was noted at 2 hours after PCI, however, patients in BMS group had higher expression of TLR4. Four hours after PCI, TLR4 expression was significantly lower in DES group than BMS group (30.1 ± 3.3% vs 39.2 ± 3.2%, P< 0.05).

Conclusion: Our findings suggest that eluted drugs can decrease PCI related inflammation by partial reduction of TLR4 expression on the surface of monocytes. Systemic use of these drugs may be a new field of research in order to decrease the likelihood of thrombosis formation.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Physician-prompts in an electronic medical record improve secondary prevention best-practices for cardiac risk factor targets and reduce utilization and costs.

Abstract nr. 18
Author Sheikh, Khalid, Health First Medical Group, Merritt Island, United States of America
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Guidelines, Lipids, Risk Factor

Fewer than 50% of patients with known coronary heart disease (CHD) achieve best-practices for risk factor control. We tested the hypothesis that physician-prompts incorporated into an electronic medical record (EMR) that reinforce established clinical guidelines for best-practices in patients with established CHD would be superior to usual care.

Over a 4 year period, 808 patients with known stable CHD, in a single managed care plan were followed. Clinical data was recorded in the GE Centricity EMR. Health care utilization and medical cost data were extracted from the managed care plan database. During 2009, 432 consecutive patients were assigned to usual care (UC). In 2011, the EMR was modified to post an immediate “pop-up” notification during the clinic visit if any parameter fell out of best-practices range. In this prompted care group (PC), 376 consecutive patients were enrolled.

Results:
PC UC p
(n=376) (n=432)
Risk factor targets reached:
Ideal HgA1c 48% 32% 0.001
Ideal LDL-C 82% 51% 0.001
Ideal BMI 12% 6% 0.001
Ideal Mean BP 68% 39% 0.001
Tobacco cessation 84% 77% 0.01
Hospital Utilization & costs:
ED visits 10.3% 16.4% 0.01
Hospitalizations 13.1% 17.4% 0.01
Length of stay (days) 0.39 0.66 0.01
ED costs (per pt.) $ 22 $ 38 0.01
Hospital costs (per pt.) $776 $908 0.01

The use of physician-prompts incorporated into an EMR in patients with established CHD is superior to usual care for improving parameters of cardiovascular risk and reducing health care utilization and costs.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Chronomics of BP/HR in terms of Double Amplitude, Acrophase, Hyperbaric Index and its relation with cortisol in night shift workers.

Abstract nr. 19
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Co-author(s) - Tiwari, Sandeep
Co-author(s) - Singh, Ranjana
Co-author(s) - Mahdi, Abbas A
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Blood pressure, Cardiovascular Disease, Lifestyle, Risk Factor

Objective: The present study was aimed to investigate the effects of rotating night shift on 24 hours chronomics of BP/HR in terms of Double amplitude, Acrophase and Hyperbaric index and its relation with salivary cortisol.

Material and Methods: 62 healthy nursing professionals, aged 20-40 year, performing day and night shift duties were recruited from the Trauma Center, GM and Associated Hospitals, King George’s Medical University, Lucknow, UP, India. BP and HR were recorded at every 30 min intervals in day time and each hour in night time synchronically with circadian pattern of salivary cortisol during shift duties.

Results: Highly Significant difference was found in double amplitude (2DA) of blood pressure between night and day shift (p<0.001). In night shift, hyperbaric index (HBI) of mean systolic blood pressure was found to be increased at 00-03 am (midnight) while during day shift, peak was found at 06-09 am. Highly significant difference was found in night cortisol levels among night (4.34 ± 3.37) vs day shift (2.70 ± 2.32), (p<0.001) due to recovery during day shift. Alteration in mean morning cortisol level was also found between night (3.73 ± 2.47) vs day shift (5.00 ± 2.73). Alterations in Acrophase of BP/HR were very common among most of the night shift workers and persistent during night and day shift due to incomplete recovery.

Conclusion: Ecphasia was also found in few night workers caused by internal desynchrononization which may be a risk factor of Cardiovascular diseases.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Microalbuminuria is Associated with Endothelial Function and Vascular Arteriosclerosis.

Abstract nr. 20
Author Ueda, Tomohiro, Hiroshima City, Japan
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Co-author(s) - Teragawa, Hiroki
Co-author(s) - Nomura, Syuichi
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Chronic Kidney Disease, Endothelium, Renal function

Background: Kidney function and cardiovascular disease are closely connected, and microalbuminuria is a proven marker of cardiovascular risk. The purpose of this study was to evaluate the relation of vascular parameter, renal function and microalbuminuria. Methods: Endothelial function was assessed by flow mediated vasodilatation (FMD) in the brachial artery by a novel semi-automatic vessel chasing system (UNEXEF18G), and nitroglycerin mediated vasodilatation (NMD) was used as a control test for FMD. Urinary albumin excretion rates (UAER), vascular and biochemical parameters were evaluated. Results: A total 119 patients enrolled in this study. The mean age was 67±11 years and 77 patients (65%) were men. Log UAER inversely correlated with FMD ($r=-0.21$ $p=0.024$) and NMD ($r=-0.33$ $p=0.0004$), but it was not correlated with eGFR ($r=-0.04$ $p=0.650$). Univariate analysis revealed that FMD correlated with age, blood sugar, log UAER and NMD correlated with log UAER. Multivariate analysis revealed that FMD independently correlations with log UAER, and NMD was independently correlations with log UAER. In addition, log UAER correlated with age, glucose, HbA1c, FMD, NMD, Carotid intima-media thickness (cIMT), carotid plaques (CP), Cardio Ankle Vascular Index (CAVI). Conclusions: These results suggest that the microalbuminuria might correlate the endothelial function and vascular arteriosclerosis, but not renal function.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Frequency dispersion on the vessel wall - the primary event in atherosclerosis.

Abstract nr. 21
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Co-author(s) - Beraia, Merab
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Imaging, Pathogenesis, Thrombosis

Purpose: The aim is to study the blood flow and vessel wall viscoelastic alterations at the boundary layer.
Methods and Materials: Peak velocity, net flow and the flow acceleration has been investigated in aorta by Magnetic Resonance Angiography in 40 healthy volunteers (age 18-51).
Results: At the outer curvature of the isthmus, flow acceleration in the initial diastole is 8.7 times higher than that in systole. Net flow from systole to diastole increases 2.5± 0.5 folds. From the end systole to the initial diastole flow separates into the opposite directed streams and there is a plateau on the net flow graph. Opposite flowing wave oscillation frequencies are 0.8Hz and 1.6Hz. Womersley number from ascending to abdominal aorta decreases from 13.2 to 8. At the outer curvature of isthmus, group wave at the boundary reflection, changes phase at 1800.
Conclusion: During the heart cycle, blood motion at the boundary layer, due to viscoelasticity of this system, forms the surface wave. At the end systole, at the outer curvature of the isthmus, pulse pressure at the reflection is in the resonance with the end systolic pressure drop and amplitude of the wall stress increases. In the initial diastole in the bulk flow, group wave, due to the frequency dispersion facilitates to the structural rearrangement of the cell aggregates, while at the boundary reflection, it shears the vessel wall.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Abstract nr. 22
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Dyslipidemia, Inflammation, Reverse Cholesterol Transport

**Objectives:** To investigate the effects of cytokines on macrophage foam cell formation and to determine the underlying molecular mechanisms.

**Background:** Atherosclerosis is an inflammatory disorder of the vasculature regulated by cytokines. The effect of various cytokines on foam cell formation in human macrophages is poorly understood and was hence investigated using both classical cytokines, such as interferon-gamma (IFN-gamma) and transforming growth factor-beta (TGF-beta), and those identified more recently, such as tumour necrosis factor-like protein 1A (TL1A) and interleukin-33 (IL-33).

**Methods:** Foam cell formation was investigated in human macrophages, bone marrow-derived macrophages from wild type and knockout mice and apolipoprotein E^{-/-} mice. Molecular mechanisms were delineated using a combination of RT-qPCR, western blot analysis, promoter analysis, pharmacological inhibitors, biochemical assays and RNA interference.

**Results:** IFN-gamma and TL1A stimulated foam cell formation by inducing the uptake of modified lipoproteins and inhibiting the efflux of cholesterol. Extracellular signal-regulated kinase was integral to the action of IFN-gamma. In contrast, TGF-beta and IL-33 attenuated foam cell formation by inhibiting the uptake of modified lipoproteins and storage of cholesterol, and stimulating the intracellular trafficking and efflux of this sterol. The action of these cytokines correlated with changes in the expression of key genes implicated in these processes. A dominant role for Smad2 in the action of TGF-beta was identified.

**Conclusions:** These studies provide novel insights into the regulation of macrophage cholesterol homeostasis by key cytokines implicated in atherosclerosis.

**Funding:** British Heart Foundation

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Serum lipid goal attainment in chronic kidney disease (CKD) under the Japan Atherosclerosis Society (JAS) 2012 guideline

Abstract nr. 23
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Chronic Kidney Disease, Guidelines, Lipoproteins, Risk stratification

Background:
In the Japan Atherosclerosis Society 2012 guideline (JAS2012-GL), chronic kidney disease (CKD) has newly been added to the high risk group for atherosclerotic cardiovascular diseases. We, therefore, have explored the lipid target level achieving rates under the JAS2012-GL in the real-world clinical practice.

Methods:
We retrospectively reviewed medical charts of patients who were hospitalized in the Nephrology Department at Kobe City Medical Center General Hospital from April 1, 2012 to May 31, 2013, and the serum lipid target level achieving rates were explored. Patients without lipid data, or undergoing regular dialysis because of chronic renal failure, were excluded. In this study, the CKD group (CKD-G) does not contain CKD patients with secondary prevention for coronary heart disease (CHD) or diabetes mellitus (DM).

Results:
CKD-G patients were 146 (81.1%) among 180 enrolled patients. According to the JAS2012-GL, 100% of the CKD-G patients were categorized into the high risk group, although only 12.1% of the CKD-G patients were at high-risk, according to the JAS2007-GL. Under the JAS2012-GL, LDL cholesterol (LDL-C) and non-HDL cholesterol (non-HDL-C) target level achieving rates for CKD-G were 71.4% and 68.1%, respectively. According to the JAS2007-GL, these rates were 81.3% and 79.1%, respectively. Under both guidelines, these rates were 71.7% and 72.1% for primary prevention DM (DM-G), and were 66.7% and 66.7% for CHD-G, respectively.

Conclusion:
After the revision of the JAS-GL in 2012, LDL-C and non-HDL-C target level achieving rates for CKD-G were reduced approximately by 10%; however, they remained similar to DM-G and higher than CHD-G.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Abnormal plasma lipidome in metabolic syndrome modified by weight loss plus exercise

Abstract nr. 26
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Intervention,Lipids,Metabolism,Nutrition

Rationale. Dyslipidemia that characterises Metabolic Syndrome (MetS) responds to weight loss.
Superior comprehensive description of the dyslipidemia through lipidomic analysis and the effects
of weight loss may identify further therapeutic targets.

Objectives. We have carried out 2 studies: firstly, comparing lipidomes in 95 subjects with MetS
and 40 matched healthy subjects (HS) by liquid-chromatography-tandem mass spectrometry;
secondly, in MetS subjects measuring effects of dietary weight loss n=19 (WL) or weight loss plus
exercise n=17(WLEX) during 12 weeks’ supervised intervention, 17 serving as controls (C).

Results. 334 lipid species were measured representing 23 classes and subclasses. MetS versus
HS showed significantly higher (+43% to +58%) di- and tri-acylglycerols (DAG, TAG,) and
cholesteryl esters (CE) (P<0.001 for all), but lower (-9% to -24%) di-and trihexosylceramides, lyso-
, alkyl and alkenylphospholipid subclasses (P<0.001 for all) including plasmalogens with potential
anti-oxidant property. Logistic regression odds ratios for MetS versus HS lipids were <0.5 for
hexosylceramides, plasmalogens, lysophospholipids, (lower in MetS). Following WL and WLEX
(similar falls in weight, glucose) lipid classes in MetS resembled HS values especially after WLEX
(P<0.05 for DAG, TAG, CE, several ceramides and phospholipids).

Conclusions. Plasma lipidomic analyses of subjects with metabolic syndrome and healthy subjects
showed higher values for DAG, TAG, CE, but significantly lower values for hexosylceramides and
phospholipid species (as in diabetes). Improvement in these lipids occurred with dietary weight
loss and even more with similar weight loss through an additional exercise program. Lipidomic
analysis may improve therapeutic targeting.
Funding: National Health & Medical Research Council, Diabetes Australia.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Epicardial fat volume is related to atherosclerotic calcification in multiple vessel beds

Abstract nr. 27
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Epidemiology, Imaging

Objective
Large amounts of epicardial fat have been associated with coronary artery atherosclerosis. It is unclear whether the amount of epicardial fat is also related to atherosclerosis in other vessels, and thereby represents a marker of systemic vascular risk. We explored relationships of epicardial fat volume with atherosclerosis in multiple vessel beds.

Methods
From the population-based Rotterdam Study, 2,298 participants underwent computed tomography examinations to quantify epicardial fat volume, and atherosclerotic calcification volume in the coronary arteries, aortic arch, extracranial and intracranial internal carotid arteries. Using linear regression modeling we investigated relationships of epicardial fat volume with atherosclerotic calcification volume in each vessel bed, adjusting for conventional cardiovascular risk factors. To test whether associations of epicardial fat with calcification per vessel bed were independent of calcification elsewhere, we created a model in which all vessel beds were entered together.

Results
We found that larger epicardial fat volume was associated with larger calcification volumes in all vessel beds. After adjustment for cardiovascular risk factors, larger epicardial fat volume was related to coronary and extracranial carotid artery calcification volume [Difference in standardized calcification volume per SD increase in epicardial fat volume: 0.10(95%C.I.:0.05;0.15), and 0.13(95%C.I.0.08;0.18)]. These associations remained present after entering all vessel beds in one model.

Conclusion
Larger volumes of epicardial fat are associated with larger amounts of coronary and extracranial carotid artery atherosclerosis, independent of cardiovascular risk factors. Epicardial fat may thus not only influence the local formation of atherosclerosis, but may also exert a more systemic effect on atherosclerosis development.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Gelsolin (GSN) induces cardiomyocyte hypertrophy and BNP expression via p38 signaling and GATA-4 transcriptional factor activation.

Abstract nr. 28  
Author Hu, Wei-Syun, Taipei, Taiwan  
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium  
Keywords Atherosclerosis, Metabolism

Cardiomyocyte hypertrophy is an adaptive response of the heart to various types of stress. Gelsolin (GSN) is a member of the actin-binding proteins (ABPs), which regulate dynamic actin filament organization by severing and capping. Moreover, GSN also regulates cell morphology, differentiation, movement, and apoptosis. In this study, we used H9c2 and H9c2-GSN stable clones, in an attempt to understand the mechanisms of GSN overexpression in cardiomyocytes. This data showed that the overexpression of GSN in H9c2 induced cardiac hypertrophy and increased the pathological hypertrophy markers atrial natriuretic peptide (ANP) brain natriuretic peptide (BNP). Furthermore, we found that E-cadherin expression decreased with the overexpression of GSN in H9c2, but β-catenin expression increased. These data presume that the cytoskeleton is loose. Further, previous studies show that the mitogen-activated protein kinase (MAPK) pathway can induce cardiac hypertrophy. Our data showed that p-p38 expression increased with the overexpression of GSN in H9c2, and the transcription factor p-GATA4 expression also increased, suggesting that the overexpression of GSN in H9c2-induced cardiac hypertrophy seemed to be regulated by the p38/GATA4 pathway. Moreover, we used both the p38 inhibitor (SB203580) and GSN siRNA to confirm our conjecture. We found that both of these factors significantly suppressed gelsolin-induced cardiac hypertrophy which through p38/GATA4 signaling pathway. Therefore, we predict that the gene silencing of GSN and/or the downstream blocking of GSN along the p38 pathway could be applied to ameliorate pathological cardiac hypertrophy in the future.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Gelsolin (GSN) enhance cardiomyocyte apoptosis during hypoxia via reducing survival protein p-Akt and increasing HIF-1α.

Abstract nr. 29
Author Hu, Wei-Syun, Taipei, Taiwan
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Inflammation

Cytoskeletal filaments play an important roles in cells such as cell shape, cell motility, intracellular transport and cellular division. Actin binding proteins (ABPs) have a lot of functions one of that is regulate the structure formation of actin to filament nucleation, elongation, severing, capping, crosslinking and actin monomer sequestration. Gelsolin (GSN) is one of actin binding proteins and it regulate actin filament formation and disassembly such as severing, capping, uncapping, nucleation of actin filament, and it regulate by pH, Ca2+, phosphoinositides (PIP2). Moreover, GSN also regulates cell morphology, differentiation, movement, and apoptosis. In our study, we used H9c2 and H9c2-GSN stable clones, in an attempt to understand the mechanisms of GSN overexpression in cardiomyocytes. This data showed that overexpression of GSN in H9c2 reduces the expression of survival markers p-Akt and Bcl-2. In hypoxia condition, overexpression GSN further reduce p-Akt expression and increase GSN, cleavage-GSN and HIF-1α expression more obviously. Moreover, overexpression GSN was more serious apoptosis compare with H9c2 cell during hypoxia.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Genistein suppresses the isoproterenol-treated H9c2 cardiomyoblast cell apoptosis associated with P-38, Erk1/2, JNK and NFκB signaling protein activation

Abstract nr. 30
Author Hu, Wei-Syun, Taipei, Taiwan
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atrial fibrillation, Chronic Kidney Disease, Inflammation

Heart disease (HD) is great associated with gender and menopausal status because of estrogens. In addition, clinical evidence shows that increased level of serum norepinephrine is found in patients with HD. Therefore this study aimed to investigate the cardio-protective effect of genistein, a selective estrogen receptor modulator (SERM) from soy bean extract, in H9c2 cardiomyoblast cells treated with isoproterenol (ISO, a norepinephrine analog). In this in vitro model, image data and results from western blotting shown that ISO treatment was capable of inducing cellular apoptosis, especially mitochondrial dependent pathway. Treatment of genistein could suppress the expression of mitochondrial pro-apoptotic proteins including Bad, caspase-8, caspase-9 and caspase-3 in H9c2 treated with ISO. By contrast, several survival proteins were expressed in H9c2 treated with genistein, such as phosphor (p)-Akt, p-Bad and p-Erk1/2. Furthermore, we confirmed that the protective role of genistein was partially mediated through the expression of Erk1/2, Akt and NFkB proteins by adding several pathway inhibitors. These in vitro data suggest that genistein may be a safe and natural SERM alternative to hormone therapy in cardio-protection.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Antioxidant enzymes in hyperlipidemic patients treated with lovostatine

Abstract nr. 37
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Dyslipidemia, Hypolipidemic Drugs, Therapy, Triglycerides

Lovostatine belongs to the class of hypolipidemic drugs which are prescribed for lowering cholesterol levels in patients with hyperlipidemia or at risk of cardiovascular disease. The aim of our study was to determine the activity of antioxidant enzymes in hyperlipidemic patients treated with lovostatine.

Material and methods: Material of our study was the whole blood from 30 male subjects on lovostatine therapy and from 110 male healthy subjects. The enzyme activities were determined in erythrocyte lysate employing standard spectrophotometric methods. Lipid parameters were measured in serum by commercial kits. Statistical analyzes was done using WinStat statistical program.

Results: Gluthatione peroxidise activity was statistically significantly lower in the examined group in comparison to the control group (p<0.001). There was a significantly positive correlation between low density lipoprotein cholesterol level and glutathione peroxidise and superoxide dismutase activity (p<0.05 and p<0.01 respectively). Triglycerides negatively correlate with the superoxide activity (p<0.01). The activity of catalase and superoxide dismutase was statistically significantly increased in smokers in comparison with non smokers patients (p<0.05 and p<0.01 respectively).

Conclusion: We may conclude that determining the activity of gluthatione peroxidise and superoxide dismutase in patient on hyperlipidemic therapy with statins could serve as a useful parameter in the evaluation of the effectiveness of the therapy.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
ASSOCIATIONS BETWEEN ANTIOXIDANT FOOD AND BEVERAGE INTAKES AND CORONARY ARTERY DISEASE (CAD) IN ELDERLY AND NON-ELDERLY JAPANESE PATIENTS

Abstract nr. 38
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease,Elderly,Lifestyle

Green tea and soybeans are popular in Japanese, but such intakes may differ between elderly and non-elderly populations. We investigated antioxidant foods (soybeans, vegetables, fruits)(<3, 3-4, >4 times/week) and beverages (green tea, black tea, coffee, wine)(<1, 1-3, >3 cups/day) intakes by questionnaires in 725 Japanese patients undergoing coronary angiography, of whom 373 were elderly (>65 years old). CAD was found in 517 patients, of whom 225 had myocardial infarction (MI). Percentages of patients taking soybeans >4 times/week, fruits >4 times/week, and green tea >3 cups/day were higher in elderly than in non-elderly (39%, 53% and 38% vs. 28%, 38% and 31%, P<0.025). Among non-elderly patients, percentages of patients taking soybeans and fruits <3 times/week were higher in patients with CAD than without CAD (37% and 48% vs. 28% and 34%, P<0.05), especially high in CAD patients with MI (42% and 56%). Among elderly patients, percentage of green tea nondrinkers (<1 cup/day) tended to be higher in patients with CAD (18%) than without CAD (13%), but highest in MI patients (25%, P<0.01). In multivariate analysis, among non-elderly, soybeans intake was a factor for CAD and MI. Odds ratios were 1.8 (95%CI=1.1-2.8) and 1.9 (95%CI=1.1-3.2) for soybeans <3 times/week. Among elderly, green tea intake was a factor for MI. Odds ratio was 2.3 (95%CI=1.3-4.0) for green tea <1 cup/day.

CONCLUSIONS: Elderly patients had more soybeans and green tea than non-elderly. In elderly, green tea intake was a factor for MI, whereas, in non-elderly, soybeans intake was a factor for CAD and MI.
Hypolipidemic effect of Emblica officinalis seeds in diabetes-induced experimental rats

Abstract nr. 39
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Diabetes, Dyslipidemia, Hypolipidemic Drugs

The antidiabetic and hypolipidemic potential of *E. officinalis* seeds have been reported for the first time by our research group. The present study was aimed to investigate the hypolipidemic effect of *Emblica officinalis* aqueous seed extract in long-term treatment of severely diabetic rats for 28 days. Rats were treated with the most effective dose of 300 mg kg\(^{-1}\) body weight of the extract identified in case of sub and mild-diabetic rats. Diabetes-induced enhanced levels of triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) were brought down significantly to 43.6, 40.3, 53.0 and 43.6% from *E. officinalis* seed extract. Glipizide was used as a reference drug for comparison. The extract has gained much attention due to potential improvement of 35.1% in high density lipoprotein-cholesterol (HDL-C) level from the extract in severely diabetic treated group. Since, lipid abnormality along with premature atherosclerosis is the major cause of cardiovascular diseases in diabetic patients, therefore the ideal treatment for diabetes, in additional to glycemic control, should have a favorable effect on lipid profile. The study has clinical implications since *E. officinalis* seed extract may reverse dyslipidemia associated with diabetes and prevent cardiovascular complications which are extensively prevalent in diabetic patients.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Obesity Paradox Still Exists After Percutaneous Coronary Intervention Independent of Metabolic Status

Abstract nr. 40
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Co-author(s) - Shin, Eak Kyun
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Intervention, Obesity

**Background:** We aimed to investigate the impact of obesity and its relation with metabolic parameters on clinical outcomes in patients undergoing PCI.

**Methods:** A total of 714 consecutive patients who underwent PCI were divided into three groups according to BMI: normal weight (BMI < 23.0 kg/m$^2$, n=211), overweight (23.0 ≤ BMI < 27.5 kg/m$^2$, n=375), and obese groups (BMI ≥ 27.5 kg/m$^2$, n=128). Lipid profile, fasting glucose, insulin, HbA1c, and insulin sensitivity were measured. Primary endpoint was 1-year major adverse cardiovascular events (MACEs), defined as the composite of all-cause death, non-fatal myocardial infarction (MI), stroke, revascularization, or admission for heart failure.

**Results:** The overweight or obese group had a higher incidence of hypertension, diabetes, and hyperlipidemia than the normal weight group. Left ventricular systolic dysfunction was more frequent in the normal group compared to the overweight or obese group (14.1% vs. 7.3% vs. 8.6%, p=0.026, respectively). Fasting glucose, HbA1c, LDL-cholesterol did not differ significantly between groups. However, obese group had higher levels of fasting insulin than normal weight group (12.5±13.6μU/mL vs. 8.6±12.4μU/mL, p=0.007). In addition, there was a significant difference of QUICKI between normal, overweight and obese groups (0.345±0.048 vs. 0.336±0.040 vs. 0.321±0.038, p<0.05 for each groups, respectively). The cumulative incidence of MACEs at 1 year was 15.6% for the normal group, 5.9% for the overweight group, and 3.9% for the obese group (p<0.001).

**Conclusions:** Overweight or obese patients had better clinical outcomes compared with normal weight patients after PCI although overweight or obese patients have more metabolic abnormality.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
The Prognostic Value of Mean Platelet Volume for Clinical Outcomes in Patients with Coronary Artery Disease Undergoing Percutaneous Coronary Intervention

Abstract nr. 41
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Co-author(s) - Kang, Woong Chul
Co-author(s) - Ahn, Taehoon
Co-author(s) - Shin, Eak Kyun

Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Thrombosis

Background: Platelet size, measured as mean platelet volume (MPV) has been reported to be correlated with platelet reactivity. Thus, we aimed to investigate the impact of MPV on 1-year clinical outcomes in patients undergoing PCI.

Methods: A total of 738 consecutive patients who underwent PCI were divided into two groups: the high MPV group (MPV ≥9.0fL, n=340, 46%) and the low MPV group (MPV <9fL, n=398, 54%). MACEs at 1 year, defined as the composite of all-cause death, non-fatal myocardial infarction (MI), stroke, revascularization, or admission for heart failure, were compared.

Results: The mean level of MPV was 9.0±1.1fL. The prevalence of acute MI was significantly higher in the high MPV group than in the low MPV group (69.1% vs. 30.4%, p<0.001). The high MPV group had higher level of CK-MB than the low MPV group (median [interquartile range], 31.7 ng/mL [3.0-143.7] vs. 2.5 ng/mL [1.3-11.2], p<0.001). In addition, systolic dysfunction was more frequent in the high MPV group compared with the low MPV group (13.3% vs. 5.6%, p<0.001). The cumulative incidence of MACEs at 1 year was significantly higher in the high MPV group than in the low MPV group (11.5% vs. 5.8%, p=0.004, respectively). By multivariate analysis adjusting for age, sex, peak level of CK-MB, or the presence of diabetes, acute MI or systolic dysfunction, the high MPV level was independently associated with 1-year MACEs (adjusted HR 2.52, 95% CI 1.34-4.76, p=0.004).

Conclusion: The level of MPV can be a powerful, independent predictor of 1-year MACEs in patients undergoing PCI.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
ROLE OF SERUM LIPIDS AND CARDIOVASCULAR DISEASE IN PATIENTS WITH HYPERHOMOCYSTEINEMIA.

Abstract nr. 42
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Lipids, Lp(a)

Introduction: Hyperhomocysteinemia is associated with increased risk of atherosclerotic disease especially stroke. Its relation with serum lipids has not been completely clarified. Our aim is to study serum lipids in patients with hyperhomocysteinemia.

Methods: Patients with serum homocysteine higher than 15 mg/dL were included. Total cholesterol, HDL, LDL, VLDL, triglycerides (TG) and Lp(a) were measured. BMI, smoking, obesity, hypertension, type-2 diabetes, coronary artery disease (CAD), cerebrovascular disease (CVD), chronic kidney disease (CKD), and peripheral artery disease (PAD) were reported. Descriptive statistics as well as correlation test and ANOVA one-way were performed.

Results: A total of 38 (22 women/16 men) patients were included. Mean age 65 (range 41-87) years-old. Obesity was found in 50%, smoking 28.9%, hypertension 55.3%, type-2 diabetes 62.6%, CAD 36.8%, CVD 28.9%, CKD 3.2%, and PAD 36.8%. Mean values of serum lipids were as follows: total cholesterol 258.76±22.85; TG 162.26±72.38; LDL 191.78±22.94; HDL 37.60±9.19; VLDL 11.15±7.78 and Lp(a): 7.55±10.02. All results are shown in mg/dl with standard deviation. Homocysteine: 20.94±3.25 μmol/l. Correlation test results: men smoked more than women (p=0.001), had more prevalence or PAD (p=0.035), and hypertensive patients had higher levels of VLDL (p=0.024). An ANOVA oneway test showed association between high level of Lp(a) and hyperhomocysteinemia (p=0.001).

Conclusions:
1.- In this hyperhomocysteinemic patients, the most common associated clinical conditions were type-2 diabetes, hypertension, obesity, CAD and PAD.
2.- Hypercholesterolemia and hypertriglyceridermia were predominant in these patients.
3.- Lp (a) was significantly increased in this group although few patients had a Lp (a) level higher than 30 mg/dL.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
HIV protein Nef causes dyslipidemia and atherosclerosis

Abstract nr. 44
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Cardiovascular Disease, HDL, Lipoproteins

HIV resides in a limited number of very specific cell types, but complications of HIV disease include metabolic abnormalities in tissues that are not infected by the virus. We hypothesized that viral proteins secreted from infected cells impair metabolism in uninfected cells and systemically. We investigated the effects of Nef, a secreted HIV protein responsible for the impairment of cholesterol efflux, on the development of atherosclerosis in two animal models. In apoE^{−/−} mice, injections of recombinant Nef increased the size of atherosclerotic lesions and caused vessel remodelling. Nef caused elevation of total cholesterol and triglyceride levels, while reducing high-density lipoprotein cholesterol levels in the plasma. These changes were accompanied by a reduction of Abca1 abundance in the liver but not in the vessels. In C57BL/6 mice on high fat/high cholesterol diet Nef caused a significant number of lipid-laden macrophages presented in adventitia of the vessels. Nef caused elevations of plasma triglyceride levels and obesity. We further investigated the lipoprotein profile of HIV patients infected with Nef-deficient virus (ΔNefHIV). The prevalence of hypoalphalipoproteinemia in these patients was reduced compared to patients infected with full virus approaching that of uninfected subjects. The size distribution of high-density lipoprotein particles was shifted toward smaller particles in HIV patients compared to uninfected subjects; this was absent in patients infected with ΔNefHIV. Our findings suggest that Nef causes dyslipidemia and accumulation of cholesterol in macrophages within the vessel wall, supporting the role of Nef in pathogenesis of atherosclerosis in HIV-infected patients.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Atherosclerosis in multiple vessel beds is related to a higher mortality risk: the Rotterdam Study

Abstract nr. 45
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Epidemiology, Imaging, Risk Factor

Objective
Atherosclerosis is a major contributor to global morbidity and mortality. We investigated whether atherosclerosis in different vessel beds contributes differentially to mortality, specifically focusing on cardiovascular mortality.

Methods
Between 2003 and 2006, a random sample of 2,413 participants from the population-based Rotterdam Study underwent computed tomography to quantify atherosclerotic calcification in the coronary arteries, the aortic arch, and the extracranial and intracranial internal carotid arteries. Follow-up for mortality was complete until January 1, 2012. We investigated relationships of calcification volume in each vessel bed with mortality using Cox regression models, adjusting for age, sex, and conventional cardiovascular risk factors. We additionally constructed a model in which we entered all vessel beds to investigate whether associations found per vessel bed were independent of calcification elsewhere.

Results
During 15,775 person-years of follow-up, 283 participants died, of whom 89 due to a cardiovascular cause. Larger calcification volumes in all vessels were related to a higher risk of all-cause mortality, cardiovascular mortality, and non-cardiovascular mortality, independent of cardiovascular risk factors. The strongest association was found for aortic arch calcification with cardiovascular mortality [age- and sex-adjusted hazard ratio per SD increase in calcification volume: 2.45(95% CI: 1.69; 3.57)]. The association between aortic arch calcification and cardiovascular mortality was independent of the amount of calcification elsewhere. We found no sex-specific differences.

Conclusion
Atherosclerotic load in major vessel beds is associated with an increased risk of death, independent of cardiovascular risk factors. In particular, aortic arch calcification yields unique information in addition to atherosclerosis elsewhere with regard to mortality.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Pigment Epithelium-Derived Factor is Associated with Necrotic Core Progression During Statin Therapy

Abstract nr. 46
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Prevention, Risk Factor, Vulnerable Plaque

Background: Pigment epithelium-derived factor (PEDF) is a potent inhibitor of angiogenesis and an important target molecule for preventing the progression of atherosclerosis. However, the relationship between PEDF and coronary atherosclerosis has not been fully examined. The aim of the present study is to evaluate the effects of statins on serum PEDF levels and the association between PEDF and coronary atherosclerosis.

Methods: Coronary atherosclerosis in non-culprit lesions of patients undergoing percutaneous coronary intervention (PCI) was evaluated using virtual histology intravascular ultrasound in 99 patients during PCI and after 8 months of statin therapy.

Results: Serum PEDF levels at baseline and at the 8-month follow-up did not differ. A significant decrease in the fibro-fatty component (-0.24 mm³/mm, p = 0.0003) and increases in the necrotic core (0.13 mm³/mm, p = 0.02) and dense calcium components (0.11 mm³/mm, p < 0.0001) were observed during the 8-month statin therapy. On univariate regression analyses, serum PEDF levels (r = 0.291, p = 0.004) and unstable angina pectoris (r = 0.203, p = 0.04) showed significant positive correlations with the percentage change in necrotic core volume. Multivariate regression analysis showed that serum PEDF level was a significant independent predictor associated with necrotic core progression during statin therapy (β = 0.218, p = 0.04).

Conclusions: Statin therapy had no effects on serum PEDF levels. Serum PEDF was a useful biomarker for predicting necrotic core progression during statin therapy, and its levels could be elevated as a counter-regulatory response mechanism to protect against necrotic core progression.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Different Regulation of Complement Activation by Native and Acetylated LDL Influences LDL and acetylated LDL Binding to Complement Receptor 1

Abstract nr. 47
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Immunity, Inflammation, LDL, Lipoproteins

Introduction: Lipoproteins can induce complement activation potentially resulting in opsonization and binding of these complexes to complement receptors. We investigated the binding of native LDL and acetylated LDL (acLDL) to the complement receptor 1 (CR1).

Methods: Binding of complement factors C3b, IgM, C1q, mannose-binding lectin (MBL) and properdin to LDL and acLDL were investigated by ELISA. Subsequent binding of opsonized LDL and acLDL to CR1 on CR1-transfected Chinese Hamster Ovarian cells (CHO-CR1) was tested by flow cytometry.

Results: Upon incubation with normal human serum both native LDL and acLDL induced complement activation with subsequent C3b opsonization. Opsonized LDL and acLDL bound to CR1. Binding to CHO-CR1 was reduced by EDTA, whereas MgEGTA only reduced the binding of opsonized LDL, but not of acLDL, suggesting involvement of the alternative pathway in the binding of acLDL to CR1. In vitro incubations showed that LDL bound C1q, whereas acLDL bound to C1q, IgM and properdin, an initiator of the alternative pathway. MBL did neither bind to LDL nor to acLDL. The relevance of these findings was demonstrated by ex vivo upregulation of CR1 on human leukocytes by LPS with a concomitant increased binding of apolipoprotein B containing lipoproteins to these leukocytes.

Conclusion: CR1 is able to bind C3b-opsonized native LDL and acLDL. C3b opsonization of LDL
is mediated via the classical pathway, whereas opsonization of acLDL is mediated via both the classical and alternative pathways. Binding of lipoproteins to CR1 may be of clinical relevance due to the ubiquitous cellular distribution of CR1.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
LincRNA-p21 Regulates Neointima Formation, Vascular Smooth Muscle Cell Proliferation, Apoptosis and Atherosclerosis by Enhancing p53 Activity

Abstract nr. 48
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Cardiovascular Disease, Genetics

**Background** -- Long non-coding RNAs (lncRNAs) recently have been implicated in many biological processes and diseases. Atherosclerosis is a major risk factor for cardiovascular disease. However, the functional role of lncRNAs in atherosclerosis is largely unknown.

**Methods and Results** -- We identified lincRNA-p21 as a key regulator of cell proliferation and apoptosis during atherosclerosis. The expression of lincRNA-p21 was dramatically down-regulated in atherosclerotic plaques of ApoE−/− mice, an animal model for atherosclerosis. Through loss- and gain-of function approaches, we showed that lincRNA-p21 represses cell proliferation and induces apoptosis in vascular smooth muscle cells (VSMCs) and mouse mononuclear macrophage cells in vitro. Moreover, we found that inhibition of lincRNA-p21 results in neointimal hyperplasia in vivo in a carotid artery injury model. Genome-wide analysis revealed that lincRNA-p21 inhibition dysregulated many p53 targets. Furthermore, lincRNA-p21, a transcriptional target of p53, feeds back to enhance p53 transcriptional activity, at least in part, via binding to mouse double minute 2 (MDM2), an E3 ubiquitin-protein ligase. The association of lincRNA-p21 and MDM2 releases MDM2 repression of p53, enabling p53 to interact with p300 and bind to the promoters/enhancers of its target genes. Finally, we show that lincRNA-p21 expression is decreased in coronary artery disease patients.

**Conclusions** -- Our studies identify lincRNA-p21 as a novel regulator of cell proliferation and apoptosis and suggest that this lncRNA could serve as a therapeutic target to treat atherosclerosis and related cardiovascular disorders.

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Rosuvastatin induced carotid plaque regression in patients with inflammatory joint diseases: The RORA-AS study

Abstract nr. 49
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Inflammation, Lipids, Prevention

Objective: Patients with rheumatoid arthritis (RA) and carotid artery plaques (CP) have increased risk of acute coronary syndromes. Statin treatment with low density lipoprotein cholesterol (LDL-c) goal ≤ 1.8 mmol/L is recommended for patients with CP in the general population. In the ROSuvastatin in Rheumatoid Arthritis, Ankylosing Spondylitis and other inflammatory joint diseases (RORA-AS) study, the aim was to evaluate the effect of 18 months intensive lipid lowering with rosuvastatin on change in CP height.

Methods: Eighty-six patients (60.5% female) with CP and IJD [RA (n=55), ankylosing spondylitis (n=21) and psoriatic arthritis (n=10)] were treated with rosuvastatin to obtain LDL-c goal. CP height was evaluated by B-mode ultrasound.

Results: Age was 60.8±8.5 years (mean±SD). Compliance of rosuvastatin use was median (IQR) 97.9% (96.0, 99.4). At baseline, median number and height of CP was 1.0 (range 1-6) and 1.80 mm (IQR 1.60, 2.10), respectively. Change in CP height after 18 months rosuvastatin treatment was -0.19±0.35 mm (p<0.001). Baseline and change in LDL-c was 4.0±0.9 mmol/L and -2.3±0.8 mmol/L (p<0.001). Mean LDL-c level during 18 months rosuvastatin treatment was 1.7±0.4 mmol/L (area under the curve). The degree of CP height reduction was independent of the LDL-c level exposure during the study period (p=0.36) (adjusted for age/gender/blood pressure). Attainment of LDL-c ≤ 1.8 mmol/L or the level of improvement in LDL-c did not influence the degree of CP height reduction (p=0.44 and p=0.46).

Conclusion: Intensive lipid lowering with rosuvastatin induced regression of CP height and reduced LDL-c significantly in patients with IJD.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Geraniol a major component of essential oil ameliorates endothelial dysfunction induced by high-fat diet fed rats.

Abstract nr. 53  
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention  
Keywords Animal model, Cardiovascular Disease, Endothelium, Prevention

Purpose of the study: Geraniol a major component of geranium oil and also found in essential oils of various spices and aromatic herbs. Geraniola monoterpene is known for potent anti-inflammatory, antihypertensive, hypoglycemic and antioxidant activity. Hence, the present study was designed to explore the vasorelaxant and cardioprotective property of geraniol in high fat diet (HFD) induced metabolic complications in rats.

Methods: Metabolic complications were induced by feeding HFD containing 20 % fat (Tallow) for 12 weeks. After confirmation of hypercholesterolemia (total cholesterol >150 mg/dl) at the end of 6 weeks, different doses of geraniol (100, 200, 400 mg/kg p.o) were administered for next 6 weeks. At the end of study plasma glucose, insulin, OGTT, lipid profile, antioxidants levels, lipid peroxidation, serum NO level, NOS activity in aorta, ECG changes, mean arterial pressure and endothelial dependent and independent vascular function in aorta were assessed.

Main finding: Administration of geraniol in HFD fed rats exhibited a significant decline in glucose, insulin resistance, triglyceride, total cholesterol, LDL levels and increase in HDL levels. Decreased serum NO level and NOS activity, increased oxidative stress along with impaired glucose tolerance associated with HFD were restored significantly by geraniol in dose dependent manner. Also increased MAP and ECG changes were normalized and reduced acetylcholine-induced, endothelium-dependent relaxation was improved significantly in geraniol treated rats (89.26 ± 0.17%).

Conclusions: Geraniol ameliorates endothelial dysfunction and metabolic complications in HFD fed rats by repressing insulin resistance, oxidative stress, lipid lowering effect with normalizing MAP, ECG changes & NO bioavailability.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference: Oral presentation  
Additional information
Role of Arterial AT1 Receptor on the Regulation of GRK4 in Hypertension

Abstract nr. 54
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Blood pressure, Cardiovascular Disease, Hypertension

G protein-coupled receptor kinase 4 (GRK4) gene variants, via impairment of renal dopamine receptor and enhancement of renin-angiotensin system functions, cause sodium retention and increase blood pressure. Whether or not GRK4 and the angiotensin type 1 receptor (AT1R) interact in the aorta is not known. We report that GRK4 is expressed in vascular smooth muscle cells (VSMCs) of the aorta. Heterologous expression of the GRK4γ variant 142V in A10 cells increased AT1R protein expression and AT1R-mediated increase in intracellular calcium concentration. The increase in AT1R expression was related to an increase in AT1R mRNA expression via the nuclear factor κB (NF-κB) pathway. As compared with control, cells expressing GRK4γ 142V had greater NF-κB activity with more NF-κB bound to the AT1R promoter. The increased AT1R expression in cells expressing GRK4γ 142V was also associated with decreased AT1R degradation, which may be ascribed to lower AT1R phosphorylation. There was a direct interaction between GRK4γ wild-type (WT) and AT1R that was decreased by GRK4γ 142V. The regulation of AT1R expression by GRK4γ 142V in A10 cells was confirmed in GRK4γ 142V transgenic mice; AT1R expression was higher in the aorta of GRK4γ 142V transgenic mice than control GRK4γ wild-type (WT) mice. Angiotensin II-mediated vasoconstriction of the aorta was also higher in GRK4γ 142V than WT transgenic mice. This study provides a mechanism by which GRK4, via regulation of arterial AT1R expression and function, participates in the pathogenesis of conduit vessel abnormalities in hypertension.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
Targeting MG53-mediated cell membrane repair for treatment of acute lung injury

Abstract nr. 55
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Inflammation

Injury to lung epithelial cells participates in the pathogenesis and progression of multiple lung diseases. Our previous studies identified mitsugumin 53 (MG53) as a key component of the cell membrane repair machinery in striated muscle cells. Here we present evidence that MG53 is also expressed in lung tissue. The mg53-/- mice show increased susceptibility to ischemia-reperfusion and over-ventilation induced injury to the lung when compared with wild type mice. In vitro studies demonstrate that extracellular application of the recombinant human MG53 (rhMG53) protein protects against injuries to lung epithelial cells. In vivo delivery of rhMG53 by intravenous or inhalation routes reduces symptoms in rodent models of acute lung injury and emphysema. Repetitive administration of rhMG53 improves pulmonary structure associated with chronic lung injury. Our data indicate a physiological function for MG53 in the lung and suggest that targeting membrane repair may be an effective means for treatment or prevention of lung diseases.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Calpastatin counteracts pathological angiogenesis by inhibiting suppressor of cytokine signalling 3 degradation in vascular endothelial cells

Abstract nr. 58
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Angiogenesis, Endothelium

Pathological angiogenesis is known to exacerbate cancer growth and chronic inflammatory diseases including atherosclerosis. Janus kinase/signal transducer and activator of transcription (JAK/STAT) signals and their endogenous inhibitor suppressor of cytokine signalling 3 (SOCS3) in vascular endothelial cells (ECs) reportedly dominate the pathological angiogenesis. However, how these inflammatory signals are potentiated during pathological angiogenesis has not been fully elucidated. We suspected that an intracellular protease calpain, which compose the multifunctional proteolytic systems together with its endogenous inhibitor calpastatin (CAST), contributes to the JAK/STAT regulations. Our present data showed that the loss of CAST is detectable in neovessels in murine allograft tumours, some human malignant tissues and oxygen-induced retinopathy (OIR) lesions in mice. EC-specific transgenic introduction of CAST caused down-regulation of JAK/STAT signals, up-regulation of SOCS3 expression and depletion of vascular endothelial growth factor (VEGF)-C, thereby counteracting unstable pathological neovessels and disease progression in tumours and OIR lesions in mice. Neutralizing antibody against VEGF-C ameliorated pathological angiogenesis in OIR lesions. Small-interfering RNA-based silencing of endogenous CAST in cultured ECs facilitated μ-calpain-induced proteolytic degradation of SOCS3, leading to VEGF-C production through amplified interleukin-6-driven STAT3 signals. Interleukin-6-induced angiogenic tube formation in cultured ECs was accelerated by CAST silencing, which is suppressible by pharmacological inhibition of JAK/STAT signals, antibody-based blockage of VEGF-C and transfection of calpain-resistant SOCS3, while transfection of wild-type SOCS3 exhibited modest angiostatic effects. Hence, loss of CAST in angiogenic ECs facilitates μ-calpain-induced SOCS3 degradation, which amplifies pathological angiogenesis through interleukin-6/STAT3/VEGF-C axis. (Miyazaki T. et al., Circ Res., In press)
Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
Improved endothelial function is associated with reduced arterial stiffness and atherosclerotic regression in rosuvastatin treated patients with inflammatory joint diseases.

Abstract nr. 59
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Keywords Atherosclerosis, Endothelium, Hypolipidemic Drugs, Inflammation

Background Endothelial dysfunction is the first step in the formation of atherosclerotic lesions and can be quantified by the degree of flow mediated vasodilation (FMD) of the brachial artery. Low FMD is a predictor of cardiovascular events. In addition, FMD is lower in patients with inflammatory joint diseases (IJD) compared to the general population. Our aim was to investigate if long-term rosuvastatin treatment in IJD patients with carotid plaques (CP) improves FMD. Furthermore, associations between change in FMD (ΔFMD) and change in CP height, arterial stiffness [aortic pulse wave velocity (aPWV) and augmentation index (AIx)], lipids and inflammatory variables were evaluated.

Methods Eighty five statin naïve IJD patients (rheumatoid arthritis: 53, ankylosing spondylitis: 24, psoriatic arthritis: 8) with ultrasound verified CP received treatment with rosuvastatin for 18 months to obtain low density lipoprotein cholesterol goal ≤1.8 mmol/L. All patients underwent assessment of FMD, aPWV, AIx and carotid ultrasound at baseline and at study end.

Results The mean±SD FMD was significantly improved from 7.10±3.14% at baseline to 8.70±2.98% at study end (p<0.001). Multiple linear regression analyses with ΔFMD as the dependent variable revealed a significant association with area under the curve erythrocyte sedimentation rate (p= 0.04), improvement in AIx (p=0.05) and CP height regression (p=0.001). The final linear regression model explained 31.1% of the variance in ΔFMD (R²=0.311).

Conclusion Long-term intensive lipid lowering with rosuvastatin improved FMD in IJD patients with atherosclerotic disease. The improved endothelial function was associated with reduced arterial stiffness, CP height decrement and level of inflammation.
Rosuvastatin improves arterial stiffness in patients with inflammatory joint diseases and established atherosclerosis: Results from the RORA-AS study

Abstract nr. 60
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Hypertension, Hypolipidemic Drugs, Inflammation

Background Arterial stiffness, as pulse wave velocity (PWV) and augmentation index (AIx), are early risk markers of cardiovascular disease (CVD). Intensive statin treatment induces carotid plaque (CP) regression in patients with inflammatory joint diseases (IJD). We evaluated the effect of rosuvastatin treatment on arterial stiffness in IJD patients with CP.

Methods The study population included 89 statin naïve IJD patients (rheumatoid arthritis: 55, ankylosing spondylitis: 23, psoriatic arthritis: 11). All patients had ultrasound verified CP and received rosuvastatin therapy over 18 months. PWV and AIx were measured at baseline and end of the study. Change in PWV and AIx from baseline was assessed with paired t-tests. Logistic regression analyses were performed with PWV and AIx as outcome variables, defined as decrease or no change/increase during the study, to assess for associations with other outcome measures.

Results From baseline to study end, mean (SD) AIx and PWV was significantly improved from 27.9 (7.7) % and 8.1 (1.6) m/s² to 26.2 (8.2) % (p=0.03) and 7.8 (1.5) m/s² (p=0.03), respectively. The logistic regression models revealed associations between: 1) PWV and change in systolic blood pressure (sBP) (p=0.008) and a lower area under the curve sBP (p=0.03), adjusted for antihypertensive medication. 2) AIx and change in CP height (p=0.03) and rosuvastatin dose (p=0.01). All associations were robust to adjustments for traditional CVD risk factors.

Conclusion Rosuvastatin therapy significantly improved arterial stiffness in IJD patients with CP. The improvement was associated with sBP change, rosuvastatin dose and atherosclerotic regression.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Mechanisms underlying the vasorelaxation of human internal mammary artery induced by epicatechin

Abstract nr. 61
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Aim: Many studies have indicated an association of an improved cardiovascular prognosis with flavanol-rich diets, which a major bioactive constituent seems to be epicatechin. Pure epicatechin has been reported to induce vasodilation and inhibit the vascular expression of proinflammatory and proatherogenic markers. Because the exact mechanisms by which epicatechin causes vasodilation are uncertain, we aimed to investigate vasorelaxant effect of epicatechin on the isolated human internal mammary artery (HIMA) and its underlying mechanisms.

Methods: Discarded segments of HIMA were collected from patients undergoing coronary artery bypass grafting and studied in organ baths.

Results: Epicatechin induced a concentration-dependent relaxation of HIMA rings pre-contracted by phenylephrine. Four-aminopyridine and margatoxin, blockers of voltage-gated K⁺ (Kᵥ) channels, and glibenclamide, a selective ATP-sensitive K⁺ (KᵥATP) channels blocker, partly inhibited the epicatechin-induced relaxation of HIMA, while iberiotoxin, a most selective blocker of large conductance Ca²⁺-activated K⁺ channels (BKCa), almost completely inhibited the relaxation. In rings pre-contracted by 80 mM K⁺, epicatechin induced partial relaxation of HIMA, whereas in Ca²⁺-free medium, epicatechin completely relaxed HIMA rings pre-contracted by phenylephrine and caffeine. Finally, thapsigargin, a sarcoplasmic reticulum Ca²⁺-ATPase inhibitor, slightly antagonized epicatechin-induced relaxation of HIMA pre-contracted by phenylephrine.

Conclusions: These results suggest that epicatechin induces strong endothelium-independent relaxation of HIMA pre-contracted by phenylephrine whilst 4-aminopyridine- and margatoxin-sensitive Kᵥ channels, as well as BKCa and KᵥATP channels, located in vascular smooth muscle, mediate this relaxation. In addition, it seems that epicatechin could inhibit influx of extracellular Ca
2+, interfere with intracellular Ca^{2+} release and re-uptake by the sarcoplasmic reticulum.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Impaired dopamine D1 receptor-mediated vasorelaxation of mesenteric arteries in obese Zucker rats

Abstract nr. 62
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease

Background: Obesity plays an important role in the pathogenesis of hypertension. Renal dopamine D1 receptor-mediated natriuresis is impaired in the obese Zucker rat. The role of arterial D1 receptors in the hypertension of obese Zucker rats is not clear.

Methods: Plasma glucose and insulin concentrations and blood pressure were measured. The vasodilatory response of isolated mesenteric arteries was evaluated using a small vessel myograph. The expression and phosphorylation of D1 receptors were quantified by co-immunoprecipitation and immunoblotting. To determine the effect of hyperinsulinemia and hyperglycemia on the function of the arterial D1 receptor, we studied obese Zucker rats fed vehicle or rosiglitazone, an insulin sensitizer and lean Zucker rats, fed high-fat diet to induce hyperinsulinemia or injected intraperitoneally with streptomycin to induce hyperglycemia.

Results: In obese Zucker rats, the vasorelaxant effect of D1 receptors was impaired that could be ascribed to decreased arterial D1 receptor expression and increased D1 receptor phosphorylation. In these obese rats, rosiglitazone normalized the D1 receptor expression and phosphorylation and improved the D1 receptor-mediated vasorelaxation. We also found that D1 receptor-dependent vasorelaxation was decreased in lean Zucker rats with hyperinsulinemia or hyperglycemia. The ability of insulin and glucose to decrease D1 receptor expression and increase its phosphorylation were confirmed in studies of rat aortic smooth muscle cells.

Conclusions: Both hyperinsulinemia and hyperglycemia caused D1 receptor dysfunction by decreasing arterial D1 receptor expression and increasing D1 receptor phosphorylation, which is involved in the pathogenesis of obesity-related hypertension.

Keywords: D1 receptor, Vasorelaxation, Hyperinsulinemia, Hyperglycemia, Obesity-related hypertension

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
Development of A TransAtlantic Cardiovascular risk Calculator for Rheumatoid Arthritis (ATACC-RA) on behalf of the ATACC-RA consortium

Abstract nr. 63
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Risk Factor, Risk stratification

Purpose: Patients with rheumatoid arthritis (RA) have an increased risk of cardiovascular disease (CVD), which is not accurately predicted by risk calculators designed for the general population. Our aim was to develop a RA specific CVD risk calculator.

Methods: RA patients from 8 centres in 7 countries were included. CVD outcomes (MI, revascularization, angina, stroke, TIA, PAD and CVD death) were collected prospectively. RA characteristics (duration, seropositivity, disease activity (DAS28) and CRP/ESR) and traditional CVD risk factors were collected at baseline. Cox models stratified by centre were used to develop a CVD risk calculator considering traditional CV risk factors and RA characteristics. Model performance was assessed using discrimination and calibration.

Results: In total 3176 RA patients without prior CVD were included (mean age: 55 [SD: 14] years, 73% female). During a mean follow-up of 7.8 years (24733 person years), 314 had a CVD event. The multivariable risk evaluation revealed 2 models including either seropositivity or DAS28 along with age, sex, current smoking, presence of hypertension, and ratio of total cholesterol to high-density lipoprotein. Both 10-fold cross validation and multiple imputation analyses confirmed these findings. Both models demonstrated good discrimination (c-statistic: 0.76 and 0.74) and calibration (observed/predicted ratio: 1.00; 95% confidence interval: 0.89, 1.12). The ATACC-RA (mean: 11.5%, SD 14.1%) showed significantly improved discrimination compared to either Framingham (c-statistic: 0.71, p<0.001) or SCORE (c-statistic: 0.72, p<0.001) risk algorithms.

Conclusions: Development of an RA-specific CVD risk calculator is feasible by pooling resources from many centres. Further development including external validation is underway.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
ENDOTHELIAL DYSFUNCTION IN STREPTOZOTOCIN INDUCED DIABETES AND INFLUENCE OF GERANIUM OIL.

Abstract nr. 64
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Animal model, Diabetes, Endothelium, Inflammation

Aim: Endothelial dysfunction is a critical factor during the initiation of diabetic cardiovascular complications and antioxidant effect plays a pivotal role in this setting. The present study aimed to investigate whether the essential oil therapy with geranium oil and its major component like citronellol can provide protection against diabetes-associated endothelial dysfunction and elucidate the possible mechanism(s) underlying this effect.

Methods: Diabetes was induced by intraperitoneal injection of Streptozotocin (45 mg/kg) in rats. Influence of geranium oil (GO) and citronellol (each 100, 200 & 400 mg/kg, orally, 1 month) on TNF-α, oxidative stress parameters, NOS activity and vascular function were evaluated.

Results: Our results showed a marked increase in aortic superoxide anion (O2−) production and serum malondialdehyde level alongside attenuating antioxidant enzyme capacities in diabetic rats. This was associated with a significant increase in TNF-α serum level of diabetic rats alongside reducing aortic NOS activity and nitric oxide (NO) bioavailability. Administration of GO & its components citronellol significantly inhibited these changes. However, the vascular endothelium-dependent relaxation with acetylcholine in aorta of diabetic rat was significantly ameliorated by citronellol (90.16 ± 0.17%). Citronellol proves more promising component for beneficial effect of essential oil.

Conclusions: Collectively, our results demonstrated that the essential oil therapy of GO affords beneficial effects through its major component citronellol against diabetes-associated endothelial dysfunction, probably through normalizing the deregulated NOS and reducing the inflammation and oxidative stress in diabetic rats. It is noteworthy; that the essential oil therapy exhibited a significant response over the diabetic complications.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Heterogeneity of traditional and un-traditional Cardiovascular Disease Risk Factors and Events in Patients with Rheumatoid Arthritis across 10 Countries

Abstract nr. 65
Author Semb, Anne Grete, Diakonhjemmet Hospital, Oslo, Norway
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Risk Factor, Risk stratification

**Purpose:** Cardiovascular disease (CVD) risk calculators for the general population do not accurately predict CVD events in rheumatoid arthritis (RA). We aimed to compare the impact of classical CVD risk factors and RA characteristics on CVD outcomes in RA patients across 10 countries.

**Methods:** CVD risk factors and RA characteristics from 13 rheumatology centers were collected at baseline. Cox-models were used to compare the impact of CVD risk factors and RA characteristics on events, and were adjusted for age/sex and age/sex/CVD risk factors.

**Results:** 5685 RA patients without prior CVD were included (mean age: 55 [SD: 14] years). During a mean follow-up of 6.1 years (31155 person-years), 476 patients developed CVD events. Mean age varied: 37-61 years. Norway and UK had the lowest CVD event rates, and South Africa, Netherlands, US-Mayo and Sweden the highest. Age effects were fairly consistent (hazard ratios [HR] from 1.6-1.8 per 10-year increase in age), but male sex varied from no effect to a doubled effect (HR=1.0-2.3). Varied effects were also seen for current smoking (HR=1.1-2.1), hypertension (HR=0.6-2.0), total cholesterol:high-density lipoprotein ratio (HR=0.9-1.2) and diabetes mellitus (HR=0.7-2.8). Effects varied also for RA characteristics, including rheumatoid factor and/or anti-citrullinated protein antibody seropositivity (HR=0.7-1.4), joint disease activity (HR=0.9-1.2) and RA disease duration (HR=0.7-1.1).

**Conclusions:** Major heterogeneity exists in CVD event rates and in impact of classical CVD risk factors and RA characteristics on CVD outcomes among RA patients across different countries, and is a challenge when developing an RA specific CVD risk calculator for international use.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Mini-oral presentation
Additional information
Impact of asymptomatic atherosclerosis on cardiovascular risk stratification and consequences for lipid lowering prevention in patients with inflammatory joint diseases

Abstract nr. 66
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Prevention, Risk Factor, Risk stratification

**Purpose:** Intensive lipid lowering treatment (LLT) is recommended for patients with carotid plaque (CP). Patients with inflammatory joint diseases (IJD) have a high frequency of CP, and we aimed to evaluate the impact of CP on cardiovascular disease (CVD) risk stratification and consequences for lipid lowering prevention in patients with IJD.

**Methods:** CVD risk stratification in IJD patients (n= 334) was performed using the SCORE algorithm and by performing ultrasound of the carotid arteries. Cross-tabulations, Chi$^2$ and ROC-curves were used to calculate sensitivity/specificity for the SCORE algorithm. The ROC curves closest point (0.1) and 80 % sensitivity were used for optimizing CVD risk classification.

**Results:** In 249 patients with IJD and a calculated risk <5%, 98 (39.4%) had CP and should receive intensive LLT. In patients with a calculated risk >5% & <10% + LDL>2.5 mmol/L (n=58), 38 (65.5 %) patients had CP and should receive intensive LLT. Thus, patients with CP who were wrongly classified to receive no or only moderate instead of intensive LLT, was 39.4% and 65.5%, respectively. Taken together, 136/307 (44.3%) of these patients would receive inadequate LLT. The sensitivity (correctly classifying patients with IJD + CP) was 0.39 and the specificity was 0.83. Optimizing SCORE cut off for very high risk, by area under the ROC curves' closest point (0, 1) or 80% sensitivity did not improve correct CVD risk stratification in congruence with recommended standards.

**Conclusion:** Carotid ultrasound contributes to optimized CVD risk classification with consequences for CVD preventive LLT in patients with IJD.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
A healthy diet is associated with less endothelial dysfunction and less low-grade inflammation over a 7-year period

Abstract nr. 67
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Endothelium, Epidemiology, Inflammation, Nutrition

A healthy diet rich in fish, fruit and vegetables, but low in alcohol, dairy products and meat, has been associated with less incident cardiovascular disease (CVD). Endothelial dysfunction and low-grade inflammation play important roles in CVD. A healthy diet might modify these phenomena. We investigated the association between the above food groups and biomarker scores of endothelial dysfunction and low-grade inflammation. In 557 participants with increased CVD risk (baseline age 59.6 ± 6.9 years) we measured diet by FFQ. Biomarkers of endothelial dysfunction (von Willebrand factor, and soluble vascular cell adhesion molecule 1, endothelial selectin, thrombomodulin, intercellular adhesion molecule 1 (sICAM-1)) and low-grade inflammation (C-reactive protein, serum amyloid A, interleukin 6, interleukin 8, tumor necrosis factor α and sICAM-1) were measured and combined into overall scores (higher scores indicating worse function). Longitudinal data, at baseline and after 7 years, were analyzed with generalized estimating equations and adjusted for confounders. Higher consumption of fish (per 100 g/wk), but not vegetables, fruit, alcohol-containing beverages, dairy products or meat, was associated with a lower endothelial dysfunction score over 7 years: β(95% CI) -0.027(-0.051; -0.004). No associations were observed with the overall low-grade inflammation score. Food component analyses indicated that more lean fish and raw vegetables, and less high-fat dairy products were associated with less endothelial dysfunction. Consumption of more fresh fruit, wine and poultry, and less high-fat dairy products was associated with less low-grade inflammation.

This suggests that dietary modification of endothelial dysfunction and low-grade inflammation, processes that are important in atherothrombosis, is possible.

Subdivision 4. Not applicable. Abstract matches with track c
Presentation Preference Oral presentation
Additional information
Blend of Unrefined Sesame and Rice bran oils Exhibits Lipid-lowering and Anti-hyperglycemic Potentials in Patients with Type 2 Diabetes Mellitus

Abstract nr. 68
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes, Lipids, Nutrition, Therapy

By considering the substantial health benefits of sesame and rice bran oils, the current study is to examine the extent to which the daily use of the blend of sesame and rich rice bran oils (20:80) as cooking oil beneficial in type 2 diabetes mellitus (T2DM). This open-label, randomized, dietary intervention comprised of 300 patients with T2DM and 100 normoglycemic subjects. Oils blend was supplied to T2DM patients, with (n=100; glibenclamide (5mg/d)) or without (n=100) anti-diabetic medication, and normoglycemic subjects while 100 T2DM patients were treated with glibenclamide (5mg/d) only. The groups supplied with the oils blend were instructed to use it as the only cooking oil for 60 days. Fasting and postprandial blood glucose was measured at days 0, 30 and 60. HbA₁C and lipid profile (TC, TG, LDL-C and HDL-C) were measured at days 0 and 60. Blood glucose was significantly lowered from 30 days, and HbA₁C and lipid profile were significantly improved at 60 days in T2DM patients substituted with the oils blend only while no significant changes were observed in normoglycemic subjects. Glibenclamide alone treatment significantly lowered blood glucose and HbA₁C only where as glibenclamide plus oils blend treated group showed a remarkable reduction of glucose from 30 days, and significantly improved HbA₁C and lipid profile after 60 days. The study reveals the fact that the blend of sesame and rice bran oils exhibits lipid-lowering and anti-hyperglycemic potentials and also exhibits additive effect with anti-diabetic drug for the effective management of T2DM.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Prevalence of low high-density lipoprotein cholesterol stratified by risk category and gender among Arabian Gulf patients in the CEPHEUS

Abstract nr. 69
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, HDL, Hypolipidemic Drugs, LDL

Objective: To estimate the prevalence of low HDL-C among patients in the Centralized Pan-Middle East Survey on the undertreatment of hypercholesterolemia (CEPHEUS) stratified by risk category (according to the joint Consensus Statement of the American Diabetes Association (ADA) and American College of Cardiology (ACC) Foundation) and gender.

Methods: CEPHEUS was conducted in patients (≥18 years of age) in six Middle Eastern countries between November 2009 and July 2010 on lipid lowering drugs (LLDs). Serum samples were used to measure lipid parameters.

Results: The overall mean age of the cohort were 56±11 years and majority were males (58%). The overall prevalence of low HDL-C was 49%. Low HDL-C was more prevalent in female compared to male (53% vs 46%; p<0.001). In the high risk group the prevalence of low HDL-C was 40% compared to 53% in the highest risk group patients (p<0.001). The prevalence of low HDL-C in the high risk group in patients who achieved LDL-C target of <2.6 mmol/l was 48% compared to 52% in those who didn't achieved LDL-C target of <2.6 mmol/l (p=0.073). In the highest risk group the prevalence of low HDL-C in patients who achieved LDL-C target of <1.8 mmol/l was 28% compared to 72% in those who didn't achieved LDL-C target of <1.8 mmol/l (p<0.001).

Conclusions: Patients in the Middle East on LLDs have a high prevalence of low HDL-C and is more seen in the highest risk group and female patients. These patients may remain at increased residual risk for CVD.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Hyperglycaemic spikes increase monocytes and atherosclerosis in normoglycaemic mice through a RAGE dependent mechanism.

Abstract nr. 70
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Diabetes

Introduction
Postprandial hyperglycaemic spikes are a major risk factor for cardiovascular disease in diabetes. We hypothesized that hyperglycaemic spikes increase circulating monocyte levels and atherosclerosis through receptor for advanced glycation endproducts (RAGE) signaling on monocyte precursors.

Methods
We injected wildtype female C57Bl/6 mice 4 subsequent times with glucose to induce hyperglycaemic spikes (~25mmol/l) and sacrificed them after 1 and 7 days of normoglycaemia. We quantified common myeloid (CMP) and granulocyte-monocyte precursors (GMP) and circulating monocyte subsets (Ly6C<sup>hi</sup> and Ly6C<sup>lo</sup>) with flow cytometry. To determine if this translated into accelerated atherosclerosis, we induced weekly hyperglycaemic spikes for 10 weeks in Apoe<sup>−/−</sup> mice. To examine the role of haematopoietic RAGE, we transplanted Rage<sup>−/−</sup> bone marrow into irradiated wildtype mice.

Results
We found in the bone marrow that the CMP and GMP were increased 2.2 and 1.3 fold after 1 day and normalized 7 days after glucose spiking, resulting in a significant increase in monocytes after 7 days (especially Ly6C<sup>hi</sup> subset). Hyperglycaemic spikes increased atherosclerotic burden in the aortic arch 2-fold. Interestingly, 1 day after glucose spiking, the expression of RAGE ligand S100A8/A9 in white blood cells and expression of RAGE on CMPs were increased. Subsequently, mice transplanted with Rage<sup>−/−</sup> bone marrow were protected against monocytosis induced by hyperglycaemic spikes.

Conclusion
These results reveal potential harm of hyperglycaemic spikes by acting on haematopoietic progenitors, stimulating monocyte production. This is dependent on RAGE signaling, with S100A8/A9 as a potential ligand. Preventing hyperglycaemic spikes or RAGE signaling may reduce cardiovascular risk in people with diabetes.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Metalloproteinases behaviour in epicardial adipose tissue and its association with coronary artery disease

Abstract nr. 71
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Angiogenesis, Cardiovascular Disease, Risk Factor

Background: Epicardial adipose tissue (EAT) is a metabolically active visceral adipose tissue surrounding and infiltrating myocardium and coronary arteries. An excessive amount of EAT may represent injury for the myocardium. Metalloproteinases are endopeptidases involved in expansion of adipose tissue as well as in atherosclerosis; however, there is no evidence about MMP’s behavior in EAT. Objective: evaluate MMP-2 and -9 in EAT from patients with coronary artery disease (CAD) and their relationship with morphologic EAT characteristics. Subjects and Results: EAT and subcutaneous adipose tissue (SAT) were obtained from patients undergoing heart surgery for coronary artery bypass graft (CAD, N=17) or valve replacement (No CAD, N=15). In EAT and SAT, MMP-2 and -9 localization and activity, vascular density (VD), size and adipocyte density and inflammatory cell infiltration were determined. MMP-2 activity was significantly increased in EAT from CAD compared to No CAD patients (1.86±0.54 vs 1.43±0.23 RU, p=0.031). In EAT from CAD patients, we observed an increase in MMP-2 (1.86±0.54 vs 1.39±0.33 RU, p=0.038) and MMP-9 (1.53±0.61 vs 1.19±0.28 RU, p=0.049) activities compared to SAT. VD was significantly higher in EAT from CAD compared to No CAD (p=0.015) and it was directly correlated with MMP-2 (p=0.006) and MMP-9 (p=0.02) activity. Conclusions: EAT from CAD patients presents higher gelatinases activity than EAT from No CAD patients and it could be responsible for the higher VD observed. The increased of MMPs activity and inflammatory infiltrate in EAT could be linked to the plaque vulnerability and the higher cardiometabolic risk in CAD.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Role of SN1 Lipases on Plasma Lipids in Metabolic Syndrome and Obesity

Abstract nr. 72
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Lipoproteins,Metabolism,Obesity

Objective: To assess the phospholipase activity of endothelial (EL) and hepatic lipase (HL) in postheparin plasma of subjects with metabolic syndrome (MS)/obesity and their relationship with atherogenic and antiatherogenic lipoproteins. Additionally, to evaluate lipoprotein lipase (LPL) and HL activity as triglyceride (TG)-hydrolyses to complete the analyses of SN1 lipolytic enzymes in the same patient.

Results: Plasma EL, HL, and LPL activities were evaluated in 59 patients with MS and 36 controls. A trend toward higher EL activity was observed in MS. EL activity was increased in obese compared with normal weight group (P=0.009) and negatively associated with high-density lipoprotein–cholesterol (HDL-c) (P=0.014 and P=0.005) and apolipoprotein A-I (P=0.045 and P=0.001) in control and MS group, respectively. HL activity, as TG-hydrolase, was increased in MS (P=0.025) as well as in obese group (P=0.017); directly correlated with low-density lipoprotein–cholesterol (P=0.005) and apolipoprotein B (P=0.003) and negatively with HDL-c (P=0.021) in control group. LPL was decreased in MS (P<0.001) as well as in overweight and obese compared with normal weight group (P=0.015 and P=0.004, respectively); inversely correlated %TG-very low-density lipoproteins (P=0.04) and TG/apolipoprotein B index (P=0.013) in control group. These associations were not found in MS.

Conclusions: We describe for the first time EL and HL activity as phospholipases in MS/obesity, being both responsible for HDL catabolism. Our results elucidate part of the controversies about SN1 lipases in MS and different grades of obesity. The impact of insulin resistance on the activity of the 3 enzymes determines the lipoprotein alterations observed in these states.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
EFFECT OF EMAP-II TO CARDIAOHEMODYNAMICS IN HYPERTENSION.

Abstract nr. 73
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Endothelium, Hypertension

Endothelial monocyte-activating polypeptide-II (EMAP-II) is a multifunctional polypeptide with proinflammatory and antiangiogenic activity. Enhancing of low-grade chronic inflammation is associated with cardiovascular diseases and hypertension. However, the role of this cytokine in hypertension is not understood.

The aim of study was investigate effect of EMAP II on heart function of spontaneously hypertensive rats (SHR). The researches were conducted on six-month SHR male rats. The functional cardiohemodynamic indicators registered via Pressure-Volume System.

It was shown that stroke volume increased by 18.2%, cardiac output – by 22% after EMAP II in SHR. The end-diastolic myocardial stiffness reduced in 4.7 times, arterial stiffness decreased by 23.2 in SHR after EMAP II.

Thus, in hypertension EMAP II has the positive effect on the reduction of arterial stiffness, end-systolic- and maximum myocardial stiffness, increase indices of heart pump function, improvement of left ventricular relaxation.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Targeted Next-Generation Sequencing to Diagnose Abnormalities of HDL Cholesterol

Abstract nr. 74
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Genetics, HDL, Lipids, Lipoproteins

Background: A low level of high-density lipoprotein cholesterol (HDL-C) is the most common lipid abnormality in patients with premature coronary artery disease. Individuals with very low or very high levels of HDL-C frequently have rare mutations in one of several genes, suggesting that a molecular diagnosis could be made in these patients. However, identification of the specific molecular abnormality in patients with extreme HDL-C is rarely performed in clinical practice. The objective of this study was to investigate the analytic validity and diagnostic yield of a targeted next-generation sequencing (NGS) assay for extreme levels of HDL-C.

Methods and Results: We developed a targeted NGS panel to capture the exons, intron/exon boundaries and untranslated regions of genes with highly penetrant effects on plasma lipid levels. We sequenced 92 patients with very low or very high levels of HDL-C recruited from a large specialty lipid clinic. We also included 6 patients with known Mendelian disorders of HDL in whom pathogenic mutations had previously been identified, and 1 family with a suspected Mendelian disorder of HDL in whom no mutation had previously been identified. We prioritized variants in accordance with medical genetics guidelines. All variants subsequently underwent validation by bidirectional Sanger sequencing. Overall, we established a molecular diagnosis in 40% of patients with low HDL-C and 8% of patients with high HDL-C. One hundred percent of prioritized variants were confirmed by Sanger sequencing and all previously known variants in patients with Mendelian disorders of HDL were accurately detected by NGS. Functional studies on a subset of these variants confirmed that they resulted in loss-of-function of the encoded proteins.

Conclusions: Our results suggest that a molecular diagnosis can be established in a substantial proportion of patients with low HDL-C, but in only a small percentage of patients with high HDL-C. Our customized NGS assay has positive predictive value and sensitivity approaching 100% for identifying these variants. Molecular diagnosis of disorders of lipid metabolism has the potential to refine diagnostic categories and may lead to new therapeutic strategies.
Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Different effect of statins on inducing diabetes mellitus: An experimental study

Abstract nr. 77
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Diabetes, Intervention

Background: To determine whether individual statins had differing effects on inducing diabetes mellitus process.
Methods: Four kinds of statins, atorvastatin, pravastatin, rosuvastatin, and pitavastatin were selected to determine the insulin secretion and resistance. The cytotoxicity, insulin secretion and glucose-stimulated insulin secretion were investigated in human pancreas islet β cells. The decrease of glucose uptake in human skeletal muscle cells was also investigated.
Results: Human pancreas islet β cells treated with 100 μM of atorvastatin, pravastatin, rosuvastatin, and pitavastatin. Cell viability was reduced by 32.12%, 41.09%, 33.96%, 29.19% relative to control cells, and insulin secretion rate was reduced by 34.07%, 30.06%, 26.78%, 19.22% relative to control cells, respectively. The insulin secretion stimulated by high glucose concentration (28 mmol/L) was significantly higher than by the physiological concentration (5.6 mmol/L) all the treatment groups, which ranged from 53.44 ng/mL to 78.32 ng/mL vs. from 35.78 ng/mL to 54.22 ng/mL. The glucose uptake rates of human skeletal muscle cells treated with 100 μM of the four statins, which were atorvastatin (58.76%) < pravastatin (60.21%) < rosuvastatin (72.54%) < pitavastatin (89.96%). The atorvastatin and pravastatin inhibited GLUT2 expression and induced p-p38 MAPK expression in human pancreas islet β cells. The atorvastatin, pravastatin and rosuvastatin inhibited GLUT4, p-AKT, p-GSK-3β, and p-p38 MAPK in human skeletal muscle cells.
Conclusions: Differences between individual statins likely exist that may cause to different effect of inducing islet cells damage and insulin resistance. Pitavastatin may have the lowest diabetes mellitus inducing effect among the four statins.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
Nurse interventions for early restarting of physical activity in people with prior acute myocardial infarction: the Fitwalking® Project.

Abstract nr. 78
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease,Lifestyle,Prevention

Aim: To evaluate effect of nurse-led aerobic exercise (Fitwalking® (FW), a brisk walking conceived by Maurizio Damilano) on body size and exercise tolerance in people with prior Acute Myocardial Infarction (AMI).

Methods: 17 consecutive people (13 males, 58.4±6.5 years) from Coronary Unit (CU), performed a 40-minutes long FW session twice a week. Inclusion criteria: prior AMI; CU admission at least 6 months before; ejection fraction >45%; age <70 years; negative stress test for ischemia/angina; no disabling diseases; consent by attending cardiologist. For educational purposes, 9 people were included in Group 1 (G1; 17 sessions performed), 8 people in Group 2 (G2; 22 sessions performed). Body size, distance walked (DW) and, only in G2, Borg Scale score (BS) were collected at baseline and at the end of study. Results were compared within each group and whole sample (WS) by T-test variance analysis.

Results: Study showed significant improvement of performance in G1 (DW: 2.8±0.0 vs 5.3±0.6 km, P=0.025), not significant improvement of exercise tolerance (G2, WS) and not significant reduction in body size (G1, WS).

Conclusion: In a short observational period and small sample, a positive trend in reducing body size and improving performance was seen. In contrast with literature, with increasing distance a reduction in BS was seen: the better shape, the better wellness? It may be likely, since some people in G1, feeling fit, continued practice with G2. A nurse-led pedagogical approach based on educational, relational and motivational competence, able to highlight trainees’ progress, made the Project possible.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
Maternal and infant adiposity and lipid profile are associated with maternal and cord blood inflammation.

Abstract nr. 79
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Epidemiology, Inflammation, Metabolism

Lipid dysregulation is exacerbated by inflammation. We investigated associations between maternal and infant lipid profiles, adiposity and inflammation.

Materials/Methods
Paired maternal (28-weeks gestation) and infant (umbilical cord) blood samples were collected from a population-derived birth cohort (Barwon Infant Study, n=1074). Data on maternal co-morbidities, infant birth weight and adiposity (standardised skin-fold thickness) were compiled. In a randomly selected subgroup of term infants (n = 227 pairs), matched maternal and cord lipids and high sensitivity C-reactive protein (hsCRP) were measured.

Results
Mean maternal age was 32.4 years (sd 4.2) with a pre-pregnancy BMI median 24.4 kg/m² (IQR 21.7-28.1); 92% of the cohort was caucasian. Caesarian births accounted for 33% of deliveries, and mean birth weight was 3.6kg (sd 0.44).

Preliminary data show that maternal cholesterol and LDL were elevated compared to non-pregnant normal ranges. Infant lipid concentrations were higher in female infants (e.g cholesterol mean difference 0.4 (95%CI 0.1-0.6) mmol/L). Infant cholesterol (r=0.40, p<0.001), HDL (r=0.30, p<0.001), LDL (r=0.37, p<0.001), ApoA (r=0.46, p<0.001) and ApoB (r=0.43, p<0.001) appeared to correlate with infant hsCRP.

After adjusting for maternal age, smoking status, mode of delivery and infant gender, multivariate regression analysis demonstrated an association between increased maternal hsCRP and increasing pre-pregnancy BMI (β=0.3 mg/L per kg/m²; p<0.001), birth weight (β=0.003 mg/L per kg, p<0.001) and infant skin fold thickness (β=0.8mg/L per mm; p=0.003). For infant hsCRP, similar patterns were observed.

Conclusion
Increased infant cholesterol positively correlated with increased infant hsCRP, whilst higher pre-pregnancy BMI and markers of infant adiposity are associated with higher maternal hsCRP. These
findings are in keeping with a relationship between inflammation, adiposity and lipid metabolism in the perinatal period.

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Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
Impact of five novel mutations and five known mutations in endothelial lipase on HDL cholesterol levels.

Abstract nr. 82
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Genetics,HDL,Risk stratification

High density lipoprotein (HDL) has multiple functions. High HDL-cholesterol (HDL-C) concentrations, generally viewed as favourable, may be due to several causes that may have different effects on composition and function. Endothelial lipase (LIPG) mutations were found to cause increased HDL-C concentrations with unresolved impact on cardiovascular disease. We aimed to seek LIPG mutations in a cohort of patients with high HDL-C and to determine their prevalence in a range of HDL-C concentrations in patients attending a referral clinic for dyslipidaemia.

Patients with hypercholesterolaemia >7.5mmol/L and consenting to research were assessed clinically in conjunction with a fasting lipogram and tests for secondary dyslipidaemia. Mutations in LIPG were first sought in those with HDL-C >2.5mmol/L, whereafter these mutations were sought in categories of 200 random samples in HDL-C ranges 1.2-1.6, 1.6-2.0, 2.0-2.5mmol/L. Polymerase chain reaction products were analysed by high resolution melting and heteroduplex patterns were analysed by sequencing.

Five unreported mutations and five known mutations in LIPG were identified in the following numbers of patients: Q249L(1), A277D(1), T298S(9), S310G(2), R315H(1), N396S(18), E417Q(1), R442W(1), R448L(1), R450G(3). Two common mutations were found across the range of HDL-C levels. Six uncommon mutations were found in the higher HDL-C categories.

Mutations in LIPG were identified. The known N396S and T298S mutations appear not to raise HDL-C powerfully while six of the mutations are likely powerful modulators of HDL-C. Further investigation is required to determine the functional and clinical impact of these mutations.

Funding: South African Medical Research Council

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
The influence of exenatide on monocytes/macrophages' phenotype, TNF alpha release and ROS generation – an in vitro study.

Abstract nr. 83
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Diabetes, Inflammation

Introduction: Diabetic patients experience accelerated atherosclerosis. Macrophages are key cells accelerating the development of atherosclerotic plaques. There are at least two major subpopulations of macrophages: M1 - associated with atherosclerosis progression (high inducible nitrous oxide synthase [iNOS] expression) and M2 – anti-inflammatory and healing promoting cells (high arginase 1 [arg1] expression). Effects of novel antidiabetic incretin-based therapies exceed its hypoglycaemic properties (e.g. a decrease in markers of low-intensity inflammation). Therefore authors aimed at the assessment of the influence of in vitro exenatide (a glucagon-like peptide-1 [GLP-1] receptor agonist) on macrophages' phenotype, inflammatory and oxidative stress markers.

Materials and Methods: Monocytes were isolated from 18 patients with recently diagnosed type 2 diabetes. Cells were exposed in vitro to exenatide, LPS and combination of the both compounds. The impact of exenatide on the phenotype of macrophages (iNOS and arg1), TNFalpha and ROS level was studied.

Results: Exenatide alone did not affect iNOS expression but it significantly inhibited iNOS expression in LPS pre-treated cells (376±76RODvs.478±112ROD; p<0.05). Compared to controls, exenatide effectively induced arg1 expression in macrophages pre-treated (100±31RODvs.379±51ROD; p<0.05) or untreated with LPS (100±31RODvs.212±66ROD; p<0.05). Exenatide diminished the level of ROS (321.5±65RUvs.401.3±75RU; p<0.05) and TNFalpha (379.3±51pg/ml vs. 451.1±42pg/ml; p<0.05) in LPS pre-treated macrophages. However no effect of exenatide was observed in cells unexposed to LPS.

Conclusion: We showed that exenatide influenced basic markers of the macrophage phenotype, skewing the population toward alternative activation and limiting classical activation induced by LPS. Additionally a reduction in inflammatory and oxidative stress markers was achieved.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Coronary artery disease risk estimation with combination of visual and biochemical markers

Abstract nr. 84
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Co-author(s) - Yarovaya, Elena
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Lipoproteins, Risk Factor, Visceral Fat

Aim: To investigate whether carotid artery dopplerography parameters and blood biomarkers concentrations are associated with coronary artery disease (CAD).

Methods: This study included 205 consecutive patients (M/F 136/69; mean age 62.8±9.0 yrs). All patients were underwent coronary angiography and carotid artery ultrasound dopplerography with the mean common carotid artery intima-media thickness (IMT) and carotid atherosclerotic plaques (ASP) assessment; 70.7% of patients had CAD; 92.4% of patients were treated with statins.

Results: CAD patients didn’t differ by lipid profile parameters, but exhibited significantly lower serum levels of apolipoprotein (apo) AI (154.5±26.3 vs. 166.4±29.7 mg/dl; p=0.000), apo B (87.7±24.4 vs. 98.1±25.3 mg/dl; p=0.000), as well as that for leptin (28.5±22.6 vs. 44.4±59.4 ng/ml; p=0.000), as compared to the patients without CAD. Meanwhile, there were significant differences in leptin (p=0.000), apo AI (p=0.000) and apo B (p=0.009) levels between patients with single-, two, and multiple-vessel disease upward in group without atherosclerotic coronary lesion. Logistic regression analysis indicated that the IMT >0.9 mm (OR=2.7, 95% CI=1.4-5.4, p=0.004) and presence of carotid ASP ≥3 (OR=4.6, 95%CI=1.9-11.4, p=0.001) independently associated with CAD. Multiple regression analysis demonstrated that presence of carotid ASP ≥3 (OR=2.5; 95%CI=1.0-5.9; p=0.045) and blood adiponectin level below median (8.0 mkg/ml) (OR=3.1; 95%CI=1.3-7.6; p=0.014) were associated with CAD.

Conclusion: Combination of three and more carotid ASP with low blood adiponectin level <8.0 mkg/ml can be used as a risk marker of atherosclerotic burden and might be helpful in non-invasive CAD prediction.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Complement receptor 1 (CR1) is involved in the binding of apo B-containing lipoproteins to circulating leukocytes.

Abstract nr. 85

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Co-author(s) - Castro Cabezas, Manuel

Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Inflammation, Triglyceride-Rich Proteins

**Background:** Apolipoprotein (apo) B is present on the surface of blood cells. Several candidate receptors may be involved. Emerging evidence suggests a role for complement activation. Complement Receptor 1 (CR1) is a candidate receptor to contribute to cell-bound apo B-containing lipoproteins. We investigated whether apo B-containing lipoproteins bind to CR1 on native leukocytes.

**Materials and methods:** Complement activation *ex vivo* (15 minutes at 37°C) was induced by bacterial lipopolysaccharides (LPS) and artificial triglyceride-rich lipoproteins (Lipoplus®). The expression of apo B and CR1 by monocytes and neutrophils was measured using flow cytometry. The expression of apo B and CR1 was expressed as mean percentage ± SD from baseline.

**Results:** Whole blood incubation with LPS resulted in an increase in apo B expression by both monocytes and neutrophils (211% ± 40%, p<0.05; 180% ± 18, p<0.05). A simultaneous increase in CR1 expression by monocytes and neutrophils was observed (586% ± 258% p<0.05; 1042% ± 758%, p<0.05), but no changes in LDL-R expression were observed. Similar results were obtained with Lipoplus, suggesting that triglycerides may induce complement activation *in vivo*.

**Conclusions:** Stimulation of leukocytes by LPS or Lipoplus resulted in a simultaneous increase in apo B and CR1 expression. This provides indirect evidence of CR1-mediated binding of apo B-containing lipoproteins to blood cells. Triglyceride-rich lipoproteins stimulate CR1 expression on leukocytes and may be the physiological trigger for the binding of apo B-containing lipoproteins to CR1 *in vivo*.

Subdivision 1. Basic Science
Presentation Preference Oral presentation
Additional information
Complement Receptor 1 may be responsible for the binding of apolipoprotein B to circulating erythrocytes providing protection against atherosclerosis.

Abstract nr. 86
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Co-author(s) - Prinzen , Lenneke
Co-author(s) - Zwan , Ellen van der
Co-author(s) - Castro Cabezas , Manuel
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins,Atherosclerosis,Genetics

Background: Apolipoprotein (apo) B is present on the surface of circulating erythrocytes. A high presence of apo B on erythrocytes (ery-apoB) has been associated with protection against cardiovascular disease (CVD). Since erythrocytes do not carry classical LDL-receptors, other mechanisms must be involved. We aimed to investigate the role of Complement Receptor 1 (CR1) in the binding of apo B to erythrocytes.

Materials and methods: Subjects with and without CVD were included. Ery-apoB and CR1 expression were measured by flow cytometry. Functional CR1 polymorphisms (Pro1827Arg) were determined.

Results: In total 431 subjects were included. Ery-apo B was lower in males than in females (1.00±0.75 au vs. 1.18±0.96 au, p=0.035). Subjects with clinical CVD had lower ery-apoB (0.99±0.80 au) than healthy subjects (1.17±0.90 au, p=0.028). Ery-apoB was lower in subjects with high intima media thickness (IMT, >0.700mm) (0.94±0.81 au) than subjects with low IMT (1.15±0.87 au, p=0.016). Ery-apoB was inversely correlated with BMI (Spearmans rho=-0.138, p=0.004), diastolic blood pressure (rho=-0.108, p=0.026), triglycerides (rho=-0.137, p=0.004), complement C3 (rho=-0.144, p=0.003) and CRP (rho=-0.135, p=0.007) and positively correlated with serum HDL-C (rho=0.119, p=0.019). In 99 subjects functional CR1 polymorphisms were determined. Subjects with the wildtype CR1 polymorphism, associated with high erythrocyte CR1 expression, had higher levels of ery-apoB (n=65; 1.354±1.17 au) than subjects with a mutated CR1 polymorphism (n=34; 0.955±0.88 au; p=0.085).

Conclusions: These data suggest a role of CR1 in the binding of atherogenic lipoproteins to circulating erythrocytes, reflecting an anti-atherogenic mechanism. Surprisingly, several cardiovascular risk factors seem to be associated to ery-apoB.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Korean traditional Chungkookjang improves lipid profile and atherogenic indices in overweight/obese subjects: a clinical trial

Abstract nr. 88
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Apolipoproteins, Lipids, Nutrition, Obesity

Chungkookjang is Korean representative fermented food with soybean, like Ganjang, Doenjang, Gochujang, and so on. This study was investigated to develop awareness and preference about Korean traditional Chungkookjang among young generation, by validating its health promoting benefits especially against lipid profile and atherogenic indices. There were 166 subjects as cross-over design; 83 subjects of 120 volunteers completed all procedure; aged 19–29 years old. They were randomized to double-blind treatment with either Chunkookjang 35g or placebo on a regular daily basis for 12 weeks. After 12 weeks wash-out period, the groups were crossed over. Chungkookjang group showed a significant decrease in serum levels of hs-CRP and LDL-C/HDL-C. Moreover, Apo A1 and Apo B were significantly improved in the Chungkookjang group. These results indicate that Chungkookjang has favorable effects on preventing and improving lipid profile and atherogenic indices in overweight and obese individuals. [This research was supported by the Globalization of Korean Foods R&D program, funded by Ministry of Food, Agriculture, Forestry and Fisheries, Republic of Korea.]
Keywords: Chungkookjang, fermented food, lipid profile and atherogenic indices

Presentation Preference Electronic poster presentation
Additional information
High-sensitivity C-reactive protein and cardiovascular risk factors in Type 2 diabetic patients with acute coronary syndrome

Abstract nr. 89
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords ACS, Diabetes, Risk Factor

Introduction. Elevated high-sensitivity C-reactive protein (hs-CRP) levels have frequently been shown to be associated with type 2 diabetes (T2D). Additionally, hs-CRP has also emerged as a powerful predictor of cardiovascular disease. Inflammation, indicated by hs-CRP and hyperglycemia, indicated by glycosylated haemoglobin (HbA1c), jointly contribute to the cardiovascular risk of patients with advanced atherosclerosis.

Objective. In this study, we assessed the correlation between hs-CRP and cardiovascular risk factors such as obesity, serum lipids and HbA1c in our local setting.

Methodology. A cross sectional study was conducted on 81 male patients with T2D who were admitted for acute coronary syndrome in the medical ward. This population was ethnically diverse comprising patients of Malay, Chinese and Asian Indian descent. Variables tested included HbA1c, serum triglyceride, high density lipoprotein (HDL) and body mass index (BMI). Hs-CRP was measured using ELISA technique.

Results. There was no significant correlation between hs-CRP and BMI (p=0.985), hs-CRP and HDL(p=0.939) and hs-CRP and triglyceride(p=0.404). However, there was significant correlation between hs-CRP and HbA1c (95% CI 0.18-0.32 and p<0.001). The observed correlation coefficient, r=0.645, suggests positive and moderate correlation.

Conclusion. Our study showed that there was a positive correlation between hs-CRP and HbA1c levels in our local population. There was no significant correlation between hs-CRP and obesity and serum lipids. Future studies are required to evaluate the influence of modulators including genetic variations on the elevation of hs-CRP levels in this population subgroup.
Leukocyte Thrombomodulin Mediates Leukocyte Adhesion to Endothelium in Vascular Inflammation

Abstract nr. 90
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Co-author(s) - Shi, Guey-Yueh
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease

Thrombomodulin (TM) is a transmembrane glycoprotein composed of multiple domains and expressed in a variety of cell types. In response to vascular injury, endothelial cell-expressed TM can modulate inflammation through thrombin and protein C-dependent pathways. However, it is not clear whether leukocyte TM is involved in inflammation. We previously demonstrated that the lectin-like domain of TM can bind Lewis^Y (Le^Y), which is upregulated in endothelial cells upon vascular inflammation and mediates cell adhesion. In this study, we investigated the interaction of leukocyte TM and Le^Y in facilitating the adhesion of leukocyte under inflammation. To test the role of TM in regulating leukocyte recruitment, carotid artery ligation was performed to induce vascular injury. Reduced leukocyte recruitment and neointima formation were observed in myeloid-specific TM deficient mice compared with those of wild-type mice. Knockdown of TM expression in human monocytic THP-1 cells resulted in decreased adhesion to activated endothelium under shear flow. We further investigated the involvement of Le^Y in the TM-dependent adhesion. Knockdown of TM or treatment of TM specific antibodies reduced the adhesion of THP-1 cells to Le^Y-immobilized surface under shear flow, indicating that the binding of TM and Le^Y facilitates THP-1 adhesion. The phosphorylation of p38, as well as, the activation of β2-integrin in THP-1 cells was induced by the addition of soluble Le^Y. In contrast, the effects were ameliorated when TM was knocked down. In conclusion, our results show that leukocyte TM interacts with Le^Y to elicit signal transduction leading to leukocyte adhesion to endothelium under vascular inflammation.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Initiation and progression of coronary atherosclerosis in WHHLMI rabbits

Abstract nr. 91
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Pathogenesis

**Objectives:** Myocardial infarction-prone Watanabe heritable hyperlipidemic (WHHLMI) rabbits show severe coronary atherosclerosis spontaneously. It is unclear that the detailed process of the initiation and progression of coronary atherosclerosis in WHHLMI rabbits.

**Methods:** Coronary arteries were excised from 136 WHHLMI rabbits (1-29 months old). These arteries were fixed by neutral buffered formalin and sliced at 500 micro meter intervals. Sections were stained histopathologically and immunohistochemically (CD31 for endothelial cells, 1A4 for smooth muscle cells, or SMC and RAM-11 for macrophages), and lesion thickness and cross-sectional narrowing (CSN) were evaluated.

**Results:** Early lesions showed mainly intimal thickening with SMC proliferation at the bifurcation of arteries. However, infiltrations of macrophages were relatively dominant in the trunk compared to lesions at the bifurcation. Fibrous plaques were mainly observed in 30-<50% CSN. Layered plaques consisting of fibromuscular components and lipids/foam cells were increased with plaque growth. Pre-fibroatheroma, which has foam cell accumulation instead of lipid core, were decreased, and fibroatheroma were increased with further progression of plaques, respectively. Thin-capped fibroatheroma were frequent in >90% CSN. Vasa vasorum were observed in plaques with >50% CSN. Although macrophage/foam cell-rich lesions were rare in WHHLMI coronary plaques, foam cell-clusters layered on the plaque surface were observed occasionally.

**Conclusions:** This study suggest that the development of coronary lesions in WHHLMI rabbits are as follows; 1) the initiation of coronary lesions is intimal thickening, 2) macrophage infiltration and increase in fibromuscular components promote coronary plaques; 3) these lesions transfer to fibroatheroma and thin-capped fibroatheroma over time.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Thrombomodulin interacts with fibronectin and promotes angiogenesis

Abstract nr. 92
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Angiogenesis, Atherosclerosis

Objective: Angiogenesis in an atherosclerotic plaque may contribute to the growth of lesions and lead to the recruitment of inflammatory cells to the plaque, resulting in chronic inflammation. Interaction of endothelial cells with extracellular matrix is critical for angiogenesis. Thrombomodulin (TM) is a cell surface glycoprotein; however, it remains unknown whether TM binds to extracellular matrix. Here, we investigate the interaction of TM with extracellular matrix and its biological roles in angiogenesis.

Methods: Solid-phase binding assays were used to assess recombinant TM lectin-like domain 1 (rTMD1) binding to extracellular matrix. The effects of TM expression on cell adhesion and migration were determined. We examined the association of TM in endothelial cells with fibronectin and analyzed the effects of TM knockdown on endothelial cell tube formation in vitro.

Results: The solid-phase binding assays showed that rTMD1 bound directly to the extracellular matrix fibronectin. Mapping analysis using various fibronectin fragments identified the N-terminal 70-kDa region of fibronectin as the TMD1-binding site. Overexpression of TM in TM-negative A2058 melanoma cells enhanced cell adhesion and migration on fibronectin, and led to increased focal adhesion kinase phosphorylation. Up-regulation of TM expression in endothelial cells promoted tube formation on Matrigel, whereas knockdown of TM expression by RNA interference reduced the tube formation. In addition, confocal microscopy analysis revealed that TM colocalized with fibronectin at tube-like structures during endothelial tube formation.

Conclusions: TM promotes cell adhesion, migration and angiogenesis by interacting with fibronectin. This implies that TM may play a role in angiogenesis-related diseases such as atherosclerosis.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
CD40-Filamin A interactions are required for translocation of CD40 to lipid rafts in endothelial cells and for endothelial cell activation

Abstract nr. 93
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Endothelium, Inflammation

Background and Aim: CD40 is a member of the co-stimulatory tumor necrosis factor receptor superfamily that is constitutively expressed not only on professional antigen presenting cells like macrophages dendritic cells and B-cells, but also on endothelial cells. Interactions between CD40 and its ligand, CD40 ligand (CD40L) are crucial for proper immune cell activation. Activation of CD40 signaling plays a role in chronic diseases such as rheumatoid arthritis, inflammatory bowel disease and atherosclerosis. However, since CD40-CD40L interactions are also important in thrombosis, blocking these interactions has severe adverse side-effects. Therefore, in search for new therapeutic targets, we aimed to identify new CD40-binding partners.

Methods and Results: We created a cDNA library of murine aortas containing atherosclerotic plaques at various stages and performed a yeast-two-hybrid with the C-terminal domain of CD40 as bait. We identified filamin A as a novel CD40 binding partner in atherosclerosis, which was confirmed by co-immunoprecipitation. By confocal microscopy we showed in endothelial cells that, upon activation of CD40, filamin A was recruited to CD40. Filamin A binds to the intracellular domain of CD40, near the transmembrane domain, at a site distinct from the tumor necrosis receptor associated factor (TRAF) binding sites. Previous studies have shown that disruption of the lipid rafts in endothelial cells disrupts part of the CD40 signaling cascade and we found that knock-down of filamin in endothelial cells using siRNAs inhibits the translocation of CD40 to lipid rafts upon activation of CD40 signaling. This inhibition of CD40 translocation resulted in repression of CD40-mediated Akt signaling but not of CD40-mediated JNK signaling and subsequent inhibition of VCAM-1 and CCL-2.

Conclusions: We show that CD40 interacts with filamin A in endothelial cells. This interaction is involved in translocation of CD40 to the lipid rafts and CD40-mediated activation of the Akt pathway. The reduced upregulation of VCAM-1 and CCL-2 makes this an interesting target for novel therapies where reduced leukocyte recruitment is favorable.

Funding: The Netherlands Organization for Scientific Research, a Vici grant to E.L.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
In vitro oxidation of LDL and its in vivo implication: A comparative study of Iranians living in IRAN and in India

Abstract nr. 94
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, HDL, LDL, Lifestyle

According to the Cholesterol-Diet-Heart theory, Low Density Lipoprotein associated cholesterol (LDL-C) was the cause of atherosclerosis. However, over three decades of rigorous efforts to reduce LDL-C have not resulted in any significant decrease in the mortality rates due to heart diseases. On the contrary, oxidized LDL (ox-LDL) correlated with the risk of heart diseases and was a far superior marker than all other lipid markers.

Since in vitro studies aim at understanding the in vivo mechanisms, we undertook a study to compare the in vitro oxidation on serum LDL of Iranians living in Iran and in India. The isolated LDL was subjected to oxidation in vitro by water soluble and fat soluble antioxidants.

Iranians living in Iran had lower levels of oxidized lipids in their serum compared with Iranians living in India. The lag phase of oxidation was significantly longer (P<0.05) and they had higher levels of antioxidants in their serum compared with the Iranians living in India. Iranians living in India had higher levels of oxidized lipids in the serum, the lag phase of LDL oxidation was short, there were protein oxidation products in the LDL and the antioxidant levels in the serum were low. The LDL oxidation profile resembled that of Indians living in similar conditions. The High Density Lipoprotein (HDL) of Iranians living in India was highly oxidized and was comparable to that of the Indians.

A major significant difference in the lifestyle of Iranians living in Iran and in India was the dietary oil they used. Iranians in India used popular brands of cooking oils. These oils are rich in polyunsaturated fatty acids and in ω-6 fatty acids. These fatty acids would become incorporated into the LDL and would be highly susceptible to oxidation. Although the HDL could prevent the LDL oxidation, since it is already oxidized it may be unable to prevent LDL oxidation.

Taken together, these results suggest that the dietary oil may be responsible for higher level of oxidized LDL in Iranians living in India and of Indians.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Probucol rescued litter size and gender ratio in reproduction of hypo-lipoproteinemia model mice

Abstract nr. 95
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords HDL, Lipoproteins

Low plasma HDL mice, such as ABCA1-null mice, Lcat-null and ABCA1 inactivator, probucol-fed mice have significantly low cholesterol content in their adrenal glands and ovaries. Upon reproduction between heterozygotes, the reproduction of ABCA1-null weaned pups were reduced to 30% of that expected by Mendelian genetics, and ABCA1 heterozygote male pups was reduced to 75% of female heterozygote pups. Lcat-null male pups was also reduced to 31% of that expected by Mendelian genetics. Gender ratio in reproduction of wild type, and 0.2% probucol fed mice were 1 to 1 in the same mating condition. Interestingly, although 0.2% probucol-fed mice reduced its plasma HDL only to 5% within 2 weeks, probucol-fed mice reproduced normally. This result suggesting some beneficial functions of probucol may protect pups’ from death by this chow treatment. Thus, we fed probucol containing chow to ABCA1 heterozygote or LCAT heterozygote mice upon mating to the lactation period. Here, significance in the Chi- squared analysis disappeared on the gender ratio in these weaned mice as well as the litter size. HMGCoA reductase expression was increased in adrenal of probucol-fed wild type mice indicate increased cholesterol de novo synthesis may supply enough cholesterol for steroid synthesis during their reproduction in these low HDL mice. Probucol-HDL (1.00 µg probucol / µg HDL-protein) derived probucol into NCI-H295 human adrenal cortex cells (0.73±0.12 µg probucol / mg cellular protein). The expression of HMGCoA reductase was significantly increased compared to b-actin. The further mechanism to increase cellular cholesterol synthesis by Probucol will be examined.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
The differential roles for Nox isoforms in the development of diabetes associated atherosclerosis

Abstract nr. 96

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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium

Keywords Atherosclerosis, Cardiovascular Disease, Diabetes

Individuals diagnosed with diabetes have accelerated development of atherosclerosis; however, the mechanisms are poorly understood. Oxidative stress appears to play a significant role, specifically Nox-derived ROS, for which there are two principal isoforms Nox1 and 4 which are upregulated in activity by glucose. The aim of this study was to delineate the role of Nox-derived oxidative stress in the development of diabetes-related atherosclerosis.

Nox isoform specific-ApoE−/− double knockout mice, Nox1−/−ApoE−/− and Nox4−/−ApoE−/− mice were rendered diabetic by streptozotocin (55mg/kg/day for 5 days), with non-diabetic wildtype mice serving as controls. Animals were diabetic for 20 weeks at which point aortas were removed and cleaned for analysis.

After 20 weeks, diabetic wildtype mice had a significant elevation in atherosclerosis compared to non-diabetic counterparts. Deletion of the Nox1 isoform in diabetes resulted in a 50% reduction in atherosclerosis development. In contrast, deletion of the Nox4 isoform in diabetes resulted in a 65% increase in atherosclerosis development. Aortic RT-PCR demonstrated a significant reduction in gene expression of markers for oxidative stress, inflammation (MCP-1, IL1β, TNFα) and fibrosis (Collagen I) in Nox1−/−ApoE−/− diabetic mice, which were significantly elevated in diabetic Nox4−/−ApoE−/− diabetic mice. Immunohistochemistry analysis of the aorta identified a significant decrease in pro-oxidant markers and macrophage infiltration in diabetic Nox1−/−ApoE−/− mice, which were significantly elevated in diabetic Nox4−/−ApoE−/− mice.

These data demonstrate opposing effects of two Nox isoforms in diabetes associated atherosclerosis, Nox1 playing a pathological role, where in turn Nox4 derived ROS plays a vasculo-protective role in atherosclerosis development.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Pharmacological inhibition of Nox as both a Primary and Delayed Intervention Attenuates Atherosclerosis Development in Diabetes.

Abstract nr. 97
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis,Diabetes,Prevention

The development of atherosclerosis in diabetes is significantly accelerated, contributing to increase incidence of stroke and heart attack. The cause for the acceleration in atherosclerosis development in diabetes is largely unknown; however, oxidative stress appears to be an essential mediator in its development. Members of the NAD(P)H oxidase (Nox) family have been identified to play a causative role in the promotion of atherosclerosis development. It has been demonstrated that Nox1, when deleted in diabetic mice attenuates atherosclerosis development. Targeting these enzymes pharmacologically is of novel interest to attenuate atherosclerosis development, particularly in diabetes.

We aimed to explore the pharmacological potential of Nox inhibition using GKT137831 in attenuating the development of atherosclerosis in diabetes. ApoE<sup>−/−</sup> mice were rendered diabetic at 6 weeks of age using streptozotocin injections, saline injected ApoE<sup>−/−</sup> mice served as controls. Mice were randomly allocated into three groups, vehicle treated, primary GKT137831 intervention (30mg/kg/day, 0wks to 20wks) and delayed GKT137831 intervention (30mg/kg/day, 10wks to 20wks). After 20 weeks animals were culled with aortas removed for assessment of atherosclerosis development, immunohistochemical analysis, RT-PCR and ELISA.

After 20wks of diabetes, atherosclerosis was significantly increased compared to controls. Administration of GKT137831 as both a primary and delayed intervention was able to attenuate the development of atherosclerosis in diabetes. Assessment of the oxidative stress marker Nitrotyrosine demonstrated a significant increase in vehicle treated diabetic mice; with a significant attenuation in diabetic mice administered GKT137831 as both primary or delayed intervention. Analysis of pro-inflammatory and chemotaxis markers by RT-PCR identified significant upregulation in MCP-1 and TNFα expression in vehicle treated diabetics, which was attenuated with GKT137831 treated as both a primary and delayed intervention.

Taken together, these results highlight the potential of targeted Nox inhibition in the prevention of
atherosclerosis and attenuation of established atherosclerosis.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Abnormal HDL particles distribution in coronary artery disease patients

Abstract nr. 98
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, HDL, Lipoproteins

Low concentration of HDL-C is associated with higher cardiovascular disease risk. HDL are composed of heterogeneous particles with different in size, density, composition and functions.

Aim. To study the HDL particles distribution in patients differing by coronary artery disease (CAD) severity.

Materials and methods. Patients with CAD verified by coronary angiography (n=130; 30-80 yrs) were included into study. All patients were treated with statins. HDL subfractional distribution was analyzed using Lipoprint System (Quantimetrix, USA).

Results. Patients were divided into three groups according to CAD severity estimated by Gensini score (GS): group 1 - GS=0, n=40; group 2 - GS =1-34, n=40; group 3 - GS ≥35, n=50. No differences between groups in HDL-C level were found. CAD patients (groups 2 and 3) as compared to group 1 had higher portion of small HDL (20,3±7,5 and 19,0±7,2 vs 16,1±5,9%; p<0,01) and intermediate HDL (46,4±4,8 and 46,2±4,6 vs 44,6±4,5%; p<0,05) and the lower portion of large HDL (32,9±7,5 and 34,6±9,0 vs 39,2±9,0%; p<0,01). The negative Spearman rank correlation between TG level and large, intermediate and small HDL particles was obtained in CAD-free patients (R= -0,354; -0,509; and -0,435; p=0,005). In group 2 the correlation was found only between TG level and intermediate HDL particles (R= -0,369; p<0,02).

Conclusions. The difference in HDL particles distribution is associated with cardiovascular manifestations severity with the predominance of smaller HDL particles and lower portion of large HDL particles. Thus, an abnormal distribution of HDL particles was found already in patients with mild-to moderate atherosclerosis (GS 1-34).

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Efficacy and Safety of Pitavastatin at Adult Doses in Children between 6 and 17 years at High Future Cardiovascular Risk

Abstract nr. 99
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Dyslipidemia, Familial Hypercholesterolemia, Pharmacology, Prevention

Objectives:
Elevated low-density lipoprotein cholesterol (LDL-C) is a risk factor for coronary heart disease (CHD) in adults, but the underlying atherogenesis begins in childhood. Therefore guidelines recommend consideration of statin therapy in children at high future CHD risk. The aim of the study was to assess the safety and efficacy of the adult dose range of pitavastatin, a relatively new member of the statin class, in hyperlipidemic children and adolescents, the youngest starting at the age of 6.

Study design:
A total of 106 hyperlipidemic children and adolescents, (48 boys and 58 girls; 43 between 6-9 years; 50 between 10-14 years; 13 ≥15 years) were enrolled in a 12 week randomized, double blind, placebo controlled study and randomly assigned to pitavastatin 1 mg, 2 mg, 4 mg or placebo. During a 52 week extension period, subjects were up-titrated from 1 mg pitavastatin to a maximum dose of 4 mg in an effort to achieve an optimum LDL-C treatment target of <110 mg/dL (2.8 mmol/L). Safety was assessed in terms of adverse events rates, including abnormal clinical laboratory variables, vital signs and physical examination.

Results:
Mean baseline LDL-C was 232.9 (±52.0) mg/dL and 97.2% of the children had genetically confirmed familial hypercholesterolema. At 12 weeks LDL-C was reduced by 24.5%, 31.1% and 40.3% against placebo in the 1 mg, 2 mg and 4 mg group, respectively. In the open label study 20.5% of the subjects reached the LDL-C goal < 110 mg/dL (2.8 mmol/L). Drug-related treatment emergent adverse events were present in 10 subjects (8.9%) of the open label study, most commonly musculoskeletal/connective tissue disorders (2.7%) and nervous system disorders (2.7%). All were considered of mild (7.1%) or moderate intensity (1.8%) by the investigator and not dose related. No clinically significant differences in clinical laboratory variables, vital signs and
physical examination were observed.  

**Conclusion:**  
The whole adult dose range of pitavastatin is well tolerated and efficacious in hypercholesterolemic children and adolescents aged 6-17 years.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Gender differences in the adipose tissue macrophage subpopulation

Abstract nr. 100
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Elderly, Inflammation, Visceral Fat

Aim: The presence of pro-inflammatory macrophages (CD14+CD16+) in inflamed tissue has been documented in several inflammatory conditions including atherosclerosis. The aim of the study is to analyse the proportion of pro-inflammatory macrophages in the perirenal fat of men and women of pre-menopausal and menopausal age.

Methods: Samples of perirenal fat were obtained while kidneys were isolated in living donors, then the samples were dissected into small pieces and exposed to collagenase. Stroma vascular fraction (SVF) was eluted and analysed using flow cytometry. Mononuclear cells expressing CD14 were identified as macrophages and further divided according to the co-expression of CD16.

Results: We found no differences in the total macrophage content between men (n=15) and women (n=28). However, we observed a higher proportion of double positive CD14+CD16+ macrophages in post-menopausal women (age<51 years, n=14) than pre-menopausal women (n=14) (45±14 vs. 58±8%; p<0.01). In addition, a correlation (p<0.05) between CD14+CD16+ macrophage content and age was found in post-menopausal women, whereas no such relationship was found in the other groups.

Conclusion: The macrophage subpopulation in adipose tissue may depend on gender and age.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
UNVEILING THE PLAYERS OF OBESITY

Abstract nr. 101
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Diabetes, Obesity

BACKGROUND
Obesity is a silent killer and a forerunner of many complications if persists long. Various studies with animal model have identified the role of leptin, the hormone of adipose tissue; in obesity and its associated complications like diabetes and atherosclerosis in later stages. The exact mechanism to know how leptin influences insulin action in body and thereby leading to diabetes or post diabetic atherosclerosis is still not completely evaluated. Hypercholesterolemia was only found common to all these three states. The present study, therefore, evaluated the role of obesity on the expression of LDLR receptor, INSULIN receptor and LEPTIN receptor.

METHOD
Receptor expression was done by immunohistochemistry/ western blot. The serum level of lipids were measured by enzyme based kit method. The serum level of insulin and leptin and its soluble receptor were measured by elisa based kit.

RESULTS
The blot for insulin expression shows no change with body weight; the blot for leptin receptor shows decrease expression with weight gain and blot for LDLR shows decrease expression with weight gain. The serum levels of insulin and leptin are increased with weight gain but soluble receptor for leptin did not change significantly. Even the obese group showed decrease tyrosine phosphorylation of insulin receptor.

CONCLUSION
This study has given some possible reasons of the inter-association of hyperleptinemia, hypercholesterolemia and hyperinsulinemia by showing possibilities of inactivation of insulin and LDLR receptor with leptin resistance.

Subdivision 3. Clinical Studies
Presentation Preference Mini-oral presentation
Additional information
P-Hydroxybenzyl alcohol-containing biodegradable nanoparticle improves functional blood flow perfusion through angiogenesis in a mouse model of hindlimb ischemia

Abstract nr. 102
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Angiogenesis

Therapeutic angiogenesis has achieved promising results for ischemic diseases or peripheral artery disease in preclinical and early-phase clinical studies. We examined the therapeutic angiogenic effects of HPOX, which is biodegradable polymer composing the antioxidant p-hydroxybenzyl alcohol (HBA), in a mouse model of hindlimb ischemia. HPOX effectively stimulated blood flow recovery, compared with its degraded compounds HBA and 1,4-cyclohexendimethanol, via promotion of capillary vessel density in the ischemic hindlimb. These effects were highly correlated with levels of angiogenic inducers, vascular endothelial cell growth factor (VEGF), heme oxygenase-1 (HO-1), and Akt/AMPK/endothelial nitric oxide synthase (eNOS) in ischemic mouse hindlimb muscle. Blood perfusion and neovascularization induced by HPOX were reduced in eNOS-/- and HO-1+/- mice. HPOX also elevated the endothelial cell markers VEGF receptor-2, CD31, and eNOS mRNAs in the ischemic hindlimb, indicating that HPOX increases endothelial cell population and angiogenesis in the ischemic muscle. However, this nanoparticle suppressed expression levels of several inflammatory genes in ischemic tissues. These results suggest that HPOX significantly promotes angiogenesis and blood flow perfusion in the ischemic mouse hindlimb via increased angiogenic inducers, along with suppression of inflammatory gene expression. Thus, HPOX can be used potentially as a noninvasive drug intervention to facilitate therapeutic angiogenesis.
HPOX increases angiogenesis-regulatory gene expression in the ischemic mouse hindlimb.

HPOX improves blood flow recovery and neovascularization in the ischemic mouse hindlimb.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
THE SIGNIFICANCE OF TRACE ELEMENTS IN THE DEVELOPMENT OF CAROTID ATHEROSCLEROSIS

Abstract nr. 103
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Pathogenesis

Purpose: Study of relationships of intima - media thickness of common carotid artery (IMT CCA) and trace elements in hair at patients with carotid atherosclerosis (CA) Methods and Materials: 132 patients with CA and 18 healthy individuals(mean age 61,2 and 64,4 years) were included in study. IMT CCA is measured at duplex scanning by HD3 ultrasound Phillips) with linear transducer 5-7.5 MHz. The levels of trace elements (TE) in hair were determined by ICP -OES (Optima-2400DV (USA)). Examined trace elements Fe, Cu, Al, Zn, Cd, Se, Ca, K, Na, Mg and the relationship Cu / Fe, Mg / Fe, Zn / Fe, Mg / Zn, Ca / Mg, K / Na, Zn / Cu, Cu / Zn in hair. Results: IMT CCA in patients was higher than in healthy group (0.98 ± 0.37 mm vs. 0.78 ± 0.27 mm, p <0.005). Revealed a significant negative correlation between IMT CCA and the level of Zn in hair (r = -0.48, p <0.05), while a positive correlation between IMT CCA and Cu levels in hair (r = 0.42, p <0.05), Mg (r = 0.47, p <0.05), Cd (r = 0.44, p <0.05) and the ratio of Cu / Zn (r = 0.41, p <0.05). Linear regression analysis was confirmed that the levels of Mg and Cd in hair and the ratio of Cu / Zn positively correlated with IMT CCA. Conclusion: Identified relationships can be useful to clarify the pathogenetic mechanisms of atherosclerosis

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Association between Carotid Arteriosclerosis and Cardio Ankle Vascular Index (CAVI)

Abstract nr. 104
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis

[Aim] CAVI is a simple and useful examination to estimate arterial stiffness, but carotid arteries are not included among the target arteries in this examination. The aim of this study was to determine the relationship between carotid arteriosclerosis and CAVI.

[Methods] The study population was comprised of 1418 individuals (839 men and 579 women; mean age 55.9±11.5 years) who underwent annual health checks at our institute. This study was a cross-sectional analysis using clinically-relevant demographic and biochemical data. Carotid arteriosclerosis was estimated by intimal-media thickening (IMT) using ultrasonography; IMT value of <1.1 was considered normal. The thickest IMT of 6 sections of right and left common carotid artery, bifurcation, and internal carotid artery was defined as max IMT. CAVI was measured using pulse wave velocity. A mean of right and left CAVI value of <9 was considered normal. Data were analyzed using t-test, chi-square test, and multiple regression analysis; significance was considered at p<0.05.

[Results] Among various laboratory data, age, mean blood pressure, fasting plasma glucose (FPG), and max IMT were significantly higher in the high CAVI group (n=146). The correlation ratio of max IMT with CAVI was 0.351. Multiple regression analysis showed that age (β=0.616), waist circumference (β=-0.168), mean blood pressure (β=0.187), FPG (β=0.062), HDL-cholesterol (β=-0.067), and max IMT (β=0.044) were independent predictors of CAVI (R²=0.482).

[Conclusion] Carotid arteriosclerosis is associated with CAVI

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Perivascular adipose tissue and coronary atherosclerosis

Abstract nr. 105
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Pathogenesis

Ectopic fat plays an important role in the development of insulin resistance and pro-atherogenic metabolic conditions. Recently, studies have shown a more direct effect of adipose tissue surrounding artery.

Methods: We analyzed perivascular adipose tissue size around the left coronary artery as well as macrophage content of this tissue and the coronary artery wall in 96 explanted hearts during heart transplantation. Two groups with different reasons for heart failure were compared – coronary heart disease (CHD n=49) and dilatation cardiomyopathy (DCMP n=47).

Results: Perivascular adipose tissue size around the left coronary artery was not different in CHD (96.4 ± 83.1 mm²) and DCMP groups (91.1 ± 77.1 mm²). This result is in disagreement with indirectly analyzed literary data using computer tomography. The fraction area occupied by macrophages (CD68+ cells) in the coronary artery was five times higher in CHD patients (1.27 ± 1.68) compared to patients with DCMP (0.26 ± 0.28, p<0.001), whereas we detected no difference in the surrounding adipose tissue. During analysis of the correlation between macrophages in the arterial wall and the surrounding adipose tissue, we found no correlation in the DCMP group (A) but a highly significant correlation in the CHD group (p<0.005) (B).

Conclusion: Perivascular adipose tissue size does not play any role in the development of atherosclerosis in the coronary artery. It is supposed that an interplay between adipose tissue and artery wall macrophages is very important in CHD but is not involved in DCMP.

Supported by grant IGA MZ CR NT 14009/3.
Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
HOMOCYSTEINEMIA AND DEVELOPMENT OF CORONARY ARTERY DISEASE

Abstract nr. 106
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis

Objective. The aim of the study was to establish if there is correlation between total plasma homocysteine (tHcy) levels with occurrence and development of coronary artery disease (CAD).

Material and methods. Total number od 165 patients were examined which were divided into 3 groups based on 10 years risk for CAD established according ATP III and Framingham criteria: high risk group consist 60 patients with CAD risk above 20%; group of 49 patients with angiographyally proven CAD and 56 patients, control group, with CAD risk less than 10%. All patients were evaluated for the following risk factors and markers: sex, age, smoking status, hypertension, family history of CAD, lipids, lipoproteins, glucose, white blood cells, urea and creatine.

Results. Mean plasma tHcy levels in high risk group were 16.0 micromol/L (p<0.04), in the group with CAD, 15.3 micromol/L respectively (p<0.02) vs. control (13.0 micromol/L). There was correlation between tHcy and total CAD risk (p<0.04) and white blood cells count (0.02) in high risk group. In the group with CAD, tHcy correlated with the frequency of high grade of coronary artery stenosis, >95% of arterial lumen (0.04).

Conclusion. We concluded that elevated tHcy correlated with the total CAD risk and the stage of coronary artery disease.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Intraplaque hemorrhage of basilar artery atherosclerosis: Prevalence and Clinical Relevance

Abstract nr. 107
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Vulnerable Plaque

Purpose: The aim of this study was to evaluate the prevalence and clinical relevance of intraplaque hemorrhage (IPH) in patients with basilar artery (BA) atherosclerosis using high-resolution magnetic resonance imaging (HRMRI).

Methods: We retrospectively analyzed the HRMRI and clinical data of 74 patients (44 symptomatic and 30 asymptomatic) with >50% BA stenosis. High-signal intensity within a BA plaque on magnetization-prepared rapid acquisition with gradient echo (MPRAGE) and/or simultaneous noncontrast angiography and intraplaque hemorrhage imaging (SNAP) was defined as an area with an intensity >150% of the signal of adjacent muscle. The relationship between IPH within BA plaque and clinical presentation was analyzed.

Results: IPH was revealed on HRMRI in 30 patients (42.3%, 24 symptomatic and 6 asymptomatic). IPH of BA plaque in symptomatic patients was significantly higher prevalence compared with asymptomatic patients (54.5% vs 20%, p = 0.006). The stenotic degree of BA plaque between IPH group and non-IPH group was significantly different (72.9 ±8.7 vs 62.2 ± 31.2, p = 0.001).

Conclusions: IPH within BA plaque on HRMRI is relatively high prevalence and associated with acute stroke.
ERECTILE DYSFUNCTION IS ASSOCIATED WITH LOW TOTAL SERUM TESTOSTERONE LEVELS AND IMPAIRED FLOW-MEDIATED VASODILATION

Abstract nr. 108
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Endothelium

Background: the value of testosterone levels and erectile dysfunction (ED) as early markers of atherosclerosis is not well understood.
Objectives: to analyze the relationship between plasma testosterone levels in men with both endothelial function (EF) and ED.
Methods: we enrolled 802 asymptomatic, intermediate cardiovascular risk patients, according to the Framingham Risk Score, aged 40 to 80 years, who underwent the study of EF, evaluation of ED and dosage of plasma testosterone.
Results: Testosterone levels correlated both with FMD (r = 0.85; p<0.0001) and IIEF-5 score (rs =0.65; p<0.0001). At multivariable logistic regression analysis, lower serum testosterone levels were strongly associated (p < 0.001) with severe (OR 0.78; CI 0.62 -0.86), and moderate ED (OR 0.85; CI 0.72 - 0.97), while worse EF was strongly associated (p < 0.001) with severe (OR 0.68; CI 0.59- 0.79), moderate (OR 0.76; CI 0.63 to 0.83) and mild to moderate ED (OR 0.8; CI 0.69 to 0.94). Even mild ED resulted statistically associated worse EF (OR 0.94; CI 0.82 - 1.07; p=0.03) but not with serum testosterone levels. These relations were not substantially affected by adjustments for further potential confounders including smoking status, hypertension, diabetes mellitus and body mass index.
Conclusions: we demonstrated a significant correlation between ED, worse EF and testosterone plasma levels in a primary prevention population, therefore low testosterone levels may be considered as early markers of atherosclerosis.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Interaction of the Adipophilin with Acyl-coenzyme A:Cholesterol Acyltransferse 1 and Neutral Cholesterol Ester Hydrolase in Lipid-loaded Macrophages

Abstract nr. 109
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Lipids,Metabolism

**Aim** To explore whether adipophilin interact with acyl-coenzyme A:cholesterol acyltransferse 1 (ACAT1) and neutral cholesterol ester hydrolase (NCEH) in lipid-loaded RAW264.7 cell induced by oxidized low density lipoprotein (ox-LDL). **Methods** RAW264.7 cells were incubated with 50 mg/L ox-LDL for different time. The expression of mRNA and proteins of adipophilin, ACAT1 and NCEH were detected by semi-quantitative reverse transcription-polymerase chain reaction and western blot respectively. Interactions between adipophilin and ACAT1 or NCEH were detected by co-immunoprecipitation. **Results** As the incubation time of ox-LDL was extended in RAW264.7 cells, the expression of mRNA and proteins of adipophilin, ACAT1 and NCEH were significantly increased compared with 0 h group ($P < 0.05$, $n=3$). Co-immunoprecipitation showed that there were interactions between adipophilin and ACAT1 in RAW264.7 macrophages with ox-LDL treatment at 0, 0.5, 1 h and 3 h, and no interactions at 6 h. There were not interactions between adipophilin and NCEH in RAW264.7 macrophages with ox-LDL treatment at 0, 0.5 h and 1 h, and interactions at 3 h and 6 h. **Conclusion** There were interactions between adipophilin and ACAT1 or NCEH in RAW264.7 macrophages with ox-LDL treatment. It suggests that adipophilin may have synergistic effects with ACAT1 and NCEH in lipid-loaded RAW264.7 cell.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Association between the estimated glomerular filtration rate and subclinical atherosclerosis in patients with and without hypertension

Abstract nr. 110
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Cardiovascular Disease, Hypertension, Prevention

Purpose: Atherosclerosis as a chronic, progressive, inflammatory disease with a long asymptomatic phase can be undiagnosed through years, until the occurrence of acute cardiovascular and/or cerebrovascular events. Revealing of factors, which are simple and cheap to assess and might indicate subclinical atherosclerosis, have highest importance. Aim of the study was to investigate relationship between existence and severity of coronary and carotid artery atherosclerosis and glomerular filtration rate (GFR).

Methods: 447 patients (mean age±SD, 63.9±11.6 years), 238 females and 209 males were included in the study. 334 of them had arterial hypertension (AH) and 113 normal blood pressure. Assessment of GFR, coronaroangiography and carotid artery ultrasound was performed in all patients. Gensini score was used for assessment of coronary artery disease severity.

Results: In comparison with hypertensives, normotensive subjects had significantly higher GFR (78.6±19.2 vs 70.8±20.8; P=0.001). GFR showed negative correlation with the stage of AH (r=-0.195; P=0.000). According to the Gensini score level, hypertensive patients had more severe atherosclerotic lesions of coronary arteries than normotensives (43.47±49.41 vs 21.81±37.00; P<0.05). GFR showed strong negative correlation with the Gensini score independent from the having of AH (r=-0.252, P=0.000 in Hypertensives and r=-0.297, P=0.000 in Normotensives, consequently). Right carotid artery (RCA) was more frequently and markedly damaged with atherosclerosis in comparison with left carotid artery (LCA). GFR showed significant correlation with the level of carotid artery damage in hypertensive and normotensive populations (r=-0.258, P=0.000 in hypertensives and r=-0.362, P=0.000 in normotensives, consequently). There was revealed strong positive correlation between coronary artery atherosclerosis expressed in Gensini scores and carotid artery atherosclerotic damage severity in both, hypertensive and normotensive populations (r=0.791, P=0.000 LCA, r=0.802, P=0.000 RCA in normotensives and r=0.745, P=0.000 LCA, r=0.769, P=0.000 RCA in hypertensives, respectively).

Conclusions: Results of our study point out that existence and severity of coronary and carotid artery atherosclerosis is significantly related with GFR. Possibility of coronary and carotid artery atherosclerosis rises with decrease of GFR. Therefore, patients with decreased GFR may be considered as a high risk group for subclinical atherosclerosis and undergo carotid and coronary artery atherosclerosis assessment examinations.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Liposomal prednisolone inhibits vascular inflammation and enhances maturation of arteriovenous fistulas in mice

Abstract nr. 111
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Animal model, Inflammation, Intervention

BACKGROUND
Arteriovenous fistulas (AVFs) for hemodialysis access have a 1-year primary patency of only 60%, mainly as a result of maturation failure that is caused by insufficient outward remodeling (OR) and intimal hyperplasia (IH). The exact pathophysiology remains unknown, but the local inflammatory vascular response is thought to play an important role. Corticosteroids are powerful inhibitors of inflammation that suffer from unwanted side effects when given systemically. In the present study, we evaluated the effect of prednisolone on AVF maturation using a targeted liposomal delivery method in a murine model of AVF failure.

METHODS
First, the effect of liposomal prednisolone on vascular smooth muscle cells (VSMCs) and macrophages was evaluated in vitro. Next, AVFs between the jugular vein and common carotid artery were created in and end-to-side manner in C57BL/6 mice. The animals were then injected (dose 10 mg/kg) with liposomal prednisolone phosphate, liposomal PBS, prednisolone phosphate or PBS at days 0, 2, 5 and 10. Fluorescent-labeled liposomes were injected in a separate group of mice. At time of scarification (day 14), the labeled liposomes were visualized using near-infrared fluoroscopy. In addition, histomorphometric analysis of the venous outflow tract was performed and the composition of the venous wall was evaluated using immunohistochemistry.

RESULTS
Incubation with liposomal prednisolone resulted in a strong reduction of IL-6 and MCP-1 in cultured macrophages while no effect of VSMC proliferation was observed. The in vivo studies revealed that the fluorescent liposomes were mainly detected in macrophages in the anastomotic
area of the AVF (Fig 1). Histomorphometrically, mice treated with liposomal prednisolone had an increased venous circumference and lumen (p<0.01; p<0.03) when compared to the PBS group (Fig 2). Furthermore, we observed a strong reduction in infiltrating CD45+ cells in the liposomal prednisolone group (p<0.01).

CONCLUSION
Liposomes proved to be an effective delivery method to target vascular inflammation in AVFs. Liposomal prednisolone results in enhanced outward remodeling of murine AVF.

**Figure 1**
Green-fluorescent-labeled liposomes accumulated at the anastomosis of the AVF. a: carotid artery. v: jugular vein
colocalization of gold-labeled liposomes and CD68+ macrophages in the anastomotic area of murine AVF.

**Figure 2**
Representative HPS-stained sections of the venous outflow tract of murine AVF of (A) PBS-treated, (B) prednisolone-treated and (C) liposomal prednisolone-treated mice.

**Presentation 2. Translational Research**

**Presentation Preference** Oral presentation

**Additional information**
Cardiometabolic risk factors and epicardial adipose tissue in children and adolescents

Abstract nr. 112
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Lipids, Obesity, Risk Factor, Visceral Fat

Background: Epicardial adipose tissue (EAT) is the visceral fat deposit around the heart and is commonly increased in obese subjects. EAT is related to cardiometabolic risk factors and non-alcoholic fatty liver disease (NAFLD) in adults, but this relationship is not well known in children.


Study groups and methods: In 25 (mean age 13.0 ± 2.3) overweight and obese subjects and 24 lean controls, blood pressure (BP), WC, fasting plasma glucose and insulin, lipids, uric acid and hepatic enzymes were measured. EAT thickness was measured by transthoracic echocardiography.

Results: In overweight and obese subjects, EAT was significantly higher compared to normal weight children. Overweight and obese children had significantly higher body mass index (BMI), WC, BP, triglycerides (TAG), low-density lipoprotein and total cholesterol, hepatic enzymes alanine aminotransferase (ALT) and g-glutamyl transferase, and lower high-density lipoprotein cholesterol (HDL-C). EAT correlated significantly with BP, TAG, uric acid, HDL-C, apoprotein B and ALT. Correlation coefficients were similar or better than for WC, but similar or lower than for BMI.

Conclusion: EAT thickness in children is associated with an unfavourable cardiometabolic risk profile including biochemical signs of NAFLD and hyperuricaemia, but is not a stronger indicator than BMI.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Correlation of some markers of inflammation, thrombosis and homocysteine with carotid arteries stenosis in patients with heart ischemic disease

Abstract nr. 113
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Prevention, Thrombosis

The purpose of the work was to find correlation which may exist between IL1β, IL6, HSCRP, fibrinogen, D dimer, SFMC and homocystein, levels and the degree of Carotid Arteries (CA) stenosis in patients with heart ischemic disease.

According to CA stenosis, 75 patients (45 female and 30 male) with chronic heart disease (mean age 55.7±1.6 years) were divided into 2 groups. Group I comprised 33 patients, who had hemodynamically insignificant stenosis of CA (<50%). Group II consisted of 42 patients with hemodynamically significant stenosis (>50%). CA intima media thickness of all patients was 1.18±1.02 mm. D dimer, SFMC, as well as homocystein, interleukins, fibronogen and HSCRP were defined.

In Group I, The value of D dimer was 900.0±3.0 ng/ml, SFMC 8.0±0.03 g/l. Homocystein was 18.8±0.06 mkmol/l, IL1β 53.3±1.0 pg/ml, IL6 49.3±0.8 pg/ml, HSCRP 6.9±0.02 mg/l and fibrinogen 5.9±0.07 g/l accordingly. The relation between these parameters and CA stenosis degree appeared to be positive (from r=0.473, to r=0.533). In Group II, The value of D dimer was 1100.9±3.0 ng/ml, SFMC 9.0±0.0.6 g/l. Homocystein increased up to 26.0±0.3 mkmol/l, IL1β up to 59.3 and IL6 55.4 pg/ml, HSCRP was 9.1±0.3 mg/l, fibrinogen 6.1±0.06 g/l. Correlation between these parameters and CA stenosis was positive (from r=0.500 to r=0.621).

Taking into consideration the result obtained, we think it is possible to use positive correlation between the degree of CA stenosis, D dimer, SFMC, homocystein and inflammation parameters as the markers of development of carotid atherosclerosis in the patients with heart ischemic disease.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Relationships between Protectin CD59 positive mononuclears and lipid levels in progressing atherosclerosis

Abstract nr. 114
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords ACS, Atherosclerosis, Immunity, Lipoproteins

Background. It's known that the complement activation is involved in the pathogenesis of clinical complications of coronary atherosclerosis via Membrane Attack Complex (MAC) formation and following cell lysis. Simultaneously a defense mechanism against the damaging effect of complement is activated. An important factor of the complement inhibition is Protectin CD59 which binds to cell membranes and stops MAC formation.

Purpose. The aim of this study was to investigate the relationship between the number of circulating CD59-"positive" (CD59 +) mononuclear cells and lipid levels in patients with acute coronary syndrome.

Methods. Blood samples of 87 (53 male and 34 female) patients with clinical and instrumental signs of acute coronary syndrome (ACS) and blood samples of 43 volunteers as controls were examined in this study. The control group was corresponding on average age, sex and lipid profile however with no or minimal signs of atherosclerosis progression. Flow cytometry with fluorescently labeled monoclonal antibodies was used to determine CD59 + peripheral mononuclears during admittance and within two weeks after discharge. Antibodies to oxidized LDL (ab-oxLDL) have been determined by ELISA. Blood levels of HDL-cholesterol, LDL-cholesterol and Lipoprotein(a) were measured with usage of routine lab kits.

Results. It has been shown that the mean quantity of CD59 + cells was significantly higher in group with documented ACS in comparison with control group. In patients with transmural myocardium infarction the expression of CD59 + was more intensive then in those with unstable angina. During treatment the levels of CD59 + mononuclears were decreased. We found in patients with acute coronary syndrome the negative correlation between the number of circulating CD59 + mononuclear cells and HDL-cholesterol levels (r = -0.64). On the other hand the significant positive correlation (r = +0.61) was found between CD59 + and Lipoprotein(a). The correlation between LDL-cholesterol levels and CD59 + was absent, while the positive correlation between ab-oxLDL and CD59 + was significantly high (r = +0.63)

Conclusion. The expression of CD59 - lipid-anchored inhibitor of complement lysis is correlated with lipoprotein blood levels in patients with clinical manifestation of atherosclerosis.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Potential therapeutic use of anti-electronegative LDL single chain fragment variable vectorized in nanocapsules on atherosclerosis

Abstract nr. 115
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis,Inflammation,LDL, Therapy

The electronegative low-density lipoprotein [LDL(-)], a native LDL modified subfraction, plays a key role in atherosclerosis, since its modifications are capable of inducing foam cells formation. The immune system is crucial in atherogenic process and therapeutic strategies directed to the immunoregulation of this process have been used. In this context, it is suggested that antibody fragments such as scFv (single chain fragment variable) may be used as new alternatives in prevention of development and/or progression of atherosclerosis. In order to increase efficiency of scFv, nanoparticles have been combined with these fragments.

Given the role of LDL(-) in atherosclerosis, this study aimed to evaluate the effects of a nanostructured system containing anti-LDL(-) scFv fragments derivatized on the surface of nanocapsules (NC-scFv) and to determine endocytic mechanisms related to its internalization by murine and human primary macrophages.

Foam cell formation was evaluated by LDL(-) uptake and the impact of different endocytic pathways were determined by NC-scFv uptake in the presence of specific endocytosis inhibitors. It has been demonstrated that treatment of primary murine and human macrophages with NC-scFv significantly decreased the uptake of LDL(-) (84.67% and 86.50%, respectively) and that this formulation is internalized by macrophages through different mechanisms of endocytosis (phagocytosis, macropinocytosis and dynamin dependent endocytosis). In human primary macrophages, both scFv anti-LDL(-) and the formulation NC-scFv significantly decreased the gene expression of IL1B (interleukin 1 beta) and MCP1 (monocyte chemoattractant protein-1).

These results provide evidence for the atheroprotective action of the NC-scFv, suggesting it as a therapeutic strategy with potential use in atherosclerosis.
Funding: FAPESP (grant to D.S.P.A. and scholarship to M.F.C. and S.M.K.), CAPES/DAAD/PROBRAL and CNPq/INCT_if.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Renal Oxidation of ESS diabetic rats is minimized by EPA

Abstract nr. 116
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model,Blood pressure,Diabetes,Dyslipidemia

Salt sensitivity (SS) is associated with increased cardiovascular risk in diabetic patients due to increased renal oxidation and decreased urinary sodium excretion. Western diet has limited content of w3 polyunsaturated fatty acids, with antioxidant capacity, such as ecoisapentaneoico acid (EPA). Therefore we hypothesized that nutritional supplementation with EPA, prevents SS in DM rats by decreasing renal oxidative stress. **Methods:** Wistar rats were used as healthy controls. Type II diabetic rat group (eSS), 3 months old, were divided in 3 groups, diabetic control (eSS), eSS treated with arachidonic acid (pro-oxidant)(2.5mg/ip, monthly) (eSS+AA) and eSS treated with EPA (2.5mg/ip month) (eSS+EPA). Animals were treated during 1 year, then placed in metabolic cages and subsequently underwent 2 subsequent experimental periods of 7 days each with normal sodium diet (0.4% NaCl)(NNaD) and high salt diet (4% NaCl)(HNaD). At the end of each period, weight, systolic blood pressure (SBP), HbA1c, triglycerides (Trig), cholesterol (Chol), creatinine (Cre), kidney γ-glutamyl transpeptidase activity (γGTP), urinary protein excretion (UprotV) were assayed. **Results** eSS rats had elevated HbA1c, Tri, Chol and reduced body weight vs. Wistar control group. The eSS+AA group also showed elevation of HbA1c, Trig, with no change in Cre and Chol. During NNaD SBP was 119±3mmHg and after HNaD 125±1mmHg (p<0.05). In contrast, in EPA+eSS lower values of HbA1c (5.6±0.3% vs 7.0±0.2%, p<0.05), Trig (175±1mg/dl vs 245±12mg/dl p<0.05), and increased body weight (424±11gr vs 511±22gr, p<0.05) vs eSS were observed. EPA supplementation prevented the increase of SBP during the HNaD (126±2mmHg vs. 128±2mmHg p>0.5). eSS+AA did not differ from eSS group. No significant difference was observed in UprotV among groups. Renal interstitium γ-GT activity in eSS group was 0.65±0.04au and 0.61±0.02au in eSS+AA group, while in EPA+eSS 0.5±0.03 (p<0.05). **Conclusion.** The salt sentitive eSS rats have hypertriglyceridemia and elevated HbA1c. Supplementation with w3 EPA prevented the salt sensitivity, increment of HbA1c and triglycerides. These beneficial changes were associated with lower kidney γ-GT activity, suggesting that the EPA dietary supplement can be used in diabetic patients to prevent salt sensitivity and improve metabolic abnormalities.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Homocysteine-lowering effects of VCRESC® (a vitamin micronutrient beverage) in patients with coronary artery disease

Abstract nr. 117
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Nutrition

INTRODUCTION: Increased homocysteine (Hcy) level is a risk factor of atherosclerotic cardiovascular diseases (ASCVD). One of possible approach to reduce Hcy levels is an oral supplementation of B-vitamins and folic acid. However, it would be hard to take sufficient amount of these micronutrients by altering daily food consumption. Aim: In the present study, we examined long-term effects of B-vitamins (B6, B12) and folic acid supplementation by commercially-available beverage on Hcy level in patients with ASCVD. Subjects and Methods: Fifteen patients with angiographically-proven stable coronary artery disease (54.4±3.0 years old) were enrolled. Fasting blood levels of vitamin B6, B12, and folic acid were measured before and after over six month-intervention period with consumption of 125 ml of VCRESC® (Nutri Co. Ltd, Mie, Japan) after breakfast. Fasting plasma Hcy levels were also measured. Results: Mean levels (±SD) of vitamin B6 (pyridoxal) significantly increased (9.2±1.0 to 31.8±3.3 ng/ml, p<0.01), and B12 levels remained unchanged (556±140 to 538±39 pg/ml, n.s.). Baseline folic acid levels remained low, and increased significantly after the period with considerable variation (6.7±0.5 to 16.2±1.8 ng/ml, p<0.01). In response to these changes, Hcy levels significantly decreased from 10.2±0.5 to 7.9±0.4 nmol/ml (p<0.01). None of adverse events potentially related to VCRESC® consumption was noted. Conclusion: Levels of vitamin B6 and folic acid significantly increased by six-month consumption of VCRESC® in association with significant reduction of Hcy, suggesting that a vitamin micronutrient beverage appears to be a novel alternative to reduce plasma Hcy levels in patients with ASCVD.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation

Additional information
A VERY LOW CALORIE DIET AMELIORATES ENDOTHELIAL FUNCTION AND INFLAMMATION MARKERS IN OBESE PATIENTS WITH TYPE 2 DIABETES

Abstract nr. 118
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Diabetes, Endothelium, Nutrition, Obesity

Introduction: Obesity and type 2 diabetes (T2D) increase the risk of cardiovascular diseases. This increased risk is associated with impairment of endothelial function and increased systemic inflammation. We investigated the effect of diet-induced weight loss on biomarkers of endothelial function and inflammation in overweight patients with T2D.

Methods: 132 T2D patients with BMI > 27 were put on a 750 kcal/day diet for 2 months, followed by 1000-1300 kcal/day diet for another 2 months. At baseline and at the end of the intervention, the endothelial markers sICAM-1, sVCAM-1, vWF, and inflammation markers CRP and IL-6 were measured in plasma.

Results: The diet intervention resulted in a 9.8±5.2 % weight loss. sICAM-1, vWF and CRP levels were significantly reduced (p < 0.01), while sVCAM-1 and IL-6 remained unaffected. The intervention reduced the number of patients with a high-risk CRP level (>3 mg/L; p=0.033) and increased the number of patients with a low-risk CRP level (<1 mg/L; p<0.0001) (figure 1). In multiple linear regression models, the diet-induced reduction in sICAM-1 level was significantly associated with baseline sICAM-1 (p<0.0001), reduction in CRP levels (p<0.0001) and reduction in bodyweight (p=0.016). The decrease in vWF was associated with baseline vWF (p<0.0001), fall in waist circumference (p=0.016) and fall in fasting glucose (p=0.028). The reduction in CRP was associated with baseline CRP (p<0.0001).

Conclusion: In conclusion, a 4-month (very) low calorie diet is a therapeutic option for improving endothelial function and reducing systemic inflammation in overweight and obese type 2 diabetes patients, ameliorating cardiovascular risk. The positive effect of this dietary regimen was most clear in the patients that presented with the highest sICAM-1, vWF and CRP plasma levels before initiation of the diet.

This study is internally funded by the Erasmus Medical Center within the funding program: ‘zorgonderzoek Erasmus MC’.
Figure 1: The effect of the dietary intervention on the distribution of participants among CRP risk categories
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
ASYMPTOMATIC PATIENTS WITH SEVERE VASCULAR DISEASE AND FIBROCALCIC PLAQUES DO NOT EVIDENCE INTRAPLAQUE INFLAMMATION WHEN STUDIED BY 18-FDG PET.

Abstract nr. 119
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Co-author(s) - De Pierris, Carlos
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Imaging, Inflammation, Pathogenesis

Background: Recent although scarce evidence indicates that symptomatic patients (P) with peripheral or carotid artery disease with levels of high ultra sensitive C-reactive protein (US-CRP) and/or echo-translucent fibrous-lipid plaques present high 18-FDG uptake in PET studies. However, there is no clinical information as to whether patients with severe vascular disease who are asymptomatic and evidence fibrocalcic plaques present the same inflammatory pattern and 18-FDG uptake. With this purpose we designed a metabolic study protocol for this type of patients by correlating PET with US-CRP findings.

Materials and Methods: A total of 18 consecutive asymptomatic P were followed up, 14 males and 4 females, with an average age of 69 ± 12 years old, with multiple vascular risk factors (hypertension: 18, dyslipidemia: 15, diabetes: 8, tobacco: 10, obesity: 10), with evidence of severe fibrocalcic lesions in carotid (n: 18), aortic (n: 4), iliofemoral (n: 4) territories with an average of 2.3±0.7 lesions per patient. US-CRP was determined (normal reference value ≤ 4 mG/liter) and all underwent 18-FDG PET-CT metabolic study, administering 15 mCi/patient and obtaining images after 90 minutes.

Results: 1-. US-CRP: 1, 97±0.5 mG/l; 2-PET-CT: no patient evidenced uptake of 18-FDG in territories with severe vascular lesions with fibrocalcic plaques.

Conclusion: Unlike what was observed in symptomatic P or P with echo-translucent fibrous-lipid plaques, these sampling patients, who were stable and showed no laboratory-based evidence of inflammation, did not present uptake of the radioactive tracer, this being in agreement with the clinical response, though severe lesions were present.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Validation of carotid artery disease as marker of coronary disease and inducible ischemia in asymptomatic patients with multiple risk factors.

Abstract nr. 120
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Co-author(s) - Martire, Maria Victoria
Co-author(s) - Perelestein, Sergio
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Imaging, Risk stratification

Background: The carotid intima-media thickness (IMT) as a long-term risk marker and the correlation between carotid plaques (CP) and SYNTAX score, have been well demonstrated. However, it has recently been known that there is information about the incidence of myocardial ischemia depending on the different degrees of severity of the carotid artery disease (CAD).

Objective: To assess the incidence and severity of inducible myocardial ischemia in patients (P) with different degrees of CAD, using quantitative data of Carotid Doppler (CD) and of the functional study of radioisotope myocardial perfusion (SPECT) as assessment test of ischemia.

Materials and Methods: A total of 397 consecutive asymptomatic P were followed up, 251 males and 146 females, with an average age of 65 ± 9 years old, with multiple cardiovascular risk factors and high pre-test probability for coronary artery disease according to clinical scores, They were indicated color Doppler echocardiography CDE and SPECT and divided into 5 groups (G) according to the degree of CAD, assessed by means of the thickness summation in mm in both carotid territories: Plaque Score (PS).

G1 (Control, n: 50): With no carotid alterations: PS: <1.1 mm; G2 (n: 150): With thickening of IMT: PS: between 1.1-1.5 mm; G3 (n: 88): PS: between 1.5-6, G4 (n: 62): PS: between 6-12; G5 (n: 47): PS: > 12. Determination of ischemic incidence in each group, correlation (r) between PS by means of CDE with summed difference score (SDS) by means of SPECT and ROC curve.

Results: From the total of 397 P, 169 (42%) developed ischemia under SPECT. For each group: G1:14 (28%), G2:41 (26%), G3:30 (34%), G4:45 (72%) *, G5:39 (83%) *. Correlation: r=PS/SDS: G1:0.13. G2:0.23. G3:0.25. G4:0.47 *. G5:0.65 *. ( *= p: <0.01). ROC curve: 0.72 ± 0.04 (PS cutting line SP: ≥ 6).

Conclusion: The quantification of carotid vascular disease and its increasing complexity predicted elevated occurrence and severity of inducible ischemia in patients with higher risk, reasserting the clinical value as an additional risk marker to the scores available at present.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Incidence and severity of alterations in calcium phosphorus metabolism in patients with multiple cardiovascular risk factors and high Framingham score.

Abstract nr. 121
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Co-author(s) - Martire, Maria Victoria
Co-author(s) - Perelestein, Sergio
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Metabolism, Risk Factor

**Background:** Contradictory and non-concluding data refer to the clinical and therapeutical importance of alterations in calcium phosphorus metabolism (CPM) in patients with multiple cardiovascular risk factors (CVRF) and high Framingham point score (PFS) (≥ 20).

**Objective:** To assess the incidence and severity of CPM alterations on a first model of clinical high risk, through the determination of parathormone (PTH), Vitamin D (Vit D), plasma calcium (P-Ca), fasting (F-Ph) and postprandial phosphatemia (Pp-ph).

**Material and Methods:** Having successively studied 88 patients (61 males aged 60±9 years), all of them with FPS ≥ 20, PTH was determined through chemiluminescence (normal reference value (RV): 7-53 pG/ml), Vit D through electrochemiluminescence (RV: 20-100 nG/ml), calcemia and phosphatemia through colorimetric, complexometric and UV methods (RV: 8,6-10,2 mgs/dl and 2,5-4,5 mgs/dl respectively). Those patients with renal clearance < 40ml/min, calcium supplement or Vit D intake, and hypercalciuria remained excluded.

**Results:**

A-Incidence. 84/88 patients (95%) presented abnormal determination (elevated) of PTH, 82/88 (93%) severe lack of Vit D, 0/88 (0%) abnormal calcemia, and 88/88 (100%) abnormal postprandial response to dietary phosphate (F-Ph, Pp-Ph, 3 h after intake). (F: fast, Pp: post prandial)

B-Magnitude of monitored alterations. PTH: 91±27 pG/ml, Vit D: 10±6 nG/ml, Ca-P: 9,01±0,25 mgs/dl, F-Ph: 3.26±0.47 mgs/dl, Pp-Ph: 4.03±0.65 mgs/dl (p< 0.01). (pG: picograms, nG: nanograms, mgs: milligrams, dl: deciliter.)

**Conclusion:** In this model to identify clinical high risk there is evidence of a very high incidence and severity of parathormone and Vit D alterations, as well as abnormal postprandial management of dietary phosphate, showing the need of a physiopathological and eventually therapeutic new approach in this insufficiently explored area.
Subdivision 3. Clinical Studies

Presentation Preference: Electronic poster presentation

Additional information

Monitored alterations. (scale log 2)

$\text{PTH}: \text{pg/ml}$
$\text{Vit D}: \text{ng/mL}$
$\text{Ca, Ph}: \text{mg/dl}$

Box plots for various parameters.
Herbal Commiphora Mukul Flavonoids Improves Chymase Enzyme Inhibition in Human Chymase Transgenic Mice a Novel Therapeutic Regimen against Atherosclerosis

Abstract nr. 122
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Pharmacology, Therapy, Vulnerable Plaque

BACKGROUND: This insilico study was designed to investigate the effects and mechanism of inhibition action of the Indian herbal plant flavonoids, Phytosterols, Gugulipids and Guggulsterones from Commiphora mukul, on chymase enzyme inhibition through its validation on human chymase transgenic mice

METHODS: We will evaluate and study on downloaded different 3D X-ray crystallographic structures of chymase Enzymes, 3N7O, 1T31, 3SON, and 2HVX to incorporate molecular docking techniques using Commiphora mukul herbal Flavanoids: Phytosterols, Gugulipids and Guggulsterones into the active site of chymase enzyme. The Molecular dynamics simulations of chymase with Commiphora mukul Flavanoids were performed to reveal its binding orientations to depict any conformational changes in the active site. At last, validation studies implies specificity, effects of computational active herbal extract of Commiphora mukul Flavanoids on the signal transduction pathway in heart remodeling of human chymase transgenic mice

RESULTS: The inhibition mechanism of chymase gives key structural focus conceptualizing rational design of novel herbal inhibitors of the enzyme. The results conferring about 3D chymase structure as well as the novel benefits of Commiphora mukul Flavanoids Phytosterols, Gugulipids and Guggulsterones in hydrolase function

CONCLUSIONS: Novel Commiphora mukul herbal flavanoids benefits in chymase inhibition for the vulnerability and treatment of cardiovascular diseases, allergic inflammation, and fibrotic disorders. Binding mode prediction reveals substitution of a heavier atom on most active site inferring about to change of its variation and orientation causing other groups to interact with Phytosterols, Gugulipids and Guggulsterones residues. Dynamics simulations depict conformational variation in inhibitor regions, binding convinced alteration, thus changing its interactions with it. Chymase with the active Phytosterols, Gugulipids and Guggulsterones inhibitors utilizing pharmacophore modeling which is enforced in databases screening for other novel potent herbal drugs. Finally, hits which constrained best at the active site, presented key interactions and favourable electronic features for chymase and clinical validation studies on human chymase transgenic mice shows how individual mutation and variation deals pathologies or "silent" protein changes in Atherosclerosis

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Association between body mass index and existence and severity of coronary artery disease in normotensive patients

Abstract nr. 123
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Cardiovascular Disease, Obesity

Introduction: Link between obesity and coronary artery disease (CAD) was proved in clinical trials and like hypertension, it is considered as a strong risk factor for CAD. Therefore, association between body mass index (BMI) and existence and severity of atherosclerotic lesion of coronary arteries still has interest in different ethnic groups.

Objectives: Study purpose was to investigate relationship between existence and severity of CAD and body mass index in patients with normal blood pressure values without any history of high blood pressure and its treatment.

Materials and Methods: 80 patients (mean age±SD, 58.15±13.69 years), 47 females and 33 males, with normal blood pressure values and no history of hypertension were included in the study. Calculation of BMI and coronaroangiography for assessment atherosclerotic process and its severity was performed in all study participants. Gensiny score was used for assessment of CAD severity. Patients with diabetes mellitus, smokers as well as with renal/liver insufficiency were excluded from the study.

Results: According to the BMI, all the study participants were divided into three groups: patients with normal weight (n=26, mean BMI = 21.34±2.66 kg/m²), overweight (n=29; mean BMI = 27.03±1.45 kg/m²) and obese (n=25; mean BMI = 35.6± 6.13 kg/m²). In comparison with normal weight patients, overweight and obese patients had significantly higher rate of CAD (46.15% vs. 62% and 60%; P<0.05, respectively). Diffuse type atherosclerotic lesion of coronary arteries was significantly frequent in obese patients in comparison with normal and overweight (36% vs. 15.4% and 1.65%). Local atherosclerotic lesions i.e. plaques were more frequent in overweight patients, than in obese and normal weight (55 % vs. 24% and 30.8%). Therefore, highest level of Gensini score appeared in patients with obesity in comparison with normal and overweight subjects (44.8±21.9 vs. 23.43±27.6 and 38.43±24.8).

Conclusions: Results of our study points out that severity of coronary atherosclerosis is significantly related with BMI. Namely, as high is BMI, as severe is atherosclerotic damage – diffuse or local. Therefore, possibility of existence and severity of coronary artery atherosclerosis rises with an increase of BMI.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
“New thiazolidine compounds improve metabolic syndrome”

Abstract nr. 124
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Diabetes, Dyslipidemia, Hypolipidemic Drugs, Obesity

The thiazolidinediones (TZDs) are anti-diabetic oral drugs used for type 2 diabetes treatment. However, clinical practice shows important adverse effects, such as weight increase and bone density loss. Thus, new thiazolidine compounds (NTC) have been developed to identify more effective drugs with less adverse effects. In this study we investigated biological effects promoted by NTC (GQ-02, GQ-11, GQ-177 and Lyso-7) on a metabolic syndrome animal model. C57BL/6J and C57BL/6J LDLr -/- mice high fat diet fed were treated with NTC, water, vehicle and pioglitazone (control). Relative gene expression in epididymal adipose tissue was quantified by RT-PCR (ΔΔct analysis method). Glucose tolerance test (GTT) was done according to protocol guidelines, serum leptin and insulin were measured by ELISA and lipid profile was evaluated by enzymatic/colorimetric assays.

All mice treated with NTC showed decreased values for GTT (AUC) and serum insulin, besides increase of adiponectin and glut-4 expression in adipose tissue, indicating hypoglycemic and insulin sensitizer effects. Treatment with NTC also showed decreased serum leptin and leptin mRNA in adipose tissue, indicating improvement of leptin resistance and hyperleptinemia condition. Moreover, one of NTC (GQ-…) modulated lipid profile by increasing HDL-cholesterol and decreasing LDL-cholesterol. Moreover, srebp expression was dow- regulated in hepatic tissue.

Our data show improvement of metabolic syndrome with beneficial effects on insulin, glucose and leptin resistance besides hypercholesterolemia. These results warrant further studies with these NTZ that can be drug candidates helpful for metabolic syndrome treatment.

Funding: FAPESP (grant to D.S.P.A. and scholarship to J.A.C. and M.F.C.), CNPq/INCT_if.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Increased aortic valve calcification in familial hypercholesterolemia: Prevalence, extent and associated risk factors in a case-control study

Abstract nr. 125
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Familial Hypercholesterolemia, Imaging, Pathogenesis

Background
Severe aortic valve calcification is seen in patients with extreme LDL-C levels as are seen in patients with homozygous familial hypercholesterolemia (FH). Although patients with heterozygous FH have lower levels of LDL-C, the prevalence of AoVC in heterozygous FH is unknown. We quantified AoVC and compared the results with controls without FH, using cardiac CT.

Methods
145 asymptomatic, statin treated, patients with FH (93 men; mean age 52, SD=8) and 131 controls without FH (78 men; mean age 56, SD=9) underwent cardiac CT calcium scoring. The amount of calcium at the aortic valve leaflets was expressed in Agatston units as the AoVC-score. The AoVC-score was compared between patients with and without FH.

Results
Prevalence of AoVC and the AoVC-score (median, IQR) were higher in FH than in controls: 41%, 51(9-117) and 21%, 21(3-49), (P<0.001 and P=0.007). LDLR-negative mutational FH was associated with the highest prevalence of AoVC (53%, P<0.001) that was generally more severe (OR 3.17 (CI 1.43-7.02; P=0.004). Age, maximum untreated total cholesterol, diastolic blood pressure, LDLR-negative mutational FH and coronary artery calcification were independently associated AoVC.

Conclusion
This is the first study to show that heterozygous FH is associated with high prevalence and extent of subclinical AoVC, and that there seems to be an LDL-C gene dosage effect as causative factor for this development.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Lipoprotein (a) is not associated with carotid plaque presence and carotid intima media thickness in statin treated FH patients

Abstract nr. 126
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia, Imaging, Lp(a), Risk stratification

Background:
Familial hypercholesterolemia (FH) leads to elevated low density lipoprotein cholesterol and thereby increases risk of premature cardiovascular disease (CVD). An additional risk factor in FH is lipoprotein (a) (Lp(a)). However, it is unknown if Lp(a) causes a higher atherosclerotic burden in these patients. Subclinical atherosclerotic burden can be visualized by carotid ultrasound measurements, and we investigated whether carotid plaque presence, and the carotid intima media thickness (C-IMT) are associated with Lp(a) in statin treated FH patients.

Methods and results
191 FH patients were included in this study, and were split into two groups. The first, with high Lp(a) (≥0.3g/L) and the second, with low Lp(a) (<0.3g/L). These groups did not differ at baseline.
Carotid plaque presence was evaluated, and C-IMT was measured twice from two different angles at both common carotid arteries. For the analysis the mean of these four measurements was used.
The association between Lp(a) and plaque presence was tested using a Chi-Square test showing no differences in plaque presence between the groups (p=0.448). C-IMT was tested with an ANOVA, and was the equal in the high Lp(a) group (CIMT= 0.588±0.133 mm) as in the low Lp(a) group (C-IMT=0.593±0.127 mm) (p=0.842).
Finally the results did not change when other cut-off values (Lp(a) 0.5g/L and 1.0g/L) were used or when Lp(a) was used as a continuous variable (data not shown).

Conclusions
Lp(a) is not associated with subclinical atherosclerosis defined as plaque presence or an increased C-IMT in statin treated FH patients. A possible explanation is that adequate statin treatment results in normalisation of C-IMT.

Subdivision 3. Clinical Studies
Presentation Preference Mini-oral presentation
Additional information
**Inter observer variability and intra observer variability of automatic carotid intima media thickness measurements**

Abstract nr. 127
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**Topic** Epidemiology of CVD; The Risk Factor Concept

**Keywords** Imaging, Risk stratification

**Introduction:** Evaluation of subclinical atherosclerosis can be done with the use of carotid ultrasound, in which the presence of carotid plaques can be evaluated and the carotid intima media thickness (C-IMT) can be measured. The aim of this study was to evaluate the measurement error of the Panasonic CardioHealth station when used for carotid plaque scan and C-IMT measurement.

**Methods and Results:** Two experienced physicians performed plaque scans and C-IMT measurements. Plaque scans were done bilaterally in the internal, external, and common carotid arteries. C-IMT was measured bilaterally from two different angles. For the intra observer variability both physicians measured 15 separate individuals twice in a blinded matter. Both physicians measured 50 patients for the inter observer variability. Intra observer plaque presence was excellent with a 100% accuracy for both observers. Inter observer plaque presence agreed in 92% of the cases, with an equal error for the observers.

The ICC of both observers where similar 0.93 (0.89-0.96) vs 0.90 (0.84-0.94). Furthermore the LOA were also showed a similar SD change (0.0049±0.074) vs (-0.015±0.075).

The intra class coefficient (ICC) between the two observers was 0.92 (0.89-0.94), and the line of agreements (LOA) where -0.0057 (±0.076).

**Conclusion:** The Panasonic CardioHealth station shows reliable reproducible results between different and individual observers. The LOA were higher than expected per measurement, which can be caused by the device. However, since no big differences were found between observers it is more likely that the difference is caused by the unreliability of the C-IMT measurement per sé.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Dunaliella salina modulates the adhesion molecules of endothelia and the cell migration of vascular smooth muscles to ameliorate inflammatory responses

Abstract nr. 128
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Inflammation, Pharmacology

The inflammatory responses of blood vessels involve up-regulation of vascular adhesion molecules such as vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1). Migration of vascular smooth muscle cells (VSMC) via MMP activities also occurs at inflammation so becomes a marker of atherosclerosis. Dunaliella salina has shown its capability to prevent hyperlipidemia-induced atherosclerosis. However, the molecular mechanisms in its prevention effects are still not yet explored. The aim of this study is to investigate the effects of Dunaliella salina extracts on expression of biomarkers that participates in atherosclerosis.

RAW246.7 macrophages were activated by LPS (1ug/ml). Endothelial cells (SVEC4-10 cell line) and VSMC (A7r5 cell line) were treated with 50% RAW-conditioned medium (i.e. 50% DMEM culture medium plus 50% LPS-activated macrophage culture medium) with and without various concentrations of Dunaliella salina extracts (from 0.01 to 1 mg/ml). Production of nitric oxide, TNF-alpha and Monocyte chemoattractant protein-1 (MCP-1) in macrophages and production of VCAM-1 and ICAM-1 in SEVC cells were measured by ELISA assay. Matrix metalloproteinases (MMP)-2 and MMP-9 protein levels and cell migration in VSMC were evaluated by Western blotting and wound healing assay, respectively.

Results showed that production of MCP-1, ICAM-1 and VCAM-1 and expression of MMP-2 were significantly suppressed by Dunaliella salina extracts (at both 0.5 and 1 mg/ml). Furthermore, VSMC migration was also decreased by Dunaliella salina extracts. These data indicate that Dunaliella salina extracts provides protective effects against proinflammation-induced atherosclerosis via prevention of adhesion molecules production and migration of VSMC.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
K-877, a potent and selective PPARα modulator, increases plasma FGF21 and improves triglyceride metabolism in Zucker fatty rats.

Abstract nr. 129
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Hypolipidemic Drugs,Lipids,Triglycerides

In clinical trials of hypertriglyceridemia (triglyceride (TG) up to 500 mg/dL), K-877 showed reduction of plasma triglycerides and markedly increased plasma fibroblast growth factor 21 (FGF21) levels, whereas fenofibrate had little effect on FGF21. We investigated the effect of K-877 on TG metabolism, using Zucker fatty (ZF) rats as a model of severe hypertriglyceridemia (TG>500 mg/dL).

After 2 weeks of treatment with K-877 3 mg/kg, significant reduction (67%) in plasma TG was observed compared with fenofibrate 100 mg/kg (41%). We examined TG clearance in blood and TG secretion rate from liver, using soybean-oil emulsion and tyloxapol (Triton WR-1339), respectively. K-877 accelerated TG clearance ($t_{1/2}$:16.3 min) compared with fenofibrate (23.7 min) and control (25.7 min). Meanwhile, TG secretion rate was reduced compared to control (892.9 mg/dL/h) but there was no significant difference between K-877 (593.6 mg/dL/h) and fenofibrate (663.0 mg/dL/h). These data suggest that accelerated TG clearance has an important role in TG-lowering by K-877 in this model.

FGF21 is known as a hormonal regulator of lipid metabolism, and decreases plasma TG while enhancing TG clearance. K-877, but not fenofibrate, increased liver FGF21 mRNA and plasma FGF21. Moreover, in primary human hepatocytes, K-877 increased secretion of FGF21 compared with fenofibric acid.

In conclusion, these results suggest that the action of K-877 on FGF21 may contribute to its potency in improving dyslipidemia. We intend to verify the effects of K-877 on severe hypertriglyceridemia in human in future clinical studies.

Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
Effect of Diets With Different Protein Composition in Weight and Lipids in Overweight and Obese Women: A Randomized Controlled Study

Abstract nr. 131
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Lifestyle, Lipids, Nutrition, Obesity

Background: High-protein hypocaloric diets have been shown to be effective in promoting weight loss in overweight and obesity, however, the protein percentage to achieve a better efficacy and acceptability has not been established.

Objective: To assess the effect of three energy-reduced diets with different high-amount of protein (20, 27 and 35% of whom around 50% coming from animal source) on weight loss and lipid metabolism. Secondary outcome involved acceptability, compliance and palatability.

Methods: Three-months randomized controlled study including women meeting the following criteria: aged ≥ 18 and < 80 years, body mass index (BMI) ≥ 27.5 and < 40 kg/m², steady weight in previous 3 months and not taking lipid-lowering drugs and/or sterols supplements. We randomly assigned 91 women to one of three calorie-reduced diets with the following distribution of calories from protein, carbohydrates and fat, respectively: 20%, 50% and 30%; 27%, 43%, and 30%; 35%, 35%, and 30%. Each participant’s caloric prescription represented a deficit of 600 kcal/day as calculated from energy expenditure. Individual visits with a nutritionist were performed every 2 weeks. Clinical, biochemical and anthropometric outcomes were assessed at baseline and at the end of dietary intervention.

Results: 80 women with a mean (±SD) age of 44.0±9.08 years and a BMI of 37.7±3.39 kg/m² completed the study. Weight loss was of -8.16±4.18, -9.66±5.28 and -10.7±4.28 kg in 20%, 27% and 35% protein-diets groups respectively (P = 0.164 among groups and P = 0.041 comparing 20 vs. 35% diets). Significant decreases were observed in triglycerides, total cholesterol and LDL cholesterol in all groups although they were higher in the 35% than in 20% group (P<0.05 in all of them). HDL cholesterol increased especially with the highest protein-diet (P=0.028 comparing 20 vs. 35% diets). Lipid profile improvement was not correlated to weight loss. Dietary compliance was higher and exercise change was homogeneus in all groups. Acceptance, palatability and saciety questionnaire showed similar results among groups.

Conclusions: A high-protein, energy-restricted diet confers weight-loss benefit. Lipid profile improved specially in those women following 35%-protein diet regardless of weight loss.
Acceptability, palatability and compliance assessed by participants were similar in all diets.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Rare APOE gene mutations in primary hyperlipidemias

Abstract nr. 132
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Genetics

Apolipoprotein E has an important role in the cellular uptake of lipoproteins. APOE ε2/ε2 genotype produces dysbetalipoproteinemia, but other rare APOE mutations have been associated with other types of dyslipidemias. The objective was to identify rare APOE variants and to establish their contribution in the pathogenesis of primary hyperlipidemias.

Methods: A total of 1,112 unrelated subjects were recruited in the Lipid Unit at Hospital Universitario Miguel Servet, Zaragoza (Spain): 509 with isolated hypercholesterolemia (LDL cholesterol >95th percentile), 505 with mixed hyperlipidemia (total cholesterol and triglycerides >90th percentile) and 98 with isolated hypertriglyceridemia (triglycerides >95th percentile and total cholesterol <90th percentile). In addition, 183 normolipemic subjects were analyzed as control group. Exclusion criteria were: body mass index >30 Kg/m², diabetes, renal or liver disease and ε2/ε2 APOE genotype. Exon 4 of APOE was sequenced in all subjects and in all available family members when a rare variant was found in the index patient.

Results: The following APOE rare variants have been identified: p.Gly145Asp (rs267606662) in two subjects with mixed hyperlipidemia; p.Arg163Cys (rs769455) in 3 subjects, one in each hyperlipidemia group; p.Leu167del in 5 subjects with mixed hyperlipidemia and in 2 subjects with isolated hypercholesterolemia; p.Arg154Ser (rs121918393) in 2 subjects with mixed hyperlipidemia. Moreover, p.Ala217Ala variant (rs72654468) was found in 2 controls, one subject with isolated hypercholesterolemia and one subject with mixed hyperlipidemia. It has also been identified 2 non previously described variants: p.Met82Ile in 1 subject with isolated hypertriglyceridemia, and p.Gly191Cys in one subject with isolated hypercholesterolemia. Family studies confirmed co-segregation of the rare APOE variants with hyperlipidemia, except for p.Ala217Ala.

Conclusions: We have found five different APOE variants in 16 subjects with different hyperlipidemias that were not found in controls. Our results support an important role of APOE gene rare mutations in the etiology of several hyperlipidemias other than dysbetalipoproteinemia.
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Direct atherogenic effects of sodium: Molecular mechanisms and shear stress pattern dependency in vitro and in vivo.

Abstract nr. 133
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Endothelium, Hypertension, Pathogenesis

Background: Increased consumption of sodium is a risk factor for hypertension and cardiovascular diseases. In vivo studies indicated that high dietary sodium may have a negative influence on endothelium. We investigated the direct effect of high sodium on endothelial-monocytic cell interactions in the regions of non-uniform shear stress both in vitro and in vivo.

Methods: Human umbilical vein endothelial cells grown in a model of arterial bifurcations were exposed to shear stress for 18h in the presence of normal or high (+ 30 mmol/L) sodium, followed by stimulation with TNF-α for 2h and a dynamic adhesion assay. Adherent THP-1 cells and the adhesion molecule expression were quantified. Sodium channel blockers, pathways’ inhibitors, and siRNA against tonicity-responsive enhancer binding protein (TonEBP) were used to identify the mechanisms of sodium effects. ApoE-deficient mice on low-fat diet received water containing normal or high salt (8% w/v) for four weeks, followed by intravital microscopy and serum cytokine/chemokine analysis using magnetic bead-based multiplexing technology.

Results: In vitro, high sodium dramatically increased the endothelial responsiveness to TNF-α under non-uniform shear stress, reflected by a significant enhancement of adhesion molecules expression and monocytic cell recruitment. This effect was prevented by the laminar flow, and was slightly reversed in the static conditions. The blockade of sodium-calcium exchanger using NiCl₂ abolished the stimulatory effect of sodium under non-uniform shear stress, whereas blocking epithelial sodium channel with amiloride had no effect. Sodium-induced increase in monocytic cell adhesion was mediated by reactive oxygen species and the endothelial NO-synthase, and was sensitive to the knockdown of TonEBP.

The direct sodium effects on endothelium were subsequently confirmed in the ApoE-deficient mice. As compared with normal-salt group, high salt intake significantly enhanced the adhesion of circulating CD11b⁺ cells to carotid bifurcations, but not to the straight segment of common carotid artery. No significant effects of high salt intake on the blood cell counts, lipid levels, or cytokine profile were observed.

Conclusions: Elevated sodium has a direct effect on endothelial activation under atherogenic shear-stress in vitro and in vivo, and promotes the endothelial-leukocyte interactions even in the absence of increased lipid concentrations.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Longer receptor residence times improve the effectiveness of CCR2 antagonists in the prevention of atherosclerosis

Abstract nr. 134
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Therapy

Background: The chemokine receptor CCR2 is known to be critically involved in atherosclerosis development, rendering blockade of the CCL2-CCR2 interaction of therapeutic interest. CCR2 receptor antagonists have, however, limited clinical success. Interestingly, it was shown for other drug targets that a measure for the dissociation of the drug-receptor complex, the so-called residence time (RT), can have a crucial impact on a drug’s efficacy. In this study, we thus aimed to determine whether an increased RT improves the therapeutic effectiveness of CCR2 antagonists.

Methods: Carotid artery atherosclerosis was induced by perivascular collar placement in apoE−/− mice, followed by daily treatment with the short RT CCR2 antagonist 15a (RT=15 min, 150 µg/day), the long RT CCR2 antagonist 15b (RT=714 min, 150 µg/day) or vehicle control. After four weeks, atherosclerotic plaques were analyzed for size and composition.

Results: During the study, treatment with the CCR2 receptor antagonists did not affect total body weight or plasma total cholesterol levels compared to the controls. At sacrifice, numbers of circulating CCR2+ monocytes were only significantly reduced in the long RT 15b-treated mice (controls: 14.9±3.2×10³, 15a: 9.1±3.1×10³ and 15b: 4.5±1.0×10³ cells/mL, *P<0.05). Atherosclerotic plaque size was reduced from 64.4±11.8×10³ µm² in control mice to 33.2±6.8×10³ µm² in 15a-treated mice (-49%, *P<0.05), and even up to 17.6±4.1×10³ µm² in 15b-treated mice (-73%, **P<0.01). Interestingly, relative plaque macrophage content was only decreased in 15b-treated mice compared to both control and 15a-treated mice (controls: 46±4%, 15a: 45±6%, 15b: 25±8%, *P<0.05). In the aortic root, 15a did not significantly affect plaque size (controls: 25±2×17×10³ µm² versus 15a: 196±17×10³ µm²), while the long RT CCR2 antagonist 15b inhibited plaque development to 157±15×10³ µm² (-38%, **P<0.01). Furthermore, also at that site of lesion development, macrophage area was only significantly reduced in the 15b treated mice (controls: 35±4%, 15a: 32±3%, 15b: 23±4%, *P<0.05 compared to controls).

Conclusion: Our data demonstrate that the CCR2 antagonist with a long residence time was more...
effective in inhibiting atherosclerotic plaque development compared to the short RT antagonist, which implies that receptor residence time is an important parameter in drug development.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Accumulation of circulating superparamagnetic iron oxide nanoparticles (SPIONs) in endothelial cells: Effects on endothelial viability and monocytic cell adhesion.

Abstract nr. 135
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Endothelium, Therapy

Background: Magnetic drug targeting is considered a promising method to accumulate drug-carrying nanoparticles at the atherosclerotic lesions. However, little is known about the biological effects of magnetic nanoparticles on the vascular cells. In this study, we analysed the endothelial accumulation of circulating SPIONs (superparamagnetic iron oxide nanoparticles), without or with external magnetic force. Moreover, the effects of SPION uptake on endothelial morphology, resistance to physiologic levels of shear stress, and TNF-α-induced monocytic cell adhesion were investigated.

Methods: Human umbilical vein endothelial cells (ECs) were grown in the bifurcating flow-through slides. Subsequently, the cells were perfused at 10 dyn/cm² for 18 h with medium containing SPIONs at a concentration of 30 µg/mL (without magnet), or 3 µg/mL (with magnet). The iron content of ECs was estimated using Prussian blue stain. In further experiments, the effects of SPION uptake on monocytic cell recruitment in response to TNF-α were analysed. EC morphology and resistance to physiologic levels of shear stress were investigated by extending the exposure to shear stress in the absence of SPIONs for up to 96 h, following the initial 18 h perfusion with SPION-containing media.

Results: In the absence of magnetic force, endothelial SPION uptake was independent of hemodynamic conditions, indicating that no increased accumulation of SPIONs occurs at non-uniform shear stress region at the outer walls of bifurcation. Application of external magnet allowed enhanced accumulation of SPIONs at the regions of non-uniform shear stress even at 10-fold decreased nanoparticle concentrations, accompanied by a reduced endothelial uptake in laminar shear stress regions. Increased uptake of SPIONs at non-uniform shear stress region was well tolerated by ECs and did not affect endothelial cell viability or resistance to prolonged shear stress exposure. At the tested concentrations, SPIONs were largely metabolized within 3 days post-application. Importantly, no significant differences in TNF-α-induced monocytic cell recruitment were detected between controls and SPION-treated ECs.

Conclusions: Magnetic targeting allows localized accumulation of increased amounts of SPION at the region of interest under physiologic-like flow conditions. These findings indicate that magnetic
targeting can constitute a suitable technique for the delivery of imaging and therapeutic nanoparticles to atherosclerotic lesions.

Accumulation of circulating SPIONs by external magnetic force in the region of interest.

Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation

Additional information
Circadian activity of cholesterol 7α-hydroxylase is determined by -203A/C polymorphism of CYP7A1 gene

Abstract nr. 137
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Genetics, Lipids, Metabolism

The -203A/C polymorphism of CYP7A1 gene encoding cholesterol 7α-hydroxylase (CYP7A1) plays an important role in determination of cholesterolemia responsiveness to the diet. Importantly, the CYP7A1 activity displays a considerable diurnal variation. Therefore, we analyzed whether -203A/C polymorphism is involved in circadian regulation of CYP7A1 activity.

The three experiments lasting 15 hours were carried out in 16 healthy male volunteers, 8 homozygous for -203A and 8 homozygous for -203C variant. First of these experiments was carried out after one day treatment with bile acid sequestrant (Questran®), the second after one day treatment with chenodeoxycholic acid (Chenofalk®) and the third one without any treatment (control). The concentration of 7α-hydroxy-4-cholesten-3-one (C4), a serum marker of CYP7A1 activity, was measured from 7 AM to 10 PM in 90min intervals. The experiments were carried out in at least three weeks intervals and their order was randomized.

The treatment with bile acid sequestrant resulted in fourfold and eightfold increase of CYP7A1 activity during the day in A and C allele homozygous carriers, respectively. The treatment with chenodeoxycholic acid resulted in a pronounced decrease in CYP7A1 activity in carriers of both variants. Importantly, the homozygous carriers of -203A allele manifested a noticeable peak of an enzyme activity around 1 PM whereas no such peak could be observed in -203C allele carriers.

It can be concluded that -203A/C polymorphism of CYP7A1 has a substantial impact on diurnal variation of enzyme activity. The mechanism behind such an effect and its possible role in determination of cholesterolemia responsiveness to the dietary fat and cholesterol remains to be determined.

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Carriers of the PCSK9 R46L variant are characterized by an anti-atherogenic lipoprotein profile assessed by nuclear magnetic resonance spectroscopy

Abstract nr. 138
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Inflammation, Lipoproteins, PCSK9

Background — Carriers of the PCSK9 R46L genetic variant are characterized by low levels of low-density lipoprotein (LDL) cholesterol and a decreased risk of cardiovascular disease (CVD). Whether these individuals are characterized by other features of a more beneficial lipoprotein-lipid profile is unknown.

Methods and Results — We measured the lipoprotein-lipid profile of 2373 participants of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study by nuclear magnetic resonance spectroscopy. Among them, 77 participants carried at least one allele of the R46L genetic variant. Carriers and non-carriers had comparable clinical characteristics (age, body mass index, blood pressure, smoking and diabetes prevalence). As expected, carriers had lower LDL cholesterol levels compared to non-carriers (3.75±0.99 vs. 4.16±1.01 mmol/L, p<0.001 in carriers vs. non-carriers, respectively). Carriers were characterized by a lower very low-density lipoprotein (85.8±26.2 vs. 99.0±33.3 nmol/L, p<0.001 in carriers vs. non-carriers, respectively) particle concentration and a lower LDL particle concentration of any size (1479.7±396.8 vs. 1662.9±458.3 nmol/L, p<0.001 in carriers vs. non-carriers, respectively). Total high-density lipoprotein (HDL) particle concentration was not different in carriers vs. non-carriers. However, carriers had a higher concentration of large HDL particles (6.4±3.3 vs. 5.5±3.5 nmol/L, p=0.04 in carriers vs. non-carriers, respectively) and a higher mean HDL particle size (9.0±0.4 vs. 8.9±0.5 nm, p=0.04 in carriers vs. non-carriers, respectively). We also found that carriers were characterized by a lower secretory phospholipase A2 (sPLA2) activity (4.21±0.88 vs. 4.61±1.26 nmol/ml/min p=0.004 in carriers vs. non-carriers, respectively) and a lower lipoprotein-associated phospholipase A2 (Lp-PLA2) activity (47.5±14.1 vs. 52.4±16.2 nmol/ml/min, p=0.008 in carriers vs. non-carriers, respectively).

Conclusions — Results of this study suggest that on top of having low LDL cholesterol levels, carriers of the PCSK9 R46L genetic variant have a lower VLDL and LDL particle concentration, a higher concentration of large HDL particles and lower sPLA2 and Lp-PLA2 activity. This anti-atherogenic profile may explain to a certain extent the reduced CVD risk observed in carriers of the PCSK9 R46L genetic variant.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Severe childhood infection is associated with adverse adult cardiovascular and metabolic risk phenotypes: The Cardiovascular risk in Young Finns Study

Abstract nr. 139
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, Inflammation, Obesity

Background: Childhood infections are ubiquitous and elicit repeated inflammatory responses. The relationships between the severity and timing of infection and later cardiometabolic risks are largely unknown.

Methods: Using fully adjusted multiple regression models, we investigated associations between infection-related hospitalisation (IRH, a marker of the severity of infection) and anthropometric, metabolic, and cardiovascular parameters in childhood and adulthood in 1376 participants from the Young Finns Study who had lifetime IRH data available. We also examined whether socio-economic status (SES) influenced the relationship between childhood infection and adult cardiometabolic outcomes.

Findings: By a mean age of 35.1 years, 597 (43.4%) individuals had ≥ 1 IRH, of which 181 (30.3%) occurred before 5 years of age. Early childhood IRH correlated with adverse adult (but not childhood) metabolic parameters; increased body mass index (BMI) (P=0.02) and metabolic syndrome (odds ratio 1.56, 95% CI 1.03-2.35, P=0.03), adjusting for age, sex, childhood BMI, and family income. Brachial flow-mediated dilatation (FMD) was significantly lower in those with early child IRH (mean±SEM 8.15±0.37 vs. 9.10±0.16%, P=0.03). These individuals had a 1.84% (95% CI 0.64-3.04, P=0.002) greater decrease in FMD between adult follow-ups at mean ages of 27 and 33 years. Childhood IRH was associated with increased asymmetrical dimethylarginine in adulthood (0.62±0.01 vs 0.59±0.01 μmol/l, P=0.04), adjusted for age, sex, adult BMI, and creatinine. Early childhood IRH was associated with lower carotid distensibility (1.95±0.06 vs. 2.09±0.02 %/10 mmHg, P=0.02), but not with carotid intima-media thickness (0.601±0.006 vs. 0.596±0.003 mm). The frequency of childhood IRH did not differ significantly between those of low and high SES. However there were significant interactions between childhood IRH & low SES, and adverse adult cardiometabolic parameters.
**Conclusions:** Childhood infections may contribute to causal pathways leading to adult cardiometabolic diseases. Childhood infection may be one mechanism underpinning the social gradients observed in cardiometabolic non-communicable diseases.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Validation of the Pooled Cohort Equations in a Hong Kong Chinese Cohort

Abstract nr. 140
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Risk stratification

Objective: The 2013 American College of Cardiology and the American Heart Association guidelines recommended the Pooled Cohort equations for evaluation of atherosclerotic cardiovascular disease risk of individuals. We investigated the usefulness of the Pooled Cohort equations in Chinese by validating this risk prediction model using the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) cohort.

Methods: The Hong Kong CRISPS is a population-based prospective cohort study of cardiovascular risk factors among 2895 Chinese men and women (aged 25-74 years) initiated in 1994. Cardiovascular (CV) events were ascertained until December 2013. The discrimination and calibration of the Pooled Cohort equations was evaluated and compared with the Framingham risk equation. A Hosmer-Lemeshow Chi-square statistic ($X^2 <20$) indicated good calibration.

Results: The discrimination power of the 2 models in both men and women was moderate (C-statistic $>0.7$). However, the calibration score of both models was unacceptable in men (Pooled Cohort $X^2=24.1$, Framingham $X^2=20.1$). Since the Framingham model systematically over-estimated CV risk [average predicted risk 18.3% (95% CI 15.5-21.0) versus average observed risk 13.4% (95% CI 11.0-15.8)], this can be corrected by recalibration of the model using the CRISPS data [average predicted risk 11.5% (95% CI 9.2-13.7) versus average observed risk 11.8% (95% CI 9.6-14.1)]. Recalibration cannot be applied to the Pooled Cohort model because the degree of miscalibration varied across the different risk categories. In women, although calibration of Pooled Cohort ($X^2=10.1$) and Framingham model ($X^2 =12.1$) appeared similar, the accuracy of the Framingham model was better [average predicted risk 6.2% (95% CI 4.6-7.7) versus average observed risk 6.3% (95% CI 4.8-7.9)] than the Pooled Cohort model [average predicted risk 4.8% (95% CI 3.3-6.3) vs average observed risk 6.9% (95% CI 5.1-8.7)].

Conclusions: Risk prediction models should be able to discriminate between individuals with and without disease, and also well-calibrated so that predicted risk estimates matches as closely as possible the observed risk in the population. The Pooled Cohort equations provide poor calibration and moderate discrimination in Hong Kong Chinese, especially in men. The Framingham risk equation can be applied to the Hong Kong population but requires recalibration in men.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Mini-oral presentation
Additional information
The effect of simvastatin treatment in hypercholesterolemic patients: a prospective study using lipidomic profiling

Abstract nr. 141
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease,Dyslipidemia,Lipids

Background: Statins are potent cholesterol-lowering drugs, widely prescribed to reduce cardiovascular risk reduction. While reduction of mevalonate pathway metabolites, including coenzyme Q10 (CoQ10) have been well-documented, the effect of statins on other lipid-related metabolites included shpingolipids and phospholipids is less well-studied. We hypothesize that statins significantly affect levels of lipid-related metabolites, beyond cholesterol, and these effects are independent of their reduction in CoQ10.

Aim: To investigate the effects of simvastatin, with/without CoQ10 (ubiquinol) supplementation, on serum lipidomic profiles in patients with hypercholesterolemia.

Methods: In this prospective double-blind study, serum lipidomic profiles were compared between baseline and after 12-week treatment of simvastatin (20mg daily), in 40 patients randomized to receive either supplementation of ubiquinol (Gp-A) or placebo (Gp-B). Lipidomic profiling was performed using UPLC and mass spectrometry and analysis using SIMCA 13.0 software, including principal component analysis (PCA), and orthogonal partial least-squares discriminant analysis (OPLS-DA). Univariate statistical tests (Paired t-test and Man-Whitney U test) were performed on SPSS software. The p values less than 0.05 were considered significant.

Results: Mean age (1SD) of patients in this study was 46.2 (12.0) years. All patients received simvastatin 20mg daily, and there was no significant differences, in terms of age, weight, baseline lipid profiles) between the groups receiving ubiquinol or ubioquinol-placebo. Baseline low-density-lipoprotein-cholesterol (LDL-C) was 4.35 (0.92) mmol/1, reducing by 30% with simvastatin treatment, with no attenuation of LDL-C lowering with ubiquinol supplementation. CoQ10 levels were significantly lowered with simvastatin treatment (Gp-B) and but not Gp-A who received supplementation of ubiquinol. Simvastatin treatment resulted in significant lowering of glycerolipids, sphingomyelins and phospholipids, and increase in lysophophotidylcholine (20:4), lysophophotidylethanolamine (20:4/0:0) and lysophophotidylethanolamine (22:6/0:0). Although the levels of some glycerolipids and ceramides were higher in Gp-A at 12-weeks compared to those in
Gp-B, ubiquinol supplementation had relatively small effect on simvastatin-induced reduction of these lipids.

**Conclusion:** In patients with hypercholesterolemia, simvastatin significantly lowers serum phospholipids and sphingolipids, and this was independent of CoQ10 supplementation for majority of lipid-related metabolites. Future molecular studies are required to study the functional significance of these changes at the cellular and tissue levels.

Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
**Statin-fenofibrate combination therapy for hypertriglyceridemia in statin-treated patients at high cardiovascular risk**

Abstract nr. 142  
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins  
Keywords Dyslipidemia, Hypolipidemic Drugs, Triglycerides

**Background:** Statin-fibrate combination therapy was recommended by several lipid guidelines as an option for patients with hypertriglyceridemia after statin monotherapy to reduce the residual CV risk related to hypertriglyceridemia. However, clinical experience about the efficacy and safety of statin-fibrate combination therapy in China is insufficient due to concern about the safety profile.  

**Method:** 506 subjects were enrolled in 28 sites in 14 cities of China. After at least 2-month statin monotherapy (including 7 types of statins) with standard dose, patients with coronary heart disease (CHD) or CHD risk equivalent and TG≥1.7mmol/L were enrolled and given fenofibrate 200mg daily on top of statins for 8 weeks. Lipid and safety parameters were compared between baseline and after treatment.  

**Results:** After 8 weeks of fenofibrate add-on treatment, mean TG level decreased from 3.00mmol/L at baseline to 1.77mmol/L (-38.1%), mean VLDL-C level decreased from 0.95mmol/L to 0.63mmol/L (-22.7%) and mean HDL-C was increased from 1.07mmol/L to 1.22mmol/L (17.4%). Neither creatine kinase (CK) increased to ≥ 5 times of upper limit of normal (ULN) nor cases of rhabdomyolysis were reported. 6 subjects (1.22%) were reported with ALT and/or AST elevated to ≥ 3 times of ULN, all of them recovered shortly after discontinuation of statins and fenofibrate.  

**Conclusion:** In patients at high CV risk with hypertriglyceridemia after standard statin therapy, add-on fenofibrate therapy effectively further improved lipid profile with acceptable safety profile.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation  
Additional information
Low levels of apoB-100 autoantibodies are associated with increased risk of coronary events

Abstract nr. 144
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Apolipoproteins, Cardiovascular Disease, Immunity, LDL

Aim: Immune responses against oxidized low density lipoproteins (LDL) play a key role in atherosclerosis development. Previous studies have indicated inverse associations between autoantibodies to oxidized LDL epitopes and cardiovascular disease (CVD). The purpose of this study was to investigate associations between autoantibodies against the apolipoprotein B-100 (apoB-100) peptides p45 and p210 and risk of CVD.

Methods and Results: In a prospective study, including 5400 individuals belonging to the cardiovascular arm of the Malmö Diet and Cancer cohort, apoB-100 autoantibodies were analyzed by ELISA. The analysis revealed significantly lower levels of IgM autoantibodies recognizing the native and malondialdehyde (MDA) apoB-100 peptides p45 and p210 and also lower IgG levels recognizing native p210 in individuals with a later incidence of CVD compared to controls. The autoantibodies were further analyzed in relation to coronary and stroke events. The same pattern was detected for coronary events, whereas the differences disappeared for incidence of stroke. No significant correlations between the autoantibodies and common and bulb carotid intima-media thickness were detected after adjustment for common risk factors (age, sex, LDL/HDL ratio, triglycerides, systolic blood pressure, smoking and diabetes). On the other hand, in a logistic regression model a significant association was found between high levels of IgG-p210\textsubscript{native} (OR = 0.811, 95% CI 0.69-0.94, \( P=0.007 \)) and occurrence of carotid plaques after adjustment for the risk factors. When tertiles of autoantibody levels were entered into a Cox proportional hazard regression model, a significant association was identified between high levels of IgM-p45\textsubscript{MDA} (Hazard ratio (HR) [95%CI]: 0.73 [0.56, 0.96], \( P=0.022 \)) or IgG-p210\textsubscript{native} (Hazard ratio (HR) [95%CI]: 0.74 [0.56, 0.97], \( P=0.030 \)) and a lower risk of incidence of coronary events after adjustment for the risk factors.

Conclusion: Taken together, the present findings suggest that high levels of apoB-100 autoantibodies protect against incidence of coronary events.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Salsalate attenuates diet induced non-alcoholic steatohepatitis by decreasing lipogenic and inflammatory processes.

Abstract nr. 145
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Inflammation, Metabolism, Prevention

Background and aims. Salsalate is an anti-inflammatory drug that was recently found to exhibit beneficial metabolic effects on glucose and lipid metabolism. Although its utility in the prevention and management of a wide range of vascular disorders as well as of type 2 diabetes and metabolic syndrome has been suggested before, the potential of salsalate to protect against non-alcoholic steatohepatitis (NASH) remains unclear. The aim of the present study was therefore to ascertain the effects of salsalate in the development of NASH.

Methods. Transgenic APOE*3Leiden.CETP mice were fed a high fat high cholesterol diet with or without salsalate for 12 and 20 weeks. The effects on body weight, plasma parameters, liver histology and hepatic gene expression were assessed.

Results. Salsalate prevented weight gain, improved dyslipidemia and insulin resistance and ameliorated diet-induced non-alcoholic steatohepatitis, as shown by decreased hepatic micro- and macrovesicular steatosis, reduced hepatic inflammation and reduced development of fibrosis. Salsalate affected lipid metabolism by increasing β-oxidation and decreasing lipogenesis, as shown by the activation of PPAR-α, PGC1-β, RXR-α and inhibition of MLXIPL/ChREBP controlled genes, respectively. Inflammation was reduced by down-regulation of the NFκB pathway and fibrosis development was prevented by down-regulation of TGF-β signaling.

Conclusions. Salsalate was shown to exert a preventive effect on the development of NASH and progression to fibrosis. These data suggest a clinical application of salsalate in preventing NASH.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Clinical relevance of morphological features of intracranial atherosclerosis in high resolution MRI vessel wall imaging

Abstract nr. 146
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Imaging,Vulnerable Plaque

Introduction: Stroke in Chinese and other Asian populations is unique, in which intracranial arterial disease (ICAD), rather than extracranial carotid stenosis, accounts for the pathophysiology. The study aimed to explore the clinical relevance of morphological features of intracranial atherosclerosis evaluated by High resolution magnetic resonance imaging (HRMRI) vessel wall imaging in Chinese stroke patients.

Subjects and Methods: International Review Board approval was obtained for this retrospective study. All patients gave written informed consent. Imaging was performed on a 3 teslar Achieva MR system (Philips Healthcare, Cleveland, OH, USA) with an 8-channel SENSE head coil. The protocol included a T1-weighted (T1w) volumetric isotropic turbo spin-echo acquisition (VISTA) vessel wall sequence, before and after (83% of patients) contrast administration, and a Time-Of-Flight Magnetic Resonance Angiography (TOF-MRA) sequence. Before acquisition of the contrast enhanced T1w VISTA sequence, 0.1 mL/kg of a gadolinium-containing contrast agent (Gadobutrol, Gadovist 1.0 mmol/mL, Bayer Schering Pharma, Newbury, UK) was administered to the patient.

Results: Nineteen patients (7 females; mean age 67 years, range 47-81 years) with an MCA stenosis were recruited. Different degrees of intracranial vessel wall lesions were identified in 18 patients, totaling 57 lesions (12%, 57/494). The morphological comparison between symptomatic and asymptomatic lesions was demonstrated in Table 1. The rate of luminal stenosis of intracranial large arteries detected by MRA was higher in symptomatic lesions than those in asymptomatic lesions (36.9% vs. 10.5%, P=0.001). There was a trend that diffuse lesions along the arterial longitudinal axis was more frequent in symptomatic lesions than those in asymptomatic lesions (42.1% vs. 18.4%, P=0.062). The enhancement of lesions after contrast administration was similar between symptomatic and asymptomatic lesions (47.4% vs. 42.1%, p=0.781).
**Conclusions:** Intracranial vessel wall imaging using a 3D T1w VISTA vessel wall sequence could provide detailed morphological assessment of plaque features. The findings based on this pilot study suggest that both luminal stenosis and morphological features of individual lesions may play a synergetic effect on stroke occurrence.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
PBMC gene expression of inflammatory markers after an acute bout of resistance exercise in young and elderly subjects

Abstract nr. 147
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis,Inflammation,Prevention

Regular physical activity promotes an anti-inflammatory response in the body. This may be one of the explanations why exercise protects against several diseases. In contrast, an acute bout of resistance exercise temporarily increases the levels of inflammatory cytokines in the muscle. How exercise influences peripheral mononuclear blood cells (PBMCs) is less clear. We wanted to investigate how an acute bout of resistance exercise affects the inflammatory gene expression in PBMCs.

Twenty-two young (25.0 ± 3.4 yrs) and fifteen elderly (74.2 ± 3.8 yrs) healthy men and women performed an acute bout of resistance exercise – 4x8 repetition maximum (RM) of leg press and knee extension, with a new set starting every third minute. Based on RNA isolated from PBMCs, 48 genes were analysed using RT-qPCR.

We will present data from the study, and discuss the possible impact of these, related to the development of chronic low grade inflammation, at the meeting.

Funding and conflict of interest: The project is funded by the Norwegian Research Council and the involved institutions.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Intracranial atherosclerotic lesion characteristics correlate with cerebrovascular lesion load after TIA or ischemic stroke: a 7.0 tesla MRI study

Abstract nr. 148
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Imaging

Introduction. Intracranial atherosclerosis (ICAS) is denoted as one of the most prevalent cause of stroke worldwide. Assessing both macroinfarcts and cortical microinfarcts (CMIs) in patients with a history of cerebrovascular disease may provide additional information on the spectrum of parenchymal brain injury caused by ICAS. In this study we investigated the presence of CMIs at 7T MRI in patients with TIA or ischemic stroke of the anterior circulation and explored the relationship between ICAS, CMIs and macroinfarcts.

Methods. Eighteen patients presenting with ischemic stroke (n=12) or TIA (n=6) underwent 7T MR imaging; the protocol included a FLAIR- and the MPIR-TSE intracranial vessel wall sequence. ICAS lesions and their characteristics, as well as infarcts (CMIs and macroinfarcts), were scored by two raters (Fig. 1). The relationship between ICAS lesions, calculated ratios of characteristics and infarcts were examined using linear regression analyses.

Results. A total number of 101 CMIs (78% of patients), 31 macroinfarcts (67%) and 75 ICAS lesions (100%) were found. Seventy-six and sixty-five percent of CMIs and macroinfarcts, respectively, were found in the same vascular territory as the ICAS lesions. A positive correlation existed between the number of macroinfarcts and CMIs (p<0.05) and between a concentric configuration and macroinfarcts (p<0.01); for CMIs no correlation was found. A diffuse thickening pattern was positively correlated to macroinfarcts (p<0.05); a weak trend was found for CMIs (p=0.09).

Conclusion. This study shows that in patients with TIA or ischemic stroke CMIs represent a relevant portion of the total cerebrovascular lesion load and coexist with macroinfarcts. These results demonstrate that the spectrum of parenchymal damage caused by ICAS is not restricted to macroinfarcts alone but also include CMIs.

References. 1Van der Kolk AG. et al., Stroke, 2011; 2Dieleman N. et al., Neurology, 2014
Fig 1. (A) FLAIR shows infarct and CMls; zoomed view (B). Vessel wall images show thickening (C+D) and enhancement (E) of the right M1 (C) and M2 (D) segments.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Discriminating host defense to fungal and bacterial infections by genome-scale metabolic modeling of human PBMCs

Abstract nr. 150
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Inflammation, Metabolism

This study aims at discriminating the metabolic pathways induced during induction of host immune responses by the fungal pathogen *Candida albicans* or the bacterial stimuli *Escherichia coli*-derived LPS, *Borrelia burgdorferi*, and *Mycobacterium tuberculosis* (MTB). We have developed PBMC (Peripheral Blood Mononuclear Cell) -specific Genome-scale Metabolic models (GEMs) for all immune challenges mentioned above. PBMC-specific GEM describes PBMC’s metabolic physiology with appropriate biochemical, genetic and genomic knowledge of PBMC. In this study, a PBMC-specific GEM was reconstructed for each immune challenge by applying the tINIT algorithm (Agren et al. 2014) based on proteomics and RNA sequencing data of unstimulated PBMCs together with PBMC microarray data stimulated by *Borrelia*, LPS, MTB or *Candida*. Within each metabolic network, the “hot regions” where significant gene expression changes occurred were identified. Through comparing such “hot metabolic regions” between fungal and bacterial stimulation, we identified de novo purine synthesis and cholesterol biosynthesis as the metabolic signatures of *Candida*-induced inflammation. We also propose that squalene 2,3-oxide and 5-aminoimidazole ribonucleotide (AIR), the intermediary metabolites in de novo purine synthesis and cholesterol biosynthesis can be used as biomarkers for diagnosing a *Candida*-induced inflammatory response. All the predictions are currently being validated using metabolomics-based approaches.

Keywords: *Candida albicans*, host defense, PBMCs, genome-scale metabolic models, biomarkers

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Assessment of Lipoprotein Particle Number by High-Performance Gel Permeation Chromatography in Patients with Cholesteryl Ester Transfer Protein Deficiency

Abstract nr. 151
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, HDL, Lipoproteins, Metabolism

Background: Recent studies showed that elevated plasma HDL-cholesterol (HDL-C) levels treated by CETP inhibitors were not associated with incidence of cardiovascular events. Therefore, we should take other parameters other than HDL-C levels into account.

Methods and Results: In this study, 9 CETP-deficient (CETP-D) patients, whose serum CETP mass was <0.1μg/mL, were compared with 9 normolipidemic controls. Free cholesterol, cholesteryl ester, triglyceride and phospholipid levels in each 20 lipoprotein subclass were determined by computer-assisted high-performance gel permeation chromatography (HPLC). Furthermore, we calculated the particle number of each subclass by using HPLC data, serum lipids and apolipoproteins, which is newly-developed LipoSEARCH® system (Skylight Biotech Inc, Akita). As we reported previously, serum HDL-C levels were markedly elevated in CETP-D patients compared with controls. The number of very large and large HDL particles in CETP-D patients was markedly higher than that in controls (4237.3±2353.4nM vs 213.4±55.7nM, 7672.2±1368.3nM vs 1720.2±536.6nM, respectively; p<0.001), while the number of small and very small HDL, which have anti-atherogenic function, was significantly lower (4339.1±937.4nM vs 5690.3±467.8nM, 1999.4±514.8nM vs 3256.5±294.0nM, respectively; p<0.001). The number of large and medium LDL was significantly lower in CETP-D patients than that in controls (158.3±36.4nM vs 240.6±51.1nM, 349.1±69.9nM vs 557.3±94.8nM, respectively; p<0.001), whereas the number of very small LDL, which is known to be atherogenic, was significantly higher (233.2±64.8nM vs 171.4±22.1nM, p=0.016).

Conclusion: The lipoprotein particle numbers calculated by this newly-developed HPLC method are dramatically changed in CETP-D patients, suggesting a proatherogenic lipoprotein profile by CETP deficiency.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Heritability Associated with Lp(a) and Apo(a) Differ Across Apo(a) Allele/Isoform Sizes and Ethnicity

Abstract nr. 153
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Genetics, Lp(a)

**Background:** Levels of lipoprotein(a), Lp(a), an independent cardiovascular risk factor, are under strong genetic regulation through the apolipoprotein(a), apo(a), gene. However, the degree of heritability of Lp(a) level and apo(a) allele/isofrom within and across families of different ethnic background is not well understood. The goal of this study was to investigate the heritability of Lp(a) level and apo(a) allele/isofrom in a family-based cohort, consisting of Caucasians and African-Americans, where a substantial difference in the distributions of Lp(a) levels and apo(a) sizes exists.

**Methods:** Families with two parents and two biological children were recruited from the general population (239 Caucasians, 88 African-Americans, 6-74 years). We determined 1) Lp(a) level [apo(a)-size insensitive ELISA], 2) apo(a) allele sizes (pulsed field electrophoresis), 3) apo(a) isoforms (Western blotting), and 4) allele-specific apo(a) level (ASL), the amount of Lp(a) carried by individual apo(a) isofrom. Heritability of these traits was estimated by measuring the relative contribution of the genetic component of variance responsible for parent-offspring resemblance.

**Findings:** For the entire study population, the estimated heritability ($h^2$) of Lp(a) level, adjusted for ethnicity, was 0.95 ± 0.07. We then assessed heritability of smaller and larger apo(a) sizes for given allele pairs. The heritability estimates of ASL for the smaller apo(a) allele were greater than those of the larger allele (0.91 vs. 0.59, p=0.0173). Similarly, although not statistically significant, heritability estimates of apo(a) isoforms (0.90 vs. 0.70) and alleles (0.98 vs. 0.82) for the smaller apo(a) were higher than those of the larger apo(a). When analyzed by ethnicity separately, an overall lower heritability estimate was observed in African-Americans vs. Caucasians for all traits. Notably, the heritability estimate for the larger apo(a) allele was lower in African-Americans vs. Caucasians (0.28 vs. 0.95, p=0.0012).

**Conclusions:** Overall, Lp(a) level as well as all traits associated with the smaller apo(a) allele were more strongly determined by genetic factors, although with a varying degree of ethnic influence. Ethnic differences in heritability attributed to genetic variation of the larger apo(a) allele warrants further investigations.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
The expression of FceR receptors for IgE in human endothelial cells and its possible role in the endothelial integrity regulation

Abstract nr. 154
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Endothelium, Immunity, Inflammation

Background and aims: The presence of IgE and its FcεR receptors in human atherosclerotic plaques suggests the potential involvement of IgE in the pathogenesis of atherosclerosis. The aim of the study was to assess the FcεRs mRNA expression in human endothelial cells (EC) and their functional effect on the endothelial integrity.

Material and methods: FcεR mRNA presence in EC was evidenced by the sequencing and mRNA expression in real-time PCR. To increase the FcεRs mRNA expression, EC were induced with IL-4 (1, 10 and 100 ng/ml) for 48 hours. In the next step, the involvement of FcεRs in the regulation of the endothelial integrity was assessed upon pre-stimulation of EC with IL-4, and following induction, firstly, with anti-DNP-BSA IgE (250ng/ml) which is supposed to bind to FcεRs and, secondly, with the specific antigen DNP–BSA (100, 250, 500ng/ml) binding to IgE in the Real-time Cell Electric Impedance Sensing (RTCA-DP) system.

Results: Our results revealed the presence of FcεRI and FcεRII mRNA expression in EC both in sequencing and real-time pcr. IL-4 (1 and 10 ng/ml) induced 2-fold increase of FcεRI mRNA expression as compared to the unstimulated control (p<0.001 and p<0.05, respectively) and 3-fold increase of FcεRII mRNA expression (p<0.05). In EC pre-induced with IL-4, linking of IgE with DNP-BSA caused the significant 20% increase of endothelial integrity observed in RTCA-DP system as compared to the unstimulated control (p<0.01).

Conclusion: FcεRI and FcεRII mRNA expression in endothelial cells suggests the existence of a potential mechanism of IgE-mediated response of endothelium to allergenic antigens.
Fig. 1 Cell-electrode impedance detection of the endothelial cells
Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
Cholesterol Efflux Capacity in Patients with Familial Hypercholesterolemia

Abstract nr. 155
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Familial Hypercholesterolemia, HDL, Reverse Cholesterol Transport, Risk Factor

Cholesterol efflux capacity from macrophages has been found to have a strong inverse association with carotid intima-media thickness (cIMT) and the likelihood of cardiovascular disease (CVD) independent of the high-density lipoprotein cholesterol (HDL-C) levels, supporting the importance of HDL function over the simple measurement of HDL-C levels. Familial hypercholesterolemia (FH) is a prevalent inherited disorder characterized by marked elevation of plasma low-density lipoprotein cholesterol concentrations and premature CVD. To date, residual risks in statin-treated FH have been rarely assessed. Accordingly, the present study was performed to investigate the relationships between cholesterol efflux capacity and clinical features including Achilles tendon thickness, subclinical atherosclerosis, and the presence of CVD in FH patients treated with statins. The subjects were 148 ethnic Japanese with clinically or genetically diagnosed as heterozygous FH previously treated with statins. Age ranged from 22 to 85 years with a mean age of 61 years [standard deviation ± 15], and 56 (37.8%) patients were known to have CVD. Serum cholesterol efflux capacity was measured in 3H-cholesterol-labeled J774.1 cells and incubated with 2.8% apolipoprotein B-depleted serum. Significant inverse relationships between cholesterol efflux capacity and Achilles tendon thickness as well as cIMT were observed after adjustment for age, sex and traditional cardiovascular risk factors (the presence of diabetes, hypertension, and/or obesity, low-density lipoprotein cholesterol levels, smoking history). However, subsequent adjustment for HDL-C attenuated these relationships. In a logistic-regression analysis adjusted for age, sex, and traditional cardiovascular risk factors, an increased cholesterol efflux capacity was associated with a decreased risk of CVD even after the addition of HDL-C level as a covariate (odds ratio per 1% increase, 0.94; 95% CI, 0.88 to 0.99; P < 0.05). The results were similar when the apolipoprotein A-I level was substituted for the HDL cholesterol level (odds ratio per 1% increase, 0.94; 95% CI, 0.88 to 1.00; P < 0.05). Among known cardiovascular risk factors, the presence of hypertension was also associated with the presence of CVD. These results suggest that cholesterol efflux capacity might be a novel biomarker as well as a therapeutic target for atherosclerosis in statin-treated FH patients.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Estimation of Vasodilatory Effect
of Hydrogen sulfide (H2S) using ultrasound on Rat Abdominal aorta

Abstract nr. 158
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease

Although hydrogen sulfide (H2S), a colorless gas with a strong odor of rotten eggs, has been recognized as a toxic gas affecting living organisms for nearly 300 years, it is now considered the third member of a family of endogenous biologically active gaseous transmitters, termed gasotransmitter or gasomediator family, along with nitric oxide (NO) and carbon monoxide (CO). H2S plays important protective roles in regulatory mechanisms of multiple systems, including cardiovascular, nervous, and immune systems. It causes relaxation of vascular smooth muscle, which results in increased organ blood flow, and hence lowers the systemic blood pressure. In addition, it has been shown to be involved in angiogenesis, energy metabolism and inflammation by acting specifically on ATP-sensitive K+ channels. Thus, abnormality in metabolism of hydrogen sulfide could lead to several diseases. The present study describes the effects of exogenous hydrogen sulfide on abdominal aorta of adult rats by using ultrasound machine. The H2S donor NaHS (5 mg/kg for 10 min) was injected in an infusion rate of (0.5 mg/kg/min), and the luminal aortic diameter during and after injection for about 60 min was measured by using ultrasound machine with assistance of computer software. It was found that the maximum dilatation was achieved after full dose injection as a concentration response carves and after 45 min from injection as a time response carve, suggesting the blood vessels are significantly dilated when exposed to H2S. As H2S is required for the physiological control of vascular function, it may be used therapeutically in hypertension and coronary heart disease.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Baseline fibroblast growth factor 21 (FGF21) levels can predict total cardiovascular disease events in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study. This study investigated the relationship of plasma FGF21 levels with baseline and on-study microvascular disease in patients with type 2 diabetes from the FIELD study. Plasma FGF21 levels were measured by enzyme-linked immunosorbent assay in 9,697 study participants at baseline. Total microvascular disease was defined as the presence of any nephropathy, retinopathy, neuropathy, and/or microvascular amputation. We assessed the association of FGF21 levels with both baseline and the development of new on-study total microvascular disease during the 5-year follow-up. Higher baseline FGF21 levels were found in patients with baseline total microvascular disease (P<0.001). The associations remained significant after further adjusting for confounding factors (OR [95% CI] = 1.13 [1.08-1.19] per SD increase in ln-transformed FGF21 levels, \( P <0.001 \)). Among 6,465 patients without baseline microvascular disease, 1,517 patients developed on-study total microvascular disease over 5 years of follow-up. Higher baseline ln-transformed FGF21 levels were associated with a higher risk of new on-study total microvascular disease after adjusting for confounding factors (OR [95% CI] = 1.09 [1.02-1.16] per SD increase in ln-transformed FGF21 levels, \( P=0.01 \)). The addition of FGF21 levels in a model of new on-study total microvascular disease with established risk factors significantly increased the integrated discrimination improvement and the net reclassification improvement (both \( P<0.01 \)). Higher baseline plasma FGF21 levels are seen in patients with type 2 diabetes and established microvascular disease, and predict the future development of new microvascular disease over 5 years of follow-up, suggesting a potential role of FGF21 in microvascular disease. FGF21 may be
useful as a potential biomarker for monitoring the progress of microvascular disease in high risk patients. The measurement of FGF21 levels was supported by a Grant-in-Aid (G 12S 6681) from the National Heart Foundation of Australia. K.L.O. was supported by Program grants (482800 & 1037903) from the National Health and Medical Research Council (NHMRC) of Australia, and a Vice-Chancellor’s Postdoctoral Fellowship from the University of New South Wales. A.C.K. was supported by NHMRC Program grant (1037786) and Fellowship grant (1024105).

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Are intracranial arteries athero-protected?

Atherosclerosis of the intracranial arteries is a major but underestimated cause of ischemic stroke and has been related to dementia. Whereas most literature focuses on the epidemiology and therapy of intracranial atherosclerosis, we provide a literature review focusing on disease etiology. Based on this review and the documented observation that intracranial atherosclerosis develops 20 years later than extracranial atherosclerosis we hypothesize that intracranial arteries are athero-protected and developed the following working model: Intracranial arteries and especially the small circle of Willis arteries have a specific constitution. They are muscular type arteries that contain only few medial elastic fibers, a thick and dense internal elastic lamina, few adventitial vasa vasorum and lack an external elastic lamina. A low endothelial permeability, a special glycocalyx and enhanced protective mechanisms against oxidative stress suggest the presence of a barrier function. Early in life, the compliance of the aorta and carotid arteries maintains a low pulse pressure in the intracranial arteries retarding the development of intracranial atherosclerosis. With increasing age and accelerated by hypertension, diabetes and an enhanced stiffness of aorta and carotid arteries the protective effect of a low pulse pressure is lost and the enhanced pulse wave propagation may become a major driver of intracranial atherosclerosis and explain the exponential increase in its incidence. Intracranial atherosclerotic lesions show special features such as fibrosis, small lipid pools, and a low grade of inflammation. This so-called stable plaque morphology may also explain the relatively low numbers of ruptured or eroded plaques in intracranial arteries. The underlying mechanisms remain largely unknown, but may be related to the above mentioned characteristics of the intracranial arteries such as the sparsity of vasa vasorum, a high antioxidant capacity, low inflammatory activity and response and protective effects of the cerebrospinal fluid. These characteristics suggest that the effect of specific atherogenic stimuli, but also of specific drug therapies may differ between extra- and intracranial arteries and is suggestive of divergent disease etiologies.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation

Additional information
Glucocorticoid-mediated immunosuppression underlies the cholestasis-induced atherosclerosis resistance

Abstract nr. 163
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis

Background & Aims: Hypercholesterolemia is an established risk factor for atherosclerosis and cardiovascular disease in the general population. Interestingly, cholestatic liver disease pathology in humans is associated with marked hypercholesterolemia but not an increased susceptibility to atherosclerosis or associated cardiovascular events. Here we aimed to provide mechanistic insight in the apparent resistance of cholestasis patients to hyperlipidemia-associated atherogenesis.

Methods: Hyperlipidemic apolipoprotein E knockout mice were fed a chow diet with or without alpha-naphthylisothiocyanate (ANIT; 0.025%) for 8 weeks to induce cholestasis.

Results: ANIT-fed mice exhibited extensive liver fibrosis, compensatory cholangiocyte proliferation, and marked increases in established plasma cholestatic indices, i.e. taurocholic acid levels (24-fold; p<0.01), alanine transaminase activity (2.7-fold; p<0.05) and bilirubin levels (+60%; p<0.01). Cholestatic mice displayed a reduced atherosclerotic lesion size (-28%; P<0.05) despite a marked rise in the free cholesterol (+31%; p<0.01) and cholesterol ester (+42%; p<0.001) levels associated with pro-atherogenic very-low-density lipoproteins and low-density lipoproteins. Macrophage and collagen contents of lesions were similar. In contrast, lesional T cell numbers were 47% (p<0.05) lower upon ANIT treatment. Importantly, cholestatic mice displayed a 72% increase (p<0.01) in plasma levels of the immunosuppressive molecule corticosterone, which could explain the concomitant 50% decrease (p<0.001) in circulating lymphocyte numbers (correlation coefficient R=-0.66; P<0.01).

Conclusions: We have shown that cholestasis is associated with elevated glucocorticoid levels, lymphocytopenia, and reduced atherosclerosis susceptibility in mice. Our findings for the first time highlight that an enhanced endogenous glucocorticoid function may contribute to the atherosclerosis resistance observed in cholestatic subjects.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Haloperidol inhibits the development of atherosclerotic lesions in LDL receptor knockout mice

Abstract nr. 164
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis

Background and Purpose: Antipsychotic drugs have been shown to modulate the expression of ATP-binding cassette transporter A1 (ABCA1), a key factor in the anti-atherogenic reverse cholesterol transport process, in vitro. Here we evaluated the potential of the typical antipsychotic drug haloperidol to modulate the macrophage cholesterol efflux function in vitro and susceptibility to atherosclerosis in vivo.

Experimental Approach: Thioglycollate-elicited peritoneal macrophages were used for in vitro studies. Hyperlipidemic low-density lipoprotein (LDL) receptor knockout mice were implanted with a haloperidol-containing pellet and subsequently fed a Western-type diet for 5 weeks to induce the development of atherosclerotic lesions in vivo.

Key Results: Haloperidol induced a 54% decrease (P=0.043) in the mRNA expression of ABCA1 in peritoneal macrophages. This coincided with a 30% (P<0.001) decrease in the capacity of macrophages to efflux cholesterol to apolipoprotein A1. Haloperidol treatment stimulated the expression of ABCA1 (+51%; P=0.021) and other genes involved in reverse cholesterol transport, i.e. CYP7A1 (+98%; P=0.004) in livers of LDL receptor knockout mice. No change in splenic ABCA1 expression was noted. However, the average atherosclerotic lesion size was significantly smaller (-31%; P=0.039) in the context of a mildly more atherogenic metabolic phenotype upon haloperidol treatment. Importantly, haloperidol markedly lowered MCP-1 expression (-70%; P<0.001) and secretion (-28%; P=0.018) by peritoneal macrophages.

Conclusions and Implications: These studies show that haloperidol treatment lowers the susceptibility for atherosclerotic lesion development in hyperlipidemic LDL receptor knockout mice. Our findings suggest that the beneficial effect on atherosclerosis susceptibility can be attributed to a haloperidol-induced inhibition of macrophage chemotaxis.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Membrane cholesterol is known to modulate a variety of cell signaling pathways and functions. While cholesterol depletion by High-Density Lipoproteins (HDL) has potent anti-inflammatory effects in various cell types, its effects on inflammatory responses in macrophages remain ill defined.

Pre-incubation of human and murine macrophages in vitro with human reconstituted (apolipoproteinA-I/phosphatidylcholine) or native HDL significantly decreased LPS-induced anti-inflammatory IL-10 production, while the opposite was observed for the pro-inflammatory mediators IL-12 and TNF-α. We show that these effects are mediated by passive cholesterol depletion and lipid raft disruption, without involvement of ABCA1, ABCG1, SR-BI or CD36. These pro-inflammatory effects are confirmed in vivo in peritoneal macrophages from ApoA-I transgenic mice, which have high circulating HDL levels. Native and reconstituted HDL enhances Toll Like Receptor-induced signaling by activating protein kinase C (PKC), since inhibition of PKC ablated the observed HDL effects. Using macrophages from NF-κB luciferase mice, we observed that HDL induces NF-κB activation. Western blot analyses showed that in particular the p65 subunit was activated. Using specific knock-out mice for the upstream activation pathways, we show that the observed HDL effects are IKK, NIK and CKII independent. Furthermore, using STAT1 knock-out mice we observed that also STAT1 is involved in the pro-inflammatory HDL effects on IL-10 and IL-12 secretion. On the other hand, using pharmacological inhibitors, we show that HDL enhances ADAM protease activity, thereby mediating TNF-α release.

Such pro-inflammatory activities on macrophages could at least partly underlie the disappointing therapeutic potential of HDL raising therapy in current cardiovascular clinical trials.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Autophagy dysfunction correlated with low cystatin C levels in atheroma is associated with plaque progression in human atheroma

Abstract nr. 167
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Pathogenesis, Vulnerable Plaque

Autophagy dysfunction in mouse atherosclerosis models has been associated with increased lipid accumulation, apoptosis and inflammation. Expression of cystatin C (CysC) is decreased in human atheroma and CysC deficiency enhances atherosclerosis in mice. Here we first investigated the association of autophagy and CysC expression levels with atheroma plaque severity in human Atheros atherosclerotic lesions is decreased while dysfunctional markers of autophagy p62/SQSTM1 and ubiquitin are increased together with elevated levels of lipid accumulation and apoptosis. The expression of LC3β and Atg5 were positively associated with CysC expression. We next investigated whether CysC expression is involved in autophagy in atherosclerotic apoE deficient mice, demonstrating that CysC deficiency (CysC−/−) in these mice results in reduction of Atg5 and LC3β levels and induction of apoptosis. Thirdly, macrophages isolated from CysC−/− mice displayed increased levels of p62/SQSTM1 and higher sensitivity to 7-oxysterol-mediated lysosomal membrane destabilization and apoptosis. Finally, CysC treatment minimized oxysterol-mediated cellular lipid accumulation. We conclude that autophagy dysfunction is a characteristic of the progression of human atherosclerotic lesions and associated with reduced levels of CysC. The deficiency of CysC causes autophagy dysfunction and apoptosis in macrophages and apoE deficient mice. The results indicate that CysC plays an important regulatory role in combating cell death induced by oxysterols via the autophagic pathway.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Pro-inflammatory cytokine IFNg modulates hepatic Sortilin expression and hepatocyte uptake capacity.

Abstract nr. 168
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Inflammation, Lipoproteins, Metabolism

Aim: Dysregulation of lipid metabolism and immunity are the two major risk factors for atherosclerosis. Yet, we reported recently increased atherosclerosis upon Treg (regulatory T lymphocytes) depletion or induced death. The effect was associated with elevated circulating VLDL (very low density lipoprotein) particles and decreased expression of hepatic Sortilin. At the same time, the Sortilin inhibitor, Atf3 mRNA was increased. The specific objective is to define how immunity, especially Treg, can regulate Sortilin and lipid metabolism.

Methods: To reproduce the inflammatory milieu, in vitro cytokine treated hepatocytes (mouse hepatocyte cell line AML-12) are used. qPCR and western blotting are used to follow expression of Sortilin Atf3 and Stat1. In silico method is used to find conserved binding sites of Stat1 on Sortilin between humans and mice, confirmed by Chip (chromatin immune precipitation) assays. Radioactive lipoprotein cultured hepatocytes are used to assess the lipid uptake capacity in inflammatory conditions.

Results: While anti-inflammatory cytokine treatments (IL-10, TGFb) don’t have any effects on Sortilin, pro-inflammatory cytokine IFNg (which can be released upon cell death or induced by the absence of Treg) decreases Sortilin mRNA and protein level when added to the culture of hepatocytes. At the same time, Atf3 is upregulated. Sortilin is decreased only after 12h of culture. Thus suggesting that IFNg can regulated Sortilin at a transcriptional level. In hepatocyte cultures, STAT1 is phosphorylated only after IFNg treatment. In silico experiment reveals 4 putative binding sites for STAT1 on Sortilin between humans and mice, confirmed by Chip assays. Upon IFNg treatment, hepatocytes have decreased uptake capacity for VLDL and LDL particles.

Conclusion: these first results suggest that IFNg can reduce Sortilin which is described to modulate VLDL secretion. Treg by reducing inflammation may inhibit IFNg effects on Sortilin.

Key words: Inflammation, lipid metabolism.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Distinctive proteomic profiles among different regions of human carotid plaques

Abstract nr. 169
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Cardiovascular Disease,Pathogenesis,Vulnerable Plaque

Purpose: Revealing the mechanisms leading to plaque vulnerability, different regions of human carotid plaques may have differential roles in development of atherosclerotic plaques. The aims of this investigation were to create a comprehensive proteomic profile from different regions of the human carotid atherosclerotic plaque, and to establish a protein reference map that could help identify functional roles of different plaque regions in plaque development.

Experimental Design: Two-dimensional gel electrophoresis was used to separate extractable proteins from five different regions of the human carotid endarterectomy samples; internal control, fatty streak, plaque shoulder, plaque centre and fibrous cap. Identification of proteins was made using matrix-assisted laser desorption/ionisation-time of flight mass spectrometry, confirmed by nano-liquid chromatography tandem-mass spectrometry.

Results: Protein mapping resulted in the successful identification of 52 unique proteins, including 15 previously unmapped proteins with regards to atherosclerosis. Expression levels of 13 proteins in atherosclerotic lesions significantly differ from the expression in internal control tissue, including overexpression of apolipoprotein A-IV in plaque centre and of fibrinogen β chain fragment in all three plaque regions. In addition, reduced levels of 9 proteins implicated in remodeling of the extracellular matrix and functions of smooth muscle cells were found in plaque regions.

Conclusions and clinical relevance: The unique sampling method of the atherosclerotic plaque was found to be successful in revealing newly mapped proteins and significant differential expression among different regions of human carotid plaques. The protein profiles with distinct functional implications are of importance for understanding and modulation of plaque biology in atherosclerosis.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Atherogenic shifts in lipoprotein profile: the relationship with biological and chronological vessel aging

Abstract nr. 170
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Dyslipidemia, LDL, Lipoproteins

Aim: to explore the relationship between atherogenic shifts in lipoprotein profile and vessel aging.
Materials and methods. Totally 202 subjects of both sexes aged 30-75 years without clinical manifestations of atherosclerosis-related diseases which didn't receive regular cardiovascular therapy were included into the study. Depending on biological age of vessels (flexible or stiff) and chronological age (years) patients were divided into 4 groups. Pulse wave velocity (PWV) > 10 m/s was used as a measure of arterial stiffness. Lipid levels were determined by routine laboratory methods. LDL subfractional distribution was analyzed using Lipoprint LDL System (Quantrimetrix, USA).

Results. In more younger subjects (≤ 45 years) with stiff vessels in comparison with younger ones with flexible vessels higher levels of total C and LDL-C were found. In older subjects (> 45 years) TG concentration was higher and that of Lp(a) - lower in those with stiff than with flexible vessels. The subfractional LDL analysis revealed elevated VLDL portion in subjects with stiff arteries irrespectively on age. In older subjects with stiff vessels the portion of the largest particles within IDL range was the lowest as compared to all other groups. Older subjects with elevated arterial stiffness had significantly more small particles of IDL-B and, especially IDL-A subfractions. Conclusion. The increased vessel stiffness as a marker of biologically old vessels in chronologically old patients was associated with elevated portion of small dense IDL particles, by the size close to potentially atherogenic LDL.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
"LRP1 and human HDL metabolism: from gene to function"

Abstract nr. 171
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Genetics, Lipoproteins, Metabolism

Aim: The LDL-receptor related protein 1 (LRP1) is the largest member of the LDL receptor gene family and has been shown to play a key role in the hepatic clearance of apoE-containing lipoproteins. Surprisingly, recent studies in liver-specific LRP1 knock-out mice have indicated that LRP1 has a direct role in HDL biogenesis and cholesterol levels. We questioned whether this also holds true in humans.

Methods: Targeted-sequencing in individuals with extremely low HDL-C levels identified the first 3 non-synonymous variations in the LRP1 gene, i.e. c.3644G>A (p.Gly1215Glu), c.11949G>T (p.Glu3983Asp) and c.9730G>A (p.Val3244Ile). To evaluate the functional importance of these variations they were introduced in LRP1 GFP-tagged expression constructs in HEK293/Huh7 cells for mRNA/protein and cellular localization studies through fluorescence microscopy. Efflux studies using respectively, fibroblasts isolated from index cases and knock-out/in hepatic cells engineered by CRISPR-Cas9 were carried out to assess the effect of these mutations on HDL metabolism.

Results: All variants involve highly-conserved nucleotides and are predicted to be deleterious to protein structure/function. Mutant LRP1 protein levels are significantly decreased by 40-60% when compared to wild-type whilst mRNA levels are not affected. In addition, measurements of LRP1 stability and localization studies have shown that mutants are less stable and co-localizing with early-endosomes.

Conclusion: Three LRP1 variants that are likely to be of functional importance have been identified in individuals with low HDL-C levels. A comprehensive approach is currently used to test for functionality and elucidate how LRP1 affects HDL metabolism in humans.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Effect of a Chronic High Cholesterol Diet on Arterial Atherosclerotic Burden in Nonhuman Primates with Advancing Age

Abstract nr. 172
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Inflammation, Lipids

Background
Both aging and a high cholesterol diet (HCD) remodel inflammatory signaling pathways, predisposing the arterial wall to metabolic vulnerability, facilitating the pathogenesis of atherosclerosis. However, synergic interactions of age and HCD remain to be clarified.

Main findings
A 2-year chronic HCD in rhesus monkeys (n=16) markedly (~2-fold, p<0.001) increases plasma cholesterol by the 6th month in both young (≤ 15 yrs) and old (>15 yrs) groups compared to a standard diet (SD, n=10). HCD dramatically increases atherosclerotic burden (a percentage of the cap perimeter to corresponding arterial circumference) along the direction of blood flow, in particular, from thoracic and abdominal aorta to iliac artery (Figure). HCD significantly increases atherosclerotic burden to a greater extent in the old vs young group (Figure) (Two-way ANOVA p<0.001). The increased plaque burden in old monkeys appears as a significant increase in plaque area, enriched with an abundant inflammatory lipid deposition of intra-and-extra macrophages.

Conclusions
HCD increases the atherosclerosis burden in both young and old groups. However, the effects of HCD and aging are additive on inflammatory responses and subsequently synergistically amplify the atherosclerotic burden with aging. Thus, targeting age/diet-associated arterial remodeling is an evidence-based approach to curb an increased prevalence of atherosclerotic burden in the elderly.
Atherosclerosis Burden
Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
FUNCTIONAL MUTATION IN ABCA1, PLASMA LEVELS OF APOLIPOPROTEIN E AND RISK OF ALZHEIMER DISEASE AND CEREBROVASCULAR DISEASE

Abstract nr. 173
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Dyslipidemia, Genetics, HDL

Aim and background: The ATP Binding Cassette Transporter A1 (ABCA1) is a major cholesterol transporter highly expressed in liver and brain. In brain, ABCA1 lipidates apolipoprotein E (apoE) and thus facilitates clearance of β-amyloid and maintenance of the blood-brain-barrier via apoE mediated pathways. Whether functional mutations in ABCA1 are associated with decreased levels of plasma apoE and increased risk of Alzheimer disease and cerebrovascular disease in the general population is unknown. We tested this hypothesis.

Methods: In a prospective study of the general population (N=92,726), we tested whether a functional mutation in ABCA1, N1800H, was associated with plasma levels of apoE and with increased risk of Alzheimer disease and cerebrovascular disease.

Results: N1800H AC versus AA was associated with a 13% decrease in plasma levels of apoE (p=1·10^-11). Multifactorially adjusted hazard ratios (HRs) for N1800H AC versus AA were 4.13 (1.32-12.9) and 2.46 (1.10-5.50) for Alzheimer disease and cerebrovascular disease, respectively. For subtypes of cerebrovascular disease, multifactorially adjusted HRs for N1800H AC versus AA were 8.28 (2.03-33.7) and 1.81 (0.67-4.84) for hemorrhagic stroke and ischemic stroke, respectively. Multifactorially adjusted HR for N1800H AC versus AA was 0.33 (0.05-2.33) for myocardial infarction.

Conclusions: A functional mutation in ABCA1, presenting with a frequency of 2:1,000, is associated with decreased plasma levels of apoE, and increased risk of Alzheimer disease and cerebrovascular disease. N1800H is not associated with increased risk of myocardial infarction, suggesting that the present observations between N1800H and Alzheimer disease and cerebrovascular disease are not due to atherosclerosis but rather may be attributed to brain and blood-brain-barrier specific effects.

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Metformin lowers plasma triglycerides by promoting VLDL-triglyceride clearance by brown adipose tissue in mice

Abstract nr. 174
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes, Dyslipidemia, Therapy, Triglycerides

BACKGROUND AND AIM
Metformin, a member of the biguanides family of antidiabetic drugs, is currently considered as the first-line drug treatment for type 2 diabetes. Metformin is well-known for its antihyperglycemic properties, which mainly involve the suppression of hepatic gluconeogenesis and subsequent lowering of hepatic glucose production. Importantly, metformin has also been shown to lower plasma very low-density lipoprotein (VLDL) triglyceride (TG) levels, via a yet unknown mechanism. Therefore, the current study was aimed at unraveling the mechanism underlying the lipid-lowering effect of metformin using APOE*3-Leiden.CETP mice, a well-established model for human-like lipoprotein metabolism.

METHODS AND RESULTS
Twelve-week old female APOE*3-Leiden.CETP mice were fed a Western-type diet for 4 weeks, followed by a Western-type diet with or without metformin (200 mg/kg BW/day) for another 4 weeks. We show that metformin markedly lowered plasma total cholesterol (-36%, P<0.05) and TG (-38%, P<0.05) levels. Importantly, metformin did not affect hepatic VLDL-TG production, VLDL particle composition, and hepatic lipid content. Metformin did, however, selectively enhance clearance of glycerol tri[3H]oleate-labeled VLDL-like emulsion particles into brown adipose tissue (BAT) (+58%, P<0.05). Importantly, BAT mass and lipid droplet content were reduced in metformin-treated mice, pointing to increased BAT activation. Both AMP-activated protein kinase (AMPK) α1 expression and activity (+38% and +19% respectively) were increased in BAT, as was the expression of hormone-sensitive lipase (HSL) and various subunits of the mitochondrial electron transport chain complex. Finally, therapeutic concentrations of metformin increased AMPK and HSL activities and promoted lipolysis in T37i differentiated brown adipocytes in vitro.

CONCLUSION
To our knowledge, this study is the first to identify BAT as a new important player in the lipid-lowering effect of metformin, by enhancing VLDL-TG uptake, intracellular TG lipolysis, and subsequent mitochondrial fatty acid oxidation. Targeting BAT might therefore be considered as a future therapeutic strategy for the treatment of dyslipidemia.

Note: This study was conducted at the Leiden University Medical Center. The presenting author (Janine Geerling) is currently employed at the Leiden Academic Center for Drug Research.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
RELATIONSHIPS BETWEEN POLYMORPHISM OF PARAOXONASE 1 AND APOLIPOPROTEIN A1 GENES IN PATIENTS WITH CORONARY ATHEROSCLEROSIS

Abstract nr. 175
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Genetics, HDL

Background. Apolipoprotein A₁ (ApoA₁) is an essential part of high density lipoproteins (HDL) responsible for the reverse cholesterol transport. Antiatherogenic action of HDL is implemented also due to antioxidative effect which is attributed to its paraoxonase 1 (PON1) enzymatic activity.

Purpose. The aim of this research was to study the common genetic variation in the PON1 and APOA1 genes in patients with coronary artery disease (CHD).

Methods. Blood samples of 177 patients with clinical and instrumental signs of CHD were examined in this study. PON1 activity was determined towards substrate – paraoxon. Polymorphisms in the PON1 and ApoA1 were analyzed by means of polymerase chain reaction assay.

Results. PON1 activity was considerably higher in patients with 192RR/QR and 55LL genotypes of PON1 and (-75)AA/AG genotypes of ApoA1. In patients with CHD a modified genetic variant (192RR or 192QR) of PON1 gene was revealed. Earlier manifestation of CHD was also typical for the patients with genotype (-75)AA/AG of ApoA1. More pronounced atherosclerotic lesions verified by the coronary angiography were associated with the presence of allele R in 192QR genes PON1, as well as allele A in (-75)GA and allele C in 83CT gene ApoA1. The ApoA1 (-75)AG polymorphism was connected with 192QR and 54LM PON1 polymorphism; genotype 192RR and 54MM was significantly more commonly detected in patients with genotype (-75)AG/AA of ApoA1.

Conclusions. The obtained findings prove the association of the PON1 192RR/QR and the Apo A1 (-75)AG/AA polymorphisms in patient with coronary atherosclerosis.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Molecular genetics of familial hypercholesterolemia in Israel – revisited

Abstract nr. 176
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia, Genetics, Metabolism

Background
In 1996, the first summary of molecular genetics of familial hypercholesterolemia (FH) in Israel was published (Reshef A, Hum Gen, 1996). Since then, new technology for mutation screening became available making genetic diagnosis easier more accurate, and more comprehensive. We performed systematic mutation screening of index cases of 68 pedigrees to reanalyze the profile of mutations among Israeli FH patients.

Methods and results
In 68 individuals the entire coding region, promoter and intron-exon junctions of the LDLR and part of exon 26 of the APOB gene were screened for variants using High Resolution Melting, with Sanger sequencing of any identified melt shifts. Mutations of the LDLR gene were found in twenty-three patients (34% of the total) with seventeen different mutations. The most common mutation was p.(V827I) (47% of the detected mutations), not previously reported in Refech et al but reported to occur in patients from Russia. Three variants, two in exon 4 and one in the promoter region were not reported before; p.(C121S), p.(E140A) and c.-191 C>A. One patient carried the common APOBp.(R3527Q) mutation. Two families from Druze ancestry carried a novel deletion of exons 7 to 14. Seventy percent of the cohort were negative for an LDLR or APOB mutation. It was hypothesized that FH can be caused by an accumulation of LDL-C raising alleles each having a small contributive effect (Talmud et al., 2013). Using this published gene score method derived from twelve common LDL-C raising SNPs in eleven genes, the mean polygene score was significantly higher (p=0.03) among mutation negative Israeli FH patients compared with a European control population.

Conclusion
We identified mutations in 30% of a new Israeli FH cohort. In these we found 3 novel LDLR mutations. The high prevalence of p.(V827I) likely reflects the change in demographics of the
Israeli population since 1996. In the remaining mutation free cohort, there is likely to be a polygenic cause of their elevated LDL-C and FH diagnosis. These data can help design a future strategy for early screening for FH in our population.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Insulin-like growth factor type 1 in children and adolescents with hypertension and prehypertension

Abstract nr. 177
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Hypertension, Obesity

Background: The insulin-like growth factor type 1 (IGF-1) system is associated with growth, differentiation and tissue development, and non-physiological activation of this system plays a role in atherogenesis. Decreased levels of IGF-1 in adults are independently associated with glucose intolerance, 2nd type diabetes mellitus, abdominal obesity and atherogenic dyslipidemia.

Material and methods: The study sample consisted of 84 patients (49 boys) with a mean age of 14 years. The analyzed outcomes were plasma levels of IGF-1, growth hormone, uric acid, insulin, glucose, total cholesterol, HDL, LDL, triglycerides, and systolic and diastolic blood pressure measured at rest. Blood pressure levels were divided into groups - normotensive, prehypertensive and hypertensive according to height percentile for age and sex.

Results: The values of circulating IGF-1 in the study group of children were positively correlated with age (p<0.001) and negatively with BMI percentile (p=0.039) and percentile for both systolic (p=0.004) and diastolic blood pressure (p=0.024). In the comparison of the groups of normotensive children (systolic blood pressure <95 percentile, n=38) and children with systolic blood pressure in the range of prehypertension to hypertension (≥95 percentile, n=28) we found a statistically significant difference in mean IGF-1 between the groups: 400.05±129.31 ng/ml versus 299.29±148.97 ng/ml (p=0.005).

Conclusion: Low IGF-1 may represent a potential marker for the presence of cardiometabolic risk factors in the pediatric population.
Apolipoprotein A-I restores endothelial function in rats with arthritis

Abstract nr. 178

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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Apolipoproteins, Endothelium, HDL, Inflammation

Endothelial dysfunction is a key event in the development of atherosclerosis and has been identified in patients with rheumatoid arthritis and in rats with experimental arthritis. We have recently shown that apolipoprotein A-I (apoA-I), the most abundant apolipoprotein in high density lipoproteins (HDL), and reconstituted HDL [(A-I)rHDL] consisting of apoA-I complexed with phosphatidylycholine inhibit streptococcal cell wall peptidoglycan-polysaccharide (PG-PS)-induced arthritis in female Lewis rats. This study asks if apoA-I also improves endothelial dysfunction in rats with arthritis. A single intraperitoneal injection of PG-PS (15 mg/kg) or an equivalent volume of saline (control) was administered to female Lewis rats. After four days the PG-PS-treated animals had acute joint inflammation, elevated circulating inflammatory cytokine levels, and had aortic endothelial dysfunction. Intravenous infusions of lipid-free apoA-I (8 mg/kg) 24 h pre- and 24 h post-PG-PS administration decreased the acute joint inflammation, reduced plasma TNF-α, IL-6 and IL-1β levels, and restored aortic endothelial function with a 39±9.2% improvement in aortic vasorelaxation and a 53±17% increase in guanosine 3’,5’-cyclic monophosphate (cGMP) production at day 4 post-PG-PS injection. In ex vivo studies, incubation of aortic rings from control female Lewis rats with TNF-α (10 ng/mL) for 6 h impaired aortic vasorelaxation by 82±2.2% and decreased cGMP production by 81±5.5%. Pre-incubation of the aortic rings for 16 h with (A-I)rHDL (final apoA-I concentration 0.5 and 1.0 mg/mL) improved the TNF-α-induced impaired aortic vasorelaxation by 1.9±0.3- and 3.2±0.9-fold, cGMP production by 2.1±0.4- and 3.4±0.4-fold, respectively. In addition, (A-I)rHDL induced endothelial nitric oxide synthase (eNOS) expression in human coronary artery endothelial cells (HCAECs) in a time- and dose-dependent manner. This effect was abolished by knockdown of scavenger receptor-B1. Incubation of HCAECs with TNF-α (1 ng/mL) for 6 h reduced HCAEC eNOS expression by 67±9.9%. Pre-incubation of the HCAECs for 16 h with (A-I)rHDL (final apoA-I concentration 0.5 and 1.0 mg/mL) restored the TNF-α reduced HCAEC eNOS expression by 2.2±0.2- and 2.7±0.2-fold, respectively. These findings establish that apoA-I improves endothelial dysfunction in rats with arthritis by, at least partly, inhibiting inflammatory cytokine induced endothelial dysfunction. This work was supported by National Health and Medical Research Council of Australia.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Validation for risk assessment chart of Japanese Atherosclerosis Society by a large external population: EPOCH-JAPAN

Abstract nr. 179
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Cardiovascular Disease, Epidemiology, Risk Factor

Some assessment tools for future individual risk for atherosclerotic diseases had been constructed and applied in developed countries, which should be originally established because the absolute risk of atherosclerotic diseases varies among different ethnic groups. However, there exists a time lag between baseline surveys of cohort studies that provided coefficients of risk assessment and the time when the relevant risk assessment tools are utilized. Consequently, it is important to validate the risk assessment tools by external cohort with more recent baseline surveys than the original ones.

In Japan Atherosclerosis Society guideline 2012 (JAS2012), NIPPON DATA80 risk assessment chart (ND80RAC) was adopted to estimate 10-year probability of coronary artery disease (CAD) mortality for the adequate management for dyslipidemia. In order to validate this chart, we used one of the largest pooled cohorts in Japan, the Evidence for Cardiovascular Prevention from Observational Cohorts in Japan (EPOCH-JAPAN), as an external population of which baseline survey was almost 10 years newer than that of ND80RAC. We analyzed the dataset of 15,091 men and 18,589 women aged 40-74 years without a history of cardiovascular disease. The probability of 10-year risk for CAD/stroke mortality was estimated using ND80RAC. The participants were divided into decile according to the estimated mortality and the mean estimated 10-year risk and the actual cumulative mortality for CAD/stroke were compared, respectively. The mean estimated 10-year risk of CAD/stroke mortality was higher than the actual mortality in higher risk group, however, which was concordant with the actual mortality in low/moderate risk group in both sexes.

The causes of overestimating the mortality in ND80RAC could be explained as followings: i) decreased morality in the high-risk elderly during last decade, ii) remarkable medical cure progress, iii) high concern of one’s prevention and control, especially in higher risk individuals.

ND80RAC is useful tool to predict mortality for CAD/stroke although it overestimates mortality in high-risk individuals.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Deletion of progranulin exacerbates atherosclerosis in ApoE knockout mice.

Abstract nr. 180
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation

Progranulin (PGRN) is a multifunctional glycoprotein involved in cellular proliferation, survival, migration and wound repair. Moreover, some mutations in the PGRN gene have been reported to result in frontotemporal lobar degeneration. We previously reported that progranulin (PGRN), which is secreted from human monocyte-derived macrophages, is bound to apolipoprotein A-I (apoA-I), a major component of HDL. Although PGRN is also known to be involved in inflammation, there is no information available on the direct effect of PGRN expression on atherosclerosis. Therefore, we generated PGRN-/-ApoE-/- (double knockout, DKO) mice to explore the role of PGRN in atherogenesis. DKO mice that were fed high fat diet for 12 weeks developed severe atherosclerosis compared to ApoE KO mice fed the same diet, suggesting that PGRN has an atheroprotective role in the development of atherosclerosis. The increase in atherosclerotic lesions in DKO mice was in part due to the enhanced expression of adhesion molecules, as well as the decreased expression of endothelial nitric oxide synthase (eNOS) in the aortic lesions. Moreover, lack of PGRN leads to accumulate excessive cholesterol in the macrophages and alter HDL-associated proteins. Recently, PGRN has been reported to bind directly to TNF receptors and suppress inflammation by disrupting TNF-alpha signaling. PGRN has a variety of atheroprotective functions, therefore PGRN can be a promising therapeutic target for atherosclerosis.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
ASSOCIATION BETWEEN SMALL DENSE LDL-CHOLESTEROL AND CORONARY ARTERY DISEASE IN NORTH INDIAN POPULATION- A CASE CONTROL STUDY

Abstract nr. 181
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease,Dyslipidemia,LDL,Risk Factor

Background –Indian population studies with established CAD often show LDL levels within normal range in patients with proven CAD. We hypothesized that Small dense LDL-cholesterol (sd-LDL-c) being more atherogenic might correlate to occurrence and severity of CAD.
Objective- The aim of the study was to evaluate the association between sd-LDL-c and severity of coronary artery disease.
Method- 125 patients with coronary stenosis on angiography, 25 patients without coronary stenosis and 40 healthy controls were studied. Direct quantitative measurement of sd-LDL-c was done by standard method.
Results- There was no significant difference between triglyceride (TG) levels and HDL-c levels among the three groups. Mean sd-LDL-c levels were higher in patients with coronary stenosis than patients without and also healthy individuals viz (16.3 + 6.8 mg/dl vs 12.7 + 7.1mg/dl vs 8.5 + 3.9 mg/dL respectively, (p<0.0001). There was a linear relation between mean sdLDL and severity of CAD with mean sd LDL in single vessel disease, double vessel disease and triple vessel disease being 14.3 + 5.8 mg/dL, 16.1 + 6.7 mg/dl and 18.2 + 7.3 mg/dl respectively ( p value <0.0001). A cut off value of 10.02 mg/l was associated with presence of CAD.(95% CI 0.82-0.93, p< 0.001).
Conclusion- Indian patients with established CAD have higher sd-LDL-c levels compared to individuals without CAD despite having comparable LDL-c levels.Beyond traditional lipid profile estimation of sd-LDL-c in Indian patients as a routine which may assist in identifying a higher risk population even treated with statin.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Cholesterol in remnant-lipoproteins as measured by different methods

Abstract nr. 182
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Co-author(s) - Tomassini, Joanne E.
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Co-author(s) - Polis, Adam B.
Co-author(s) - Tershakovec, Andrew
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords LDL, Lipids, Lipoproteins, Risk Factor

Background: Elevated remnant-lipoprotein cholesterol (RLP-C) levels increase the risk of ischemic heart disease (IHD). The concurrence of RLP-C assessment by different separation methods is not well described. This analysis examined RLP-C assessed by immunoseparation (IM) and vertical auto profile (VAP [IDL+VLDL₃]) methods using samples from a previously reported randomized, clinical trial.

Methods: This analysis assessed fasting RLP-C in hyperlipidemic patients (n=2,382) treated with ezetimibe/simvastatin (E/S)10/20 mg, E/S + niacin (N) 2g and N 2 g during 24 weeks, and E/S 10/20 mg and E/S + N 2g during 64 weeks. Pearson correlation and Bland-Altman plots were used to evaluate the degree of similarity between the 2 methods used to measure RLP-C levels, and change and % change from baseline across treatments.

Results: Cholesterol mass at baseline measured by RLP-C VAP was ~4X higher than by IM; both declined with treatment by 24 weeks with little further reduction at 64 weeks. RLP-C change and % reduction from baseline were larger when measured by VAP vs IM. Although the 2 methods were moderately correlated (r=0.54 - 0.59) for RLP-C levels and changes, Bland-Altman plots showed little agreement between the methods for RLP-C levels but slightly better agreement for RLP-C changes.

Conclusion: RLP-C defined by IM and VAP methods differs in mass and response to pharmacologic intervention. Given the relationship between RLP-C and IHD risk, standardization of methods is needed for RLP-C use in risk assessment.
### Subdivision 3. Clinical Studies

**Presentation Preference** Oral presentation

**Additional information**

<table>
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IM=immunoseparation method; RLP-C=remnant lipoprotein cholesterol; VAP=vertical auto profile (IDL+VLDL) method
Ezetimibe does not increase fasting glucose levels more than statins alone in non-diabetic, hypercholesterolemic patients

Abstract nr. 183
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease,Diabetes,LDL

Background: Statin (St) therapy can be associated with a slightly increased risk of diabetes mellitus (DM) and insulin resistance in nonDM patients. In prior studies, ezetimibe (Eze)+St did not increase fasting serum glucose (FSG) more than St alone in St naïve, nonDM, hypercholesterolemic (HC) patients for up to 96 wks. This analysis assessed the effects of Eze on FSG changes when given to nonDM, HC patients on stable St therapy.

Methods: Data was pooled from 2 randomized, double blind, add on‡ (Eze added to stable St [n=1506] vs placebo [n=851] studies) for 8 wks in nonDM patients at baseline (BL). Mean FSG changes from BL were estimated for each treatment group (LDA model¶) and between treatment differences calculated. Numbers of patients with FSG ≥126 mg/dl and effect of BL cofactors were also assessed.

Results: No significant FSG increases from BL were observed with St and Eze+St in add on studies; the between treatment group difference was also not significant (p>0.05; Figure). The lack of an Eze effect on FSG is consistent with prior findings in St naïve subjects comparing Eze+St vs St. FSG changes were not related to age, and BL BMI, HDL-C and TG, nor to changes from baseline in LDL-C, BMI, HDL-C, TG and ApoB. Proportions of patients with FSG ≥126 mg/dl during the trial were low, similar for St and Eze+St, and highest in those with BL FSG ≥100≤126 mg/dL.

Conclusion: In HC patients on stable St therapy, addition of Eze did not increase FSG levels more than St therapy, consistent with Eze+St therapy effects in St naïve patients.
1st line: 7 studies in St-naive/drug wash-out HC patients treated with Eze 10 mg + St vs St (10 to 80 mg of simvastatin and atorvastatin, 10 to 40 mg lovastatin and pravastatin) during 12 weeks; 2nd line Add-on: 2 studies in HC patients on St therapy (10 to 80 mg of atorvastatin, simvastatin, pravastatin, fluvastatin and lovastatin, 0.2 to 0.8 mg cerivastatin) ≥6 weeks prior to study entry, randomized to Eze 10 mg vs placebo; ns=not statistically significant at the 0.05 alpha level; **p<0.01. Change from baseline assessed using longitudinal data analysis (LDA) model with terms for treatment and trial."
Characteristics of statin intolerant patients from a pooled analysis of 464 patients in phase 2 (GAUSS-1) and 3 (GAUSS-2) trials

Abstract nr. 184
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Dyslipidemia, LDL, PCSK9

Introduction: Statins effectively reduce LDL-C and cardiovascular (CVD) morbidity and mortality. However, studies indicate 5 to 15% of patients are unable to tolerate statins due to muscle-related complaints, with no similarly effective LDL-C reducing agent available. Evolocumab (AMG 145), a PCSK9 monoclonal antibody, results in large reductions in LDL-C, and has been assessed in phase 2 and 3 trials in patients with statin intolerance (GAUSS-1 and GAUSS-2). We report the characteristics of these patients from a pooled analysis of 464 patients from these two trials (Table).

Methods: GAUSS-1 and GAUSS-2 enrolled patients who were intolerant of at least one and two statin(s), respectively, who also had LDL-C above their NCEP ATP III risk levels.

Results: Baseline characteristics are reported below (Table). Mean (SD) LDL-C was 5.0 (1.5) mmol/L at entry, a majority was <65 yrs old, and there was no gender imbalance. The reasons for statin intolerance were: myalgia (84%), myositis (15%), and rhabdomyolysis (2%). Baseline median (Q1, Q3) CK was 105 (71, 170) IU/L; 100% of patients were intolerant of ≥1 statin, 92% of ≥2 statins, 46% of ≥3 statins, and 17% of ≥4 statins.

Conclusion: In this analysis of statin-intolerant patients from GAUSS-1 and GAUSS-2, most patients had existing CVD, diabetes, were considered high risk by NCEP ATP III, could not tolerate ≥2 statins, and had extremely high baseline LDL-C. These patients constitute a significant unmet need for effective and tolerable LDL-C lowering therapy.
Table. Baseline Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N = 464</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>62 (9)</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>223 (48)</td>
</tr>
<tr>
<td><strong>Lipid parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Ultracentrifugation LDL-C, mmol/L</td>
<td>5.0 (1.5)</td>
</tr>
<tr>
<td>LDL-C, mmol/L, calculated</td>
<td>5.0 (1.4)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>7.2 (1.5)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.4 (0.4)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.7 (1.2, 2.4)</td>
</tr>
<tr>
<td>Lp(a), mmol/L</td>
<td>36 (11, 133)</td>
</tr>
<tr>
<td><strong>Worst muscle-related side effect in statin intolerance history, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Myalgia</td>
<td>388 (84)</td>
</tr>
<tr>
<td>Myositis</td>
<td>68 (15)</td>
</tr>
<tr>
<td>Rhabdomyolysis</td>
<td>7 (2)</td>
</tr>
<tr>
<td><strong>Cardiac risk factors, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes mellitus</td>
<td>83 (18)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>255 (55)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>46 (10)</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>188 (41)</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>29 (5)</td>
</tr>
<tr>
<td><strong>NCEP risk categories</strong>, n (%)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>208 (45)</td>
</tr>
<tr>
<td>Moderately high</td>
<td>136 (29)</td>
</tr>
<tr>
<td>Moderate</td>
<td>99 (21)</td>
</tr>
<tr>
<td>Lower</td>
<td>21 (7)</td>
</tr>
</tbody>
</table>

* All values are mean (SD). Triglycerides and Lp(a) are median (Q1, Q3)
* In GAUSS-1, LDL-C was measured by preparative ultracentrifugation method; in GAUSS-2, LDL-C was determined by the Friedewald formula with reflexive testing via preparative ultracentrifugation when calculated LDL-C was <1.0 mmol/L or triglyceride levels were >3.9 mmol/L.
* Muscle pain, ache, or weakness without creatine kinase (CK) elevation
* Muscle symptoms with increased CK levels
* Muscle symptoms with marked CK elevation
* Risk category definitions: high, diagnosed coronary heart disease (CHD) or risk equivalent; moderately high, 2 or more risk factors and Framingham risk score 10%-20%; moderate, 2 or more risk factors and Framingham risk score <10%; lower, 0 or 1 risk factor.
SD, standard deviation; Q1, quartile 1; Q3, quartile 3; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); HDL-C, high density lipoprotein cholesterol; BMI, body mass index; NCEP, National Cholesterol Education Program

This study was funded by Amgen Inc.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Multicenter Evaluation of new LDL-Cholesterol Generation 3 assay on Roche Clinical Chemistry Analyzers

Abstract nr. 185
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, LDL, Risk Factor

**Medical Background:** Low Density Lipoprotein (LDL) is produced in the circulation from its heptic precursor, VLDL (Very Low Density Lipoproteins). Elevated LDL concentrations in blood results in the destruction of the endothelial function and a higher LDL-cholesterol (LDL-C) uptake in the monocyte/macrophage system as well as by smooth muscle cells in vessel walls. The LDL-C concentration is the most powerful clinical predictor with respect to coronary atherosclerosis, and, therefore, it is used for coronary heart disease (CHD) risk assessment and monitoring of lipid lowering therapies.

**Methods and Results:** The analytical performance of the new LDL-Cholesterol Gen.3 (LDLC3) assay was evaluated in four different laboratories using cobas c 702, cobas c 502 and cobas c 501 instruments. LDL cholesterol esters and free cholesterol in LDL are directly measured using cholesterol esterase and cholesterol oxidase in the presence of surfactants which selectively solubilizes only LDL.

Repeatability and intermediate precision was measured in the concentration range from 1.5 to 5.1 mmol/L according to the CLSI EP5 protocol using two Roche controls and three human serum pools. For the repeatability the coefficients of variation (CVs) were determined to be less than 1.5 % and for intermediate precision yielded CVs ranging between 1.3 and 3.7 % (two runs/day, 21 days). The recovery of four controls (Roche Diagnostics) was determined in three independent runs. The recovery of LDL-C target values ranged from 96.7 to 107.4 %.

Method comparison experiments were designed in compliance with CLSI EP09-A3, using > 250 serum samples. Statistical Passing-Bablok analysis of method comparisons against the Roche LDL-C Gen.2 yielded correlation coefficients $r > 0.992$ ($p < 0.0001$), slopes between 0.99 and 1.01 (95 % confidence interval: 0.98 - 1.04) and intercepts from -0.05 to 0.01 (-0.09 – 0.04) mmol/L.
The reactivity against VLDL’s was tested as well as the interference of chylomicrons appearing in non-fasting samples (up to 2000 mg/dL triglyceride). Fasting and non-fasting samples can be used.

**Conclusions:** The MCE of the LDL-Cholesterol Gen.3 assay from Roche Diagnostics proved excellent analytical performance with regard to precision, recovery, method correlations, and specificity. The assay is well-suitable for routine use.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Different profiles of intracranial atherosclerotic plaque compositions between anterior and posterior circulation

Abstract nr. 186
Author Yang, Wenjie, Chinese University of Hong Kong, Hong Kong, Hong Kong
Co-author(s) - Niu, Chunbo
Co-author(s) - Zhao, Hailu
Co-author(s) - Wong, Kasing
Co-author(s) - Ng, Hokeung
Co-author(s) - Chen, Xiangyan
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Pathogenesis

**Background:** Intracranial atherosclerotic disease (ICAD) is reaching greater clinical prominence as the most common cause of ischemic stroke, especially in Asian patients. Current understanding of ICAD has been advanced by high-resolution magnetic resonance imaging (HRMRI), but the clinical application of HRMRI is limited by insufficient pathological evidence about intracranial atherosclerosis. Based on a cohort of Chinese adult autopsy cases, the aim of the study was to investigate the distribution of intracranial atherosclerosis, the plaque compositions and to explore whether variations existed between anterior and posterior circulation in plaque features.

**Methods:** It was a hospital cohort based study collecting a series of consecutive Chinese adult autopsy cases (88 cases). Intracranial large arteries including 164 middle cerebral arteries (MCA), 25 internal carotid arteries (ICA), 35 vertebral arteries (VA) and 36 basilar arteries (BA) were collected. H&E staining, Victoria Blue staining and immunostaining for inflammatory cells were performed. The phenotype of atherosclerotic plaques, the involvement of the entire intima or not and the occurrence of complications (intraplaque hemorrhage, neovasculature, thrombus and calcification) were evaluated and compared between anterior and posterior circulation.

**Results:** A total of 88 cases were included, with a median age of 74 years old (range 23-99 years), 55.7% men. Compared with the plaques in anterior circulation, the more plaques in posterior circulation were categorized to lower level of revised AHA classification with a higher rate of adaptive intima thickening (26.8% vs. 7.9%) but lower proportion of pathological intima thickening (8.5% vs. 15.9%) and fibrocalcific atheroma (12.7% vs. 21.7%, \( p = 0.025 \)). As for the involvement of the intima for individual plaques, the rate of plaques involving the entire intima circumferential was higher than that in anterior circulation (66.2% vs. 26.5%; \( p < 0.001 \)). Thrombi was more frequently detected in plaques of posterior circulation than those of anterior circulation (21.1% vs. 7.4%; \( p = 0.03 \)).

**Conclusions:** Intracranial atherosclerotic plaques presented with different phenotypes and compositions between anterior and posterior circulation. Plaques of the posterior circulation were less atheromatous but more involvement of the entire intima and complicated with higher percentages of thrombi than those of anterior circulation.
Table 1 Plaque histological differences between anterior and posterior circulation groups

<table>
<thead>
<tr>
<th>Plaque Phenotype</th>
<th>Total cerebral arteries (N=260)</th>
<th>Anterior circulation (n=180)</th>
<th>Posterior circulation (n=71)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>12 (3.2%)</td>
<td>8 (8.5%)</td>
<td>4 (2.8%)</td>
<td>0.095</td>
</tr>
<tr>
<td>Intima xanthoma</td>
<td>15 (5.9%)</td>
<td>18 (10.5%)</td>
<td>2 (5.6%)</td>
<td>0.369</td>
</tr>
<tr>
<td>Adaptive intima thickening</td>
<td>24 (9.9%)</td>
<td>19 (10.6%)</td>
<td>5 (6.9%)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Pathological intima thickening</td>
<td>38 (14.5%)</td>
<td>30 (16.6%)</td>
<td>8 (11.3%)</td>
<td>0.222</td>
</tr>
<tr>
<td>Fibrous cap atheroma</td>
<td>30 (23.2%)</td>
<td>25 (19.0%)</td>
<td>5 (7.0%)</td>
<td>0.196</td>
</tr>
<tr>
<td>Thin fibrous cap atheroma</td>
<td>83 (31.2%)</td>
<td>59 (32.8%)</td>
<td>24 (33.8%)</td>
<td>0.765</td>
</tr>
<tr>
<td>Fibrocalcific atheroma</td>
<td>59 (23.1%)</td>
<td>41 (22.8%)</td>
<td>9 (12.7%)</td>
<td>0.214</td>
</tr>
<tr>
<td>Involvement of the entire intima</td>
<td>97 (37.3%)</td>
<td>50 (27.8%)</td>
<td>47 (66.2%)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Thrombus</td>
<td>29 (11.1%)</td>
<td>14 (7.8%)</td>
<td>15 (21.1%)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Calcification</td>
<td>79 (30.3%)</td>
<td>54 (29.5%)</td>
<td>25 (35.2%)</td>
<td>0.364</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>28 (10.7%)</td>
<td>20 (11.1%)</td>
<td>8 (11.3%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Neovascularism</td>
<td>51 (19.6%)</td>
<td>24 (13.3%)</td>
<td>7 (9.9%)</td>
<td>0.669</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>60 (23.0%)</td>
<td>42 (23.3%)</td>
<td>18 (25.4%)</td>
<td>0.822</td>
</tr>
</tbody>
</table>

*p < 0.05

Fig. 1 Comparison of histological phenotype between anterior circulation plaques and posterior circulation plaques

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Plaque components of intracranial atherosclerosis is co-existent with aorta or coronary artery atherosclerosis

Abstract nr. 187
Author Yang , Wenjie, Chinese University of Hong Kong, Hong Kong, Hong Kong
Co-author(s) - Niu , Chunbo
Co-author(s) - Zhao , Hailu
Co-author(s) - Wong , Kasing
Co-author(s) - Ng , Hokeung
Co-author(s) - Chen , Xiangyan
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Pathogenesis

Background: Atherosclerosis is a systemic disease that affects the entire body, involving aorta, coronary artery, renal artery and cerebral artery. Intracranial atherosclerotic disease (ICAD) was known as one of the most common subtype of strokes worldwide, especially in Asians. However, its pathophysiology has long been understudied due to the inaccessibility of intracranial vessel specimen. Based on our intracranial atherosclerotic plaque biobank, we aimed to explore the potential correlation between intracranial atherosclerosis and the severity of generalized atherosclerosis in a series of Chinese adult autopsy cases.

Methods: We histologically examined the pathological feature of cerebral artery atherosclerosis from 88 consecutive autopsy cases (median age, 74 years; 55.7% men). The autopsy reports were retrieved to get information about the severity of atherosclerosis in aorta, coronary arteries and renal arteries. Totally 260 intracranial large arteries were collected and H&E staining and Victorial Blue staining were examined. The atherosclerotic plaque phenotypes and components were evaluated.

Results: Compared with cases without pathological brain infarctions, cases with brain infarctions exhibited severer atherosclerosis of the coronary arteries ($p = 0.006$), but not aorta or renal arteries (Table 1). The phenotypes of intracranial atherosclerosis were correlated with the severity of generalized atherosclerosis (table 2): with the severity aggravation of coronary and aorta atherosclerosis, the phenotypes of intracranial atherosclerosis increased from I to VI (Spearman correlation coefficients = 0.310 and 0.296, respectively, all $p < 0.05$); for benign nephrosclerosis, its frequency increased gradually with increase of intracranial atherosclerosis phenotype from I to VI (Spearman correlation coefficients = 0.183, $p < 0.05$). As for plaque compositions, intraplaque hemorrhage, neovasculature and calcification were more frequently detected in cases with aorta atherosclerosis than those without aorta disease (14.8% vs. 3.4%, $p < 0.01$; 16.6% vs. 3.4%, $p < 0.01$; and 34.9% vs. 21.3%, $p < 0.05$, respectively), and in cases with coronary artery atherosclerosis than those without (15.1% vs. 1.3%, $p < 0.01$; 15.1% vs. 5.1%, $p < 0.05$; and 35.2% vs. 19.0%, $p < 0.05$, respectively).

Conclusions: Among Chinese populations, stroke patients due to intracranial atherosclerosis often
present with co-existent coronary artery atherosclerosis.

Table 2 Correlations between intracranial vasculature changes and generalized atherosclerosis

<table>
<thead>
<tr>
<th>Subdivision</th>
<th>Translational Research</th>
<th>Presentation Preference Oral presentation</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient</td>
<td>0.05**</td>
<td>0.15**</td>
<td><strong>p &lt; 0.01</strong></td>
</tr>
</tbody>
</table>

Table 1 Comparison of generalized atherosclerosis between the cases with and without brain infarctions due to intracranial atherosclerosis

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Analyses of Serum and Thrombus Fatty Acid Composition at the onset of Acute Coronary Syndrome in Japan

Abstract nr. 188
Author Yasuda, Tomoyuki, Kakogawa East City Hospital, Kakogawa, Japan
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords ACS,Lipids,Nutrition,Thrombosis

Background:
Fatty acids (FAs) can affect the pathogenesis of acute coronary syndrome (ACS). However, serum FA levels and thrombus FA composition at ACS onset have not been evaluated. In Japan, it is not mandatory to state the trans FA (TFA) content in the food. TFA has some pro-atherogenic (which are solid) and pro-inflammatory properties. The ratio of omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA)/arachidonic acid (AA) has been an established predictor of ACS. In this study, we hypothesized that the levels of these FAs may change at ACS onset, possibly contributing to the pathogenesis of ACS.

Methods:
Forty-four subjects with ACS were enrolled in this study. Serum and thrombus aspirated from occluded coronary arteries were collected from patients with ACS onset and subjected to gas chromatographic analysis. Thrombus FA ratio was calculated from the specific FA content per total FA content.

Results:
Similar to previous reports with a general population, age was positively correlated with serum EPA/AA ratio and negatively correlated with serum TFA concentrations. Serum triglyceride levels were strongly correlated with serum AA and TFA levels. Patients with high serum TFA levels exhibited the presence of coronary thrombi that contained a high TFA thrombus ratio. The thrombus/serum ratio of TFA was greater than that of EPA and AA, indicating that solid TFA was highly concentrated in the coronary thrombus.

Conclusions:
The results indicate that solid TFAs formed a part of the components of coronary thrombi, revealing their possible role in the pathogenesis of ACS. Young patients with ACS had a high TFA...
and low EPA/AA ratio. Therefore, banning the intake of TFA should be considered in Japan and all over the world to prevent the onset of ACS, particularly in the younger generation.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Prevention of ethanol induced steatosis in experimental rats by naringenin and rutin

Abstract nr. 189
Author Nalini, Nalini, Chidambaram, India
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, LDL, Pharmacology, Prevention

Alcohol is the most frequently abused psychomotor drug throughout the world and has been known in all civilizations since ancient times. The World Health Organization reports about two billion alcohol consumers worldwide and 76.3 million people with diagnosable alcohol use disorders. Chronic consumption of alcohol leads to lipid accumulation and inflammation which are early manifestations of steatosis/atherosclerosis in turn poses serious health problems. Our present investigation was aimed to evaluate the modulatory mechanism of the natural products, naringenin and rutin against ethanol induced alterations in lipids and inflammatory markers of control and experimental rats. Male albino wistar rats were randomized into four groups. Groups 1, 2 and 3 received 40% isocaloric glucose. Liver injury/steatosis was induced in groups 4, 5 and 6 by administering 20% ethanol (equivalent to 6g/kg/b.wt.,) via intragastric intubation for 60 days. In addition, groups 2 and 4 were given naringenin at the dose of 50mg/kg/b.wt., suspended in 0.5% CMC for the last 30 days of the experiment. Group 3 and 6 were given rutin at the dose of 100mg/kg/b.wt., suspended in 0.5% CMC for the last 30 days of the experiment. Ethanol administered rats showed significantly increased levels of lipids (total cholesterol, triglycerides, free fatty acids, phospholipids, HDL, LDL and VLDL), increased expression of inflammatory markers (TNF-, IL-6, NF-κB, COX-2 and iNOS) and fibrosis markers (TGF- and collagen). Supplementation with naringenin and rutin restored the ethanol induced changes. Thus, naringenin and rutin showed a protective effect against lipids and inflammatory markers mediated steatosis in alcohol induced liver injury.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Deficiency of the co-stimulatory molecule CD27 impairs regulatory T cell survival and exacerbates atherosclerosis.

Abstract nr. 190
Author Winkels, Holger, Ludwig-Maximilian University, Munich, Germany
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Co-author(s) - Spitz, Charlotte
Co-author(s) - Bürger, Christina
Co-author(s) - Weber, Christian
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Immunity

Atherosclerosis, an inflammatory disease of large arteries, is – through its clinical manifestations stroke and myocardial infarction – the leading cause of morbidity and mortality in the industrialized world. Adaptive immunity and co-stimulatory signals play a pivotal role during all stages of the disease. Recently, regulatory T cells “Treg” were attributed an anti-inflammatory and anti-atherogenic role. The interaction of CD70, a member of the tumor necrosis factor super family “TNFSF” with its receptor CD27 modulates Treg development but also affects T cell proliferation, differentiation, and activation at the sites of antigen priming and at effector function. We hypothesized an increased atherosclerotic burden and an exacerbation of disease upon CD27 deficiency.

Cd27−/− mice were crossed with Apoe−/− mice. Cd27−/−Apoe−/− and littermate controls (Cd27+/+ Apoe−/−) were sacrificed at the age of 18 and 28 weeks. Cryosections of the aortic sinus were prepared and analyzed for atherosclerotic lesion size, histology and cellular composition. 18 week-old Cd27−/−Apoe−/− mice have a trend towards bigger atherosclerotic lesions displaying a 2.5-fold higher macrophage content. Flow cytometry revealed a significant decrease in the abundance of splenic (26%) and aortic (27%) Tregs and increased apoptosis of Treg in the thymus (60%) of Cd27−/−Apoe−/− mice. In contrast, 28 week-old Cd27−/−Apoe−/− mice did not differ in splenic Treg content and had similar atherosclerotic plaque size and phenotype compared to their littermate controls. Furthermore, bone marrow transplantation of Cd27−/−Apoe−/− and littermate controls into Apoe−/− recipient mice revealed a 2.3-fold increase in atherosclerotic plaque size and a pronounced pro-inflammatory plaque phenotype accompanied by reduced frequency of aortic (54.1%) and splenic (17.7%) Tregs. Cd27−/−Apoe−/− Tregs showed the same suppressive and migratory capacity as those isolated from controls.

Taken together, our data reveal that deficiency for CD27 impairs thymic Treg development thereby...
exacerbating early atherogenesis and increasing the macrophage content of atherosclerotic lesions. However, later stages of atherosclerosis were not affected by a CD27 deficiency.

Funding resources
Deutsche Forschungsgemeinschaft (DFG), SFB1054 and SFB1123
The Netherlands Organization for Scientific Research, Vidi and Vici grant
The Netherlands Heart Foundation, Dr. E. Dekker Established Investigator grant

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
REPLACING SATURATED FAT WITH POLYUNSATURATED FAT REDUCES TOTAL CHOLESTEROL AND LDL CHOLESTEROL IN HEALTHY SUBJECTS WITH MODERATE HYPERCHOLESTEROLAEMIA

Abstract nr. 191
Author Ulven, Stine Marie, Oslo and Akershus University College of Applied Sciences, Oslo, Norway
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Co-author(s) - Christensen, Jacob
Co-author(s) - Telle-Hansen, Vibeke
Co-author(s) - Raael, Ellen
Co-author(s) - Granlund, Linda
Co-author(s) - Andersen, Lene
Co-author(s) - Holven, Kirsten

Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Intervention, LDL, Nutrition

Background and aim: Reduced intake of saturated fatty acids (SFAs) combined with increased intake of polyunsaturated fatty acids (PUFAs) is the main focus of dietary recommendations to reduce plasma cholesterol and subsequently the risk of cardiovascular disease (CVD). The objective of the present study was to investigate the effect of replacing food items with different fat quality (replacing SFAs with PUFAs) on plasma total cholesterol and LDL cholesterol among healthy subjects with moderate hypercholesterolemia.

Methods: An eight-week double-blinded randomized, controlled parallel designed trial with two groups including healthy adults aged 25-70 y with serum total cholesterol within the normal range and LDL cholesterol ≥ 3.5 mmol/L was performed. The intervention group received commercially available food items in which saturated fat was replaced by vegetable sunflower and rapeseed oil. The control group received similar commercially food items with a higher content of SFAs and lower content of PUFAs, and were chosen based on sales statistics and were among the most sold products within each food category. In both groups, the minimum daily intake of each food item was according to data from the National dietary survey in Norway assessed in men and women aged 18-70 y. Before the baseline visit, all subjects (n=99) performed a run-in period where the control food items were consumed daily for two weeks.

Results and conclusion: Preliminary data shows that daily intake of food items with improved fatty acid composition for eight weeks caused significant lowered plasma total cholesterol and LDL cholesterol levels in healthy adults with moderate hypercholesterolemia compared to the control group.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Development of new LDL-Cholesterol Generation 3 assay on Roche Clinical Chemistry Analyzers

Abstract nr. 192
Author Dr. Klima, Horst, Roche Diagnostics GmbH, Penzberg, Germany
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Co-author(s) - Sobczak, Georg
Co-author(s) - Pelloli, Jolanda
Co-author(s) - Loehr, Bernd
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Dyslipidemia, LDL, Lipids

Medical background:
LDL-C is the primary target of lipid lowering therapy, because low density lipoproteins are the most arteriosclerotic particles. LDL-C possesses the highest predictive value in the diagnosis of atherosclerosis and CHD risk. It is the leading parameter in monitoring of lipid lowering therapies.

Test principle
In the colorimetric direct assay for LDL cholesterol esters and free cholesterol in LDL are measured on the basis of a cholesterol enzymatic method using cholesterol esterase and cholesterol oxidase in the presence of surfactants which selectively solubilizes only LDL.

Development Goals LDL-Cholesterol Gen.3:
- Good correlation to reference method (BQ)
- Improvement of specificity for LDL-C
- Reduction of interference of remnants and of VLDL fractions
- Improvement of interference of turbidity (L-Index 1000)

Results
Measuring range and Lower limits of measurement
The linear assay range of the LDLC3 assay from 0.10 to 14.20 mmol/L. The LoB, LoQ and LoD are 0.10 mmol/L.

Traceability
LDLC3 assay has been standardized against the beta quantification method.

Limitations and interferences
No interference of bilirubin (conjugated and non-conjugated) up to 60 mg/dL (1026 μmol/L), L-Index of 60, no interference of lipaemia (Intralipid) up to a L-Index of 1000, no interference of hemoglobin up to 1000 mg/dL (1026 μmol/L), H-Index of 1000, and no interference by lipid lowering drugs (statin, fibrates, nicotinic acid).

Fasting / Non-fasting study: Non-fasting samples can be used in the LDLC3 assay.

Precision - CLSI EP5 – 21 days
Repeatability ≤ 1.2% and Intermediate precision ≤ 2.1%.

Conclusions
The development goals for the new LDLC3 assay were met. The use of LDLC3 method in
laboratory routine will improve quality of test results for LDL-C.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Intracranial arterial calcification is a risk factor of new onset non-cardioembolic stroke but fails to predict long-term stroke recurrence

Abstract nr. 193
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Risk Factor

Introduction: Intracranial arterial calcification (IAC) is frequently observed on computed tomography of the brain (CT brain). However, its clinical relevance to vascular events has been understudied. Based a hospital-based cohort, the objective of this study was to investigate the effects of IAC on first time non-cardioembolic stroke and long term stroke recurrence in Chinese adults.

Subjects and methods: The cohort included consecutive men and women referred for brain CT during December 2004 in Prince of Wales Hospital. The severity of IAC on 16-slice brain MDCT (multi-detector-row computed tomography) was assessed at baseline among 85 first time non-cardioembolic ischemic stroke patients and 144 age-gender-matched non-ischemic stroke patients. Traditional risk factors of atherosclerosis were recorded at baseline. Regular follow up was performed to record stroke recurrence in stroke patients till October 2014. Patients with atrial fibrillation or valvular heart disease were excluded. Patients with tumor or other disease causing mortality within one year were excluded.

Results: Logistic regression showed that diabetes (OR 2.203; 95% CI, 1.098-4.418; P=0.026), smoking (OR 2.693; 95% CI, 1.334-5.437; P=0.006), hyperlipidemia (OR 2.331; 95% CI, 1.034-5.259; P=0.041), mild calcification in intracranial internal carotid artery (IICA) (OR 2.711; 95% CI, 1.226-5.999; P=0.014) and moderate calcification in IICA (OR 5.404; 95% CI, 1.546-18.888; P=0.008) were risk factors of new onset non-cardioembolic stroke. During a follow up of mean 7.13 years, stroke recurrence was recorded in 16 patients (18.82%). The vascular risk factors and severity of IAC were compared between patients with and without stroke recurrence during follow up, which showed that diabetes (OR 3.909; 95% CI, 1.166-13.103; P=0.027) and ischemic heart diseases (IHD) (OR 6.202; 95% CI, 1.443-26.658; P=0.014) could predict recurrent non-cardioembolic stroke, while severity of IAC in patients with and without recurrent stroke was similar.

Conclusions: Intracranial arterial calcification is a risk factor of new onset non-cardioembolic stroke but fails to predict long-term stroke recurrent in Chinese adults. Diabetes and ischemic heart diseases could predict long term recurrent non-cardioembolic stroke.
Table 2. Patients with and without recurrent non-cardioembolic stroke in 10-year follow up (mean 7.13 ys/person)

<table>
<thead>
<tr>
<th>variable</th>
<th>overall N=85</th>
<th>recurrent stroke N=16</th>
<th>no recurrent stroke N=69</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (mean SD)</td>
<td>69.97(13.01)</td>
<td>68.19(12.37)</td>
<td>70.39(13.16)</td>
<td>0.335</td>
</tr>
<tr>
<td>gender(male)</td>
<td>43(52.9%)</td>
<td>9(56.3%)</td>
<td>37(53.6%)</td>
<td>0.794</td>
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<tr>
<td>hypertension</td>
<td>53(62.4%)</td>
<td>12(75.0%)</td>
<td>41(59.4%)</td>
<td>0.247</td>
</tr>
<tr>
<td>diabetes</td>
<td>29(34.1%)</td>
<td>9(56.3%)</td>
<td>20(29.0%)</td>
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</tr>
<tr>
<td>renal failure</td>
<td>4(4.7%)</td>
<td>1(6.3%)</td>
<td>3(4.3%)</td>
<td>0.573</td>
</tr>
<tr>
<td>smoking</td>
<td>27(31.8%)</td>
<td>4(25.0%)</td>
<td>23(33.3%)</td>
<td>0.766</td>
</tr>
<tr>
<td>hyperlipidemia</td>
<td>22(25.9%)</td>
<td>6(37.5%)</td>
<td>16(23.2%)</td>
<td>0.239</td>
</tr>
<tr>
<td>IHD</td>
<td>11(12.9%)</td>
<td>5(31.3%)</td>
<td>6(8.7%)</td>
<td>0.015</td>
</tr>
<tr>
<td>TIA</td>
<td>12(14.1%)</td>
<td>3(18.8%)</td>
<td>9(13.0%)</td>
<td>0.698</td>
</tr>
<tr>
<td>ICA mean rank</td>
<td>45.59</td>
<td>42.40</td>
<td></td>
<td>0.550</td>
</tr>
<tr>
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<td>3/0/9/4/0</td>
<td>81/53/7/0</td>
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<td>0.587</td>
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<tr>
<td>VA mean rank</td>
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<td>42.38</td>
<td></td>
<td></td>
</tr>
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<td>39/1/24/3/0</td>
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<td></td>
</tr>
<tr>
<td>MCA mean rank</td>
<td>39.63</td>
<td>43.78</td>
<td></td>
<td>0.315</td>
</tr>
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<td>noncalcified/minimal/mild</td>
<td>15/0/1/0/0</td>
<td>58/1/8/1/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BA mean rank</td>
<td>44.19</td>
<td>41.48</td>
<td></td>
<td>0.368</td>
</tr>
<tr>
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<td>14/0/2/0/0</td>
<td>63/0/0/0/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACA mean rank</td>
<td>43.16</td>
<td>42.96</td>
<td></td>
<td>0.945</td>
</tr>
<tr>
<td>noncalcified/minimal/mild</td>
<td>15/0/1/0/0</td>
<td>65/0/4/0/0</td>
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<td></td>
</tr>
<tr>
<td>PCA mean rank</td>
<td>43.00</td>
<td>43.00</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>noncalcified/minimal/mild</td>
<td>16/0/0/0/0</td>
<td>69/0/0/0/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intracranial artery mean rank</td>
<td>44.41</td>
<td>42.67</td>
<td></td>
<td>0.742</td>
</tr>
<tr>
<td>noncalcified/minimal/mild</td>
<td>2/0/11/3/0</td>
<td>71/52/9/0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Comparisons of baseline characteristics and IAC scores between patients with non-cardioembolic stroke and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall</th>
<th>Ischemic stroke</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (mean/SD)</td>
<td>68.48(12.18)</td>
<td>69.97(13.01)</td>
<td>67.6(11.62)</td>
<td>0.450</td>
</tr>
<tr>
<td>gender (male)</td>
<td>117(51.1%)</td>
<td>45(52.9%)</td>
<td>72(50.0%)</td>
<td>0.667</td>
</tr>
<tr>
<td>hypertension</td>
<td>118(51.5%)</td>
<td>53(62.4%)</td>
<td>65(45.5%)</td>
<td>0.012</td>
</tr>
<tr>
<td>diabetes</td>
<td>53(23.1%)</td>
<td>29(34.1%)</td>
<td>24(16.7%)</td>
<td>0.002</td>
</tr>
<tr>
<td>renal failure</td>
<td>8(3.5%)</td>
<td>4(4.7%)</td>
<td>4(2.8%)</td>
<td>0.473</td>
</tr>
<tr>
<td>smoking</td>
<td>49(21.4%)</td>
<td>27(31.8%)</td>
<td>22(15.3%)</td>
<td>0.003</td>
</tr>
<tr>
<td>hyperlipidemia</td>
<td>37(16.2%)</td>
<td>22(25.9%)</td>
<td>15(10.4%)</td>
<td>0.002</td>
</tr>
<tr>
<td>IHD</td>
<td>17(7.4%)</td>
<td>12(14.1%)</td>
<td>5(3.5%)</td>
<td>0.003</td>
</tr>
<tr>
<td>TIA</td>
<td>19(8.3%)</td>
<td>12(14.1%)</td>
<td>7(4.9%)</td>
<td>0.014</td>
</tr>
<tr>
<td>ICA (mean rank)</td>
<td>131.97</td>
<td>104.98</td>
<td>131.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>noncalcified/ minimal/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mild/moderate/severe</td>
<td>11/1/62/11/0</td>
<td>43/5/89/7/0</td>
<td>11/1/62/11/0</td>
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</tr>
<tr>
<td>VA (mean rank)</td>
<td>126.66</td>
<td>108.11</td>
<td>126.66</td>
<td>0.012</td>
</tr>
<tr>
<td>noncalcified/ minimal/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mild/moderate/severe</td>
<td>49/1/31/4/0</td>
<td>108/0/29/6/1</td>
<td>49/1/31/4/0</td>
<td></td>
</tr>
<tr>
<td>MCA (mean rank)</td>
<td>120.23</td>
<td>111.90</td>
<td>120.23</td>
<td>0.054</td>
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<td>noncalcified/ minimal/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mild/moderate/severe</td>
<td>74/1/8/1/0</td>
<td>136/1/5/1/1</td>
<td>74/1/8/1/0</td>
<td></td>
</tr>
<tr>
<td>BA (mean rank)</td>
<td>118.47</td>
<td>112.95</td>
<td>118.47</td>
<td>0.115</td>
</tr>
<tr>
<td>noncalcified/ minimal/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mild/moderate/severe</td>
<td>78/0/7/0/0</td>
<td>139/1/4/0/0</td>
<td>78/0/7/0/0</td>
<td></td>
</tr>
<tr>
<td>ACA (mean rank)</td>
<td>118.71</td>
<td>112.81</td>
<td>118.71</td>
<td>0.019</td>
</tr>
<tr>
<td>noncalcified/ minimal/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mild/moderate/severe</td>
<td>80/0/5/0/0</td>
<td>143/0/0/1/0</td>
<td>80/0/5/0/0</td>
<td></td>
</tr>
<tr>
<td>PCA (mean rank)</td>
<td>115.00</td>
<td>115.00</td>
<td>115.00</td>
<td>1.000</td>
</tr>
<tr>
<td>noncalcified/ minimal/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mild/moderate/severe</td>
<td>85/0/0/0/0</td>
<td>144/0/0/0/0</td>
<td>85/0/0/0/0</td>
<td></td>
</tr>
<tr>
<td>Intracranial artery (mean rank)</td>
<td>131.01</td>
<td>105.55</td>
<td>131.01</td>
<td>0.001</td>
</tr>
<tr>
<td>noncalcified/ minimal/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mild/moderate/severe</td>
<td>9/1/63/12/0</td>
<td>39/6/88/10/1</td>
<td>9/1/63/12/0</td>
<td></td>
</tr>
</tbody>
</table>

TIA = transient ischemic attack; VA = vertebral artery; MCA = middle cerebral artery; BA = basilar artery; ACA = anterior cerebral artery; PCA = posterior cerebral artery; Intracranial artery = calcification in the most severe intracranial artery.

Table 1. Comparisons of baseline characteristics and IAC scores between patients with non-cardioembolic stroke and controls

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation

Additional information
Framingham and PDAY Risk Scores Measured at 18-30 Years Predict Coronary Ischemia During the Next 25 Years: The CARDIA Study

Abstract nr. 195
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Co-author(s) - Colangelo, Laura
Co-author(s) - Lewis, Cora E.
Co-author(s) - Jacobs, David
Co-author(s) - Liu, Kiang
Co-author(s) - Loria, Catherine
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Epidemiology, Prevention, Risk Factor

Risk factors measured in adolescence and young adulthood predict future atherosclerosis but no studies assessing future cardiovascular events (CVD) later in life have been published. The Framingham risk score predicts CVD well in middle aged-adults. The PDAY (Pathobiological Determinants of Atherosclerosis in Youth Study) risk score, derived directly from correlations of measures of atherosclerosis in 15-35 year olds with post mortem risk factor measurement, predicts future coronary calcium accumulation. In the CARDIA (Cardiovascular Risk Development in Young Adults Study) study, we assessed the ability of the Framingham and PDAY risk scores, age adjusted and calculated at baseline, in 5016 black and white men and women aged 18 to 30 years to predict future ischemic heart disease (myocardial infarction, sudden death, angina, coronary revascularization procedure). There were 97 ischemic events during 25 years of follow up. Outcomes are presented in the Table. Both scores had high predictive values of future events with large increases in risk for every standard deviation increase in score; Framingham performed slightly better but the difference was not statistically significant. When education level (above and below a high school education) and family history of cardiovascular disease were added to make a revised PDAY score, event prediction improved only slightly. Results were similar when total CVD (189 events) was the outcome variable. This study demonstrates that CVD risk as a young adult strongly predicts premature CVD and that risk estimated by likelihood of having atherosclerosis (PDAY) has a similar predictive value as a clinically based risk score (Framingham)
<table>
<thead>
<tr>
<th>Risk score (adjusted for age)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDAY</td>
</tr>
<tr>
<td>Revised PDAY</td>
</tr>
<tr>
<td>Framingham</td>
</tr>
</tbody>
</table>
All participants (N=5016) with 97 CHD events:
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
N-acetylcysteine Prevents Lipid Peroxidation, Inflammation and Insulin Resistance Induced by Advanced Glycated Albumin in Wistar Rats

Abstract nr. 196
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Co-author(s) - Catanozi, Sergio
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Co-author(s) - Ramos Pinto, Paula
Co-author(s) - Massola Shimizu, Maria Heloísa
Co-author(s) - Mitiko Okamoto, Maristela
Co-author(s) - Correa Pinto Jr, Danilo
Co-author(s) - Fabres Machado, Ubiratan
Co-author(s) - C. Corrêa-Giannella, Maria Lúcia
Co-author(s) - Passarelli, Marisa

Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Diabetes, Inflammation, Lipids

Advanced glycation end products (AGE) contribute to the pathogenesis of chronic complications in diabetes mellitus (DM) by increasing oxidative and inflammatory stress. We investigated the effect of chronic administration of homologous AGE-albumin in rats, associated or not with N-acetylcysteine (NAC) treatment in lipid peroxidation, mRNA expression of interleukins 6 (Il6) and 10 (Il10) in periepididimal adipose tissue and insulin sensitivity.

One-month old male Wistar rats (n=8/group) were randomized to four groups receiving daily intraperitoneal injections of control (C) or AGE-albumin (20 mg/kg/day) alone or together with NAC (600mg/L drinking water), for 90 days. AGE-albumin was prepared by incubating rat albumin with 10 mM glycolaldehyde for 4 days, 37 °C and C-albumin with PBS alone. Plasma total cholesterol, triglycerides, glucose and liver enzymes were determined by enzymatic techniques; thiobarbituric acid reactive substances (TBARS; nmol/24h) in urine samples by spectrophotometric assay; gene expression by real-time quantitative RT-PCR with TaqMan system and glucose disappearance constant by the insulin tolerance test (kITT; %/min). One-way ANOVA and Newman-Keuls post-test were utilized to compare groups (mean±SD).

Body weight, blood pressure, plasma lipids, glucose and liver enzymes were unchanged after AGE or AGE+NAC treatment as compared to their respective controls. NAC reduced TBARS concentration that was increased by AGE-albumin (AGE-albumin 247±45.6 vs AGE-albumin + NAC = 166±26.1; p<0.05). Compared to AGE-albumin, NAC diminished the mRNA of Il6 (respectively, 2.0±0.4 vs 0.3±0.02; p=0.01) and Il10 (respectively, 1.8±0.1 vs 0.4±0.02; p=0.0001). A worsening in insulin sensitivity by the ITT was observed in AGE-albumin when compared to C-albumin-treated animals (2.6±0.5 vs 3.4±0.6; p<0.05), which was prevented by NAC (AGE+NAC...
3.8±0.4, NS vs C+NAC 3.6±0.7).
In conclusion, NAC reduces lipid peroxidation, inflammation in adipose tissue and improves insulin sensitivity that were adversely affect by chronic administration of AGE-albumin. These events can contribute to prevent chronic complications elicited by AGE in DM.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
The expression of genes involved in lipid flux and inflammation is downregulated by aerobic exercise training in mouse peritoneal macrophages

Abstract nr. 197
Author Ramos Pinto, Paula, Faculty of Medical Sciences University of Sao Paulo, Sao Paulo, Brazil
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Co-author(s) - Juvenal Gomes, Diego
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Co-author(s) - Tallada Iborra, Rodrigo
Co-author(s) - C. Corrêa-Giannella, Maria Lúcia
Co-author(s) - C. Corrêa-Giannella, Maria Lúcia
Co-author(s) - Catanozi, Sergio
Co-author(s) - Passarelli, Marisa
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Atherosclerosis, Lipids, Lipoproteins

Regular physical exercise effects on atherosclerosis prevention and reduction are related to improvement on lipid metabolism, reverse cholesterol transport and oxidative and inflammatory stress. We aimed at investigating how aerobic exercise training modulates the expression of genes involved in lipid flux and inflammation in macrophages.

Three-month-old C57BL/6J male mice were trained on treadmill (15m/min; 30 min/day), during 6 weeks and a control group were kept sedentary. Immediately after the last exercise bout macrophages were harvested from peritoneal cavity and RT-PCR by Taqman assay was performed to access mRNA expression of Abca1, Abcg1, Nr1h3, Nr1h2, Scarb1, Olr1, Cd36, Pparg, Il10, Il6, Tnf,. Actb was utilized as housekeeping. Plasma total cholesterol (TC), HDL cholesterol (HDLc), triglycerides (TG) and glucose were determined by enzymatic methods. Comparisons between groups were carried out by Student t test (mean±SE).

Exercise training did not change plasma levels of TC, HDLc, TG and glucose. Body weight was similar between sedentary and trained animals. In comparison to sedentary animals, exercise training reduced mRNA levels of genes involved in the uptake of LDL-cholesterol by macrophages: Olr1 (respectively, 1.0±0.01 vs 0.80±0.01; p=0.046) and Cd36 (1.0±0.03 vs 0.65±0.01; p=0.007). Genes related to cholesterol efflux were also diminished by exercise: Abca1 (1.0±0.01 vs 0.8±0.01; p=0.0006), Abcg1 (1.0±0.01 vs 0.87±0.01; p=0.038), Nr1h3 (1.0±0.02 vs 0.64±0.01; p=0.008), Nr1h2 (1.0±0.01 vs 0.81±0.01; p=0.009), while Scarb1 (1.0±0.02 vs 0.89±0.01; p=0.278) remained unchanged. In addition, there was a reduction in the expression Tnf (1.0±0.03 vs 0.76±0.01; p =0.021), Pparg (1.0±0.01 vs 0.69±0.01; p=0.016), Il10 (1.0±0.03 vs 0.62±0.01; p=0.016) and a trend for reduction in Il6 mRNA (1.0±0.01 vs 0.71±0.01; p=0.065).

In conclusion, aerobic exercise training in wild type mice reduces the mRNA levels of genes involved in the uptake of LDL-cholesterol by macrophages, which agrees with the reduction of those related to cholesterol efflux and inflammation. Our data points for a beneficial role of
physical exercise in the prevention of atherosclerosis that is reflected in peritoneal macrophages.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
PCSK9 increases Lp(a) secretion in primary human hepatocytes without modifying Lp(a) uptake

Abstract nr. 198
Author Villard , ELISE F., SANOFI, Chilly-mazarin, France
Co-author(s) - Poirier , Bruno
Co-author(s) - Guillot , Etienne
Co-author(s) - Le Bail , Jean-Christophe
Co-author(s) - Blankenstein , Jorg
Co-author(s) - Muslin , Anthony J.
Co-author(s) - Janiak , Philip
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia,LDL,Lp(a),PCSK9

PCSK9 inhibition by monoclonal antibody administration is a very effective therapeutic strategy to reduce circulating LDL-C. PCSK9 inhibition is associated with an unexpected reduction of lipoprotein (a) [Lp(a)] plasma levels in patients, but the mechanism responsible for this reduction remains to be elucidated. The objective of this study was to identify the underlying mechanism of Lp(a) regulation by PCSK9 in primary human hepatocytes. For this purpose, Lp(a) levels in cell media were quantified by ELISA after incubation with physiological concentrations of wild type or mutant forms of PCSK9. PCSK9 increased, in a concentration dependent manner, Lp(a) levels (67% at 20 nM, p<0.001). The gain of function D374Y-PCSK9 mutant was more efficient than wild type PCSK9 to increase Lp(a) levels, while the loss of function mutant R194A failed to modulate Lp(a) levels. We therefore aimed to determine whether this PCSK9-dependent increase in Lp(a) levels in hepatocyte media resulted from a modulation of Lp(a) expression or Lp(a) cellular uptake. APOA gene expression was up regulated by 1.6 fold (p=0.03) in the presence of 20 nM PCSK9. To explore Lp(a) uptake by hepatocytes, Lp(a) was purified from human plasma and labeled with BODIPY-FL. The contribution of LDL receptor [LDLR] in cellular Lp(a) uptake was evaluated by use of 3 different strategies to modulate LDLR activity. Atorvastatin, a potent inducer of LDLR expression, significantly increased LDL uptake (+40% at 1 µM, p<0.05) but did not modulate Lp(a) uptake. In addition, selective inhibition of LDLR with an anti-LDLR antibody impaired LDL uptake without modifying the uptake of Lp(a). Finally, PCSK9 failed to modulate Lp(a) uptake by human hepatocytes, while it reduced LDL uptake by 60% (p<0.05). Taken together, these results show that PCSK9 does not prevent Lp(a) uptake by human hepatocytes but instead promotes APOA expression and increased Lp(a) assembly and secretion. These findings provide new insights into the regulation of Lp(a) metabolism by PCSK9 in primary human hepatocytes and suggest that PCSK9 inhibition may reduce Lp(a) secretion.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Nonalcoholic fatty liver disease induced by apolipoprotein CIII overexpression is associated with inflammation and cell death

Abstract nr. 199
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Co-author(s) - Amarylis, Wanschel
Co-author(s) - Nardelli, Tarliza
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Hypolipidemic Drugs, Inflammation, Triglyceride-Rich Proteins

Nonalcoholic fatty liver disease (NAFLD) is the principal liver manifestation in obesity and metabolic syndrome. The natural history of the disease involves steatosis, oxidative stress, inflammation and cell death. By comparing apolipoprotein (apo) CIII transgenic mice with control non-transgenic (NTg) littermates, we show here that the overexpression of apoCIII, independent of high fat diet (HFD), results in NAFLD features including increased liver lipid content, decreased antioxidant power, increased expression of TNFα, TNFα receptor, cleaved caspase-1 and interleukin-1β, decreased adiponectin receptor-2 and increased cell death. This phenotype is aggravated and additional NAFLD features are differentially induced by HFD in apoCIII mice. HFD induced glucose intolerance together with increased gluconeogenesis, evidencing hepatic insulin resistance. Marked increases in plasma TNFα (8-fold) and IL-6 (60%) were induced by HFD in apoCIII mice compared to NTg mice. Cell death signals (Bax/Bcl2), effectors (caspase-3) and apoptosis were augmented in both low and HFD apoCIII mice. Fenofibrate treatment reversed several of the diet and apoCIII effects, but did not normalize apoCIII inflammatory traits even under fully corrected liver lipid content. These results indicate that apoCIII overexpression plays a major role in liver inflammation and cell death, increasing the susceptibility to and the severity of diet induced NAFLD.
Physical Exercise Reduces Inflammatory State of Atherosclerotic Lesions in Hypercholesterolemic Mice Fed a High Fat Diet

Abstract nr. 200
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, LDL

Increased infiltration of LDL into the subendothelial space, oxidative stress and inflammation are early atherogenesis events. Physical exercise promotes beneficial effects in the prevention and progression of atherosclerosis. These exercise effects are related to improved endothelial function and lipid profile. The aim of this study was to evaluate the effects of chronic aerobic exercise on the inflammatory state of early atherosclerotic lesions in LDL receptor deficient mice (LDLr-/-) fed a high-fat diet. LDLr-/- male mice were submitted to exercise on a treadmill for 8 weeks or remained sedentary. Then, plasma was collected to determine biochemical parameters, bone marrow-derived macrophages were isolated for migration and chemotaxis assays and gene expression (RT-PCR), and the atherosclerotic lesions were analyzed in the aortic root. As expected, exercised mice had reduced lipid laden lesion areas and adipose tissue mass. Plasma levels of glucose, triglycerides and cholesterol were similar between sedentary and exercised mice; however, FPLC plasma lipoprotein fractionation showed lower IDL/LDL-cholesterol levels in the exercised mice. By immunohistochemistry, we verified a reduction in the inflammatory markers IL-1β and MCP-1 in the atherosclerotic plaque. Consistent with these findings, we also observed in macrophages significant reductions in the mRNA expression of IL-1β, MCP-1, IL-6 and TNF-α. In addition, we show that exercise reduces the expression of the endoplasmic reticulum stress related protein CHOP/GADD153 and the presence of the oxidative stress marker, nitrotyrosine, in the plaque. In macrophages, there was a significant decrease in the expression of CD36 mRNA, suggesting a reduced oxidized LDL uptake in these cells. The macrophage migration, evaluated in vitro either in the basal or stimulated conditions, was attenuated by the exercise intervention (50%). The cell motility phenotype of these cells, determined by the interaction of RAC-1 and F-actin, was repressed by the exercise. Besides the plaque and macrophages findings, we observed that exercise also reduced the plasma concentrations of proinflammatory cytokines IL-1β, IL-6 e TNF-α. Together, these data demonstrate that chronic aerobic exercise during the early development of atherosclerosis leads to rapid and beneficial changes in size, cellular composition and inflammatory status of the plaque, as well as reduces the systemic inflammation.
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Foxp3 regulatory T cells and T regulatory type 1 cells would cooperatively suppress the development of atherosclerosis in mice.

Abstract nr. 201
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Immunity, Inflammation

**Background:** Recent studies have shown that Foxp3⁺ regulatory T cells (Tregs) may inhibit atherosclerosis development through suppressing pathogenic immune responses. However, previous studies do not provide the direct evidence for the atheroprotective role of Tregs. The purpose of this research is to clarify the role of Tregs in the development of atherosclerosis.

**Methods and Results:** We employed DEREG (Depletion of regulatory T cells) mice, which carry a diphtheria toxin (DT) receptor under the control of the foxp3 gene locus, and crossed them with LDLR-deficient (LDLR⁻⁻) mice to establish DEREG/LDLR⁻⁻ mice. In these mice, DT injection led to efficient depletion of Foxp3⁺ Tregs in spleens, lymph nodes and aortas. DEREG/LDLR⁻⁻ (n=20) or control LDLR⁻⁻ (n=22) mice fed a high-cholesterol diet were injected with DT (125ng/mouse) twice per week for 4 weeks and atherosclerosis was examined. No statistical differences in plasma lipid profiles were detected between the 2 groups. Unexpectedly, depletion of Foxp3⁺ Tregs did not aggravate atherosclerotic lesion formation in the aortic root, while increasing IFNγ producing inflammatory CD4 T cells and proliferative activity of T lymphocytes. Treg-depletion did not change the lipid content of the plaques and the accumulation of macrophages in the atherosclerotic plaques. Interestingly, we found that Foxp3⁺ Treg depletion resulted in a dramatic increase of T regulatory type 1 (Tr1) cells, which are known as IL-10 producing T cells with anti-atherogenic properties. We also found marked increased IL-10 secretion in splenic lymphocytes of Treg-depleted mice. Furthermore, blockade of IL-10 receptor (IL-10R) in vivo increased the exaggerated inflammatory response and the atherosclerotic lesion size in the Treg-depleted mice, raising an intriguing possibility that Foxp3⁺ Tregs and Tr1 cells represent alternative fates of T-cell differentiation under hypercholesterolemia, and that their anti-atherogenic function would be partially redundant.

**Conclusions:** Our data indicate that Foxp3⁺ Tregs and Tr1 cells would cooperatively inhibit atherosclerotic plaque formation in hypercholesterolemic mice. These findings suggest that modulating regulatory immune responses mediated by not only Foxp3⁺ Tregs but also Tr1 cells would be the promising strategies to prevent or cure atherosclerotic disease.

**Subdivision 1. Basic Science**

Presentation Preference Oral presentation
Additional information
Vascular PCSK9: a mediator for atherogenesis independent of LDL receptor

Abstract nr. 203
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Atherosclerosis, LDL, PCSK9

PCSK9 (Proprotein convertase subtilisin/kexin type 9) increases the LDL levels by binding to hepatocyte LDL receptors (LDLR) and subjects it to degradation. We show that PCSK9 regulates apolipoprotein B (apoB) production by inhibiting its degradation process via the autophagic pathway irrespective of the presence of LDLR. In addition to the role of PCSK9 in promoting hyperlipidemia, we propose that vascular-PCSK9 in endothelial cells (EC) plays a role in initiating atherogenesis, irrespective of the presence of LDL receptor.

Our laboratory has generated double knockout mice lacking both LDLR and Apobec1 (apoB mRNA editing enzyme), named LDb, Ldlr-/-Apobec1-/- . They develop atherosclerotic lesions spontaneous. To investigate the role of PCSK9 in atherogenesis, we deleted Pcsk9 gene from LDb mice to generate the triple knockout mice (named LTp, Ldlr-/-Apobec1-/-Pcsk9-/- ). The LTp mice had significantly decreased levels of cholesterol (29%), triglyceride (33%) and apoB (34%), compared to parental LDb mice. However, despite their high cholesterol levels at over 300 mg/dl, the atherosclerotic lesions in LTp mice were significantly decreased in comparison to LDb mice (8.8%±3.5 vs. 24%±3.3, p=0.004). We hypothesized that vascular PCSK9 regulates the development of atherosclerosis. We incubated LDL containing PCSK9 (LDL/PCSK9) on primary aortic endothelial cells (EC) obtained from LDb or LTp to study the effects of LDL/PCSK9 on inflammation. We show that LDL/PCSK9 could not induce the expressions of Lox-1, TLR-2, or ICAM-1 in EC from LTp, resulting in absence responses on proinflammatory markers and autophagic molecules. Our results suggest that vascular PCSK9 play a role in atherogenesis.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Triglyceride Rich Lipoprotein Cholesterol and Risk of Cardiovascular Events Among Patients Receiving Statin Therapy in the TNT Trial

Abstract nr. 204
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Risk Factor, Therapy, Triglyceride-Rich Proteins

**Background:** Mendelian randomization data suggest that lifetime higher triglyceride rich lipoprotein cholesterol (TRL-C) levels calculated as the difference between non-HDL-C and LDL-C are causally related to cardiovascular disease (CVD). We assessed the relationship between TRL-C and CVD risk, and whether this was modifiable in the Treat to New Targets (TNT) trial.

**Methods:** The effect of atorvastatin 10mg (AT10) on TRL-C was assessed during the open-label run-in phase and that of atorvastatin 80mg (AT80) vs AT10 assessed over 5 years (N=10001). The relationship between quintiles of baseline TRL-C and the primary endpoint (PEP) of the TNT trial was determined in the AT10 arm. The randomized effect of AT80 vs AT10 on the PEP was assessed across baseline quintiles of TLR-C. Finally the independent relationship between the % change in TRL-C levels at 3 months and subsequent risk of PEP was assessed using Cox regression models.

**Results:** AT10 reduced TRL-C from a median of 30mg/dl to 27mg/dl (p<0.0001), which was reduced further to 23mg/dl at 3 months by AT80 (p<0.0001) and maintained over time. Higher TRL-C levels were associated with higher rates of PEP, ranging from 9.72 % (Q1) at 5 years to 13.77% (Q5); hazard ratio (HR) Q5 vs Q1 1.49, 95%CI 1.15-1.92 (p trend <0.0001, AT10 arm). AT80 did not significantly alter risk of PEP in TRL-C Q1 and 2, but did reduce the PEP risk in Q3-Q5, with evidence of effect modification (p for homogeneity 0.0053) and greater absolute benefits observed in Q4 and Q5 of baseline TRL-C (Figure). Finally in multivariable models independent of reductions in LDL-C, a 1SD reduction in TRL-C was associated with lower risk (HR 0.93, CI 0.864-0.999) and of similar magnitude to that for 1SD lowering in LDL-C (HR 0.89, CI 0.831-0.953).

**Conclusion:** TRL-C levels are reduced by atorvastatin therapy in a dose dependent fashion. Higher TRL-C levels are associated with increased CVD risk which is significantly attenuated by intensive atorvastatin therapy. Independent of the reduction in LDL-C, the percentage reduction in TRL-C is associated with CVD suggesting that TRL-C is both a risk marker and a potential target for therapeutic intervention.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Longer GT repeats and rs2071746T allele in the heme oxygenase-1 gene promoter are associated with abdominal aortic aneurysm

Abstract nr. 205
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Genetics, Inflammation

Abdominal aortic aneurysm (AAA) is multifactorial disease with life-threatening complications due to mainly asymptomatic course of development. Vascular inflammation induced by oxidative stress contribute to pathogenesis. Inter-individual differences in response to oxidative stress are partially under genetic control. In this study the associations between the functional SNPs and (GT)n repeat length polymorphisms in genes involved in the vascular response to hypoxia/ischemia: HIF1A (hypoxia inducible factor-1α) and HMOX1 (heme oxygenase-1) and the development of AAA were examined.

The study encompassed a series of 518 AAA patients, 345 patients with atherosclerotic aortoiliac occlusive disease (AOID) and 498 controls. The HIF1A rs11549465C>T, rs11549467G>A and HMOX1 rs2071746A>T SNP genotyping was performed by using predesigned TaqMan SNP-genotyping assays. For simultaneous assessment of the HIF1A and HMOX1 (GT)n polymorphisms, the method based on multiplex-PCR with fluorescent-labeled sense primers and fragment size analysis using DNA sequencer has been developed.

We found, that carriers of the HMOX1 (GT)n repeat long allele (n>27) had increased risk of developing AAA (OR=1.46 for dominant model, P=.034). The frequency of carriers of both HMOX1 risk alleles: rs2071746T and/or long (GT)n repeat in AAAs (58.5%) was higher as compared to AIOD (49.0%, P=.007). On the other hand, the frequency of noncarriers in AAAs was 0.0%, as compared to 1.3% in controls (P=.010) and 0.9% in AIOD (P=.066).

In conclusion, HMOX1 gene promoter long (GT)n repeat allele and rs2071746T, allele related to decreased anti-inflammatory and antioxidant capacity of heme oxygenase-1, are associated with abdominal aortic aneurysm. Supported by Polish Ministry of Sciences grant NN403_250440 and INNOMED.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
KMUP-3 protects Cardiac Fibroblasts from apoptosis induced by hydrogen peroxide through the Adaptive Autophagy

Abstract nr. 206

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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention

Keywords Pharmacology

Autophagy is important for the turnover of organelles at low basal levels under normal conditions and it is up-regulated in response to stresses such as ischemia/reperfusion and in cardiovascular diseases such as heart failure. Apoptosis also plays important biological roles in the pathogenesis of many diseases. Oxidative stress induced by myocardial infarction is one of the major factors of heart failures. In our previous studies, 7-[2-[4-(4-nitrobenzene)piperazinyl]ethyl]-1,3-dimethylxanthine (KMUP-3) is a chemically synthetic xanthine-based derivative. It has been shown to induce autophagy in cardiac fibroblasts. The aim of this study was to investigate how KMUP-3 modulates hydrogen peroxide (H$_2$O$_2$)-induced apoptosis in neonatal rat cardiac fibroblasts and to elucidate the cellular and molecular mechanism. Preincubation of KMUP-3 significantly inhibited apoptosis and increased cell viability for neonatal rat cardiac fibroblasts under oxidative stress. Western blot showed that KMUP-3 enhanced p-eNOS, eNOS, PKG, LC3-II, Atg7 formation, and increased Bcl-2/Bax ratio in H$_2$O$_2$-treated neonatal rat cardiac fibroblasts. Moreover, KMUP-3 attenuated H$_2$O$_2$-induced MMP-2, MMP-9 and cleaved caspase-3 protein expressions. These effects were blocked by both the L-NAME and L-NIO, indicating that eNOS plays a role in the modulation of KMUP-3 in H$_2$O$_2$-induced apoptosis. However, when cardiac fibroblasts treated with Atg7 siRNA, which blocked the autophagy in the cells and resulted in a further increase in cell apoptosis. These results showed that KMUP-3 may promote autophagy to decrease oxidative stress-induced apoptosis in cardiac fibroblasts. Therefore, KMUP-3 might exert cardioprotective effects in heart diseases through regulation of autophagy and apoptosis.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Influence of ranolazine and trimetazidine on inflammatory parameters in patients with chronic ischemic heart disease

Abstract nr. 207
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Inflammation, Intervention, Therapy

Levels of tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6), that together with stimulates the synthesis of the acute-phase proteins, correlate closely with C-reactive protein (CRP) serum levels. They were all found to be independent predictors of future coronary artery events in apparently healthy males as well as in patients with coronary artery disease. The novel anti-ischemic drugs, ranolazine and trimetazidine, reduce the levels of some inflammatory parameters in patients with stable coronary artery disease.

The aim of this study was to compare the effects of ranolazine and trimetazidine on CRP, TNF-α, IL-6, interleukin 10 (IL-10) and vascular adhesion molecules (VCAM) in patients with stable coronary artery disease.

In a prospective, double blind study, 56 males aged between 32 to 65 years with chronic ischemic heart disease were randomised and submitted to 12 weeks treatment with either trimetazidine (35 mg twice daily) or ranolazine. Ranolazine was given in a dose of 375 mg twice daily for 4 weeks and was increased to 500 mg twice daily.

Ranolazine was found to lower the CRP concentration after 12 weeks from 4.5±6.26 to 1.93±3.11 mg/l (p=0.038), while no changes was observed in trimetazidine group (3.78±7.65 to 3.6±6.46 mg/l; p=0.831) (p=0.103 for comparison of interventions). Ranolazine lower IL-10 levels from 1.5±1.2 to 1.1±1.2 ng/l (p=0.031), with no changes in trimetazidine group (1.4±0.7 to 1.4±1.0 ng/l; p=0.817) with no difference between the groups (p=0.327). TNF-α decreased in ranolazine group from 18.8±28.9 to 11.7±20.5 ng/l (p=0.186) and from 27.4±34.0 to 22.6±31.1 ng/l (p=0.639) in trimetazidine group, with no difference between the groups (p=0.844). IL-6 changed in ranolazine group from 3.4±2.2 to 2.8±1.4 ng/l (p=0.109) and from 3.4±2.4 to 3.5±2.4 ng/l (p=0.631) in trimetazidine group, with no difference between the groups (p=0.116). VCAM changed in ranolazine group from 888±219 to 904±356 μg/l (p=0.688) and from 1002±345 to 993±386 ng/l (p=0.756) in trimetazidine group, with no difference between the groups (p=0.608).

Our study shows that ranolazine significantly lower the levels of CRP and IL-10 with no influence on IL-6, TNF-α and VCAM, while trimetazidine have no influence on measured parameters in patients with stable coronary artery disease.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Renal and cardioprotective effects of irbesartan via reduction of serum sodium, uric acid, LDL-C, and microalbuminuria in hypertensive patients

Abstract nr. 208
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Blood pressure, Hypertension, Renal function, Risk Factor

**Background** Irbesartan, angiotensin II receptor blocker (ARB) is a well-established first-line treatment option for hypertension. We examined the long-term effects of irbesartan beyond lowering blood pressure. **Methods and results** In this study, 211 (97 male and 114 female, mean 66.1 years) hypertensive patients treated with irbesartan were enrolled. We observed blood pressure (BP), pulse rate (PR), serum levels of sodium, potassium, uric acid, microalbuminuria and lipid profile at baseline, 6 and 12 months after administration of irbesartan. Systolic and diastolic BP were significantly reduced (-23±3 mmHg and -10±2 mmHg, respectively), while there was no significant change in PR during the period. Serum sodium was significantly reduced (141 ± 2.2 mEq/l to 140 ± 2.4 mEq/l), while there was no significant changes in serum potassium. Furthermore, urinary albumin was significantly reduced at 12 months (167 ±686 mg/g Cr to 80 ± 237 mg/g Cr). In 35 patients with uric acid more than 7.0 mg/dl without medication for hyperuricemia, serum uric acid was significantly reduced (7.9 ±1.1 mg/dl to 6.7 ± 1.1, p<0.01). LDL cholesterol was also significantly reduced (115 ± 5 mg/dl to 107 ± 5 mg/dl, p<0.01) in patients with uric acid less than 7.0 mg/dl. **Conclusion** Irbesartan therapy significantly reduced not only BP, but also other metabolic factors in patients with hypertension. Furthermore, irbesartan had sodium excretion effect in addition to angiotensin receptor blocker for lowering blood pressure. This study suggested that irbesartan might have renal and cardioprotective effects in hypertensive patients.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Epicardial adipose tissue in overweight and obese children and its relationship to cardiometabolic risk factors, insulin resistance and hyperuricemia

Abstract nr. 209
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease,Obesity,Risk Factor,Visceral Fat

Background: Epicardial adipose tissue (EAT) is the visceral fat deposit around the heart and is commonly increased in obese subjects. EAT is related to cardiometabolic risk factors and non-alcoholic fatty liver disease (NAFLD) in adults, but this relationship is not well known in children. Objectives: The aim of our study was to assess by echocardiography the EAT in overweight and obese children and its relationship to cardiometabolic risk factors, insulin resistance, NAFLD markers and hyperuricemia.

Study group and methods: In 25 (mean age 13,0 2,3) overweight and obese subjects and 24 lean controls, blood pressure (BP), waist circumference (WC), fasting plasma glucose and insulin, lipids, uric acid and hepatic enzymes were established and EAT thickness measured by transthoracic echocardiography.

Results: In overweight and obese subjects, EAT was significantly higher compared to normal weight children. Overweight and obese children had significantly higher body mass index (BMI), WC, BP, triglycerides (TAG), low density lipoprotein and total cholesterol, hepatic enzymes alanine aminotransferases (ALT) and g-glutamyl transferase, and lower high density lipoprotein cholesterol (HDL-C). EAT correlated significantly with BP, TAG, uric acid, HDL-C, apolipoprotein B and ALT. Correlation coefficients were similar or better than for WC, but similar or lower then for BMI.

Conclusions: In conclusion EAT thickness in children is associated with an unfavourable cardiometabolic risk profile including biochemical signs of NAFLD and hyperuricemia but is not a stronger indicator than BMI.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Endothelial dysfunction, inflammation and body mass index in patients with coronary heart disease in combination with nonalcoholic hepatic steatosis

Abstract nr. 210
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Endothelium, Inflammation, Obesity

Objective: To compare the relationship of endothelial dysfunction, inflammation, serum levels of aminotransferases depend from body mass index (BMI) in patients with coronary heart disease in combination with nonalcoholic hepatic steatosis.

Methods: We studied 19 patients with coronary heart disease in combination with nonalcoholic hepatic steatosis. Allocated 2 groups according to BMI: one group consisted of 10 (52%) people with first degree of obesity (BMI 30,0 to 34,9 kg/m2) mean age 53,7±5,4, group 2 - 9 (40%) who are overweight (BMI 25 to 29,9 kg/m2) mean age 57,2±7,04. Patients with diabetes were excluded. All patients not taking statins. The reactive hyperemia test for assessment of endothelial dysfunction was consecutively performed in all patients. Brachial artery enlargement by less than 10% was considered as a sign of endothelial dysfunction. Studied biochemical parameters: the levels of C-reactive protein (CRP), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT).

Results: Endothelial dysfunction was found in 9 patients (90%) in a group 1 and in 6 patients (66%) in a group 2, vasospastic response observed in 1 patient (10%) in a group 1. The mean level reactive hyperemia index changes did not significantly differ between the two groups (5,5±4,3 % and 5,4±1,4 %, p>0,05 ). The mean level CRP in a group 1 was higher (4,7±0,6mmol/l) than in group 2 (2,3±0,34mmol/l) (p<0,05); of ALT: 36,2±11,9mmol/l and 27,2±13,2mmol/l (p<0,05); of AST: 27,0±2,4mmol/l and 20,2±2,3mmol/l (p<0,05); of GGT: 60,2±9,03mmol/l and 47,2±3,7mmol/l (p<0,05). There was correlation between the endothelial dysfunction and level of AST (r=0,89; p<0,05) in a group 1 however in a group 2 such connection was not observed. The level of CRP in a group 1 associated with level gamma-glutamyl transpeptidase (GGT) (r=0,87; p<0,05) than in group 2.

Conclusion:
In patients with coronary heart disease in combination with NAFLD and first degree of obesity (BMI 30,0 to 34,5 kg/m2) inflammation were more expressed in those who had overweight (BMI 25,0 to 29,9). There was an association between endothelial dysfunction and level of AST, level of CRP and level GGT in a group 1 however in a group 2 such connection was not observed.
Presentation Preference Electronic poster presentation
Additional information
Prevalence of cardiovascular diseases among type 2 diabetic patients shifted to insulin therapy in Najaf Governorate, Iraq

Abstract nr. 212
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Diabetes, Epidemiology

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Background: Diabetes mellitus is a diseases of vascular complications particularly when it is associated with dyslipidemia. More than half of type two diabetic patients (51%) have being shifted to insulin therapy by their specialist physicians in Iraq. The prevalence of cardiovascular diseases from the start of disease exceed 30% of type two diabetic patients in Iraq. There is no local evidence about the role of insulin therapy on reducing risk of cardiovascular diseases.

Objective: To estimate the prevalence of cardiovascular diseases among patients with type 2 diabetes who are shifted to insulin treatment.

Design: A cross sectional study

Methods: From registered 7120 diabetic patients in the Popular Medical Clinics Directorate in Najaf governorate, 5248 patients diagnosed by Najaf Center of Diabetes and Endocrine Diseases, as Type 2 Diabetes Mellitus. A random sample of 2820 patients with Type 2 diabetes were selected during their attendance to Ninth popular medical clinic in Najaf from January 1st 2005 to 30 September 2014 for receiving their free prescribed treatments. The selected patients were interviewed for presence or absence of cardiovascular events regarding the duration of their started therapy.

Results: The prevalence of cardiovascular diseases in type 2 diabetic shifted to insulin in Najaf-Iraq was 34.6% after age of 60 years versus 48% of patients on oral hypoglycemic drugs. No significant difference in use of available statins (Fluvastatin and Atorvastatin,) between diabetics with and without these disease. Conclusion: cardiovascular diseases were less prevalent among type 2 diabetic patients on insulin therapy regardless of their statin use.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Effect of Saroglitazar on HbA1c level in type 2 Diabetes Mellitus: A clinical experience data

Abstract nr. 214
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes, Dyslipidemia

Background: Saroglitazar (ZYH1) is a novel peroxisome proliferator-activated receptor (PPAR) agonist with predominant PPARα and moderate PPARγ activity. It has been developed for the treatment of dyslipidemia and has favourable effects on glycaemic parameters in type 2 diabetes mellitus. Saroglitazar showed both anti-dyslipidemic and anti-diabetic effects mainly mediated via activation of PPARα and PPARγ respectively. It has also shown favorable glycaemic indices by reducing the fasting plasma glucose and glycosylated hemoglobin in diabetic patients.

Objective: To evaluate the clinical effectiveness of saroglitazar in terms of glycaemic control (HbA1c) in type 2 diabetic patients.

Method: An analysis of the clinical features and laboratory data of 200 (109 males, 91 females) patients of either gender, aged 18-70 years diagnosed with type 2 diabetes mellitus (glycosylated hemoglobin [HbA1c] > 7 to 9%) was collected. All the patients with type 2 Diabetes Mellitus treated with Saroglitazar were observed for a period of 6 months. Saroglitazar was recommended for once daily administration as 4 mg tablets. Outcome was assessed in terms of HbA1c level.

Result: Out of 200 patients, Eighty-three percentages (n=166) T2DM patients achieved significant reduction in glycemic level in terms of HbA1c level ≥0.5% from the baseline HbA1c level, whereas marked reduction of HbA1c level was not achieved in seventeen percentage (n=34) within 6 months of observation period.

Conclusion: Effects of Saroglitazar 4 mg, on HbA1c were significantly better than baseline, thus, Saroglitazar appeared to be an effective therapeutic option for improving glycemic control (HbA1c) in patients with type 2 diabetes mellitus.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Introduction: Apolipoprotein E (apoE) is an important element in the metabolism of cholesterol, lipoproteins and triglycerides. We can distinguish three types of apoE protein: apoE 2, apoE 3 and apoE 4. The fourth phenotype is associated with significantly increased risk of cardiovascular diseases, diabetes and Alzheimer's disease. The role of apoE 4 in patients with metabolic syndrome (MS) has been broadly discussed recently. MS is associated with serious lipid metabolism disorders as well as increased oxidative stress. The same pathological factors are also a cause of nonalcoholic fatty liver disease (NAFLD). There are no reports about lipids metabolism changes in apoE4 carriers with NAFLD.

Aim: The aim of the study was to compare fatty acids and their derivatives concentration in apoE 4 and the other apoE variants among patients with NAFLD.

Materials and methods: 22 patients with NAFLD were enrolled in the study. The study group included 11 apoE 4 carriers, as well as a control group, also consisting of 11 patients, with apoE 2 and apoE 3 phenotypes. The concentrations of fatty acids and their derivatives were measured in plasma. The extraction is based on solid phase extraction technique. The fatty acids analysis was performed using Agilent Technologies 7890A gas chromatography. Arachidonic and linoleic acid transformation products – Lipoxin A4 (LX A4), 16–hydroxyeicosatetraenoic (16-HETE), 13–hydroxyoctadecadienoic acid (13-HODE), 9–hydroxyoctadecadienoic acid (9-HODE), 15–hydroxyeicosatetraenoic acid (15-HETE), 12–hydroxyeicosatetraenoic acid (12-HETE), 5–oxoeicosatetraenoic acid (5-oxoETE) – were analyzed using Agilent Technologies 12600 high-performance liquid chromatography. Statistical analysis was used for statistical analysis (Statsoft 2011).

Results: ApoE 4 carriers had significantly increased plasma concentration of several fatty acids (Table 1) as well as the concentration of arachidonic and linoleic transformation products (Table 2).

Discussion: ApoE 4 carriers showed increased concentration of monounsaturated fatty acids,
which is caused by increased activity of Δ 9 desaturase. Increased activity of this enzyme and significantly higher concentration of stearic acid seems to be a consequence of increased lipogenesis in patients with ApoE4 phenotype. In the other hand, ApoE 4 carries are more exposed to inflammation because of their higher concentration of inflammatory markers (5-HETE,9-HODE,3-HODE).

<table>
<thead>
<tr>
<th>Fatty acids derivatives</th>
<th>Median (IQR) apoE 4</th>
<th>Median (IQR) control</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LX A4</td>
<td>2.579 (3.197)</td>
<td>1.472 (3.627)</td>
<td>NS</td>
</tr>
<tr>
<td>16 - HETE</td>
<td>4.837 (7.103)</td>
<td>1.235 (0.256)</td>
<td>NS</td>
</tr>
<tr>
<td>13 - HODE</td>
<td>4.053 (5.919)</td>
<td>1.860 (0.190)</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>9 - HODE</td>
<td>8.273 (8.794)</td>
<td>1.896 (0.693)</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>15 - HETE</td>
<td>1.233 (1.817)</td>
<td>0.506 (0.300)</td>
<td>NS</td>
</tr>
<tr>
<td>12 - HETE</td>
<td>16.960 (9.223)</td>
<td>13.524 (8.480)</td>
<td>NS</td>
</tr>
<tr>
<td>5-oxoETE</td>
<td>0.0134 (0.009)</td>
<td>0.0192 (0.089)</td>
<td>NS</td>
</tr>
<tr>
<td>5 - HETE</td>
<td>0.902 (1.057)</td>
<td>0.151 (0.124)</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

**Fatty acids derivatives concentrations**

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Median (IQR) apoE 4</th>
<th>Median (IQR) control</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>0.0526</td>
<td>0.0531</td>
<td>NS</td>
</tr>
<tr>
<td>Palmitoleic acid (C16:1)</td>
<td>0.0132</td>
<td>0.0144</td>
<td>NS</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>0.0652</td>
<td>0.0944</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Oleic acid (C18:1 n9)</td>
<td>0.3834</td>
<td>0.0800</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Vaccenic acid (18:1 n7)</td>
<td>0.0137</td>
<td>0.0933</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Linoleic acid (C18:2 n6)</td>
<td>0.2505</td>
<td>0.2141</td>
<td>NS</td>
</tr>
<tr>
<td>γ-Linolenic (C18:3 n6)</td>
<td>0.0337</td>
<td>0.0470</td>
<td>NS</td>
</tr>
<tr>
<td>α-Linolenic acid (18:3 n3)</td>
<td>0.0274</td>
<td>0.0243</td>
<td>NS</td>
</tr>
<tr>
<td>Arachidonic acid (C20:4 n6)</td>
<td>0.1263</td>
<td>0.1423</td>
<td>NS</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (20:5 n3)</td>
<td>0.0357</td>
<td>0.0270</td>
<td>NS</td>
</tr>
<tr>
<td>Docosapentaenoic acid (22:5 n3)</td>
<td>0.1029</td>
<td>0.0651</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Docosahexaenoic acid (22:6 n3)</td>
<td>0.0651</td>
<td>0.0456</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Fatty acids concentrations**

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Blockade of Tim-4 aggravates atherosclerosis

Abstract nr. 216
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Pathogenesis

Introduction: Proteins of the transmembrane T cell immunoglobulin and mucin domain (Tim) family are expressed by numerous immune cells, recognize phosphatidylserine (PS) exposing cells and exert either a costimulatory or coinhibitory role. Tim-4, present on macrophages and antigen-presenting cells, has been shown to play a critical role in the clearance of apoptotic cells, regulates the number of PS-expressing activated T cells, and is genetically associated with triglyceride levels. Since both apoptosis and the presence of activated T cells contribute to atherosclerotic lesion formation, we investigated whether interference in Tim-4 function would affect atherosclerosis.

Methods and Results: LDLr-/- mice were fed a Western-type diet for 4 weeks while being treated twice a week i.p. with an anti-Tim-4 (21H12) mAb that blocks PS recognition and phagocytosis or an isotype control (rat IgG1). Treatment with anti-Tim-4 increased the area of atherosclerotic lesion in the aortic root by 45% (7.76±1.07%) compared with control mice (5.37±0.51%, P<0.01), independent of plasma cholesterol and triglyceride levels. Anti-Tim-4-treated mice showed increased activated T cell numbers and 'late' apoptotic cells in the circulation. Additionally, anti-Tim-4 treatment induced splenomegaly, enhanced splenocyte proliferation and increased IFNγ+ (Th1) and IL-4+ (Th2) cells.

Conclusion: Blockade of Tim-4 aggravates atherosclerosis likely by prevention of phagocytosis of PS-expressing apoptotic cells and activated T cells by Tim-4-expressing cells.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Features psychovegetative disorders in patients with chronic cerebral ischemia and ways of their correction

Abstract nr. 217
Author Sultankhodjaeva, Nadira, Postgraduate Medical Institute, Tashkent, Uzbekistan
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Intervention, Lifestyle, Pathogenesis, Pharmacology

Modern metabolic preparations, interfering with metabolic processes in the organism, normalize first of all a biological basis of the adapted reaction of the person. The purpose of our research was studying dynamics of cognitive and emotional sphere at patients - invalids with ChIB on a background of an atherosclerosis of vessels of the brain at stages of rehabilitation. By way of complex rehabilitation we applied a preparation mildronat.

Materials and methods of research. 45 patients in the age of from 30 till 55 years (middle age 42+3.76) were surveyed. The estimation of efficiency of treatment was based on results of following researches: clinic-neurological research, psychological – emotional research, in the present work were used test of Spielberg in modification (132.) Screening scale of diagnostics of dementia were researched clinically on the basis of criteria of the American psychiatric association - DSM-IV by means of "the Brief scale of estimation of the mental status" (Mini-Mental State Examination, agant. Folstein et al., 1975) which defines quantitative and quality standard of cognitive defect, and cardiointervalography.

As a result of researches at patients was observed the high degree both situational, and to personal uneasiness and also an easy and average degree dementia before the treatment, and also result CIG have shown moderated sympaticotony. On a background of treatment at patients positive dynamics from psychological – emotional spheres was observed: the tendency to decrease as the situational and personal uneasiness, the moderate expressiveness cognitive frustrations. Dynamics of research of 2 groups has shown the to decrease both personal, and situational uneasiness under Spielberg’s test, and also obvious reduction of expressiveness of cognitive frustrations. Vegetative sphere of the given patients has sharply decreased to normotony. Therefore, the application of mildronat which possesses not only vasoactive and antioxidant, it is also neuroprotective and which’s metabolic properties which influencing to subsystem of emotional-affective reaction is pathogenetically proved.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
High-sensitivity C-reactive protein levels across countries and ethnicities

Abstract nr. 218
Author Izar, Maria Cristina, Federal University of Sao Paulo, Santana De Parnaiba, Brazil
Co-author(s) - Fonseca, Francisco Antonio
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, Inflammation, Prevention

Background: Despite substantial differences in ethnicities, habits, culture, prevalence of traditional cardiovascular risk factors and affordable therapies, atherosclerosis remains the major cause of death in developing and developed countries. However, regardless of these inequalities, inflammation is currently recognized as the common pathway for the major complications of atherosclerosis, stroke, and ischemic heart disease.

Methods: A PubMed search was conducted for 'high-sensitivity C-reactive protein' (hs-CRP) in combination with race, ethnicity, gender, prevalence, geographic, epidemiology, cardiovascular, obesity, diabetes, hypertension, cholesterol, smoking, ischemic heart disease, stroke, and mortality. There is no systematic approach implemented for the selection of articles in this review. Instead, it was based on relevance to the topic. Additional articles identified from reference lists of relevant publications were also included.

Results and Conclusions: This review described marked differences in the cardiovascular mortality across countries and ethnicities, which can be attributed to inequalities in the prevalence of classic risk factors and stage of cardiovascular epidemiological transition. However, hs-CRP seems to add prognostic information for cardiovascular risk and mortality even after multiple adjustments. Taking into account the perception of cardiovascular disease as an inflammatory disease, the more widespread use of hs-CRP appears to be a valid tool to identify people at risk, independently of the ancestrality or geographic region. Further, the large Canakinumab ANtiinflammatory Thrombosis Outcome Study (CANTOS) trial involving subjects from Europe, Asia, Africa, and Americas is ongoing and important questions will be answered, including the need for different cutoffs of hs-CRP, the relevance of this biomarker for different ethnicities and the validity of an antiinflammatory treatment for people at risk.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
The prevalence of heterozygous familial hypercholesterolemia in Tyumen region of Russian Federation

Abstract nr. 219
Author Ershova, Alexandra, National Research Center for Preventive Medicine, Moscow, Russian Federation
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Co-author(s) - Storojak, Marina
Co-author(s) - Ephanov, Alexey
Co-author(s) - Shalnova, Svetlana
Co-author(s) - Medvedeva, Irina
Co-author(s) - Boytsov, Sergey
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Familial Hypercholesterolemia

**Background:** Heterozygous familial hypercholesterolemia (FH) is an autosomal dominant disorder known to be associated with elevated cholesterol levels and increased risk of premature coronary artery disease. Historically, the community prevalence of FH is estimated to be one in 500; however, recent data suggest that this is an underestimate. The Copenhagen General Population Study determined that the prevalence in individuals classified as definite or probable FH approached one in 137. The prevalence of FH in Russia has not previously been evaluated. The aim of our study is to investigate the prevalence of FH in the Russian population.

**Materials and methods:** The study was of a randomly selected, community-based population comprising 1,630 people of Tyumen region of Russian Federation (from the ESSE-RF epidemiological study). The level of low-density lipoprotein cholesterol was measured in all participants. All subjects were interviewed to assess statin treatment. All participants who had low-density lipoprotein cholesterol (LDL-cholesterol) higher 4.9 mmol/l and who had LDL-cholesterol lower 4.9 mmol/l but had statin therapy were examined by experts in FH. The diagnosis of FH was determined using the Dutch Lipid Clinical Network Criteria (DLCN).

**Results:** We examined 126 participants from 142 who had the level of low-density lipoprotein cholesterol higher 4.9 mmol/l and 5 participants from 11 who had statin treatment (aged 59 (53-62) years). The prevalence of individuals classified with definite FH (DLCN criteria >8 points) was 0.31% (one in 323), probable FH (6–8 points) was 0.67% (one in 149), definite or probable FH combined (>5 points) 0.98% (one in 102), possible FH 6.87% (one in 15) (3–5 points).

**Conclusion:** The prevalence of FH in the Russian population is higher than estimated value.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
**Plant sterol supplementation on top of lipid-lowering therapies in familial hypercholesterolemia**

Abstract nr. 220  
Author Izar, Maria Cristina, Federal University of Sao Paulo, Santana De Parnaiba, Brazil  
Co-author(s) - Fonseca, Francisco Antonio  
Co-author(s) - Machado, Valeria  
Co-author(s) - Fonseca, Henrique Andrade  
Co-author(s) - Fonzar, Waleria Toledo  
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins  
Keywords Familial Hypercholesterolemia, Hypolipidemic Drugs, Lipids, Nutraceuticals

**Background:** Familial hypercholesterolemia (FH) is the most common inherited disorder of lipid metabolism, resulting in very high levels of LDL-cholesterol (LDL-C) from birth and increased premature coronary disease. Underdiagnosed and undertreated, this condition often requires combined lipid-lowering therapy (LLT), with room for further interventions. Plant sterols (PS) supplementation, by reducing intestinal cholesterol absorption, can further lower LDL-cholesterol in 10%, but the combination of high-dose statin, ezetimibe and PS has not been addressed yet in FH individuals. We tested the effects of plant sterols on top of two intensive LLT on LDL-C, sterols synthesis and absorption markers.

**Methods and results:** Forty-two individuals of both genders with confirmed diagnosis of FH, aged 49-60 years were prospectively included. Study design was PROBE (randomized, open label, with parallel arms and blinded endpoints). After a 4-week washout period of previous LLT, eligible subjects were randomized to receive simvastatin 80mg or simvastatin 80mg plus ezetimibe 10mg in a blinded fashion for 12 weeks. After this period, 2g of phytosterols, as free sterols were given in 500mg capsules with meals for additional 12 weeks. Both LLTs reduced total- and LDL-C, triglycerides and ApoB, while addition of phytosterols further reduced LDL-cholesterol only in the group receiving simvastatin/ezetimibe ($P=0.031$). Simvastatin increased campesterol, decreased desmosterol, while combined therapy reduced absorption markers and reduced desmosterol plasma levels ($P<0.05$ vs baseline, for all).

**Conclusions:** This study has shown that PS supplementation in FH benefited those individuals treated with simvastatin plus ezetimibe, but not those receiving simvastatin alone. In addition to ezetimibe, PS can counterbalance the increased sterols absorption besides improving lipid profile. Our study confirms the relevance of a more intensive blockade of cholesterol absorption and the validity of phytosterols supplementation for patients with FH.
Individual profiles showing variables of lipid profile by treatment

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Cardiovascular risk factors in patients with rheumatoid arthritis in combination with hypertension.

Abstract nr. 221
Author Sirenko, Oksana, Dnipropetrovsk medical academy, Dnipropetrovsk, Ukraine
Co-author(s) - Kuryata, Olexander
Co-author(s) - Lysunets', Tetyana
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Hypertension, Inflammation, Obesity, Risk Factor

The objective was to evaluate the frequency of cardiovascular risk factors and hypertension in patients with rheumatoid arthritis depending on body weight. The study involved 100 patients with rheumatoid arthritis and stably selected therapy for more than 6 months at the age from 45 to 65 years (mean age 53.19 ± 5.40 years). Traditional cardiovascular risk was assessed, taking into account risk factors by SCORE scale and amended for patients with RA. The levels of total cholesterol, triglycerides, C-reactive protein, serum creatinine, body mass index, body area index were determined. Arterial hypertension was diagnosed in 41 (41%) patients with rheumatoid arthritis and was associated with traditional risk factors (age, obesity), rheumatoid factor, hyperuricemia and the duration of glucocorticoid therapy. 10-year risk of fatal cardiovascular events matched by SCORE in patients with rheumatoid arthritis was 1.48 ± 1.94%, with consideration of 1.5 coefficient- 1.98 ± 2.53%, which is considered medium risk. Overweight and obesity have been established in 67 (67%) patients with rheumatoid arthritis. Body mass index was associated with duration of rheumatoid arthritis inflammation activity, duration of therapy with glucocorticoids. Patients overweight had the highest level of performance inflammation, the risk of cardiovascular complications. Identification of hypertension and obesity increases the information content of the risk assessment of cardiovascular events in rheumatoid arthritis.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Anti Inflammatory Effect of High Complex Carbohydrate Diet and Physical Activity in Severely Obese Volunteers

Abstract nr. 223
Author Raz, Olga, Tel Aviv Medical Center, Tel Aviv, Israel
Co-author(s) - Ori, Rogovsky
Co-author(s) - Tovit, Rosenzweig
Co-author(s) - Izhak, Shapira
Co-author(s) - Berliner, Shlomo
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Inflammation, Nutrition, Obesity

**Aim:** The presence of low grade, internal inflammation is one of the main causes for development of insulin resistance, type 2 diabetes mellitus and atherosclerosis. The aim of the study is to evaluate the effect of lifestyle modifications on the inflammatory profile of obese volunteers.

**Methods:** Blood samples were taken before and after 8 months of intensive life modification program, including consumption of high-complex carbohydrate diet and intensive physical activity in a group of apparently healthy severely obese volunteers. **Results:** Substantial improvement was noted in the biometric, metabolic and inflammatory biomarkers. A reduction was found in BMI and in the concentrations of CRP, triglycerides, LDL, total cholesterol, insulin concentration, HOMA-R, the adhesion molecule ICAM1 and the pro-inflammatory cytokines TNF-alpha and IL6. Erythrocyte Sedimentation Rate and the degree of red cell aggregation were reduced. However, a significant increment in fibrinogen concentrations was noted.

**Conclusion:** The study shows the beneficial anti-inflammatory properties of this intervention program. The pro-aggregating properties of fibrinogen following intense physical activity are probable counterbalanced by the anti-aggregatory properties of an improved lipid profile and an attenuated acute phase response. The study suggests that strenuous physical activity is not advised for untrained obese individuals.
Relationship between circulating lipoprotein lipase and remnant lipoproteins in pre-and post-heparin plasma

Abstract nr. 224
Author Nakajima, Katsuyuki, Gunma University, Maebashi, Japan
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Co-author(s) - Imamura, Shigeyuki
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Co-author(s) - Shimomura, Youichiro
Co-author(s) - Machida, Tetsuo
Co-author(s) - Sumino, Hlroyuki
Co-author(s) - Murakami, Masami
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia,Lipoproteins, Metabolism, Triglyceride-Rich Proteins

Objectives
We developed a highly sensitive and specific LPL-ELISA and reported that most lipoprotein lipase (LPL) in the circulating plasma is bound to remnant lipoproteins (RLP) specifically. We have further investigated the relationship between circulating LPL and remnant lipoproteins in plasma with and without heparin injection.

Methods
LPL mass and activity of pre-heparin and post-heparin plasma were determined in 40 healthy volunteers with TC, TG, LDL-C, HDL, RLP-C, RLP-TG and other plasma parameters. Superose 6B was used to fractionate RLP under the presence of tetrahydrolipstatin, the inhibitor of LPL. LPL activity was determined by enzymatic method (Imamura et al. J Lipid Res. 2008; 49:1431)

Results
LPL concentration was determined in post-heparin and pre-heparin and plasma in 40 volunteers (65±20ng/mL, 382±42 ng/ml, respectively). More than 70 % of LPL was found in RLP in pre-heparin plasma, while less than 30% of LPL was found but significantly increased in RLP in post-heparin plasma. Addition of tetrahydrolipstatin (1μg/mL) significantly inhibited the LPL activity and movement of LPL to HDL fraction from RLP. RLP was apoC3 and apoC1 rich as well as apoE rich. In vitro study showed that addition of apoC1 and apoC3 significantly reduced LPL activity (50% and 70 %, respectively). These results indicated that LPL binds to RLP with apoC1 and apoC3 and inhibits LPL activity in pre-heparin plasma.

Conclusion
Highly sensitive and specific LPL-ELISA assay made it possible to investigate the plasma circulating LPL in RLP with and without heparin injection. Tetrahydrolipstatin significantly inhibit LPL activity and prevented the hydrolysis of RLP in both pre-heparin and post-heparin plasma.
Low concentration of LPL in RLP kept large particle size and delayed the metabolism of remnant lipoproteins in plasma.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Dissecting molecular mechanisms of fatty liver disease that promote atherogenesis

Abstract nr. 225
Author Sivasubramaniyam, Tharini, University of Toronto, Toronto, Canada
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Co-author(s) - Li, Angela
Co-author(s) - Brunt, Jara J
Co-author(s) - Schroer, Stephanie A
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Co-author(s) - Woo, Minna
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Pathogenesis

Fatty liver is an emerging independent risk factor for atherosclerosis. However, the common association between fatty liver and other risk factors precludes definitive conclusions about the causal role of fatty liver per se in atherogenesis. Thus, the exact mechanisms by which fatty liver increases atherosclerosis risk are largely unknown. The Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway is a major signalling pathway downstream of cytokines and growth factors. In the liver, we and others previously showed that deletion of JAK2 led to spontaneous development of profound fatty liver on chow diet. Interestingly, those defects commonly associated with fatty liver including systemic insulin resistance, inflammation and glucose intolerance were absent in these mice. Thus we asked whether fatty liver in the absence of the other commonly associated risk factors affected atherogenesis. L-JAK2\textsuperscript{-/-} in both atherosclerosis-prone APOE\textsuperscript{-/-} and LDLR\textsuperscript{-/-} models, after 12wks of atherogenic diet showed similarly profound fatty liver without glucose intolerance or insulin resistance. These mice in both athero-prone models developed over a 2-fold increase in plaque burden compared to controls as assessed by Oil-Red-O staining of the descending aorta and the plaques in the aortic arch appeared more advanced with increased macrophage content and significantly less luminal smooth muscle actin. We had previously shown that JAK2 was required for GH signaling in liver resulting in low circulating insulin-like growth factor-1 (IGF-1) and hypothesized this to play a role in the increased atherosclerosis. To establish the causal role of reduced systemic IGF1 in the increased atherosclerosis observed in hepatic JAK2-deficient mice, L-JAK2\textsuperscript{-/-} APOE\textsuperscript{-/-} mice were infused with IGF-1 analog or vehicle while on an atherogenic diet for 12wks. Preliminary data show that restoring circulating IGF-1 attenuates fatty liver and atherosclerotic plaque burden in hepatic JAK2 deficient mice. Thus, our studies show an essential role of hepatic JAK2 in the protection against atherosclerosis through circulating IGF-1 and define a liver-centric mechanism in the maintenance of healthy vasculature.

Funding: This work was supported by operating grants from Canadian Institute of Health Research MOP-191501 and MOP-201188 to Minna Woo. Tharini Sivasubramaniyam and Sally Shi are supported by the CIHR Doctoral Research Award.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Diagnosis of the apoB Dyslipoproteinemias: The apoB algorithm AP

Abstract nr. 227
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Co-author(s) - Sniderman, Allan
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Cardiovascular Disease, Lipoproteins, Triglyceride-Rich Proteins

The problem: Excellence in clinical care begins with accurate diagnosis. Accurate diagnosis allows risk to be assessed most precisely and the most appropriate therapy to be chosen. Using only lipids, it is not possible to diagnose all apoB dyslipoproteinemias.

Objective: To diagnose all the major apoB dyslipoproteinemias based on total cholesterol, triglycerides and apoB.

Results: The major apoB dyslipoproteinemias are due to the elevation of one or more of the apoB containing lipoprotein particles: chylomicron particles, VLDL particles, chylomicron and VLDL particles, chylomicron and VLDL remnant particles, LDL particles and Lp(a) particles. With the exception of elevated Lp(a), all can be differentiated based on total cholesterol, triglycerides and apoB and therefore each can be specifically identified and treated.

The specific advantages are:
1. The hypertriglyceridemias- chylomicronemia, chylomicrons and VLDL, VLDL and remnant disorder can all be easily distinguished, which is essential as they differ in cardiovascular risk and treatment.
2. Remnant disorder (familial dysbetalipoproteinemia), which is highly atherogenic, can now be diagnosed in routine clinical care and is more common than appreciated.
3. In patients with the atherogenic lipoprotein phenotype with high TG and low HDL-c but normal LDL-C, elevated LDL particle number can be recognized by high apoB levels.
4. Non-HDL-C will not substitute for apoB in this approach and a series of discordance analyses have shown apoB is a more accurate marker of cardiovascular risk than non-HDL-C.

Conclusion: We present a diagnostic algorithm of the apoB dyslipoproteinemias based on apoB, total cholesterol and triglyceride. The apoB APP, which can be downloaded for free from the internet in may 2015, will produce the correct answer once total cholesterol, triglycerides and apoB are entered. The differential diagnosis, pathophysiology and treatment for all the apoB dyslipoproteinemias are also provided on the APP. Accurate diagnosis has always been a pillar of medical care. It is time for lipidology to rediscover the value of accurate diagnosis.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Statin use and low density lipoprotein cholesterol goal attainment in a high cardiovascular risk population in the Netherlands

Abstract nr. 228
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Co-author(s) - Khan, Irfan
Co-author(s) - van-Riemsdijk, Melanie
Co-author(s) - Herings, Ron
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, LDL, Prevention, Risk Factor

Background/objective: Low density lipoprotein cholesterol (LDL-C) is a key therapeutic target for cardiovascular (CV) risk reduction. Meta-analysis of statin trials as well as a trial involving a non-statin LDL-C lowering treatment, ezetimibe, suggest a linear relationship between LDL-C and risk of major CV events. The objective of the current study was to study statin treatment and LDL-C goal attainment in a real-world high-risk population in the Netherlands.

Methods: From the PHARMO Database Network, patients aged ≥18 years with an LDL-C measurement in 2012 (index date) were selected and hierarchically categorized in following mutually exclusive categories: 1) familial hypercholesterolemia (FH), 2) recent acute coronary syndrome (ACS; within 1-year pre index date); 3) coronary heart disease (CHD); 4) ischemic stroke; 5) peripheral artery disease (PAD) or 6) diabetes mellitus (DM). Statin use at index date was assessed and patients covered by a prescription within 45 days were considered to be taking the medication.

Results: Of 61,839 patients meeting the inclusion criteria, 1,132 (2%) were included in FH; 2,431 (4%) in recent ACS; 6,292 (10%) in CHD; 2,868 (5%) in ischemic stroke; 3,017 (5%) in PAD; and 46,099 (75%) in DM. Overall, 65% were taking statins. Recent ACS had the highest proportion of patients taking a statin (77%); although, only 23% were taking a high intensity statin (atorvastatin ≥40mg, rosuvastatin ≥20mg, or simvastatin 80mg). The percentage of patients achieving an LDL-C level <100 mg/dl was 54% overall and ranged from 23% for FH to 58% for ACS. When an LDL-C <70 mg/dl was considered, overall achievement was 19% and ranged from 4% for FH to 20% for DM. The likelihood of achieving an LDL-C <70 mg/dl was significantly higher among patients covered with a statin (p <0.0001).

Conclusion: Overall statin use in the cohort was modest with only 13% taking high intensity statins. The majority of high CV risk patients did not achieve an LDL-C <70 mg/dl, the goal for this population according to European Society of Cardiology guidelines. The treatment goal for this
population in the Netherlands is LDL-C <100 mg/dl, which was achieved by 54% of patients.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Novel aortic dissection model by pharmacologically-induced endothelial dysfunction

Abstract nr. 230
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Blood pressure, Cardiovascular Disease, Endothelium

Aortic dissection (AD) is life-threatening aortic disease which has only surgical operation or antihypertensive drug as therapeutic strategies. AD is considered to be based on hypertension and degradation of media as well as aortic aneurysm progression. Recently, it has been reported that endothelial dysfunction also might be necessary for AD onset. However, since appropriate AD model animals have not been developed yet, the detailed pathological mechanism of AD is still unknown. Therefore, we tried to develop a novel mice model showing a high rate of AD and examine the involvement of endothelial dysfunction in AD onset.

To induce endothelial dysfunction, Nω-nitro-l-arginine methyl ester (l-NAME), a nitric oxide synthase (NOS) inhibitor, was used. Ten mg/kg/day of l-NAME were orally administered in drinking water to C57BL/6 mice from the age of 7 weeks. Three weeks later, the administration of angiotensin II (Ang II) (1000 ng/kg/min, 6 weeks) and β-aminopropionitrile (BAPN) (150 mg/kg/day, 2 weeks) were performed with the osmotic mini pumps. This protocol, Ang II plus BAPN administration has been established as a pharmacologically-induced aortic aneurysm model (Kanematsu et. al. Hypertension. 2010). Incidence of AD was determined by the formation of false lumen under Elastica van Gieson’s staining. Incidence of AD and lethal rupture was significantly increased in Ang II, BAPN and l-NAME (ABL) group compared with Ang II and BAPN (AB) group. Vascular cell adhesion molecule-1 (VCAM-1) expression, matrix metalloproteinase (MMP)-2/9 activities and the expressions of inflammatory cytokines were also significantly increased in ABL group.

Since pitavastatin is known to have endothelial protective effect, the effect of pitavastatin on our novel AD model was examined to clarify the involvement of endothelial dysfunction on AD onset. Administration of pitavastatin significantly suppressed the incidence of AD and rupture compared to ABL group. With Griess method, it was observed that NO production in aorta was increased in pitavastatin administered mice compared to control or l-NAME treated mice.

Our present study proposed the novel pharmacologically-induced AD model mice, which might be involved in endothelial dysfunction. In the future, our AD model should be useful for understanding of AD mechanisms and developing new therapeutic strategies.
Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
Adherence to national guideline in primary and secondary prevention of cardiovascular diseases in The Netherlands: the LifeLines cohort study

Abstract nr. 231
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Co-author(s) - Kamphuisen , Pieter W
Co-author(s) - Kuivenhoven , Jan Albert
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease,Dyslipidemia,Epidemiology,Prevention

Objective: Cardiovascular disease (CVD) is the leading cause of death worldwide. While there is indisputable evidence that statin treatment reduces the burden of CVD, undertreatment remains an issue of concern in primary and secondary prevention. The aim of this study was to assess the use of lipid-lowering drugs (LLD) among 70.292 participants in The Netherlands as a proxy of adherence to the national guideline for prevention and treatment of CVD.

Methods: LifeLines is a population-based prospective cohort study in the three Northern provinces of The Netherlands. At baseline, all participants completed questionnaires, underwent a medical examination and lab testing. In those participants who did not report CVD, the 10-year risk of cardiovascular morbidity or mortality was estimated. Subsequently, we assessed how many participants were eligible to use LLD and then analyzed how many indeed reported LLD use.

Results: In primary prevention, 23% (753 of 3268) of those eligible for LLD, reported treatment with LLD, while in secondary prevention this was 69% (899 of 1302). In primary prevention, patients with diabetes mellitus were best treated (67%). Notably, of the patients with stroke, only 47% (182 of 386) reported LLD.

Conclusions: Despite clear guidelines and multiple national initiatives to improve CVD risk management, adherence to guidelines for the treatment of CVD in The Netherlands remains a major challenge. This study calls out for improving public awareness and improving primary and secondary care to prevent unnecessary CVD related morbidity and death.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Effect of a low-fat spread with added plant sterols on biomarkers of endothelial dysfunction and low-grade inflammation in hypercholesterolemic subjects

Abstract nr. 232
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Co-author(s) - Greyling, Arno
Co-author(s) - Wagner, Frank
Co-author(s) - Trautwein, Elke
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Inflammation, Intervention, Lifestyle

Aim: Plant sterols (PS) lower LDL-cholesterol, an established risk factor for coronary heart disease. Endothelial dysfunction and low-grade inflammation are two important features in the development of atherosclerosis. Data on the effect of PS on biomarkers of endothelial function and/or low-grade inflammation are scarce. The aim of the current study was to investigate the effect of regular intake of PS on biomarkers of endothelial dysfunction and low-grade inflammation.

Methods: This study was designed as a double-blind, randomized, placebo-controlled, parallel-group study. After a 4-week run-in period, 240 hypercholesterolemic but otherwise healthy men and women consumed a low-fat spread with added PS (3 g/d) or a placebo spread for 12 weeks. Endothelial dysfunction biomarkers (von Willebrand factor, soluble intracellular adhesion molecule 1 (sICAM-1), soluble endothelial-selectin and soluble vascular cell adhesion molecule 1 (sVCAM-1)) and low-grade inflammation biomarkers (C-reactive protein, serum amyloid A, interleukin-6, interleukin-8, tumour necrosis factor-α and sICAM-1) were measured using a multiarray detection system based on electro-chemiluminescence technology. Biomarkers were available for a subset (n=100) of the population and were combined into z-scores. This study was registered at clinicaltrials.gov (NCT01803178).

Preliminary results: The intake of PS did not significantly affect most of the biomarkers of endothelial dysfunction and low-grade inflammation. Only sVCAM-1 was significantly reduced (log(sVCAM-1) = -0.08 ng/mL (95%CI: -0.14; -0.02)) after PS intake compared to placebo. The z-scores for endothelial dysfunction (-0.12; 95%CI: -0.28; 0.04) and low-grade inflammation (-0.12; 95%CI: -0.32; 0.07) were not significantly changed after PS intake compared to placebo.

Conclusions: Biomarkers of endothelial dysfunction and low-grade inflammation were not significantly affected upon regular PS intake in hypercholesterolemic men and women.
Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Abstract nr. 233
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Co-author(s) - Akhvlediani , Manana
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease,Dyslipidemia,HDL,Metabolism

The purpose of study was to assess the feasibility and clinical benefit of application "Flavocitrin"-citrus extract, that contains polyphenol hesperidin and polymethoxylised flavonoids (nobiletin and tangeritin) to lipidlowering therapy of patients who fail to achive LDL cholesterol target on maximal statin dosing regimen and to statin intolerant patients.

A two-month combined therapy with flavocitrin and statins was performed in 25 CHD patients with metabolic syndrome, in whom long-term statin therapy did not provide sustained inhibition of lipid disorder (I group); 19 patients that appiered intolerant to statin therapy were given flavocitrin as monotherapy (IІ group). 32 similar patients who received only statins during two-month, formed the control group (ІІІ).

All participants underwent measurement of plasma lipoprotein profile, high sensitive CRP, fibrinogen (Fi), parameters of hemostasis, C-peptide, lipid hydroperoxide (LPO) as the direct indicator of lipid peroxidation degree.

In groups of patients receiving flavocitrin (І,ІІ) statistically significant reduction of plasma LPO meanings (P <0.001) and inflammatory markers were revealed on the background of relatively unaltered cholesterol particles, and increase (P>0.05) of HDL-cholesterol. This tendency was mostly expressed in insulin-resistant patients. In this regard, four-week flavocitrin therapy proved to be protective in prevention of oxidative stress and further progression of dyslipidemia. The opposite response was brought out in control group where average values of LPO activity, HDL, LDL, CRP and Fi remained almost unchanged.

Data presented in current study display the significant efficacy of pharmacotherapy with F when given as monotherapy as well as in combination with statines in management of dislipidemia and metabolic disorders. Action of citrus flavonoids appear to realize mainly by trigering special mechanisms of antioxidant protection through inhibition of LDL oxidation, platelet aggregation and anti-inflammatory effect on vascular endothelium.

Our results emphasize the need for further investigation of the associated therapy with citrus flavonoids and statins in patient populations with high susceptibility to LDL oxidation, notably individuals at high CV risk.

Subdivision 3. Clinical Studies
Presentation Preference Electronic poster presentation
Additional information
Analysing endothelial dysfunction in type2 Diabetes Mellitus patients using Flow Mediated Dilatation score.

Abstract nr. 235
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Co-author(s) - Patel , Feny
Co-author(s) - Kadri , Mahira
Co-author(s) - Patel , Swati
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Cardiovascular Disease,Endothelium,Inflammation

Background:
The risk of CVD in type2 diabetes mellitus is 4% higher than in healthy counterparts. The first stage to CVD is endothelial dysfunction along with vascular injury leading to atherosclerotic changes in the tunica intima of the arteries. Endothelial dysfunction can be analysed to diagnose early atherosclerotic changes and arterial stiffness. Roles of hyperglycemia, diabetic dyslipidemia and inflammation in the acceleration of vascular injury can be detected earlier and treated so as avoid severe cardiac events.

Objective:
To determine early stage CVD risk in type 2 DM patient using FMD (flow mediated dilatation) score – correlating it with acceleration of inflammatory process of vascular injury.

Method:
A total of 50 (36 F and 14 M) patients with type 2 DM of more than 10 years with age above 50years were screened for FMD score along with 30 (17 F 13 M) healthy controls, using Angiodefender ( Everist Genomics Ann Arbor, MI, USA). Pro inflammatory cytokines- TNF alpha, IL-6, IL-1 were measured using standard ELISA kits. As surrogates for disease activity C-reactive protein and ESR levels were determined. A Framingham risk score was also assessed in order to evaluate coronary heart disease risk at 10years in per cent.

Result:
The FMD score in 50 type 2 DM patients showed that 70% (n=35) patients suffered from impaired endothelial function and increased arterial thickness; 16% (n=8) patients suffered from endothelial dysfunction, arterial stiffness and atherosclerosis whereas the remaining 14% had normal endothelial function. On the other hand in the healthy counterparts, the FMD score was normal in 80% (n=24) patients. The pro inflammatory cytokines were either normal or high in the patients with impaired endothelial function. C-reactive proteins and ESR levels also varied from high to normal in patients with endothelial dysfunction. The Framingham risk score also matched with 20% high risk in patients with lower flow mediated dilatation.

Conclusion:
The flow mediated dilatation score can be effectively used as a marker to determine the vascular injury and endothelial dysfunction in patients with type2 DM.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Predicted reduction in the risk of cardiovascular events in patients treated with high-intensity statins

Abstract nr. 236
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Co-author(s) - Nicholls, Stephen
Co-author(s) - Lundman, Pia
Co-author(s) - Barter, Philip
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, LDL, Pharmacology

The 2010 Cholesterol Treatment Trialists’ Collaboration (CTTC) meta-analysis of 26 clinical trials determined the reduction in the risk of major vascular event (MVE), coronary heart disease (CHD) death, death by other cardiac causes and all-cause mortality for every 1.0 mmol/L (38.7 mg/dL) statin-mediated reduction in low-density lipoprotein-cholesterol (LDL-C). We aimed to determine the potential impact of high-intensity statin therapy on cardiovascular events by applying average statin-induced LDL-C reductions to the results from the CTTC meta-analysis. The least-squares mean (LSM) change in LDL-C was determined using 6735 patient exposures to atorvastatin 40 and 80 mg and rosuvastatin 20 and 40 mg from 12 randomised clinical trials included in the VOYAGER database. The resulting predicted risk reduction with high-intensity statin therapy was then estimated using the results from the CTTC meta-analysis. LSM reductions in LDL-C for atorvastatin 40 and 80 mg were 2.0 mmol/L and 2.2 mmol/L, respectively. LSM reductions in LDL-C for rosuvastatin 20 and 40 mg were 2.2 mmol/L and 2.4 mmol/L, respectively. The estimated rate ratios achieved with these high-intensity statins were substantial and consistently below 1.0. The table shows the individual rate ratios for atorvastatin 40 and 80 mg and rosuvastatin 20 and 40 mg. In conclusion, taking into account the considerable number of people who are candidates for statin therapy, this analysis indicates that high-intensity statins have the potential to prevent a substantial number of cardiovascular events. Additionally, these results show that the magnitude of risk reduction is dependent on the choice and dose of statin therapy.

Analysis and medical writing support was funded by AstraZeneca.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information

<table>
<thead>
<tr>
<th></th>
<th>ATV 40 mg</th>
<th>ATV 80 mg</th>
<th>RSV 20 mg</th>
<th>RSV 40 mg</th>
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<tbody>
<tr>
<td>Estimated rate ratios (95% CI) achieved using LSM change in LDL-C</td>
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</tr>
<tr>
<td>MVE</td>
<td>0.60</td>
<td>0.58</td>
<td>0.58</td>
<td>0.55</td>
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<tr>
<td>(0.56–0.66)</td>
<td>(0.53–0.63)</td>
<td>(0.53–0.63)</td>
<td>(0.50–0.60)</td>
<td>(0.50–0.60)</td>
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<tr>
<td>All-cause mortality</td>
<td>0.81</td>
<td>0.79</td>
<td>0.79</td>
<td>0.77</td>
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<tr>
<td>(0.76–0.86)</td>
<td>(0.74–0.84)</td>
<td>(0.75–0.84)</td>
<td>(0.72–0.83)</td>
<td>(0.72–0.83)</td>
</tr>
<tr>
<td>CHD death</td>
<td>0.64</td>
<td>0.61</td>
<td>0.61</td>
<td>0.58</td>
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<tr>
<td>(0.59–0.68)</td>
<td>(0.57–0.65)</td>
<td>(0.57–0.66)</td>
<td>(0.54–0.63)</td>
<td>(0.54–0.63)</td>
</tr>
<tr>
<td>Death by other cardiac causes</td>
<td>0.79</td>
<td>0.77</td>
<td>0.77</td>
<td>0.75</td>
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<tr>
<td>(0.75–0.83)</td>
<td>(0.73–0.81)</td>
<td>(0.74–0.82)</td>
<td>(0.71–0.80)</td>
<td>(0.71–0.80)</td>
</tr>
</tbody>
</table>

Published rate ratios per 1.0 mmol/L (38.7 mg/dL) LDL-C reduction: MVE=0.78 (95% CI 0.76–0.80); all-cause mortality=0.90 (95% CI 0.87–0.93); CHD death=0.80 (95% CI 0.74–0.87); and death by other cardiac causes=0.89 (95% CI 0.81–0.98).

*Calculated using the delta method; *Includes major coronary events, coronary revascularisation, or stroke.
Predicted impact of statins on 10-year atherosclerotic cardiovascular disease risk: Results from VOYAGER

Abstract nr. 237
Author Palmer, Michael, Keele, United Kingdom
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Co-author(s) - Barter, Philip
Co-author(s) - Karlson, Björn
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Dyslipidemia, LDL

**Background:** Reductions in low-density lipoprotein cholesterol (LDL-C) resulting from statin therapy have been shown to lead to reductions in atherosclerotic cardiovascular disease (ASCVD) risk. We used data from the VOYAGER meta-analysis database, comprising 32,258 patients from 37 randomised trials, to estimate the reduction in 10-year ASCVD risk as a result of lipid parameter modification with three different statins and doses.

**Methods:** 29,486 patients who would be considered candidates for high-intensity statin therapy, as defined by the 2013 American College of Cardiology/American Heart Association (ACC/AHA) blood cholesterol guideline, were selected from the VOYAGER database. Using the 2013 ACC/AHA assessment of cardiovascular risk guideline and based on a patient’s total cholesterol and high-density lipoprotein cholesterol levels, the change in ASCVD risk was calculated as a ratio of on-treatment to baseline risk for each patient. Least-squares mean (LSM) ratios of 10-year ASCVD risk were calculated for atorvastatin 10–80 mg, rosuvastatin 5–40 mg and simvastatin 10–80 mg. Hypothetical cohorts of 100,000 subjects each were created, with different baseline risks ranging from 7.5% to 80%. Within each cohort, the potential numbers of ASCVD events prevented were estimated for each statin and dose.

**Results:** The LSM ratios of 10-year ASCVD risk for atorvastatin 10–80 mg, rosuvastatin 5–40 mg and simvastatin 10–80 mg are shown in the table. With increasing baseline ASCVD risk and increasing statin dose, an associated increase in the predicted number of ASCVD events prevented over a 10-year period was observed (Figure).

**Conclusion:** Reductions in the number of predicted ASCVD events is dependent on baseline ASCVD risk and the choice and dose of statin. Effective statin therapy can potentially prevent a clinically significant number of ASCVD events, particularly in patients at high ASCVD risk. These results highlight the importance of using the appropriate intensity statin, and the benefits of the use of high-intensity statins, defined by the 2013 ACC/AHA blood cholesterol guideline as rosuvastatin 20 and 40 mg and atorvastatin 40 and 80 mg, in moderate-to-high risk patients.

Analysis and medical writing support was funded by AstraZeneca.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation

Additional information
Lipid Abnormalities Remain High among Treated Hypertensive Patients with Stable CHD: Results of the Dyslipidemia International Study (DYSIS) II Belgium

Abstract nr. 238
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Hypertension, LDL, Lipids

Background: Despite treatment with lipid lowering therapy (LLT), elevated lipid abnormalities persist among hypertensive patients with coronary heart disease (CHD), putting them at risk for future cardiovascular events. Both hypertension and hyperlipidemia are very frequent comorbidities among CHD patients and therefore tight control of both should be targeted.
Objective: To identify the prevalence of lipid abnormalities and unmet needs among hypertensive patients with stable CHD in Belgium currently receiving LLT.
Methods: DYSIS II is a multicenter, observational cross-sectional study conducted from May-September 2013 in 10 outpatient care centers in Belgium. Eligible adult patients had a documented history of CHD (past acute coronary syndrome (ACS) events >3 months before enrollment), full lipid profile available 0-12 months prior to enrollment, on LLT for ≥3 months, and were not participating in randomized clinical trials involving medication. Patient characteristics, risk factors, treatment patterns, and laboratory values were collected. LDL-C lipid target achievement was assessed based on ESC/EAS guidelines. Patients were identified as having hypertension based on data collected through the study case report form.
Results: Among 265 hypertensive stable CHD patients currently on LLT (80.4% male, mean age 70.3 ± 9.5 years), 98.5% had hypercholesterolemia, 87.9% previous percutaneous coronary intervention or coronary artery bypass graft, 59.2% history of ACS, 56.7% led a sedentary lifestyle, 51.2% family history of CHD, 50.2% were former smokers, 47.5% type 2 diabetes mellitus, and 9.8% were current smokers. Half the patients had a blood pressure measurement <140/<90 mmHG (systolic/diastolic), with 97.4% (n=258) receiving antihypertensive therapy. 113 (42.6%) patients achieved LDL-C <70 mg/dl while being treated with the following LLT: 82.3% statin monotherapy, 10.9% statin plus ezetimibe, 5.3% combination statin plus other non-statin, and
1.5% non-statin monotherapy. Mean atorvastatin equivalent dose was 28 ± 22 mg/day. Patients mean lipid values are provided in Table 1.

**Conclusion:** Overall, mean LDL-C values were approximately 5.2 mg/dl from recommended LDL-C target, with about 57% of LLT treated hypertensive stable CHD patients in Belgium not achieving the recommended target. Additional effective lipid lowering strategies are needed among these very high risk patients to prevent future cardiovascular events.

<table>
<thead>
<tr>
<th>Table 1: Mean lipid profiles</th>
<th>LLT Treated Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low density lipoprotein (LDL) cholesterol</td>
<td>75.2 ± 24.6 mg/dl</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>150.2 ± 33.1 mg/dl</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>135.2 ± 88.1 mg/dl</td>
</tr>
<tr>
<td>Non-HDL cholesterol</td>
<td>101.4 ± 32.8 mg/dl</td>
</tr>
</tbody>
</table>

**Table 1: Mean lipid profiles**

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
**LDL-C Target Attainment among Treated ACS Patients in Hong Kong and Taiwan: The Dyslipidemia International Study (DYSIS) II ACS Results**

Abstract nr. 239  
Author Yan , Bryan P., Chinese University of Hong Kong, Hong Kong, Hong Kong  
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Co-author(s) - Horack , Martin  
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Co-author(s) - Brudi , Philippe  
Co-author(s) - Balaji , Hiremagalur Parthasarathy  
Co-author(s) - Leung , Michael  
Co-author(s) - Ambegaonkar , Baishali  
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins  
Keywords ACS,Dyslipidemia,LDL,Lipids

**Background:** Patients presenting with acute coronary syndrome (ACS) remain at very high risk of future cardiovascular events. Providing optimal therapy for effective control of risk factors including dyslipidemia, hypertension and diabetes is critical to reduce future complications.  

**Objective:** To identify the prevalence of lipid abnormalities and therapeutic gaps among ACS patients receiving lipid lowering therapy (LLT) in Hong Kong and Taiwan.  

**Methods:** DYSIS II is a multinational, observational cross-sectional study conducted in 18 hospitals in Hong Kong (6 centers) and Taiwan (12 centers). Consecutive adult patients hospitalized for an ACS event with full lipid profiles available within 24 hours of admission, on LLT ≥3 months or not treated at all, not participating in randomized clinical trials involving any medication, and alive at discharge were eligible. Patient characteristics, risk factors, treatment patterns, and laboratory values were collected. Low density lipoprotein cholesterol (LDL-C) target attainment was assessed based on 2011 ESC/EAS guidelines.  

**Results:** Among 270 ACS patients (76.3% male, mean age 64.4 ± 11.9 years), 65.2% had hypertension, 50.6% hyperlipidemia, 44.7% metabolic syndrome, 40.4% led a sedentary lifestyle, 39.2% type 2 diabetes, 32.2% history of coronary heart disease (CHD), 26.7% current smokers, and 26.7% had a family history of CHD. 125 (46.3%) patients were on LLT: 92.8% statin monotherapy, 4.0% statin plus ezetimibe, 2.4% statin plus other non-statin, and 0.8% non-statin monotherapy. Mean atorvastatin equivalent dose was 14 ± 12 mg/day. Among LLT patients, only 28.8% were able to reach the <70 mg/dl LDL-C target (mean LDL-C 89.6 ± 35.0 mg/dl).  

**Conclusion:** Overall, mean LDL-C values were approximately 20 mg/dl from recommended LDL-C target with over 70% of LLT treated ACS patients in Hong Kong and Taiwan not reaching the recommended target. Additional effective lipid lowering strategies for LDL-C target attainment are needed among these very high risk patients.
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Serum matrix metalloproteinase 8 and complement system activation in acute coronary syndromes

Abstract nr. 240
Author Salminen, Aino, University of Helsinki, Helsinki, Finland
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, Immunity, Inflammation

The complement system has a central role in innate immunity and contributes to various diseases, including cardiovascular diseases. Complement is extensively activated in atherosclerotic lesions, in thrombosis, and in the myocardium of ischemic hearts. Complement component C3 is the central component in complement activation. Serum matrix metalloproteinase (MMP)-8 and tissue inhibitor of metalloproteinase (TIMP)-1 are promising novel biomarkers of cardiovascular diseases. MMP-8 is a collagen-degrading enzyme that is present in atherosclerotic lesions. The expression and activation of MMP-8 is increased during inflammation. However, the origin of serum MMP-8 is not completely clear.

We studied the association of serum concentrations of C3 and C4, and CRP, MMP-8 and TIMP-1 in 343 patients with acute coronary syndrome (ACS) and 326 healthy controls matched by age and sex.

The median concentrations of MMP-8 were 47 ng/ml in healthy subjects, 82 ng/ml in patients with unstable angina pectoris (UAP), and 124 ng/ml in patients with myocardial infarction (MI) (p < 0.001). The concentrations of TIMP-1 and MMP-8/TIMP-1 molar ratios were also higher in patients with UAP and AMI compared to controls. Mean C3 concentrations were 1.87 mg/ml in healthy subjects, 1.66 mg/ml in patients with UAP, and 1.69 mg/ml in patients with AMI (p < 0.001). Serum C3 levels were inversely correlated with serum MMP-8 levels (r = -0.18, p < 0.001). C4 concentrations did not differ between the groups and they did not correlate with MMP-8. Serum MMP-8 was correlated with CRP (r = 0.39, p < 0.001), but serum C3 did not show correlation with CRP. In a multivariate model adjusted for age, gender, and smoking, the concentrations of MMP-8 (directly), CRP (directly), and C3 (inversely) remained significantly associated with ACS.

Decreased concentrations of C3 in serum reflect the activation of the complement system in patients with ACS. Serum MMP-8 may be associated with complement activation, but it also has an independent association with ACS.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
The cut-off values of anthropometric indices for identifying subjects at risk for metabolic syndrome in Iranian elderly men.

Abstract nr. 241
Author Gharipour, Mogan, Isfahan, Iran
Co-author(s) - Sadeghi, masoumeh
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, Metabolism, Obesity

Aim: This study aimed to investigate which anthropometric indices could be a better predictor of metabolic syndrome (MetS) and the cut-off points for these surrogates to appropriately differentiate MetS in the Iranian elderly. METHOD: The present cross-sectional study was conducted on a sample of Isfahan Healthy Heart Program (IHHP). MetS was defined according to Third Adult Treatment Panel (ATPIII). In total, 206 elderly subjects with MetS criteria were selected. Anthropometric indices were measured and plotted using receiver operating characteristic (ROC) curves. RESULTS: WC followed by WHtR yielded the highest area under the curve (AUC) (0.683; 95% CI 0.606-0.761 and 0.680; 95% CI 0.602-0.758, resp.) for MetS. WC at a cut of 94.5 cm resulted in the highest Youden index with sensitivity 64% and 68% specificity to predict the presence of ≥2 metabolic risk factors. BMI had the lowest sensitivity and specificity for MetS and MetS components. WC has the best ability to detect MetS which followed by WHtR and BMI had a lower discriminating value comparatively. CONCLUSION: WC is the best predictor for predicting the presence of ≥2 metabolic risk factors among Iranian elderly population and the best value of WC is 94.5 cm. This cut-off values of WC should be advocated and used in Iranian men until larger cross-sectional studies show different results.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Oxidised low-density lipoproteins and hyperlipidaemia cause platelet activation through a pathway that requires CD36 and phospholipase C?2

Abstract nr. 242
Author Woodward, Casey, University of Hull, Hull, United Kingdom
Co-author(s) - Wraith, Katie
Co-author(s) - Naseem, Khalid
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, LDL, Thrombosis

Introduction: Dyslipidaemia and particularly increased circulating low-density lipoproteins (LDL) are contributing factors to atherosclerosis. LDL can become trapped vessel walls where it becomes oxidatively modified (oxLDL) and contributes to the formation of atherosclerotic plaques. However, recent evidence suggests that patients with established cardiovascular disease have oxLDL circulating in the bloodstream. OxLDL is ligand for the scavenger receptor CD36 which is present on the surface of platelets and macrophages. Previously we have shown that oxLDL binding to CD36 induces multiple activatory responses including secretion, shape change and aggregation through a tyrosine kinase dependent mechanism.

Aim: To assess the molecular pathways responsible for oxLDL-mediated platelet hyperreactivity with an emphasis on the role of phospholipase C γ2 (PLCy2).

Results: Immobilised oxLDL, but not nLDL, supported adhesion, activation and spreading of human platelets. The incubation of platelets with the pan src family kinase and pan phospholipase C inhibitors blocked platelet spreading but not adhesion. To determine whether PLCy2 was responsible, we immunoprecipitated PLCy2 from human platelets stimulated with oxLDL and probed for phosphorylation status. PLCy2 was phosphorylated on tyrosine (tyr)\(^{753}\) and tyr\(^{759}\) by oxLDL in a time and dose dependent manner. Using an assay for IP\(_3\) as a marker of PLC activity, we show that oxLDL triggers activation of PLC y2. Pharmacological blocking of CD36 with the antibody FA6.152 or lipid SSO prevented phosphorylation of PLCy2. Similarly the phosphorylation and activation of PLCy2 was prevented by inhibitors of Src family and Syk kinases. In order to confirm these findings, we used PLCy2\(^{-/-}\) mice. PLCy2\(^{-/-}\) mice showed diminished phosphotyrosine profiles upon stimulation with oxLDL and did not spread under static conditions compared with wild type controls.

Conclusion: This study has begun to show that oxLDL stimulates the activation of PLCy2 through a CD36-Src-Syk pathway and this may offer further insight as to how hyperlipidaemia may promote platelet hyperreactivity.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Pivotal role of microRNA-33 in metabolic syndrome

Abstract nr. 244
Author Gharipour, Mogan, Isfahan, Iran
Co-author(s) - Sadeghi, masoumeh
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Cardiovascular Disease, Genetics, Metabolism

Metabolic syndrome (MetS) is a major public health concern and increase in the incidence of MetS caused a rise in the rates of global morbidity, and mortality due to cardiovascular disease and diabetes. Lifestyle modification, a healthy diet, and pharmacological treatment and bariatric surgery are recommended in order to control this syndrome. Molecular mechanisms of metabolic disorders are essential in order to develop novel, valid therapeutic strategies. MicroRNA-33 plays imperative regulatory roles in a variety of biological processes including collaboration with sterol regulatory element-binding protein (SREBP) to maintain cholesterol homeostasis, high-density lipoprotein formation, fatty acid oxidation, and insulin signaling. Investigation of these molecules and their genetic targets may potentially identify new pathways involved in complex metabolic disease processes, improve our understanding of metabolic disorders, and influence future approaches to the treatment of obesity. This article reviews the role of miRNA-33 in metabolic syndrome, and highlights the potential of using miRNA-33 as a novel biomarker and therapeutic target for this syndrome.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
Changes in HDL Function in Acute Inflammation as Revealed by Optimized Zymogram to Study PON1 in Subclasses: Pilot Study

Abstract nr. 246
Author Alejandro, Gugliucci, Touro University-California, Vallejo, U.S.A.
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Cardiovascular Disease, Functionality, HDL

Background: Solving the HDL paradox will necessitate the development of techniques to explore HDL function that are practical and well adapted to clinical studies and eventually become useful in patient monitoring. PON1 is a key player in HDL function and its activity is modified by inflammation/acute phase. To make some inroads into studying active PON1 distribution across HDL subclasses we had previously developed a zymogram method that we validated and employed in several studies.

Objective: To modify and optimize our method and employ it in a pilot study to explore HDL PON1 function changes in acute inflammation, as a model we employed post cerebrovascular accident (CVA) patients. We hypothesize that with this methodology we can detect changes in PON1 distribution in these patients after the episode and follow their temporal course.

Material and Methods: Native lipoproteins from serum are separated in a 4-12% gradient gel and activity is detected in situ using para-nitro-phenylacetate, scanning and densitometry. A total of 10 patients (men/women = 6/4, mean age 66.0 ± 12.0 years), diagnosed with ischemic CVA were studied. The study was approved by the Ethics Committee of Showa University. Blood examinations were performed at 3 sequential points (i.e., admission, 1 day, 7 days).

Results: The new method allows for a 1 step, shorter zymogram process as compared to our previous procedure that needed a coupled reaction. When we applied it to acute post-CVA patients the method shows that the significant drops in PON1 1 day after the event (25 +/- 7 %, p 0.03) correspond to changes in PON1 distribution in HDL subclasses or to specific loss of activity in very large and very small HDL. The changes are more apparent for HDL3, with recovery after one week paralleled by changes in CRP.

Conclusions: We have developed a practical, 1 step zymogram method to measure PON1 activity in HDL subclasses. With this tool the effects of inflammation on HDL antioxidant function can be followed up. Our pilot, proof of principle data show quick deleterious effects of acute phase and inflammation on PON1 distribution in HDL particles.
Figure 3. PON1 activity in HDL subclasses separated by native gradient electrophoresis gels

PON1 zymogram of 2 representative subjects at admission (0) 1 and 7 days. HDL were separated by their hydrodynamic diameter in an 8 × 10 × 0.15 cm non-denaturing 4-12% gradient polyacrylamide gel electrophoresis (Novex® 4-12% Tris-Glycine gel, Invitrogen, Carlsbad, CA, USA). PON1 activity in lipoprotein subclasses was determined by the enzymatic detection of PON1 hydrolysis of paranitrophenylacetate in situ. Note (arrows) that the drop in PON1 total activity as shown in Figure 1 is accompanied by reduced PON1 activity in both very large HDL2 and small HDL3, suggesting changes in HDL maturation that produce dysfunction. Note that the changes are transient and tend to recover in 7 days.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Macrophage Notch1 promotes neointima formation in mechanically-injured femoral arteries

Abstract nr. 247
Author Koga, Jun-ichiro, Kyushu University, Fukuoka, Japan
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Inflammation, Intervention

Background: Restenosis remains a major complication of vascular intervention. Activated macrophages accumulating in injured vessels promote neointima formation. We previously demonstrated that the Notch ligand Delta-like 4 (Dll4) activates macrophages and accelerates atherogenesis, but the role of each Notch receptor expressed by macrophages in the pathogenesis of restenosis is unknown. The present study tested the hypothesis that macrophage Notch1 promotes neointima formation after vascular injury.

Method and results: Using the Cre/LoxP system, we established conditional mouse strains that lack or overexpress Notch1 in a myeloid cell lineage, mostly macrophages in vascular lesions (MΦ-Notch1-KO and MΦ-Notch1-Tg). To clarify the role of macrophage Notch1 in vascular disease, we used a wire injury model in these mice. Notch1 deletion suppressed neointima formation in femoral arteries, while Notch1 overexpression tended to accelerate lesion development (the intima/media ratio: Control, 1.9±0.1; MΦ-Notch1-KO, 1.0±0.2*; MΦ-Notch1-Tg, 2.5±0.2; N=8-9, *p<0.01). Macrophage accumulation to the injured arteries was lower in MΦ-Notch1-KO mice compared to Control, especially in the adventitia. The expression of monocyte chemoattractant protein-1 (MCP-1) mRNA was lower in injured arteries of MΦ-Notch1-KO mice. Peritoneal macrophages from MΦ-Notch1-KO mice showed decreased chemotactic activity to MCP-1 in vitro. In the macrophage cell line RAW264.7, enforced expression of constitutively active Notch1 induced pro-inflammatory molecules IL-1β, IL-6, MCP-1 and iNOS, typical markers of M1 macrophages. Conditioned media (CM) from control macrophages induced growth and migration of cultured smooth muscle cells (SMC), but these effects were suppressed in CM from Notch1-KO macrophages. Furthermore, we crossed MΦ-Notch1-KO and MΦ-Notch1-Tg mice with LDL receptor-deficient mice and fed a high cholesterol diet. In this hyperlipidemic condition, deletion of macrophage Notch1 also decreased neointima formation and macrophage accumulation (the intima/media ratio: Control, 3.1±0.3; MΦ-Notch1-KO, 1.4±0.3*; MΦ-Notch1-Tg, 2.4±0.3; N=9-11 each; *p<0.001 vs. Control).

Conclusion: Macrophage Notch1 promotes the development of vascular lesions, offering a new therapeutic target.
Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Combination EZ/Prava is superior to Pravastatin for suppressing Carotid atherosclerosis of patients with Hypercholesterolemia

Abstract nr. 248
Author Sawayama, Yasunori, Fukuoka, Japan
Co-author(s) - Hatashima, Azusa
Co-author(s) - Sasaki, Jun
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Dyslipidemia,Lipids,Therapy

Objective: We compared the effectiveness of pravastatin plus ezetimibe with that of pravastatin for preventing an increase of carotid artery intima-media thickness (IMT) in patients with hypercholesterolemia.

Methods: This study was a single center, open-label, parallel-group trial. Sixty subjects with hypercholesterolemia and LDL cholesterol levels ≥120 mg/dL on pravastatin therapy were randomized to either continue pravastatin at 5-10 mg/day combined with ezetimibe at 10 mg/day (EZ/Prava, n=33) or to receive a dose of pravastatin at 10-20 mg/day (Prava, n=27). The primary endpoint was the change of the carotid IMT after 24 months.

Results: LDL cholesterol showed a significant decrease from baseline in both groups after 24 months, with decreases of 25.1% (p<0.001) and 6.3% (p=0.026) in the EZ/Prava and Prava, respectively. The IMTs at 24 months were 1.10±0.21 mm (-5.2% vs. baseline, p=0.0012) and 1.09±0.30 mm (-2.1% vs. baseline, p=0.07) in the EZ/Prava and Prava, respectively. There was a significantly greater decrease in the compared with the Prava (p=0.019).

Conclusions: Compared with Prava, EZ/Prava had a superior effect on LDL cholesterol and more effectively controlled the progression of IMT during two years of treatment.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Relationship of oxidative-antioxidants changes of LDL with coronary heart disease and some atherosclerosis risk factors in men population of Novosibirsk

Abstract nr. 249
Author Ragino, Yulia, Institute of Internal and Preventive Medicine of SB of RAMS, Novosibirsk, Russian Federation
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Co-author(s) - Sherbakova, Lilia
Co-author(s) - Malyutina, Sofia
Co-author(s) - Voevoda, Mikhail
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Epidemiology, LDL, Risk Factor

Associations of coronary heart disease (CHD) and certain atherosclerosis risk factors with parameters of atherogenic oxidation-antioxidant changes in low density lipoprotein (LDL) in men population were studied.

A population-based survey of 1024 Novosibirsk men 47-73 years old was performed. Program of the survey included the questionnaires, standardized cardiological survey, anthropometry, blood pressure measurement, ECG recording. In 223 people (21.8%) had «definitely CHD» (stable angina pectoris, FC II-IV) by a validated epidemiological, clinical and functional criteria. Biochemical studies included the determinations of blood total cholesterol (CH), triglyceride (TG), high density lipoprotein cholesterol (HDL-CH), high sensitive C-reactive protein (hsCRP), baseline lipid peroxidation (LPO) and fat-soluble antioxidants in LDL, LDL resistance to oxidation, concentration of autoantibodies to oxidized LDL (oxLDL).

For the Novosibirsk male population as regional values are 10-90% cut-off point percentile distribution of studied atherogenic oxidation-antioxidant changes of LDL. Correlations were found between baseline LPO level in LDL and hsCRP level, between LDL resistance to oxidation and blood lipid profile, body mass index (BMI) and the presence of CAD, between autoantibodies to oxLDL and hsCRP level, BMI, between antioxidants content in LDL, especially alpha-tocopherol, and blood lipid profile, hsCRP level, BMI. Elevated level of LPO products in LDL, decreased antioxidants content in LDL and, especially, decreased resistance of LDL to oxidation, concentration of autoantibodies to oxidized LDL (oxLDL), independently associated with elevated levels of CH, TG, hsCRP, reduced HDL-CH, increased BMI. Positive correlations and independent associations between parameters of LDL oxidative changes, especially of decreased LDL resistance to oxidation, and CHD were revealed. Negative correlations between parameters of LDL antioxidative changes, especially of decreased LDL alpha-tocopherol content, and CHD were revealed also. The incidence of CHD is higher at index of initial LPO level in LDL >0,8 (nM MDA/mg LDL protein) and decreased LDL resistance to oxidation (at rates in initial stage of LDL oxidation >5,4 and in progressive stage of LDL oxidation >13,2). On the other hand, the incidence of CHD is lower in index of alpha-tocopherol in LDL >1,06 mg/mg protein LDL.
The results confirm the data about key atherogenic role of oxidized LDL. The study was supported by RSCF, project N 14-45-00030.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Key inflammatory biomarkers of atherosclerotic plaques instability: results of arterial wall and blood studies

Abstract nr. 250
Author Ragino, Yulia, Institute of Internal and Preventive Medicine of SB of RAMS, Novosibirsk, Russian Federation
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Co-author(s) - Polonskaya, Yana
Co-author(s) - Kamenskaya, Oxsana
Co-author(s) - Kashtanova, Elena

Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Endothelium, Inflammation, Pathogenesis, Vulnerable Plaque

The aim of the study was to revealed the key significant inflammatory, destructive, oxidative and endothelial dysfunction biomarkers of coronary atherosclerotic plaques instability and investigate their blood levels in men with coronary atherosclerosis (CA).

Concentrations of inflammatory (tumor necrotic factor, TNF-alpha, interleukins, IL-1-beta, IL-6, IL-8, IL-18, soluble ligand of CD40 receptor, sCD40L, high sensitive C-reactive protein, hsCRP, monocyte chemotactic protein-1, MCP-1, endothelial monocyte activating protein II, adhesion molecules, sICAM-1 and sVCAM-1), destructive (matrix metalloproteinase, MMP-3, MMP-7, MMP-9, tissue inhibitor of metalloproteinase, TIMP-1 and endothelin-1), oxidative (concentrations of lipid peroxidation products, LPO, including in low density lipoproteins, LDL, proteins oxidative modification, paraoxonase activity, antioxidants concentrations, lipid parameters) and endothelial dysfunction biomarkers were studied in blood and in atherosclerotic plaques of coronary artery in 84 men with CA.

Blood levels of hsCRP, IL-8, IL-6, sCD40L, oxidized LDL apolipoproteins and lipoprotein (a) were higher, but blood levels of sVCAM, TIMP-1, NO metabolites and resistance of LDL to oxidation were lower in men with prevalence of unstable atherosclerotic plaques in coronary arteries compared to men with prevalence of stable atherosclerotic plaques in coronary arteries. Blood levels of hsCRP, IL-6, IL-8, oxidized proteins, NO metabolites and sVCAM were correlated with coronary artery atherosclerotic plaques instability. Moreover, strong positive correlations of blood levels of hsCRP, IL-6, IL-8 and MCP-1 with their concentrations in coronary artery atherosclerotic plaques were revealed only.

Thus, according to study results the inflammatory biomarkers such as hsCRP, IL-6, IL-8 and MCP-1 can be a key inflammatory biomarkers of atherosclerotic plaques instability in blood.

The study was supported by Grant RFBI 09-04-00374.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Lycopene inhibits cyclic strain-induced endothelin-1 expression via induction of heme oxygenase-1 in human umbilical vein endothelial cells

Abstract nr. 251  
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Co-author(s) - Sung, Li-Chin  
Co-author(s) - Cheng, Tzu-Hurng  
Co-author(s) - Chen, Jin-Jer  
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention  
Keywords Nutrition, Pharmacology

Lycopene, the most potent active antioxidant among the major carotenoids, is associated with a reduced risk for cardiovascular diseases (CVD). Endothelin-1 (ET-1) is a powerful vasopressor synthesized by endothelial cells and plays a crucial role in the pathophysiology of CVD. However, the direct effects of lycopene on vascular endothelial cells have not been fully described. This study investigated the effects of lycopene on cyclic strain-induced ET-1 expression in human umbilical vein endothelial cells (HUVECs) and identified the signal transduction pathways that are involved in this process. Cultured HUVECs were exposed to cyclic strain in the presence or absence of lycopene, and the changes in strain-induced ET-1 expression, oxidative stress, extracellular signal-regulated kinase (ERK) phosphorylation, and heme oxygenase-1 (HO-1) induction were analyzed. Lycopene inhibited cyclic strain-induced ET-1 expression and ERK phosphorylation. Furthermore, lycopene reduced the level of cyclic strain-induced p22phox mRNA, NAD(P)H oxidase activity, and reactive oxygen species production. By contrast, lycopene treatment enhanced HO-1 expression; in addition, HO-1 silencing partially abrogated the repressive effects of lycopene on strain-induced ET-1 expression. This study reports for the first time that lycopene inhibits cyclic strain-induced ET-1 secretion via suppression of p22phox and induction of HO-1 in HUVECs. Thus, this study provides valuable new insight into the molecular pathways that may contribute to the proposed beneficial effects of lycopene on the cardiovascular system.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation

Additional information
Efficacy and safety of the PCSK9 inhibitor evolocumab in patients with mixed hyperlipidemia

Abstract nr. 252
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Co-author(s) - Djedjos , C. Stephen
Co-author(s) - Dent , Ricardo
Co-author(s) - Bridges , Ian
Co-author(s) - Pellacani , Andrea
Co-author(s) - Miller , Michael
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Dyslipidemia,LDL,PCSK9,Therapy

Background: Evolocumab (AMG 145), a fully human monoclonal antibody, inhibits PCSK9 and significantly reduces low-density lipoprotein cholesterol (LDL-C). However, the effects of PCSK9 inhibition on LDL-C lowering in patients with mixed hyperlipidemia are less well studied.

Methods: We evaluated the efficacy and safety of evolocumab in patients selected from the phase 2 and 3 trials who also had elevated triglyceride levels (≥1.70 mmol/L). Triglyceride level ≥4.5 mmol/L at screening was an exclusion criterion for these studies, but post-enrollment triglyceride levels could exceed 4.5 mmol/L. All included patients had high triglyceride values, and the mean HDL-C was low though the latter was not an inclusion criterion for the analysis.

Efficacy was evaluated in four phase 3 randomized studies (n=1148) and safety, from the phase 2 and phase 3 studies (n=2092) and their open label extension studies (n=1485). Efficacy analyses were based on 12-week studies, while safety analyses included data from all available studies.

Results: Patients with mixed hyperlipidemia taking evolocumab had mean±SD baseline LDL-C of 3.4±1.4 mmol/L, median (Q1, Q3) triglycerides of 2.0 (1.6, 2.5) mmol/L and mean±SD HDL-C of 1.2±0.3 mmol/L. The mean treatment difference in percent change from baseline in LDL-C (mean of weeks 10 and 12) with evolocumab was approximately –67% vs. placebo and –42% vs. ezetimibe (all p<0.001) (figure) compared to –65% vs. placebo and –40% vs. ezetimibe in the entire study population. Treatment differences for evolocumab vs. placebo and ezetimibe followed a similar pattern for non-HDL-C, apolipoprotein B, and lipoprotein(a) (figure). Evolocumab was well tolerated, with balanced rates of serious adverse events or adverse events leading to discontinuation of the investigational product for evolocumab vs. placebo and ezetimibe (table).

Conclusion: This analysis studied the effect of evolocumab in patients with mixed hyperlipidemia. LDL-C reduction with evolocumab in patients with mixed hyperlipidemia was similar to the LDL-C reduction seen in previous reports of the entire study population.
Figure. Reduction in Key Lipids (%) at Mean of Weeks 10 and 12 for Evolocumab vs Ezetimibe vs Placebo in Patients with Mixed Hyperlipidemia

Values are presented as mean %±standard error. All treatment differences were p<0.001. LDL-C was based on a reflexive approach. When the calculated LDL-C was <40 mg/dL or triglycerides were >400 mg/dL, calculated LDL-C was replaced with ultracentrifugation LDL-C from the same blood sample, if available.

Table. Summary of Safety Variables for Evolocumab vs. Ezetimibe or vs. Placebo in Patients with Mixed Hyperlipidemia

<table>
<thead>
<tr>
<th></th>
<th>Evolocumab</th>
<th>Ezetimibe</th>
<th>Placebo</th>
<th>Evolocumab + SOC</th>
<th>Ezetimibe + SOC</th>
<th>Placebo + SOC</th>
<th>Open-Label Extension (OSLER)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=1355)</td>
<td>(n=227)</td>
<td>(n=510)</td>
<td>(n=983)</td>
<td>(n=582)</td>
<td>(n=309)</td>
<td></td>
</tr>
<tr>
<td>All adverse events</td>
<td>607 (49.2)</td>
<td>124 (54.0)</td>
<td>236 (46.3)</td>
<td>600 (61.0)</td>
<td>275 (54.4)</td>
<td>225 (72.8)</td>
<td></td>
</tr>
<tr>
<td>Grade ≥ 2</td>
<td>253 (19.6)</td>
<td>88 (27.8)</td>
<td>120 (23.5)</td>
<td>120 (22.0)</td>
<td>133 (20.9)</td>
<td>143 (46.2)</td>
<td></td>
</tr>
<tr>
<td>Grade ≥ 3</td>
<td>55 (4.1)</td>
<td>8 (3.5)</td>
<td>23 (4.5)</td>
<td>65 (6.6)</td>
<td>33 (6.6)</td>
<td>36 (11.7)</td>
<td></td>
</tr>
<tr>
<td>Grade ≥ 4</td>
<td>7 (0.5)</td>
<td>0 (0.0)</td>
<td>4 (0.8)</td>
<td>8 (0.8)</td>
<td>3 (0.6)</td>
<td>4 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Serious adverse events</td>
<td>46 (3.4)</td>
<td>5 (2.2)</td>
<td>13 (2.5)</td>
<td>56 (5.7)</td>
<td>30 (6.0)</td>
<td>29 (9.4)</td>
<td></td>
</tr>
<tr>
<td>Leading to discontinuation of investigational product</td>
<td>21 (1.5)</td>
<td>13 (5.7)</td>
<td>8 (1.6)</td>
<td>22 (2.2)</td>
<td>NA</td>
<td>4 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Serious</td>
<td>3 (0.2)</td>
<td>0 (0.0)</td>
<td>1 (0.2)</td>
<td>6 (0.6)</td>
<td>NA</td>
<td>1 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Non-serious</td>
<td>19 (1.4)</td>
<td>13 (5.7)</td>
<td>7 (1.4)</td>
<td>15 (1.6)</td>
<td>NA</td>
<td>3 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Fatal adverse events</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (0.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

All values are presented as number of patients (%)

In the open-label extension OSler study, patients were randomized in a 2:1 ratio to evolocumab + standard of care (SOC) or SOC. At year 2, all patients randomized to evolocumab + SOC continued while SOC patients initiated evolocumab.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Is low serum HDL-C without other lipid abnormalities associated with atherosclerotic diseases? -A pooled analysis of 9 Japanese cohorts-

Abstract nr. 253
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, HDL, Lipoproteins

Background and Purpose: Serum high-density lipoprotein cholesterol (HDL-C) is known to be inversely associated with the risk of coronary artery disease (CAD). However, recent clinical trials failed to show beneficial effect by raising serum HDL-C. Because low HDL-C is usually observed in persons with other lipid abnormalities, it is necessary to examine whether isolated low HDL-C, i.e., low HDL-C without other lipid abnormalities, is associated with CAD.

Method: We conducted a large pooled analysis using individual data from nine Japanese cohorts with 41,206 participants aged 40 to 89 years who were free of CAD and stroke. We divided these participants into three groups as follows: 1) isolated low HDL-C group; defined as HDL-C <40 mg/dl in men or <50 mg/dl in women, triglycerides (TG) <150 mg/dl, and total cholesterol (TC) <240 mg/dl; 2) non-isolated low HDL-C group; defined as HDL-C <40 mg/dl in men or <50 mg/dl in women, TG ≥150 mg/dl and/or TC ≥240 mg/dl; and 3) normal HDL-C group. Sex and cohort-stratified Cox proportional hazard models were used to estimate hazard ratios (HRs) for death due to CAD and ischemic stroke after adjusting for age, body mass index, systolic blood pressure, smoking and alcohol drinking.

Results: During a 13-year follow-up, there were 355 deaths due to CAD and 286 deaths due to ischemic stroke. Multivariable-adjusted HRs (95% confidence interval) for CAD in isolated low HDL-C group, compared with normal HDL-C group, was 0.81 (0.57–1.14), which was 1.37 (1.04–1.80) in non-isolated HDL-C group. Sex-specific analysis showed almost similar results; however, in women, we observed lower risk of CAD in isolated low HDL-C group compared with normal group, of which HR was 0.51 (0.29–0.89); this might be due to its low serum TC level. We did not observe any association between low HDL-C groups and ischemic stroke.

Conclusions: Isolated low HDL-C is not associated with increased risk for CAD, even in Japanese
population of which risk for CAD due to low HDL-C is more evident than Westerners as previously reported.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Mini-oral presentation
Additional information
Phase 3 Randomized Trial Evaluating Alirocumab Every Four Weeks Dosing as Add-on to Statin or as Monotherapy: ODYSSEY CHOICE I

Abstract nr. 254
Author Roth, Eli, Sterling Research Group, Cincinnati, United States of America
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Co-author(s) - Baccara-Dinet, Marie T
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Co-author(s) - Manvelian, Garen
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, LDL, PCSK9, Pharmacology

Background: In previous Phase 3 studies, the PCSK9 monoclonal antibody alirocumab was administered at doses of 75 or 150 mg every 2 weeks (Q2W). CHOICE I (NCT01926782) evaluated the LDL-C lowering effect of alirocumab 300 mg every 4 weeks (Q4W) ± maximally tolerated statin and other lipid-lowering therapies.

Methods: CHOICE I included patients with hypercholesterolemia at (1) moderate to very high cardiovascular (CV) risk receiving maximally tolerated statin, (2) moderate CV risk not receiving statin, or (3) moderate to very high CV risk and statin intolerance. Patients were randomized to alirocumab 300 mg Q4W, alirocumab 75 mg Q2W (calibrator arm), or placebo for 48 weeks. Treatments were administered Q2W subcutaneously via two 1-mL pre-filled syringes (alirocumab 75 mg, 150 mg or placebo as appropriate). At week (W)8 patients not achieving target LDL-C levels (<70 or <100 mg/dL depending on CV risk), or if LDL-C reduction was <30% from baseline, had their dosing regimen adjusted to alirocumab 150 mg Q2W at W12 in a blinded fashion. Co-primary endpoints were percent LDL-C change from baseline to W24 and to averaged LDL-C for W21-24.

Results: Approximately two-thirds of randomized patients (n=547/803) were receiving statins. At W24, significant reductions in LDL-C from baseline were observed with alirocumab 300 mg Q4W: mean differences versus placebo were -52.4% (patients not receiving statin) and -58.7% (patients receiving concomitant statin) (p<0.0001) (Figure). Average reductions in LDL-C from baseline to W21-W24 were also significantly greater in the alirocumab 300 mg Q4W arm versus placebo in patients not receiving (55.2%) and receiving (65.0%) concomitant statin (p<0.0001). At W12, only 14.7% (no statin) and 19.3% (concomitant statin) of patients receiving alirocumab 300 mg Q4W required dose adjustment to 150 mg Q2W. Treatment-emergent adverse event rates ranged from 61.1-75.0% (placebo) and 71.5-78.1% (alirocumab 300 mg Q4W) (Table). A higher rate of injection site reactions was observed with alirocumab 300 mg Q4W versus placebo; most of these
events were mild and did not lead to treatment discontinuation.  

**Conclusions:** Alirocumab 300 mg Q4W may offer an additional treatment option in patients requiring LDL-C lowering.  

**Study funding:** Study funded by Sanofi and Regeneron Pharmaceuticals, Inc.

**Table. Safety summary (Safety population)**

<table>
<thead>
<tr>
<th>Event</th>
<th>75 mg Q2W (N=121)</th>
<th>100 mg Q2W (N=146)</th>
<th>300 mg Q2W (N=138)</th>
<th>Placebo (N=153)</th>
<th>75 mg Q2W (N=121)</th>
<th>100 mg Q2W (N=146)</th>
<th>300 mg Q2W (N=138)</th>
<th>Placebo (N=153)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEAEs, n (%)</td>
<td>30 (21.1)</td>
<td>114 (78.1)</td>
<td>54 (39.9)</td>
<td>50 (34.1)</td>
<td>223 (71.5)</td>
<td>96 (61.1)</td>
<td>16 (10.2)</td>
<td></td>
</tr>
<tr>
<td>Treatment-emergent AEs, n (%)</td>
<td>3 (6.1)</td>
<td>18 (6.6)</td>
<td>7 (0.7)</td>
<td>6 (7.7)</td>
<td>25 (8.5)</td>
<td>16 (10.5)</td>
<td>12 (7.9)</td>
<td>13 (8.4)</td>
</tr>
<tr>
<td>TEAEs leading to death, n (%)</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TEAEs occurring in ≥15% patients in any group, n (%)</td>
<td>2 (0.4)</td>
<td>10 (6.8)</td>
<td>4 (5.6)</td>
<td>3 (2.9)</td>
<td>15 (4.9)</td>
<td>10 (6.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infections and infestations</td>
<td>14 (17.8)</td>
<td>90 (41.1)</td>
<td>25 (28.0)</td>
<td>26 (33.3)</td>
<td>105 (53.7)</td>
<td>35 (22.3)</td>
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<td></td>
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<tr>
<td>Upper respiratory tract infection</td>
<td>2 (2.5)</td>
<td>13 (6.9)</td>
<td>4 (5.6)</td>
<td>5 (6.4)</td>
<td>18 (9.5)</td>
<td>5 (2.2)</td>
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<tr>
<td>Sinusitis</td>
<td>3 (10.0)</td>
<td>9 (3.0)</td>
<td>6 (3.9)</td>
<td>0</td>
<td>10 (5.3)</td>
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<tr>
<td>Nasopharyngitis</td>
<td>2 (2.0)</td>
<td>7 (4.9)</td>
<td>3 (4.2)</td>
<td>3 (3.9)</td>
<td>23 (7.4)</td>
<td>19 (6.4)</td>
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</tr>
<tr>
<td>Urinary tract infection</td>
<td>1 (0.7)</td>
<td>7 (4.9)</td>
<td>2 (2.8)</td>
<td>2 (2.8)</td>
<td>15 (8.4)</td>
<td>12 (6.2)</td>
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<td></td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>0</td>
<td>7 (4.9)</td>
<td>8 (11.1)</td>
<td>5 (6.4)</td>
<td>19 (7.6)</td>
<td>15 (9.6)</td>
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<tr>
<td>Headache</td>
<td>16 (11.0)</td>
<td>16 (11.0)</td>
<td>4 (2.6)</td>
<td>3 (2.8)</td>
<td>10 (7.0)</td>
<td>4 (2.8)</td>
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<td></td>
</tr>
<tr>
<td>Vascular disorders</td>
<td>12 (7.7)</td>
<td>7 (4.9)</td>
<td>6 (8.9)</td>
<td>4 (2.9)</td>
<td>12 (8.5)</td>
<td>10 (6.4)</td>
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<td></td>
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<tr>
<td>Hypertension</td>
<td>10 (7.7)</td>
<td>9 (6.2)</td>
<td>6 (8.9)</td>
<td>4 (2.9)</td>
<td>9 (6.2)</td>
<td>10 (6.4)</td>
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<td></td>
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<tr>
<td>Gastrointestinal disorders</td>
<td>0</td>
<td>9 (6.2)</td>
<td>5 (6.9)</td>
<td>4 (2.9)</td>
<td>12 (8.5)</td>
<td>9 (5.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0</td>
<td>9 (6.2)</td>
<td>5 (6.9)</td>
<td>4 (2.9)</td>
<td>12 (8.5)</td>
<td>9 (5.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal and connective tissue disorders</td>
<td>9 (24.3)</td>
<td>35 (24.1)</td>
<td>18 (25.9)</td>
<td>13 (16.7)</td>
<td>80 (52.6)</td>
<td>40 (25.3)</td>
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<tr>
<td>Arthralgia</td>
<td>1 (0.7)</td>
<td>10 (6.8)</td>
<td>3 (4.2)</td>
<td>4 (5.1)</td>
<td>14 (4.5)</td>
<td>5 (2.1)</td>
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<tr>
<td>Pain in extremity</td>
<td>1 (0.7)</td>
<td>10 (6.8)</td>
<td>1 (1.4)</td>
<td>2 (2.6)</td>
<td>6 (2.6)</td>
<td>2 (1.3)</td>
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<tr>
<td>Osteoarthritis</td>
<td>2 (1.6)</td>
<td>5 (3.4)</td>
<td>0</td>
<td>0</td>
<td>8 (2.6)</td>
<td>3 (1.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myalgia</td>
<td>2 (1.6)</td>
<td>4 (2.7)</td>
<td>3 (4.2)</td>
<td>2 (2.6)</td>
<td>4 (2.9)</td>
<td>19 (6.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back pain</td>
<td>1 (0.7)</td>
<td>3 (2.1)</td>
<td>5 (6.9)</td>
<td>3 (3.9)</td>
<td>23 (7.4)</td>
<td>6 (3.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General disorders and administration site conditions</td>
<td>6 (16.2)</td>
<td>39 (26.7)</td>
<td>13 (18.1)</td>
<td>17 (21.6)</td>
<td>65 (20.8)</td>
<td>27 (17.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection site reaction</td>
<td>2 (1.6)</td>
<td>7 (4.8)</td>
<td>2 (2.6)</td>
<td>0</td>
<td>6 (1.9)</td>
<td>7 (4.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injury, poisoning and procedural complications</td>
<td>4 (10.8)</td>
<td>30 (20.5)</td>
<td>11 (15.3)</td>
<td>5 (6.4)</td>
<td>38 (12.6)</td>
<td>24 (15.3)</td>
<td></td>
<td></td>
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<tr>
<td>Antipodal bula</td>
<td>3 (2.4)</td>
<td>2 (1.3)</td>
<td>2 (2.8)</td>
<td>1 (1.3)</td>
<td>2 (0.6)</td>
<td>1 (0.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confusion</td>
<td>0</td>
<td>5 (3.5)</td>
<td>0</td>
<td>1 (1.3)</td>
<td>6 (1.9)</td>
<td>4 (2.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mean calculated LDL-C levels in patients receiving (A) no statin and (B) statin

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Efficacy and safety of evolocumab (AMG 145) in patients with high LDL-C levels at baseline

Abstract nr. 255
Author Santos , Raul, University of São Paulo, São Paulo, Brazil
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Co-author(s) - Bridges , Ian
Co-author(s) - Honarpour , Narimon
Co-author(s) - Somaratne , Ransi
Co-author(s) - Wasserman , Scott
Co-author(s) - Scott , Rob
Co-author(s) - Raal , Frederick
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Dyslipidemia,Familial Hypercholesterolemia,PCSK9, Therapy

Background: Evolocumab (AMG 145), a fully human monoclonal antibody to PCSK9, has demonstrated effective and safe reductions in LDL-C across different patient populations. We analysed data from phase 2 (P2) and 3 (P3) studies to assess efficacy and safety in patients with high baseline LDL-C.

Methods: Patients with high LDL-C (≥4.1mmol/L on statin treatment or ≥6.1mmol/L not on statin) were enrolled in P2 and P3 evolocumab studies. Efficacy results (140mg Q2W and 420mg QM) were pooled from 4 P3 studies (n=438) and reported for the mean of weeks 10 and 12. Safety analysis included data from P2 and P3 as well as their open label extensions (n=737). An ad hoc analysis based on Simon-Broome criteria estimated the number of possible heterozygous familial hypercholesterolemia (HeFH) patients among those not enrolled in an FH-specific study. Most of these patients did not have a known history of FH.

Results: Evolocumab significantly reduced LDL-C relative to placebo (58%) or ezetimibe (35%) in patients with high LDL-C at baseline; Lp(a) was also significantly reduced (table). Most AEs were mild and no clinically meaningful imbalances were observed between control and evolocumab (total exposure 709 patient-years) (table). There were 78/318 (25%) possible HeFH patients among those not enrolled in an FH study (26/48 [54%] with no baseline statins, 52/270 [19%] with baseline statins). Of these patients, 11/78 (14%) had a recorded history of FH.

Conclusion:
There was potent LDL-C and Lp(a) reduction with evolocumab in patients with high LDL-C at baseline. Safety with evolocumab was similar to control. A post-hoc analysis suggests FH may be underdiagnosed in high LDL-C patients.
### Table. Lipid Changes and Safety in High LDL-C patients*  

<table>
<thead>
<tr>
<th>Mean LDL-C Values, mmol/L ± standard deviation</th>
<th>Evolocumab (N = 18)</th>
<th>Ezetimibe (N = 18)</th>
<th>Placebo (N = 110)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4.08 ± 1.44</td>
<td>5.36 ± 2.20</td>
<td>4.70 ± 1.97</td>
</tr>
<tr>
<td>Week 10</td>
<td>3.87 ± 1.39</td>
<td>4.34 ± 2.13</td>
<td>2.02 ± 1.69</td>
</tr>
<tr>
<td>Week 12</td>
<td>3.11 ± 1.69</td>
<td>4.64 ± 2.33</td>
<td>2.25 ± 1.65</td>
</tr>
</tbody>
</table>

#### Lipid Changes - change from baseline at mean of weeks 10 and 12, %

<table>
<thead>
<tr>
<th></th>
<th>Evolocumab vs Placebo</th>
<th>Ezetimibe vs Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLC</td>
<td>Mean treatment difference (95% CI)</td>
<td>-57.6 (-83.0, -32.2)</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Mean treatment difference (95% CI)</td>
<td>6.2 (3.0, 9.4)</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TG</td>
<td>Mean treatment difference (95% CI)</td>
<td>-16.5 (-23.7, -9.3)</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ApoB</td>
<td>Mean treatment difference (95% CI)</td>
<td>-47.0 (-51.1, -42.8)</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LP (Lp(a))</td>
<td>Mean treatment difference (95% CI)</td>
<td>-27.3 (-33.2, -21.4)</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

#### Safety Data, n (%)  

<table>
<thead>
<tr>
<th></th>
<th>Parent Studies</th>
<th>Open-Label Extension Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any Control</td>
<td>Evolocumab</td>
</tr>
<tr>
<td></td>
<td>(N = 244)</td>
<td>(N = 692)</td>
</tr>
<tr>
<td>Any adverse events</td>
<td>121 (49.6)</td>
<td>296 (52.7)</td>
</tr>
<tr>
<td>Grade ≥2</td>
<td>50 (20.5)</td>
<td>113 (22.9)</td>
</tr>
<tr>
<td>Serious AEs</td>
<td>5 (2.0)</td>
<td>19 (3.9)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>6 (2.4)</td>
<td>9 (1.8)</td>
</tr>
<tr>
<td>Creatinine increase &gt;5x ULN</td>
<td>1 (0.4)</td>
<td>4 (0.8)</td>
</tr>
<tr>
<td>ALT or AST &gt;5x ULN</td>
<td>0 (0.0)</td>
<td>3 (0.6)</td>
</tr>
</tbody>
</table>

*High LDL-C defined as ≥4.1 mmol/L (on statin treatment) or ≥6.1 mmol/L (not on statin)  
1LDL-C was based on a relative approach. When the calculated LDL-C was <40 mg/dL or triglycerides are >400 mg/dL, calculated LDL-C was replaced with ultracentrifugation LDL-C from the same blood sample, if available  
2Efficacy analysis includes data from 4 phase 3 studies  
3Safety analysis includes data from phase 2 and 3 studies  
4LDL-C, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; Lp(a), lipoprotein (a); QOW, every 2 weeks; QM, monthly; TG, triglycerides; SoC, standard of care.

**Funding:** Amgen, Inc.

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**Subdivision 3. Clinical Studies**

**Presentation Preference Oral presentation**

**Additional information**
Abstract nr. 256

Author Baila-Rueda, Lucía, Zaragoza, Spain
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Co-author(s) - Lamiquiz-Moneo, Itziar
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Co-author(s) - Cenarro, Ana
Co-author(s) - Civeira, Fernando

Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Familial Hypercholesterolemia, LDL, Metabolism

Autosomal dominant hypercholesterolemias (ADH) are characterized by high levels of LDL cholesterol, familial presentation and high risk of premature cardiovascular disease. The genetic cause and pathogenic mechanism of approximately 20-40% of ADH are unknown (ADH-) and probably they are a heterogeneous group of diseases. Variations in cholesterol absorption have been associated to hypercholesterolemia. However, if these variations contribute to ADH-pathogenesis is unknown. With the hypothesis that some forms of autosomal dominant hypercholesterolemia of unknown cause, are associated with cholesterol absorption, we have studied the phytosterols (sitosterol, campesterol and stigmasterol) and cholestanol, concentrations in a group of 54 unrelated subjects with LDLc (above the 95th percentile), familial presentation and exclusion of secondary causes. We have selected those subjects, n=23, that showed phytosterols and cholestanol concentrations above 90th percentile and have studied their familial sterol concentrations to test the familial cosegregation between LDLc and phytosterols and cholestanol. Sterols and cholesterol were quantified by HPLC-MS/MS in serum after 10 hours of fasting. Subjects were without lipid lowering drugs or supplements phytosterols at least 5 weeks before blood extraction. Results. Lipids and non-cholesterol sterols values for affected and non-affected subjects are described in the following table. Non-cholesterol sterols of intestinal origin were highly correlated with LDLc. Since phytosterols and cholestanol have been described as surrogate markers of cholesterol absorption, these results suggest that hyper-absorption of cholesterol is a common phenomenon in ADH-. If this increase is cause or consequence of hypercholesterolemia should be determined. Our results describe for the first time the cosegregation of cholesterol absorption in affected families. In conclusion, the absorption markers of cholesterol are positively correlated and cosegregate with LDLc in these families. These data suggest that intestinal hyper-absorption plays an important role in these hypercholesterolemias.
Table. Main clinical characteristics and non-cholesterol parameters of Autosomal Dominant Hypercholesterolemia without homozygosity, mean (SD) or median (interquartile range)

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Men, n (%)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
</tr>
<tr>
<td>Body Mass Index, kg/m²</td>
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<tr>
<td>Total Cholesterol, mg/dL</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td></td>
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<tr>
<td>Non HDL Cholesterol, mg/dL</td>
<td></td>
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<tr>
<td>HDL Cholesterol, mg/dL</td>
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</tr>
<tr>
<td>LDL Cholesterol, mg/dL</td>
<td></td>
</tr>
<tr>
<td>Lipoprotein(a), mg/dL</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein A1, mg/dL</td>
<td></td>
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<tr>
<td>Apolipoprotein B, mg/dL</td>
<td></td>
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<tr>
<td>C-reactive protein, mg/dL</td>
<td></td>
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<tr>
<td>Glucose, mg/dL</td>
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<tr>
<td>Cholesterol x 10³ / total cholesterol</td>
<td></td>
</tr>
</tbody>
</table>
Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Cigarette smoking and the pleiotropic effects of simvastatin in the CHD patients

Abstract nr. 257
Author Janczura , Miroslaw, The John Paul II Hospital, Dep. of Anesthesiology and Intensive Pulmonary Care, Krakow, Poland
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease,Hypolipidemic Drugs,Lifestyle,Prevention

**Aim** Statins account for several pleiotropic effects ensuring their clinical efficacy, although cigarette smoking and obesity additionally impact simvastatin treatment. The study aimed to assess whether cigarette smoking may adversely impact the endothelial function during simvastatin treatment in the coronary heart disease (CHD) patients.

**Methods** The study covered 60 subjects with stable CHD, aged 39-65 years, and 60 sex-matched healthy controls. Endothelial function was assessed through measuring the flow-mediated dilation (FMD) at baseline, after 6 and 12 months of simvastatin treatment (daily dose of 40 mg) in the CHD group. Serum hs-CRP, plasma TNF-α, and 8-izo-PGF$_{2α}$ concentration in urine were measured at those time points.

**Results** At baseline, mean FMD value was significantly lower in the CHD group, as compared to the controls (6.80±2.73% vs. 8.18±2.32; p<0.05). After simvastatin treatment, a significantly increased FMD value was observed in the CHD group non-smokers (6.87±2.43% vs. 8.38±2.15% vs. 8.77±2.50%; p=0.04 and p=0.01; after 6 and 12 months, respectively). Mean hs-CRP concentrations were significantly higher in the CHD smokers, as compared to other control smokers subjects (3.30±2.30 mg/L vs. 1.30±0.98 mg/L; p<0.0001), and were not significantly higher, as compared to the CHD non-smokers (p=0.32). Also at baseline plasma TNF-α concentration were higher in the CHD subjects, as compared to the controls (p<0.05). There were no differences in the urine 8-izọ-PGF$_{2α}$ between the CHD subjects and the controls. After simvastatin treatment the concentrations of hs-CRP (3.30±2.30 mg/L vs. 1.77±1.50 mg/L vs. 1.90±1.30 mg/L; p=0.011) and TNF-α (1.84±1.30 pg/mL vs. 1.56±1.20 pg/mL vs. 1.25±0.90 pg/mL; p=0.004) significantly decreased only in the CHD smokers. There was no change in urine 8-iso-PGF$_{2α}$ concentration after treatment, although it was slightly lower in the CHD non-smokers group.

**Conclusions** Restoration of endothelial function and reduction of pro-inflammatory markers are attributable to simvastatin treatment. The actual extent of these favorable results may be affected by various accompanying factors, e.g. smoking.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
The impact of Q192R PON1 gene polymorphism on the pleiotropic effects of simvastatin in the CHD patients

Abstract nr. 258
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Hypolipidemic Drugs, Lifestyle, Prevention

Aim The relationship between Q192R polymorphism of paraoxonase 1 (PON1) gene, oxidative stress and cardiovascular risk is well documented. The present study aimed to assess the impact of Q192R polymorphism of PON1 on the serum enzyme activity, and the pleiotropic effects of simvastatin in the CHD patients.

Methods The study covered 65 individuals with stable CHD, aged 42-65 years, and 65 sex-matched healthy controls. Genetic Q192R PON1 gene variants were genotyped using the PCR-RFLP technique, while PON1 activity in serum was assessed spectrophotometrically at baseline and after 12 months of treatment with simvastatin (daily dose of 40 mg) in the CHD group. Endothelial function was assessed through measuring the flow-mediated dilation (FMD) at those time points. Urine 8-izo-PGF$_{2\alpha}$ (oxidative stress marker) was measured at baseline, and after 12 months of treatment, being expressed in pg/mg of creatinine.

Results At baseline, mean FMD value was significantly lower (p=0.015), and urine 8-izo-PGF$_{2\alpha}$ (p<0.0001) concentration was significantly higher in the CHD group, as compared to the controls. Adjusted to PON1 genotypes, no significant differences in the PON1 activity at baseline, nor after simvastatin treatment, were observed. Only in the 192QQ genotype patients was there an improvement in FMD (6.82±2,34% vs. 8.44±3.11%; p=0.02), accompanied by an inhibition of oxidative stress, as exemplified by lower concentrations of urine 8-iso-PGF$_{2\alpha}$ (1130.56±415.30 pg/mg of creatinine vs. 752.65±259.24 pg/mg of creatinine; p=0.043) after 12 months of simvastatin treatment. Furthermore, favourable effects of treatment (improvement of endothelial function, urine 8-izo-PGF$_{2\alpha}$ reduction) were encountered primarily in the non-smokers and non-obese subjects with 192QQ genotype, as compared to the 192R allele carriers.

Conclusions Q192R polymorphism impacted the pleiotropic effects of simvastatin treatment, whereas smoking and obesity adversely affected its beneficial outcome.
Additional information
Fifty years experience of detection, analysis and treatment of homozygous FH

Abstract nr. 259
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Familial Hypercholesterolemia, Intervention, Therapy

This study recounts the experience of a single centre in the UK in detecting and treating homozygous familial hypercholesterolaemia (FH) over a period of 50 years, based on a retrospective analysis of case records, laboratory data and peer-reviewed publications. In 1964 the first FH homozygote to be referred to one of us (NBM), an 8 year old Iraqi girl, underwent 4 successive manual plasmapheresis procedures at Hammersmith Hospital (HH). This approach reduced her serum cholesterol from 22 to 13.6 mmol/l but the effect was transient. Subsequently she underwent an unsuccessful ileal bypass in 1966 and died soon after from myocardial ischaemia, aged 10. However, in 1975 2 homozygotes were treated successfully at HH with bi-weekly plasma exchange for 6 months using a continuous flow cell separator, which revolutionised the management of this fatal disorder. During the period 1964-2014 a total of 44 homozygotes have been treated and/or had mutational analysis at HH, of whom 16 have undergone at least one plasma exchange or lipoprotein apheresis procedure, the longest for 25 years. 3 patients underwent a liver transplant, one a portacaval shunt and one an ileal bypass. Ten patients are known to have died, the youngest aged 2 years and 11 months, the oldest aged 55. Ethnically, 25 of the homozygotes were British, 6 Asian, 6 European and the remainder from elsewhere. DNA analysis showed that 30 patients had autosomal dominant FH, 12 being genetic homozygotes, 18 compound heterozygotes and 5 had autosomal recessive hypercholesterolaemia. Death certificates are being sought in patients lost to follow up in order to relate clinical outcome to the severity of the underlying mutation(s) and the nature and timing of treatment. We conclude that the introduction at HH of lipoprotein apheresis and of DNA analysis, plus the advent of statins and ezetimibe, have markedly improved the past and present management of homozygous FH.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Systemic Leukocyte-bound Apolipoprotein B is Inversely Associated with the Presence of Clinical and Subclinical Atherosclerosis

Abstract nr. 260
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Co-author(s) - Geijn, Gert-Jan van de
Co-author(s) - Liem, Anho
Co-author(s) - Castro Cabezas, Manuel
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Atherosclerosis, Lipoproteins

Background: A high level of erythrocyte-bound apolipoprotein (apo) B in humans has been associated with a decreased prevalence of clinical and subclinical atherosclerosis. We aimed to determine the relationship between leukocyte-bound apo B in the circulation and atherosclerosis.

Methods: The level of apo B on circulating leukocytes was measured by flow cytometry in subjects with and without coronary artery disease (CAD) in a cross-sectional design, and expressed as mean fluorescent intensity in arbitrary units (au). Carotid intima media thickness (cIMT) was measured using B-mode ultrasound. Data are given as mean ± SD.

Results: 442 subjects were included, of whom 185 had a history of CAD. Compared to subjects without CAD, patients with CAD had lower levels of apo B bound to neutrophils (12.9 ± 4.8 and 14.3 ± 5.8 au, respectively, p=0.006) and to monocytes (2.6 ± 1.1 and 2.8 ± 1.3 au, respectively, p=0.02). No differences existed for lymphocyte-bound apo B. Neutrophil-bound apo B was inversely correlated with cIMT (Spearman rho: -0.102, p=0.035). Furthermore, both monocyte- and neutrophil-bound apo B were significantly and positively associated to different components of the metabolic syndrome, such as BMI, triglycerides and C3. There was also a positive association between leukocyte-bound apo B and erythrocyte-bound apo B (rho: 0.502, p<0.001; rho: 0.304, p<0.001; rho 0.190, p<0.001 for lymphocyte-, monocyte- and neutrophil-bound apo B, respectively), possibly reflecting a similar binding mechanism.

Conclusion: Unexpectedly, binding of apo B-containing lipoproteins to circulating leukocytes may represent a protective mechanism against atherosclerosis.
In Vivo Evidence for Chylomicrons as Mediators of Postprandial Inflammation

Abstract nr. 261
Author Vries , Marijke de, Sint Franciscus Gasthuis, Rotterdam, Netherlands
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Co-author(s) - Janssen , Hans
Co-author(s) - Valdivielso , Pedro
Co-author(s) - Rioja Villodres , José
Co-author(s) - Ramírez-Bollero , José
Co-author(s) - Castro Cabezas , Manuel
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Apolipoproteins, Inflammation, Triglycerides

Background: The postprandial situation is a pro-inflammatory condition most likely linked to the development of atherosclerosis. We evaluated the relationship between apolipoprotein (apo) B48 and postprandial leukocyte activation markers.

Methods: Leukocyte activation markers were measured by flow cytometry in patients with and without coronary artery disease (CAD). In 12 healthy subjects, leukocyte activation markers, triglycerides and apo B48 were determined after an oral fat load (8 hours).

Results: 94 patients participated in the study. Fasting apo B48 was significantly higher in patients with CAD (n=56, 7.9 ± 4.9 mg/L) than in subjects without CAD (n=38, 5.7 ± 3.7 mg/L, p=0.019), and higher in males (n=51, 8.0 ± 4.8 mg/L) than in females (n=43, 5.8 ± 4.0 mg/L, p=0.025). Fasting apo B48 and triglycerides correlated positively with fasting monocyte CD11b and granulocyte CD66b expression. No correlations were found between these inflammatory markers and plasma total apo B or LDL-C. Plasma apo B48 increased after an oral fat load (n=12), with the maximal increase after 2 hours, from 3.6 ± 1.9 mg/L to 6.4 ± 3.1 mg/L (p<0.001). Monocyte CD11b expression also increased, with the maximal increase after 4 hours, from 14.8 ± 2.3 au to 16.5 ± 2.2 au (p=0.043). The postprandial apo B48 response correlated positively with postprandial monocyte CD11b (Spearman’s rho: 0.615, p=0.033), but no correlations were found between postprandial triglycerides and postprandial leukocyte activation markers.

Conclusion: This study suggests that chylomicrons may be directly responsible for the postprandial leukocyte activation. The postprandial chylomicron response may be a stronger mediator of postprandial inflammation than postprandial triglyceridemia.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Whole exome sequencing of severe hypertriglyceridemia in patients negative for mutations in candidate genes

Abstract nr. 262
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Genetics, Triglycerides

The vast majority of primary severe hypertriglyceridemia (HTG) are diagnosed in adulthood and the molecular basis has not been completely elucidated. This study aimed to identify novel HTG-causing genetic variants in patients with no detectable mutation in usual candidate genes. Exomes of 2 unrelated subjects with severe HTG were sequenced. LPL, APOA5, APOE and LMF1 mutations were previously excluded by Sanger sequencing. First, exome variants were filtered by frequency <0.05% and novelty. Second, common and rare variants in genes previously associated with triglycerides (TG) levels in genome-wide association study (GWAS) meta-analysis were examined. Finally, the rest of variants were filtered by function, analysing those that were non-synonymous, stop-gain, stop-loss, indels, or splice site change with functional effect. All variants were analysed in silico by Polyphen-2 and Mutation Taster software.

In genes associated with TG in GWAS, both subjects showed variants in ANKR1C4, LRPAP1, TIMD4, LRP1 and MAP3K1. Moreover, subject A showed also rare variants in LIPC and MLXIPL, while subject B presented a JMJD1C variant. Only LIPC (p.Gln355Arg) and MLXIPL (p.Asp67Tyr) were considered as possibly pathogenic by in silico analysis. The analysis of variants considered pathogenic by in silico analysis showed variants in the following genes: Subject A, GRK4, HNF1A, FADS6, SR1H2, HSPG2, PCSK9, SDC1, ACSL1, FDFT1, NR4A3, SORC53, CPT1A, APOBR, LRP1B and HNF1B; and Subject B, CES1, COL15A1 and FADS6. Moreover, a variant previously associated with high levels of total cholesterol, LDL-cholesterol and TG was found in heterozygosis in subject A, p.Val103Met in GCKR.

According to our results, we can conclude that severe hypertriglyceridemia might be a polygenetic disease with several genes implicated and exome sequencing could help to identify these causal genes and variants.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Extreme elevations of Lp(a) and vascular presentations.

Abstract nr. 263  
Author Corral, Pablo, ICM, mar del plata, Argentina  
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins  
Keywords Cardiovascular Disease, Lipoproteins, Lp(a), Risk Factor

Elevated lipoprotein(a) (Lp(a)) is a causal genetic risk factor for cardiovascular disease. The pathophysiological role of Lp(a) is not fully understood yet but it has been reported to possess atherogenic and prothrombotic properties. The aim of this study was to describe different serious vascular manifestations, associated with extreme elevations of Lp(a) (values > 100 mg/dL, immunoturbidimetric method).

Five clinical cases with extreme elevations of Lp(a) are described (values between 148 mg/dL to 239 mg/dL). None had diagnosis of Familial Hypercholesterolemia and the rest of the basic lipid panel not explain the vascular events. None of the cases had diabetes, hypertension, chronic kidney disease or smoking. Each of the five patients, presented a different and serious vascular event. All patients had less than 55 years old (42 to 53 years); three were males. The first case had presented with a severe coronary disease; the second patient had bilateral carotid atherosclerosis. The third case had a systemic embolism from a left ventricular thrombus. The fourth patient had peripheral artery disease and the last case had suffered severe aortic valvular calcification.

This study describes five different forms of presentation of severe vascular events, associated with "normal" basic lipid panel, without other major cardiovascular risk factors, but with the presence of extreme elevations of Lp(a). Lp(a) should be measured once in all subjects with premature CVD, familial hypercholesterolemia, a family history of premature CVD and/or elevated Lp(a), recurrent CVD despite statin treatment and unusual/unexpected vascular events. Despite the strong evidence between increased Lp(a) plasma concentrations and a greater risk of CVD, there is little evidence that reduction in Lp(a) prevents CVD. Further investigation is required to assess the atherothrombotic risk due to the Lp(a) particle. Large prospective, randomized, controlled intervention trials with selective reduction in plasma lipoprotein(a) levels to reduce CVD are urgently needed in order to define more precisely who to treat, to what targets and to ensure treatment approaches.
### Lp(a) table. Patients

**Subdivision 3. Clinical Studies**

**Presentation Preference** Mini-oral presentation

**Additional information**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Lp(a) Level (mg/dL)</th>
<th>Vascular manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M</td>
<td>64</td>
<td>161</td>
<td>aortic valvular calcification</td>
</tr>
<tr>
<td>2 M</td>
<td>48</td>
<td>215</td>
<td>peripheral artery disease</td>
</tr>
<tr>
<td>3 M</td>
<td>45</td>
<td>238</td>
<td>coronary disease</td>
</tr>
<tr>
<td>4 F</td>
<td>50</td>
<td>145</td>
<td>bilateral carotid atherosclerosis</td>
</tr>
<tr>
<td>5 F</td>
<td>49</td>
<td>177</td>
<td>systemic embolism from a left ventricular thrombus</td>
</tr>
</tbody>
</table>
In Vivo Evidence of Matrix Metalloproteinases (MMPs) and Vascular Inflammation: Coronary Rotational Atherectomy Study

Abstract nr. 264
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation

[Objective] MMPs are involved in several steps in atherosclerotic plaque development. However, little in vivo evidence is available about relation with vascular inflammation. To examine the hypothesis that MMPs are regulated by inflammatory process, we measured peripheral levels of MMP2, MMP9, and hsCRP, pentaxin 3 (PTX3), before and after rotational coronary atherectomy (RA), which ablates coronary atheroma into blood stream. [Methods and Results] We enrolled consecutive 90 patients (mean age: 69 years, M/F=55/35) treated successfully with RA. MMP9 levels (mean±SD, ng/ml) significantly (p<0.05) increased 3 hours after RA (from 35±20 to 48±44), and returned to 38±41 24 hours after procedure. MMP2 levels increased behind the changes of MMP9 and reached the peak at 24 hours after procedure (from 783±258 to 867±291 ng/ml). Preprocedural levels of MMP2 showed significant and positive associations with PTX3 (r=0.231, p<0.05). By contrast, MMP9 levels showed no association with those of hsCRP nor PTX3. Associations of MMP9 increase after RA with PTX3 were modest (r=0.199, p=0.059) with its basal levels and were highly significant (r=0.422, p<0.0001) with its increase after RA. [Conclusions] These results suggest that, in advanced coronary atherosclerosis, MMP9 might be actively produced in association with vascular inflammation.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Xuezhikang can reduce arterial stiffness in patients with essential hypertension, independent of lipid lowering effects

Abstract nr. 265
Author Zheng, Jin, Beijing, China
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Dyslipidemia, Hypertension, Prevention

Objective Increased arterial stiffness occurs early in hypertension and is considered to play an important role in cardiovascular events. Xuezhikang, an extract of Cholestin, contains statin-like components. We hypothesized that Xuezhikang treatment can reduce arterial stiffness in patients with essential hypertension.

Methods: One hundred essential hypertension patients were prospectively enrolled to Xuezhikang group (1200 mg/day) and placebo group. Physical examination, lipid profiles, high sensitivity C reactive protein (hs-CRP), matrix metalloproteinases-9 (MMP-9), arterial stiffness parameters, including β, Ep, AC, AI and PWVβ measured by Echo Tracking technique, were obtained at baseline and after 6 months' therapy in all subjects.

Results: Ninety cases with completed data were included at the end of the study. The Xuezhikang group (n=46; age, 58.4±10.0 years) and placebo group (n=44; age, 57.1±10.4 years) were well balanced with respect to clinical characteristics, use of medications, lipid profiles and arterial stiffness parameters. Xuezhikang therapy significantly reduced the levels of β, Ep and PWVβ from baseline (8.4±3.1 vs. 6.8±2.1, p=0.007; 122.8±43.9 vs. 100.7±33.2, p=0.009; 6.7±1.2 vs. 6.1±1.0, p=0.013), but placebo group didn't. There were significant reductions in total cholesterol by and LDL cholesterol in Xuezhikang group (5.5±0.7 vs. 5.1±0.6, p=0.013; 3.4±0.6 vs. 2.9±0.5, p=0.001), but not in placebo group. Xuezhikang therapy also significantly reduced hs-CRP and MMP-9 from baseline [2.1 (0.4-10.0) vs. 1.4 (0.3-4.1), p=0.020; 17.2±2.4 vs. 12.7±3.8, p<0.001], but placebo group didn't. Of interest, the percent change of PWVβ in Xuezhikang group was significantly correlated with the percent change of hs-CRP or MMP-9 (r=0.144, p=0.043; r=0.278, p=0.030 respectively), but not with lipid profiles.

Conclusions: Six months' Xuezhikang treatment can reduce arterial stiffness, independent of lipid lowering effects in hypertensive patients. It seems that Xuezhikang has some statins' pleiotropic effects, such as anti-inflammation and reducing extracellular matrix degeneration, might play a role in improving arterial stiffness in patients with essential hypertension.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Anacetrapib reduces (V)LDL-cholesterol via reducing PCSK9 and enhancing hepatic remnant clearance

Abstract nr. 266
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Hypolipidemic Drugs, LDL, PCSK9

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Departments of 1Endocrinology, 2Human Genetics, 3Cardiology, and 4Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center; 5TNO-Metabolic Health Research, Gaubius Laboratory; 6Department of Pediatrics, University of Groningen.

Objective: Very recently, we showed that the CETP inhibitor anacetrapib attenuated atherosclerosis development by reducing (V)LDL-cholesterol (C) rather than by raising HDL-C (Kühnast & Van der Tuin; Eur Heart J 2014). Here, we investigated the mechanism by which anacetrapib reduces (V)LDL-C and whether this effect was dependent on the inhibition of CETP.

Methods and results: APOE*3-Leiden.CETP mice were fed a Western type diet alone or supplemented with anacetrapib (30 mg/kg bw/day). Microarray analyses of livers followed by gene-set enrichment analyses revealed a down-regulation of the cholesterol biosynthesis pathway. Subsequent pathway analyses predicted down regulation of sterol regulatory element-binding protein-1 and -2 (z-score -2.90 and z-score -2.56, respectively) controlled pathways. These data suggest an increased supply of cholesterol to the liver. We found that plasma PCSK9 was decreased (-47%), accompanied by a decreased hepatic Pcsk9 mRNA expression (-28%) and increased hepatic LDL receptor protein content (+64%). These data were consistent with the observation that anacetrapib increased the clearance rate (+54%) and hepatic uptake (+25%) of [14C]cholesteryl oleate-labeled VLDL remnants. In contrast, anacetrapib did not alter VLDL-TG production or clearance of VLDL-TG as assessed using glycerol tri[3H]oleate labeled VLDL-like particles. In APOE*3-Leiden mice that do not express CETP, anacetrapib still decreased plasma...
(V)LDL-C and PCSK9 levels, indicating that these effects were independent of CETP inhibition. 

**Conclusion:** Anacetrapib reduces (V)LDL-C by increasing hepatic remnant uptake via two mechanisms: 1) inhibition of CETP activity, resulting in remodelled VLDL that are more susceptible to hepatic uptake; and 2) a CETP-independent reduction of plasma PCSK9 level that has the potential to increase LDL receptor-mediated hepatic remnant clearance. 

This research was performed within the framework of CTMM, the Center for Translational Molecular Medicine (www.ctmm.nl), project PREDICCt (grant 01C-104). PCNR is an Established Investigator of the Netherlands Heart Foundation (2009T038).

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Central GLP-1 receptor signaling increases the uptake of plasma triglycerides and glucose by activated brown adipose tissue

Abstract nr. 267
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Co-author(s) - Romijn, JA
Co-author(s) - Rensen , PCN
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes,Dyslipidemia,Metabolism,Triglycerides

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Objective: Central GLP-1 receptor (GLP-1R) activation, implemented for the treatment of type 2 diabetes mellitus, has recently been shown to increase thermogenesis in brown adipose tissue (BAT). The aim of the present study was to investigate the effects of chronic central GLP-1R activation on the involvement of BAT in the clearance of plasma TG and glucose, under insulin sensitive and insulin resistant conditions.

Methods and results: Chow and 12-week high-fat-fed C57Bl/6J mice were continuously infused with exendin-4 (0.75 nmol/day) or vehicle into the left lateral ventricle for 5 days. In addition, a group of vehicle-treated mice was added that mice were pair fed to the exendin-4-treated group. Central infusion of exendin-4 in chow fed mice increased sympathetic outflow towards BAT, evident from tyrosine hydroxylase staining (+103%), and increased UCP-1 levels (+44%), decreased lipid content (-67%), and increased uptake by BAT of plasma TG (+276%) and glucose (+142%). These effects were largely independent of the hypophagic effect of exendin-4. Interestingly, in high-fat-fed mice, exendin-4 still increased sympathetic outflow to BAT and increased the uptake of plasma TG (+291%) and glucose (+482%) by BAT. Again, these effects were largely independent of the effects of exendin-4 on food intake, and accompanied by an increased fat oxidation and lower body weight.

Conclusion: Chronic activation of the central GLP-1R by exendin-4 enhances the sympathetic outflow to BAT and increases the uptake of TG and glucose from plasma by BAT. We anticipate that targeting the central GLP-1R will be a valuable strategy in the treatment of dyslipidemia and hyperglycemia in addition to obesity.

This research was supported from the Netherlands CardioVascular Research Initiative:
CVON2011-19. PCN Rensen is an Established Investigator of the Netherlands Heart Foundation (grant 2009T038).

Subdivision 2. Translational Research

Presentation Preference Mini-oral presentation
Additional information
Clearance of circulating low-density lipoprotein (LDL) is dependent on COMMD1 and WASH-mediated endosomal sorting of the low-density lipoprotein receptor (LDLR)

Abstract nr. 268
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Co-author(s) - Groen, Albert
Co-author(s) - Kuivenhoven, Jan Albert
Co-author(s) - Horton, Jay
Co-author(s) - Burstein, Ezra
Co-author(s) - Hofker, Marten
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Cardiovascular Disease, Genetics, LDL

The low-density lipoprotein receptor (LDLR) plays a pivotal role in clearing circulating low-density lipoprotein (LDL) cholesterol, which is a major risk factor for cardiovascular disease, but the mechanisms regulating the intracellular trafficking of LDLR remains poorly understood. In a recent study we identified COMMD1 as a novel gene in regulating plasma LDL cholesterol levels. We discovered that hepatic Commd1 deficiency results in elevated total plasma cholesterol levels in mice. This increase in circulating cholesterol is caused by raised levels of LDL cholesterol. The role of COMMD1 in cholesterol homeostasis was further validated in dogs homozygous for a naturally occurring COMMD1 loss-of-function mutation. Similar as liver specific Commd1 knockout mice, dogs deficient for COMMD1 show raised plasma LDL cholesterol levels. We identified that COMMD1 interacts, and colocalizes with LDLR, and with various endocytic proteins, including the WASH complex. WASH is a multi-subunit protein complex involved in endosomal sorting of specific cargos. Here we demonstrate that depletion of COMMD1 or WASH result both in mislocalization of LDLR, and subsequently decreased LDL uptake. Furthermore we identified that individuals with a mutation in a protein associated with the COMMD1/WASH protein complex are hypercholesterolemic. Altogether, this study show for the first time that COMMD1 is associated with a endosomal sorting machinery, which is essential for proper intracellular trafficking of the LDLR, and impaired endosomal LDLR sorting causes hypercholesterolemia in men, dogs, and mice.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Alicucumab in patients with hypercholesterolemia not on statin therapy: the ODYSSEY CHOICE II study

Abstract nr. 269
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Co-author(s) - Watts , Gerald
Co-author(s) - Manvelian , Garen
Co-author(s) - Lecorps , Guillaume
Co-author(s) - Baccara-Dinet , Marie T
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease,LDL,PCSK9,Pharmacology

Background: Observational studies report that 7-29% of patients receive sub-optimal statin doses or no statin therapy, mainly due to statin-associated muscle symptoms (SAMS). CHOICE II (NCT02023879) evaluated the LDL-C-lowering effect of the monoclonal PCSK9 antibody alirocumab at a dose of 150 mg every 4 weeks (Q4W) in patients with hypercholesterolemia not receiving statin therapy.

Methods: This study included patients with hypercholesterolemia receiving fenofibrate, ezetimibe or diet alone. Eligible patients had (a) moderate to very high CV risk and SAMS (inability to tolerate ≥2 statins due to muscle symptoms), or (b) moderate CV risk without SAMS. Patients were randomized to alirocumab 150 mg Q4W, alirocumab 75 mg Q2W (calibrator arm), or placebo for 24 weeks. Doses were adjusted to alirocumab 150 mg Q2W at week (W)12 if W8 LDL-C target levels were not met (<70 or <100 mg/dL, depending on CV risk) or LDL-C reduction at W8 was <30% from baseline. The primary efficacy endpoint was percent change in LDL-C from baseline to W24; safety was assessed throughout.

Results: 233 patients were randomized; >90% of patients did not use statins due to SAMS (Table); ~70% received fenofibrate or ezetimibe; ~30% diet alone. At W24, significantly greater LDL-C reductions from baseline were observed for alirocumab 150 mg Q4W versus placebo (LS mean difference -56.4%; p<0.0001) (Figure). At W12, 49.1% of patients in the alirocumab 150 mg Q4W arm required dose adjustment. Overall, 63.9% of patients in the alirocumab 150 mg Q4W arm achieved pre-defined LDL-C target levels at W24 versus 1.8% of placebo patients (ITT analysis) (Table). Treatment-emergent adverse events (TEAEs) occurred in 63.8% and 77.6% of placebo- and alirocumab 150 mg Q4W-treated patients, respectively. Muscle symptoms were infrequent;
the most common TEAEs were injection site reactions, arthralgia, headache and nasopharyngitis (Table).

Conclusions: Alirocumab 150 mg Q4W/150 mg Q2W significantly reduced LDL-C versus placebo on background of ezetimibe, fenofibrate or diet alone. Using individualized dosing regimens, 63.9% of patients in the alirocumab 150 mg Q4W/150 mg Q2W arm achieved their predefined LDL-C target levels in the absence of statin therapy.

Study funding: Study funded by Sanofi and Regeneron Pharmaceuticals, Inc.

Mean calculated LDL-C levels in patients with SAMS
Table. Effects of alirocumab

<table>
<thead>
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<th>Subdivision 3. Clinical Studies</th>
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<tr>
<td>Presentation Preference Oral presentation</td>
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<tr>
<td>Additional information</td>
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</tbody>
</table>

Table. Effects of alirocumab (150 mg QW/150 mg Q2W or 75 mg Q2W/150 mg Q2W) on LDL-C in patients with hypercholesterolemia

<table>
<thead>
<tr>
<th>Randomized patients</th>
<th>ALI 150 mg QW/150 mg Q2W (N=115)</th>
<th>ALI 75 mg QW/150 mg Q2W (N=110)</th>
<th>ALI 150 mg Q2W (N=58)</th>
<th>PBO (N=58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT population, n</td>
<td>32</td>
<td>115</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Statin intolerant patients, n (%)</td>
<td>53 (20.7)</td>
<td>19 (16.4)</td>
<td>51 (87.9)</td>
<td></td>
</tr>
<tr>
<td>Baseline LDL-C (mmol/L), mg/dL</td>
<td>163.9 (69.1)</td>
<td>151.5 (44.9)</td>
<td>150.5 (74.3)</td>
<td></td>
</tr>
<tr>
<td>Change from baseline to W24, LS mean (SD), %</td>
<td>-51.7 (2.3)</td>
<td>-53.5 (1.8)</td>
<td>4.7 (2.2)</td>
<td></td>
</tr>
<tr>
<td>P-value vs. PBO</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Patients requiring ALI dose adjustment to 150 mg Q2W at W12, %</td>
<td>45 (17.6)</td>
<td>84 (73.6)</td>
<td>57 (93.8)</td>
<td></td>
</tr>
<tr>
<td>Safety summary, n (%)</td>
<td>58</td>
<td>115</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Treatment emergent AEs, n (%)</td>
<td>7 (2.1)</td>
<td>6 (5.3)</td>
<td>4 (6.8)</td>
<td></td>
</tr>
<tr>
<td>Treatment leading to discontinuation, %</td>
<td>4 (6.0)</td>
<td>2 (1.7)</td>
<td>2 (3.4)</td>
<td></td>
</tr>
</tbody>
</table>

Adverse events occurring in ≥2% patients in any group, n (%)

| Infections and infestations | 12 (27.9) | 7 (27.8) | 13 (23.4) |            |
|fections with or without superinfection | 1 (3.0) | 0 (0.0) | 1 (3.0) |            |
| Upper respiratory tract infection | 1 (2.6) | 1 (3.6) | 2 (3.4) |            |
| Nervous system disorders | 12 (27.9) | 57 (41.4) | 8 (13.3) |            |
| Headache | 5 (11.6) | 15 (11.7) | 3 (5.1) |            |
| Diarrhea | 4 (8.9) | 1 (0.9) | 4 (8.9) |            |
| Gastrointestinal disorders | 10 (22.2) | 26 (19.4) | 8 (13.8) |            |
| Nausea | 3 (6.5) | 6 (5.2) | 2 (3.4) |            |
| Arthralgia | 11 (24.5) | 5 (4.3) | 7 (12.1) |            |
| Skin and subcutaneous tissue disorders | 9 (11.3) | 9 (7.8) | 6 (10.3) |            |
| Rash | 3 (6.5) | 0 (0.0) | 0 (0.0) |            |
| Mucocutaneous and connective tissue disorders | 14 (28.1) | 33 (25.7) | 12 (20.7) |            |
| Allergic reaction | 7 (15.9) | 1 (0.8) | 1 (1.7) |            |
| Muscle spasms | 2 (6.5) | 2 (6.5) | 0 (0.0) |            |
| Myalgia | 3 (6.5) | 1 (0.8) | 2 (3.4) |            |
| Pain in extremities | 2 (4.2) | 0 (0.0) | 2 (3.4) |            |
| Back pain | 2 (4.2) | 0 (0.0) | 2 (3.4) |            |
| Cardiac disorders and administration site conditions | 12 (24.5) | 26 (19.4) | 8 (13.8) |            |
| Injection site reaction | 8 (17.8) | 4 (3.5) | 0 (0.0) |            |
| Fibrillary | 4 (8.9) | 6 (5.2) | 0 (0.0) |            |
| Injury, poisoning and procedural complications | 5 (10.6) | 12 (15.5) | 6 (10.3) |            |
| Fall | 0 (0.0) | 6 (5.2) | 2 (3.4) |            |

Al, alirocumab; PBO, placebo; Q2W, every two weeks; Q4W, every four weeks

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information

*Pts were enrolled with moderate, high or very high CV risk if they were statin intolerant (unable to tolerate ≥ 2 statins, one at lowest daily starting dose, another at any dose); pts who did not meet this definition were only enrolled with moderate CV risk.
*All injections will be administered as single 1 mL injections by prefilled pen.
*The blinding will be maintained in the alirocumab 150 mg Q2W arm by alternating every two weeks with placebo Q4W.
DEVELOPMENT OF A NEW SCORE REFLECTING FAMILY HISTORY OF CARDIOVASCULAR DISEASE AND ITS ASSOCIATION TO ATHEROSCLEROSIS

Abstract nr. 270
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Co-author(s) - Veglia, Fabrizio
Co-author(s) - Castelnuovo, Samuela
Co-author(s) - Bonomi, Alice
Co-author(s) - Tedesco, Calogero C.
Co-author(s) - Tremoli, Elena
Co-author(s) - Baldassarre, Damiano
Co-author(s) - .., on behalf of the IMPROVE study group
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis,Cardiovascular Disease,Epidemiology,Risk stratification

Background and objective: In cardiovascular (CV) risk assessment, the family history (FH) of cardio, cerebral or peripheral vascular disease are often summarized under a generic yes/no “FH” definition. This completely ignores the presence of different kinds of cardiovascular pathologies in the same family. Here we interrogated the dataset of the IMPROVE study to assess whether a score of the overall FH burden ($FH_{\text{count}}$) confers an additional risk not grasped by a generic FH definition.

Methods: 3703 individuals free of vascular events (median age 64.4 years; 48% men) were followed up for 3 years. The subject’s FH profile was determined by a face-to-face interview. The $FH_{\text{count}}$ was defined as the number of different pathologies among cardio, cerebral, or peripheral vascular diseases (Items) recorded in the family history. The score ranges from 0 (no pathologies) to 3 (presence of the three kinds of pathologies in the family).

Results: In the IMPROVE cohort, 21.5%, 50.8%, 23.8% and 3.9% of subjects were exposed to zero, one, two, or three FH items, respectively. C-IMT$_{\text{mean-max}}$ increased progressively accordingly to the $FH_{\text{count}}$. The trend was significant ($P_{\text{trend}}=0.003$) even after adjustment for latitude and vascular risk factors known to aggregate at family level (i.e. dyslipidemia, hypertension, diabetes). By Cox analysis, the $FH_{\text{count}}$ was an independent associate of vascular events ($P_{\text{trend}}=0.05$), with a hazard ratio (HR), adjusted for latitude and traditional risk factors of 1.20 (95% CI: 1.00-1.45) for one step of $FH_{\text{count}}$. Conversely, the generic FH definition was not associated with cardiovascular risk (adjusted HR (95% CI) = 1.39 (0.95-2.03).

Conclusions: The count of FH items is an independent risk factor for subclinical and clinical atherosclerosis which works better than the generic definition of family history of vascular disease.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
CAROTID PLAQUES SIZE AND COMMON CAROTID IMT MEASURED IN PLAQUE-FREE AREAS SHOW ADDITIVE VALUE IN CARDIOVASCULAR-RISK PREDICTION AND INDIVIDUAL RECLASSIFICATION

Abstract nr. 271
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Co-author(s) - Frigerio, Beatrice
Co-author(s) - Ravani, Alessio L.
Co-author(s) - Castelnovo, Samuela
Co-author(s) - Sansaro, Daniela
Co-author(s) - Bonomi, Alice
Co-author(s) - Tedesco, Calogero C.
Co-author(s) - Amato, Mauro
Co-author(s) - Tremoli, Elena
Co-author(s) - 
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Cardiovascular Disease, Epidemiology, Risk stratification

Background and objective: Whether the size of carotid plaques and the mean of common carotid (CC) intima-media thickness (IMT) measured in plaque-free areas (PF CC-IMT mean) can be used as additive, rather than alternative, variables in models for cardiovascular risk refinement and reclassification remains to be established. To address this issue, in the “IMPROVE-study” European cohort (n=3703), we have evaluated the independence of the two variables in cardiovascular risk prediction as well as their capacity to mutually add in reclassification models.

Methods: Carotid plaque size was indexed as cIMT max, i.e. the size of the biggest plaque detected in the whole carotid tree. PF CC-IMT mean was assessed considering both CCs in their entire length. Quartiles of cIMT max and PF-CC-IMT mean were obtained and Hazard Ratios (HR) calculated comparing top quartiles of both PF CC-IMT mean and cIMT max vs normal values (1st, 2nd, 3rd quartiles) with Cox regression models.

Results: In this analysis stratified by center, both ultrasonographic variables were independent predictors of vascular events (VEs), even after adjustment for age, sex, vascular risk factors (LDL-C, HDL-C, systolic blood pressure, personal history of diabetes and of hypertension, family history of diabetes, family history of hypertension, pack-years), and pharmacological treatments (statins, beta-blockers, ACE inhibitors, diuretics and calcium antagonists); HR (95%CI) = 1.98 (1.47, 2.67); P<0.0001 and 1.68 (1.23, 2.29); P=0.001 for cIMT max and PF CC-IMT mean, respectively. When cerebrovascular or coronary events were considered, the Cox analysis showed that cIMT max and PF CC-IMT mean were independent predictors of cerebro VEs (P=0.0001 and P=0.005 for cIMT max and PF CC-IMT mean, respectively, whereas only cIMT max remained a significant and
independent predictor of coronary events (P=0.025). In reclassification analyses, considering the combined end-point, top quartile of PF CC-IMT_{mean} significantly adds to a model containing both Framingham Risk Score and top quartile cIMT_{max} [IDI (99% C.I.) 0.009 (0.003, 0.016); p=0.0001] and vice-versa [IDI (99% C.I.) 0.02 (0.010, 0.029); P<0.0001], thus corroborating an additive value in risk reclassification.

**Conclusion**: Carotid plaque size and CC-IMT measured in plaque-free areas are independent predictors of VEs; as such, they should be used as additive, rather than alternative, variables in models for cardiovascular risk prediction and risk reclassification.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Achilles Tendon Thickness: A More Distinguishable Benchmark than LDL-C for CAD

Abstract nr. 272
Author Tanaka, Nobukiyo, Ichinomiya Nishi Hospital, Ichinomiya, Japan
Co-author(s) - Yokoyama, Shinji
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease,Familial Hypercholesterolemia,LDL

Background and Purpose: FH is a highly prevalent hereditary disease at strong risk of CAD. It is important to estimate contribution of FH to CAD in Japan where the incidence is still relatively low to the Western World. Besides elevation of LDL-C and family history of FH/premature CAD, tendon/skin xanthomas are major symptoms of FH as one of its diagnostic criteria. We intended to evaluate the contribution of FH to CAD in Japan by measuring Achilles tendon thickness (ATT) of the patients with and without CAD.

Methods and Results: We investigated 484 patients who visited Ichinomiya Nishi Hospital from May 2010 to Jury 2014. ATT was measured on X-ray films. 173 patients were identified with CAD. LDL-C of CAD was lower than non-CAD, probably due to more extensive use of statins (57.2 % in CAD and 16.7 % in non-CAD). Two FH patients were identified based in each group by the JAS guidelines, resulting in 1.2 % vs 0.6 % in CAD and non-CAD groups, respectively, not reaching significance. Age, Family history, HDL-C, and ATT was extracted as a predictor of CAD by multiple logistic regression analysis (OR: 1.03, [1.00-1.05], P=0.021, OR: 2.29, [1.38-3.82], P=0.003, OR: 0.97, [0.94-1.00], P=0.026, OR: 1.39, [1.08-1.79], P=0.010, respectively). ATT was one of the risk markers of CAD by ROC curve (AUC: 0.60). Cut-off value of ATT in CAD is 6.7mm by positive likelihood ratio and 7.5mm by Youden index by ROC curve. More patients with CAD had ATT over 7 mm (OR: 1.69, P=0.007).

Discussion and Conclusion: FH is likely a risk of CAD in Japanese public health but more extensive survey is required to draw a quantitative conclusion. ATT is an apparent risk for CAD while an inconclusive marker for FH in general practice. The use of statins may mask FH due to substantial modification of LDL-C.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information

Patients diagnosed as FH

<table>
<thead>
<tr>
<th>Patient</th>
<th>age</th>
<th>sex</th>
<th>CAD</th>
<th>LDL-C (mg/dL)</th>
<th>Family History</th>
<th>ATT (mm)</th>
<th>statin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>M</td>
<td>-</td>
<td>199</td>
<td>none</td>
<td>10.2</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>F</td>
<td>-</td>
<td>195</td>
<td>none</td>
<td>9.3</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>M</td>
<td>OMI</td>
<td>204</td>
<td>Mother: Dyslipidemia with angina at 65yo</td>
<td>15.4</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>F</td>
<td>OMI</td>
<td>89</td>
<td>Sister: MI at 63yo</td>
<td>11.6</td>
<td>Rosuvastatin 2.5mg/day</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CAD (-) n=311</th>
<th>CAD (+) n=173</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial hypercholesterolemia (n,%)</td>
<td>2 (0.6%)</td>
<td>2 (1.2%)</td>
<td>0.620</td>
</tr>
<tr>
<td>FH hetero (population ratio)</td>
<td>(0.2%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ROC curve

<table>
<thead>
<tr>
<th>ATT Cut-off (mm)</th>
<th>TPF</th>
<th>FPF</th>
<th>Odds</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR_</td>
<td>6.7</td>
<td>0.418</td>
<td>0.572</td>
</tr>
<tr>
<td>Youden index</td>
<td>7.5</td>
<td>0.238</td>
<td>0.422</td>
</tr>
</tbody>
</table>
DECREASED IDL, LDL-1 AND HDL SUBFRACtIONS IN SICKLE CELL DISEASE PATIENTS IS ACCOMPANIED BY A REDUCTION OF APO-A1

Abstract nr. 275
Author Aslan, Mutay, Akdeniz University Faculty of Medicine, Antalya, Turkey
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Co-author(s) - Yonden, Zafer
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Co-author(s) - Kaya, Hasan
Co-author(s) - Oktay, Gonul
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, HDL, LDL, Lipoproteins

Abstract:

Aim: Although hypocholesterolemia is a reported finding in sickle cell disease (SCD), low-density lipoprotein (LDL)/high-density lipoprotein (HDL) subfractions and HDL-associated enzymes have not been determined in SCD patients.

Methods: Blood was collected from 38 hemoglobin (Hb)A volunteers and 45 homozygous HbSS patients who had not received blood transfusions in the last 3 months. Serum lipids were measured by automated analyzer while LDL and HDL subfraction analysis was done by continuous disc polyacrylamide gel electrophoresis. Serum levels of cholesteryl ester transfer protein (CETP), lecithin cholesterol acyltransferase (LCAT), apolipoprotein B (apoB) and apolipoprotein A-1 (apoA-I) were determined by enzyme-linked immunosorbent assay (ELISA).

Results: Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were significantly decreased, while TG levels were significantly increased in SCD patients compared to controls. A significant decrease in intermediate-density lipoprotein (IDL)-C, IDL-B, IDL-A and LDL-1 fractions were seen in SCD patients, while no significant difference was observed in small dense LDL particles. A significant decrease was seen in HDL-large, HDL-intermediate and HDL-small fractions in SCD patients versus controls. Levels of LCAT and ApoA-1 protein measured in SCD patients were significantly lower while no significant difference was observed in CETP and ApoB protein levels compared to controls.

Conclusions: The reduction observed in LDL- and HDL-C in SCD patients was reflected as significantly decreased IDL, LDL-1 and HDL-subfractions. Decreased HDL subfractions may possibly lead to the reduced ApoA-1 and LCAT protein levels observed in SCD patients.

Keywords: Sickle cell disease, Low-density lipoprotein, High-density lipoprotein, Lecithin-cholesterol acyltransferase, Apolipoprotein A-1.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Interaction between HIV-1 protein Nef and endoplasmic reticulum chaperone calnexin drives inhibition of activity of ABCA1

Abstract nr. 276
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Co-author(s) - Pushkarsky, Tatiana
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Lipoproteins, Pathogenesis, Reverse Cholesterol Transport

HIV-infected patients are at increased risk of developing atherosclerosis, in part due to downmodulation and impairment of activity of the ATP-Binding Cassette A1 (ABCA1) cholesterol transporter by the HIV-1 protein Nef (Mujawar et al., PLoS Biol. 2006 4:e365). The mechanism of this effect of Nef remained unknown for several years. Recently, we provided evidence that inhibition of ABCA1 activity in HIV-infected cells involves Nef interaction with an endoplasmic reticulum (ER) chaperone calnexin and disruption of calnexin binding to ABCA1, leading to ABCA1 retention in ER, degradation, and eventual suppression of cholesterol efflux (Jennelle et al., J Biol Chem. 2014 289:28870-84). However, the mechanics of this Nef effect remained unclear, in particular because the substrate-binding domain of calnexin is located within the ER lumen, whereas Nef has not been documented to enter the lumen of ER. We therefore strived to characterize Nef-calnexin interaction. Initial experiments using deletion mutants of calnexin lacking cytoplasmic, luminal, or transmembrane domains demonstrated that Nef interacts with the cytoplasmic tail of calnexin. Computer structure modeling and docking predicted that lysine residues in positions 4 and 7 of Nef are critical for this interaction. We have used publicly accessible docking servers to build a dataset of Nef-calnexin interaction models, and analyzed these models to identify the amino acid residues in Nef with the highest number of interactions. The list of these residues included Lys 7, which formed strong interaction with calnexin. Modeling of Nef showed that Lys 4 plays a key role in stabilizing structure of the Nef N-terminal region where Lys 7 is located. Thus, mutation of both lysines can have a dual effect of destabilizing structure of the binding interface and disrupting Nef-calnexin interaction. We introduced mutations of these lysine residues into a molecular clone of HIV-1 and demonstrated that mutated Nef does not interact with calnexin, ABCA1 is not downregulated in cells infected with the mutant virus, and cholesterol efflux from cells infected with such virus remains intact. This study identifies potential targets that can be exploited to block the pathogenic effect of HIV infection on cholesterol metabolism.

Subdivision 1. Basic Science
Presentation Preference Oral presentation
Additional information
HIV infection induces structural and functional changes in High Density Lipoproteins

Abstract nr. 277
Author Bukrinsky, Michael, George Washington University School of Medicine and Health Sciences, Washington, United States of America
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Co-author(s) - Sviridov, Dmitri
Co-author(s) - Fitzgerald, Michael
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, HDL, Lipids, Lipoproteins

Coronary artery disease is a growing clinical problem in HIV-infected subjects. The increased risk of coronary events in this population has been linked to low levels of HDL, but the effects of HIV infection and anti-retroviral treatment (ART) on HDL structure and function remain poorly characterized. Here, we determined the composition and function of HDL particles isolated from plasma samples collected from ART-naive and ART-positive HIV-infected patients and compared them to HDL from a convenience control group. Proteomic profiling revealed decreased levels of paraoxonase (PON) 1 and PON 3 in HDL from both treated and untreated HIV-positive patients, and PON activity of HDL from HIV-infected subjects was significantly lower than in control group. Lipidomic profiling uncovered that sphingomyelin and ether-linked glycerophospholipid species in the HDL particles correlated positively with viral load and negatively with CD4+ T cell counts in the ART-naive subjects. Consistent with analysis of lipids, the level of PLTP showed a significant positive correlation with viral load and negative correlation with CD4+ T cell counts in ART-naïve HIV-positive samples. Given the low PON1 and PON3 levels in HDL from HIV-infected subjects, we tested for the level of oxidized phospholipids in the HDL particles from a subset of HIV+ ART-naïve and treated subjects with completely suppressed viremia. No significant differences in oxidized lipids were found between HDL from control and untreated or PI-treated subjects, but a significant increase was detected in the NNRTI-treated samples. Similarly, the ratio of oxidized LDL to total LDL was significantly increased in the NNRTI-treated samples. These results suggest that reduced PON levels in HDL of HIV-infected subjects do not translate to increased levels of oxidized lipids, however, low PON may contribute to increased oxidation in NNRTI-treated patients. Functional analysis demonstrated a negative correlation between cholesterol efflux capacity of HDL and viral load in ART-naive HIV-infected group. Taken together, these results indicate that HIV infection associates with a number of both protein and lipid compositional changes in HDL particles. Moreover, HIV infection affects cholesterol efflux function of HDL, thus contributing to an increased risk of atherosclerosis in this patient population.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Modeling the VLDL delipidation cascade: apolipoprotein B and triglyceride pools have identical rate constants despite different fates and mass distributions

Abstract nr. 278
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Co-author(s) - Ginsberg, Henry
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Metabolism, Triglycerides

Labeled amino acid and glycerol are used simultaneously to determine VLDL apolipoprotein B (apoB) and triglyceride (TG) rate constants (e.g., Nagashima JCI 15:1323, 2005). Models include subpools in a delipidation cascade with the TG to B ratio decreasing along the cascade. While one can develop and fit models for VLDL-B and VLDL-TG separately, we believe it more appropriate to develop a single model for apoB and TG in VLDL. The integrated model should express the assumptions about the relationships between apoB and TG along the cascade. In brief, we assume that each pool along the cascade has a specific TG:B ratio. A simple two-pool cascade is shown in the figure (notation as in Ramakrishnan Bull. Math. Biol. 72:2019, 2010), A for apoB and B for TG. The mass distribution between big and small particles is different for apoB and TG (a much larger fraction of VLDL-TG is in pool 1): Q1/Q2 for TG is b/s times Q1/Q2 for apoB, where “b” and “s” are the TG:B ratio in big and small particles, respectively. It is also seen that the synthetic flux ratio into the two pools is different for apoB and TG, interestingly by the same ratio b/s. Less intuitive, and quite striking from a modeling perspective, is our result that the pool models for tracer enrichments are identical for apoB and TG; this is conveyed in the figure where the r parameters, relevant to enrichment modeling, are seen to be the same for apoB and TG. Thus, a combined model for apoB and TG, when written for enrichments rather than for total tracer quantities, has the same rate constants for both apoB and TG; the models differ only in the mass fractions. We have taken this approach in our publications. The widely done modeling of total tracer quantities does not make the commonality clear; this can be seen in the figure where the k parameters, relevant to total tracer quantity modeling, are not the same for apoB and TG. In conclusion, the differential equations are identical for VLDL-B and VLDL-TG, provided they are in enrichment terms.
Two-pool VLDL delipidation cascade for apoB and TG

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Apolipoprotein B kinetics modeled using prior studies’ averaged amino acid parameters rather than plasma amino acid enrichments from each study

Abstract nr. 279
Author Ramakrishnan, Sekhar, Columbia University, New York, United States of America
Co-author(s) - Holleran, Stephen
Co-author(s) - Ginsberg, Henry
Co-author(s) - Soffer, Gissette
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, LDL, Metabolism

Apolipoprotein B (ApoB) turnover kinetics are routinely studied by measuring ApoB enrichment in VLDL, IDL and LDL following a bolus injection of a stable isotope of an amino acid (AA), possibly along with a primed constant infusion of a second stable AA isotope. It is customary to measure plasma AA enrichments and model AA kinetics for each individual to improve the modeling of the apoB enrichment data even though AA metabolism is not of interest. We theorized that if, instead of measuring each subject’s plasma AA enrichments, mean AA kinetic parameter values from prior studies were used in fitting each subject’s apoB enrichment data, the resulting apoB parameter estimates for each subject would approximate closely the values obtained when that subject’s AA enrichments are measured and used. We tested this theory by reanalyzing the data from a crossover study of apoB kinetics in 8 subjects before and on treatment with pioglitazone (reported in Nagashima et al JCI 15:1323, 2005), but using the mean AA kinetic parameter values (the averages of the values obtained when individual AA enrichment data were used) for all subjects, on or off pioglitazone. We found excellent agreement: correlation coefficients, between apoB kinetic parameters estimated using individual AA enrichments vs those estimated using mean AA kinetic parameters, exceeded 0.79 for VLDL, IDL and LDL apoB fractional catabolic rates (FCR) and production rates (PR). The correlation coefficients are shown in the Table, along with the results, by paired t-tests, of comparisons of placebo vs pioglitazone with each modeling approach. The p-values are seen to be very close. We conclude that, without sacrificing validity or statistical power, considerable laboratory work can be saved by not measuring each subject’s plasma AA enrichments but, rather, using mean AA kinetic parameters from prior studies. We are in the process of confirming these findings in a different crossover study.

This work was supported by grants TR000040 from the NIH/NCATS and HL55638 from the National Heart, Lung and Blood Institute.
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n=8
Table of correlations and paired t p-values with two modeling approaches

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation

Additional information
Is the lower LDL value the less occurrence of vascular events? — Results from a Taiwan SPARCLE Registry

Abstract nr. 280
Author Wu, Chau-Chung, National Taiwan University Hospital, Taipei, Taiwan
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Co-author(s) - Jeng, Jiann-Shing
Co-author(s) - Yin, Wei-Hsian
Co-author(s) - Chuang, Shao-Yuan
Co-author(s) - Yeh, Hung-I
Co-author(s) - Chen, Jaw-Wen
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, LDL

Background / Purpose: Aggressive lipid control for patients with atherosclerotic diseases has been recommended by several guidelines. This study aimed to evaluate the vascular events in patients with different levels of low-density lipoprotein cholesterol (LDL-C) from an atherosclerotic disease registry in Taiwan.

Methods: This multicenter cohort study was conducted in 14 hospitals in Taiwan. A total of 3,434 outpatients who had a history of atherosclerotic vascular disease were recruited. Patients were categorized into 4 groups based on the LDL-C values: <70, 70-99, 100-129, and >130 mg/dL. Primary endpoints included cardiovascular death, nonfatal stroke, nonfatal myocardial infarct, and cardiac arrest required resuscitation. Kaplan-Meier method and Cox proportional hazard model were applied to assess relevant variables between groups.

Results: Of all patients (mean age, 65.6±11.8 years; male, 2,402 [69.9%]), 559 (16.2%), 1,300 (37.9%), 948 (27.6%), and 627 (18.3%) were categorized in the <70, 70-99, 100-129, and >130 mg/dL of LDL-C respectively. The mean duration of follow-up was 2.36±0.36 years. Patients with lower LDL-C were more likely to have older age, higher frequencies of diabetes and myocardial infarction, but lower frequency of ischemic stroke. Patients with LDL-C 100-129 mg/dL had the lowest risk of vascular events and death (adjusted hazard ratio, 0.30; 95% confidence intervals, 0.14-0.66; p=0.003) as compared to patients with LDL-C <70 mg/dL (Figure). Statin use can decrease the occurrence of vascular events and death (adjusted hazard ratio, 0.58; 95% confidence intervals, 0.35-0.97; p=0.037).

Conclusions: For patients with atherosclerotic vascular disease, LDL-C control in the range of 100-129 mg/dL and statin use has the lower risk of vascular events in Taiwanese.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
DPP-4 signaling in T cells contributes atherosclerotic development and renal dysfunction

Abstract nr. 281
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Co-author(s) - Kitamura, Kazutaka
Co-author(s) - Masuda, Chieko
Co-author(s) - Uto, Kenta
Co-author(s) - Hagiwara, Nobuhisa
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Pathogenesis, Renal function

**Background:** Dipeptidyl peptidase4 (DPP-4, CD26) is expressed on CD4+ memory T cells and represents a marker of T cell activation. However, it is not known whether DDP4 inhibitors for type 2 diabetes prevent atherosclerosis development. In this study, we examined the effect of DDP-4 inhibitor for the inflammatory cytokine producing-T cells in atherosclerotic model mice.

**Methods/Results:** We examined 10W ApoE−/− mouse (Cont) and compared to the mice fed with high fat diet for 8 weeks (HFD), alogliptin (DPP4 inhibitor) 20mg/kg/day, or 40mg/kg/day. In HFD, sudan staining of aorta revealed the atherosclerotic lesion was increased compared to Cont. In addition, IFNγ+Th1 and IL17+Th17 were increased by FACS, and infiltrated into the immunohistochemistry plaque of aorta and glomeruli. Furthermore, the urinary albumin/creatinine ratio was increased in HFD compared to Cont. Alogliptin treatments inhibited IFNγ+Th1 and IL17+Th17 filtrates into the plaque and glomeruli, and the proteinuria. Next, to investigate whether the inflammatory cytokine induced dysfunction of podocytes were involved in proteinuria, we investigated nephrin, podocin, and phosphorylated nephrin in the kidney by Western blots and immunohistochemistry. The decreased phosphorylated neprin in HFD were recovered in Alog. Finally, we confirmed that the morphological changes of podocytes in HFD were improved in Alog by electron microscopy.

**Conclusion:** DDP4 signaling in IFNγ+ Th1 and IL17+Th17 T cells might aggravate atherosclerosis acceleration and induce the initiation of glomerular tissue damage.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Beneficial effects of extract from Mexican genetic resources of Ganoderma lucidum on cholesterol metabolism and gut microbiota in C57BL6 mice

Abstract nr. 282
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Co-author(s) - Torres, Nimbe
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Metabolism, Nutrition, Prevention

Although the medicinal mushroom *Ganoderma lucidum* (Gl) provides health benefits, Mexican genetic resources from this species have not yet been studied. We studied the effect of the consumption of Gl standardized extract in mice fed with a high cholesterol diet on the expression of genes involved in liver cholesterol metabolism, as well as in the gut microbiota. Mice were fed 20% casein (AIN93G) diet for 42 days, with and without cholesterol (0.5%), and supplemented with Gl extract (1.0%). Consumption of Gl extract decreased the expression of the HMGCoA reductase gene involved in the endogenous synthesis of cholesterol, and increased LDL-r and PCG1β gene expression compared to mice fed with high cholesterol diet only. Moreover, Gl extract significantly reduced hepatic and serum cholesterol and triglycerides concentration compared to the cholesterol group without Gl extract. A gut microbial analysis revealed that the consumption of Gl extract increased significantly the gene expression of *Lactobacillus, Bacteroidetes, Actinobacteria, and Bifidobacteria*, while decreased *Firmicutes*, as compared to mice fed with a cholesterol diet without Gl extract. Our data showed that the consumption of Gl extract had positive effects on cholesterol metabolism and the gut microbiota, which may help in the prevention of atherosclerosis and metabolic syndrome due to its hypolipidemic and prebiotic properties.

This work was supported by CONACYT.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Sparse endothelial cell monolayer downregulates TNF-α -induced E-selectin expression through epigenetic mechanisms

Abstract nr. 283
Author Tani, Mariko, Tokyo Medical and Dental University, Tokyo, Japan
Co-author(s) - Hamada, Katsuhiko
Co-author(s) - Osaka, Mizuko
Co-author(s) - Yoshida, Masayuki
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Endothelium, Inflammation

[Objective] Activation of vascular endothelium, such as expression of adhesion molecules, plays an important role in vascular inflammation. Recent studies revealed the morphological patterns affect various cellular functions including cytokine induced cell activation. In this study we investigated the influence of cell density on TNF-α mediated-E-selectin in human umbilical endothelial cells (HUVEC).

[Methods and Results] To obtain sparse and confluent monolayer, HUVECs were seeded at a density of 7.2×10^3 cells/cm^2 and 29.2×10^3 cells/cm^2, respectively and then cultured 36 hours followed by stimulation with TNF-α. Total E-selectin protein expression was significantly greater in confluent monolayer than those in sparse monolayer. When the single cell surface expression was determined by flow cytometric analysis, confluent monolayer exhibited significantly higher E-selectin than sparse monolayer. Interestingly, neither NFkB activation nor phosphorylation of JNK and p38 was involved in this phenomenon. To our surprise, chromatin accessibility of E-selectin gene, judged from CHART-PCR, was higher in confluent monolayer than in sparse monolayer (P<0.05). Finally, ChIP assay of E-selectin promoter revealed that E-selectin proximal promoter region was highly and specifically acetylated in confluent HUVEC monolayer upon TNFa activation (p<0.01).

[Conclusion] Our data suggest that cell density regulates endothelial expression of E-selectin via epigenetic pathway that impact on the accessibility of chromatin.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
Initial oxidation of LDL by iron at lysosomal pH is due to tryptophan radicals and is not inhibited by probucol

Abstract nr. 284
Author Ahmad, Feroz, Institute of Cardiovascular and Metabolic Research, University of Reading, Reading, United Kingdom
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins,Atherosclerosis,LDL,Pathogenesis

The LDL oxidation hypothesis proposes that cells oxidise LDL in arterial interstitial fluid and macrophages take it up rapidly, becoming foam cells. LDL oxidation is inhibited by interstitial fluid, however, and large clinical trials have shown no protection by antioxidants, including probucol. We therefore proposed that LDL might be nonoxidatively modified and aggregated by enzymes, such as sphingomyelinase, in interstitial fluid, rapidly phagocyted by macrophages and oxidised by iron inside lysosomes, which have a pH of about 4.5 (Wen & Leake (2007) Circ. Res. 100, 1337). Tryptophan in apoB-100 might be involved in the initiation of LDL oxidation by copper (Giessauf et al. (1995) BBA 1256, 221). We investigated the mechanisms of LDL oxidation by iron at lysosomal pH. LDL (50 µg LDL protein/ml) was oxidised by FeSO₄ or FeCl₃ (5 µM) at 37 °C in 150 mM NaCl/ 10 mM sodium acetate buffer, pH 4.5. Lipid oxidation was measured in terms of conjugated dienes at 234 nm and tryptophan oxidation by the loss of fluorescence (Ex/Em 282/331 nm). Unexpectedly, the loss of tryptophan fluorescence was faster with Fe²⁺ than Fe³⁺. Interestingly, probucol did not inhibit lipid oxidation for about 100 min for Fe²⁺ and Cu²⁺ at pH 4.5 and did not decrease the loss of tryptophan fluorescence. In contrast, probucol inhibited entirely the initial oxidation of LDL by Cu²⁺ at pH 7.4. As probucol was unable to prevent loss of tryptophan fluorescence, but would be expected to scavenge lipid radicals, the initial oxidation of LDL at pH 4.5 might be due to the formation of tryptophan radicals which attack the lipids. This might explain why the oxidation of LDL lipids at pH 4.5 is faster with Fe²⁺ than Fe³⁺. We propose the following mechanism of LDL oxidation by Fe²⁺ at lysosomal pH.

Fe²⁺ + O₂ → Fe³⁺ + O₂⋅
O₂⋅ + H⁺ ↔ HO₂⋅ (pKa 4.8)
HO₂⋅ + TrpH → Trp⋅ + H₂O₂
Trp⋅ + O₂ → TrpOO⋅
LH + TrpOO⋅ → L⋅ + TrpOOH
L⋅ + O₂ → LOO⋅

This might help to explain why probucol was not beneficial in the clinical trials.
Funded by the Felix Trust.
Kinetics of decrease of LDL-Trp fluorescence during LDL oxidation

Measurement of conjugated diene formation during oxidation of LDL (50μg protein/ml) by 5μM FeSO4 or FeCl3 or CuSO4 at pH 4.5 or 7.4

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Antihypertensive therapy and phospholipid membrane spectrum in hypertensive patients

Abstract nr. 285
Author Smiianova, Yuliya, Sumy State University, Sumy, Ukraine
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Co-author(s) - Vynnychenko, Lyudmyla
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Hypertension, Therapy

Objective. Study the phospholipid composition of platelets’ membranes in hypertensive patients, its role in the development of inflammation and membran-destructive processes influenced antihypertensive therapy.

Methods. The study involved 58 hypertensive patients. Control group consisted of 25 people. Levels of total lipids (TL), phosphatidylserine (PS), sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylethanolamine (PE), lysophosphatidylcholine (LPC) and fosfoinozitolphosfat (FI) were determined using two-dimensional thin layer chromatography on silico-gel. All patients received basic+antihypertensive therapy (fosinopril 10-20mg, diltiazem 90-180mg).

Results. In all stages of arterial hypertension were made for the fractional composition of the lipid fractions of platelet membranes. Significantly (p<0.05) improved the content of TL in I stage to (4.64±0.28)g/l and cholesterol to (1.31±0.11)g/l, while the level of SM, PS, PC were reduced by 13.2%, 30.8%, 11.2% respectively. FI median concentration was reduced 2.03 times compared to the control in all patients, with the most pronounced changes were observed in III stage. Least fraction FI decreased in latent stage at 0.61 times. In all stages reduced the percentage of SM: I-at 1.49 times, II-1.45, III-1.53. PE fraction was reduced in all patients. The largest decline was 1.27 times, the percentage of PE was observed in III stage. PS and PE are the basic components of the inner bilayer cytomembranes, depletion of membrane phospholipids leads to a redistribution of both indoors cell membranes, which helps to change the structure and function of membranes. Pronounced increase in LPC (2.05 times) was noted in the III stage, indicating that the destabilization of their structure and high permeability.

Changing the phospholipid composition of the membranes of platelets under the influence of treatment: TL fell under the influence of fosinopril (0.03 to 0.88 mg/ml) and a combination of an ACE inhibitor with diltiazem (0.05 to 0.87 mg/ml), and this reduction was dependent both on the stage of arterial hypertension. A moderate reduction of observed and PC, while significantly reducing the level of LPC, CM, and increased the level of PC, PS and PE.

Conclusion. In the membranes of platelets lysoform phospholipids accumulate, indicating a strengthening of the processes of lipid peroxidation and accumulation of bioactive substances with membran-destructive activity.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Identification of and Alerting to High Cardiovascular Risk may lead to Positive Changes in Lifestyle and Reduced Risk Factor levels

Abstract nr. 287
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Co-author(s) - Mørch-Reiersen, Lisa T
Co-author(s) - Garstad, Kjersti W
Co-author(s) - Granlund, Linda
Co-author(s) - Thyholt, Kari
Co-author(s) - Jacobs, David R
Co-author(s) - Retterstøl, Kjetil

Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Lifestyle, Prevention, Risk Factor

Purpose: Many individuals with a high risk of cardiovascular disease (CVD) are undiagnosed. The joint aim of the present studies was to investigate the impact of identifying and alerting people to high risk on lifestyle behavior and risk factor levels.

Method: We performed two studies as part of vascular screenings in Norwegian pharmacies in 2012 (n=18,000) and 2014 (n=15,000). In study 1 (2012) there were 140 subjects with total cholesterol (TC) ≥7.8 mmol/L, of whom 59 subjects were approached, and 49, (16% men and 84% women), consented to participate in a 24-week follow-up study. There was no control group. In study 2 (2014), we measured TC, HDL-C and LDL-C, HbA1c, blood pressure and body mass index in 1,500 subjects, of whom the ~40% with the highest CVD risk participated in an 8-week randomized controlled trial (RCT). 595 subjects, (28% men and 72% women), met the inclusion criteria and were randomly assigned to the intervention group (n=204) or the control groups (n=391). The intervention group was informed about their CVD risk at the first visit, while the control groups had delayed information.

Main findings: In study 1, mean TC decreased 0.9±1.0 mmol/L (p=0.02) after 24 weeks. 29 participants (59%) reported seeing their general practitioner for a new check, of whom 13 (27%) started lipid lowering treatment. Furthermore, 27 participants (55%) reported increased intake of fruits and vegetables and decreased intake of saturated fat. Data from study 2 will be presented at the conference.

Conclusion: Identification of and alerting to TC ≥7.8 mmol/L resulted in ≥12% decrease in TC after 24 weeks, and appeared to increase use of statins and improved diet in people with high cholesterol who visited Norwegian pharmacies.

Funding: The screenings (2012 and 2014) are funded by Boots Norge AS and Mills DA. The PhD student is funded by the University of Oslo and Throne Holst Foundation.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Quantifying Stroke and Bleeding Risk: Results from Three Practice Settings

Abstract nr. 288
Author Breve , Frank, Mid Atlantic PharmaTech Consultants, LLC, Ventnor City, United States of America
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Co-author(s) - Bateman , Julie
Co-author(s) - Watson , Sharon
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Anticoagulants,Atrial fibrillation,Guidelines,Thrombosis

Purpose
The purpose of this research was to assess the management of patients with nonvalvular atrial fibrillation (NVAF) by a cardiology group, two long-term care (LTC) facilities and a hospital-based anticoagulation clinic.

Methods
This project was conducted using a retrospective cohort design. The study population included patients identified with a diagnosis of NVAF. Patient information collected manually and via electronic medical records was transcribed onto project-specific data collection forms. Information included stroke risk assessment (CHADS2), antithrombotic therapy, bleeding risk (HAS-BLED) and time in therapeutic range (TTR) for patients receiving warfarin. Data and statistical analysis was conducted by a third party using Microsoft Access and descriptive statistics reported for all parameters.

Results
There were a total of 194 patients included in the analysis, mostly from the cardiology group (54%). The mean CHADS2 score for the sample was 2.5 and 85% were classified as high risk for stroke (CHADS2 >2). Hypertension (77%) and diabetes (38%) were the most common disease-related risk factors. Overall, 73% of high risk patients received a practice guideline-recommended anticoagulant. More clinic patients (92%) received an anticoagulant than in the cardiology group (89%) and the LTC facilities (38%). Six patients (4%), all from the LTC facilities, received aspirin in combination with an antiplatelet agent. Of the high risk patients receiving no therapy, 36% lacked documentation as to why an anticoagulant was not prescribed. The mean HAS-BLED score for the sample was 2.2 and most patients were determined to be intermediate (38%) or high risk (28%) for bleeding. TTR for those patients receiving warfarin was 64%, 51% and 49% for the cardiology group, anticoagulation clinic and LTC facilities, respectively.

Conclusions
Oral anticoagulants are recommended for patients at high risk for stroke. However, approximately 27% of the high-risk patients in this analysis did not receive a guideline-recommended agent and documentation is lacking when no anticoagulants are prescribed. TTR was consistent with
published literature and varies by practice setting. Although it is generally higher in anticoagulation clinics, the cardiology group had the highest TTR in this analysis.

![Graph showing Time in Therapeutic Range](image)

**Time in Therapeutic Range**
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
Effects of Freeze-Dried Strawberry Supplementation on Metabolic Biomarkers of Atherosclerosis in Subjects with Type 2 Diabetes

Abstract nr. 289
Author Moazzen, Sara, Tabriz, Iran
Co-author(s) - Amani, Reza
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Diabetes, Endothelium, Inflammation, Nutrition

Background: To our knowledge there has been no study investigating the impact of freeze-dried strawberry (FDS) supplementation on metabolic biomarkers of atherosclerosis in subjects with type 2 diabetes (T2D).

Objective: This study was carried out to determine the effects of FDS supplementation on glycemic control, biomarkers of oxidative stress, inflammation and serum total antioxidant status in subjects with T2D compared to matched control subjects.

Methods: Thirty-six subjects with T2D (23 females; mean body mass index 27.90 ± 3.7; mean age 51.57 ± 10 years) were randomly divided into two groups. The treatment group consumed 2 cups of FDS beverage (50 g of FDS is equivalent to 500 g of fresh strawberries) or macronutrient matched placebo powder with strawberry flavor daily for 6 weeks in a randomized double-blind controlled trial. Anthropometric measurements, dietary intakes, hemoglobin (Hb)A1c, antioxidant status, C-reactive protein and malondialdehyde (MDA) levels were assessed at baseline and 6 weeks post-intervention.

Results: FDS supplementation significantly decreased C-reactive protein levels as a biomarker of inflammation (2.5 vs. 2.04 mg/l, p < 0.05) and lipid peroxidation in the form of MDA (3.36 vs. 2.7 nmol/ml, p < 0.05) at 6 weeks compared to the baseline. Moreover, supplementation led to a decreasing trend in HbA1c (~5.7%, p < 0.05) and significant increase in total antioxidant status in the FDS group (1.44 vs. 1.26 mmol/l, p < 0.01) compared to the placebo group. No significant changes were observed in serum glucose concentrations and anthropometric indices.

Conclusions: FDS improved glycemic control and antioxidant status, and reduced lipid peroxidation and inflammatory response in patients with T2D. Supplementation with freeze-dried berry products, as natural sources of antioxidants with low glycemic index, could be considered as an adjunctive therapy in ameliorating metabolic complications of T2D.

All procedures involving human subjects were approved by the medical ethics committee of Ahvaz Jundishapour University of Medical Sciences, study No. ETH_393, clinical trial registration No. IRCT201110117765N1.
Percentage of change in hs-CRP, TAC, MDA and HbA1c after 6 weeks postintervention in both the FDS and control groups. * Significant change in the tr

Presentation Preference Oral presentation

Additional information
Arterial stiffness doesn't correlate with endothelial dysfunction: Can arteriosclerosis reflect intimal atheromatous changes?

Abstract nr. 290
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Co-author(s) - Min-Kyong, Moon
Co-author(s) - Kim, Hee-Jun
Co-author(s) - Kim, Hack-Lyoung
Co-author(s) - Jae-Bin, Seo
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Endothelium

Objective: This study aimed to investigate the difference and association between Reactive Hyperemia Index (RHI) and brachial artery Pulse Wave Velocity (baPWV).

Methods: A total 142 patients (mean 61±10 years, 71% of male) who underwent RHI and baPWV were included. All patients had either more than one of atherosclerotic risk factors or coronary artery disease. A worse baPWV between both legs was selected as a representative value in each patient.

Results: 35% of patients had diabetes mellitus (DM), 8% of patients had peripheral artery disease (PAD) and 58% of patients have coronary artery disease (CAD). baPWV was not linearly correlated with RHI. (r=-0.045, p=0.62, Figure 1) In diabetic patients, both RHI and baPWV were worse than non-DM. (1.5 vs 1.7, p=0.011; 1815 vs 1545, 0.001) In PAD patients, RHI was worse than non-PAD, while baPWV showed no difference between two groups (1.2 vs 1.7, p=0.003; 1625 vs 1847 cm/sec, p=0.370) In diabetic subgroup, RHI was similar between CAD and non-CAD. (1.6 vs 1.7, p=0.163) However baPWV was better in non CAD group with borderline significance. (1715 vs 1588 cm/sec, p=0.061) In nondiabetic subgroup, RHI was lower in PAD and CAD than non-PAD, non-CAD. (1.2 vs1.8, p=0.016; 1.7 vs 1.9, p=0.035) However, baPWV was even better in CAD than non-CAD (1485 vs 1627 cm/sec, p=0.009), and comparable between PAD and non-PAD. (1730 vs 1536 cm/sec, p=0.123) Figure 2 shows baPWV and RHI in other subgroups.

Conclusions: Arterial stiffness represented by baPWV doesn't correlate with RHI. It’s more prominent in nondiabetic or CAD/PAD patients. It suggests baPWV poorly reflects atheromatous changes initiated by endothelial dysfunction.
**RHI vs baPWV linear correlation**

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<td>1651±425</td>
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<td>1822±492</td>
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**RHI vs baPWV in subgroups**

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
The effects of vitamin D supplementation on cardiometabolic biomarkers in non alcoholic fatty liver disease: Double blind randomized controlled trial

Abstract nr. 291
Author Amani, Reza, Jundishapur University of Medical Sciences, Ahvaz, Iran
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Co-author(s) - Hajiani, Eskandar
Co-author(s) - Cheraghian, Bahman
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Nutrition, Risk Factor, Therapy

Aim: Prevention of cardiovascular disease (CVD) is crucial in non alcoholic fatty liver disease (NAFLD). The aim of this study was to assess the effects of vitamin D supplementation on some cardiometabolic biomarkers in adult patients with NAFLD.

Methods: Fifty three patients with NAFLD were enrolled in a parallel double blind placebo controlled study. The patients were randomly allocated to receive either one oral pearl consisting of 50,000 IU vitamin D₃ (n=27) or a placebo (n=26), every 14 days for 4 months. Serum aminotransferases, high-sensitive C-reactive protein (hs-CRP), tumor necrosis factor α (TNF-α), malondialdehyde (MDA), lipid profile as well as grade of hepatic steatosis and homeostasis model assessment of insulin resistance (HOMA-IR) were assessed pre- and post-intervention.

Results: The median of serum 25(OH)D₃ significantly increased in the vitamin D group compared with the controls (16.2 vs. 1.6 ng/mL, p<0.001). This increase accompanied by significant decrease in serum MDA levels (-2.09 vs -1.23 ng/mL, p<0.05) and also near significant decrease in serum hs-CRP (-0.25 vs. 0.22 mg/L, p=0.06). These between group differences remained unchanged after controlling for baseline covariates. Vitamin D supplementation did not make any changes in the serum lipid profile and liver enzymes.

Conclusion: Improved vitamin D status led to improvement in serum hs-CRP and MDA in patients with NAFLD. Vitamin D supplementation might be considered as an adjunctive therapy to attenuate some risk factors of CVDs in terms of systemic inflammation and lipid peroxidation in NAFLD patients especially when vitamin D status is compromised.
CSL112 enhances the cholesterol efflux capacity of plasma to a similar magnitude in STEMI patients and normolipidaemic subjects ex vivo

Abstract nr. 293
Author Didichenko, Svetlana, CSL Behring AG, Bern, Switzerland
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Co-author(s) - Rached, Fabiana
Co-author(s) - Santos, Raul
Co-author(s) - Serrano Jr, Carlos
Co-author(s) - Cukier, Alexandre
Co-author(s) - Kontush, Anatol
Co-author(s) - Chapman, M. John
Co-author(s) - Wright, Samuel
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords ACS, HDL, Reverse Cholesterol Transport, Therapy

CSL112 is apolipoprotein AI (apoA-I) purified from human plasma and reconstituted with phosphatidylcholine (PC) to form lipoprotein particles suitable for infusion. CSL112 is being developed as a therapy to reduce the high risk of recurrent events in the weeks following an acute coronary syndrome (ACS).

Previously, we demonstrated that infusion of CSL112 dramatically increases cholesterol efflux capacity to a similar magnitude in both healthy subjects and patients with stable atherosclerotic disease. This effect is accompanied by changes in the HDL profile with a profound increase in small HDL species such as pre-β1-HDL, the preferred acceptor of cholesterol from laden cells. As ACS depresses cholesterol efflux, we investigated the ability of CSL112 to promote HDL remodelling and to elevate plasma cholesterol efflux capacity ex vivo in patients, post-acute myocardial infarction (MI).

Plasma drawn from ST segment elevation MI (STEMI) patients within 24 h of ACS or from normolipidaemic subjects was incubated with CSL112 for 1 h at 37°C. HDL particle size distribution was determined by native gel electrophoresis followed by western blotting. Ex vivo capacity of apoB-depleted plasma to support cholesterol efflux was assessed using [3H]cholesterol-loaded RAW264.7 macrophages expressing ABCA1.

STEMI plasma displayed lower levels of apoA-I (47.82% ± 8.9%, n=8), marked elevations of serum amyloid A (SAA) and an attenuated cholesterol efflux capacity (73.3% ± 6.9%, n=8) when compared to control plasma (100%, n=7). Incubation with CSL112 altered HDL particle size distribution in both control and patient plasma, raising levels of pre-β1-HDL (0.125 ± 0.043 mg/ml in STEMI; 0.37 ± 0.06 mg/ml in controls) and small HDL3C-like particles. Neither pre-β1-HDL nor small HDL3C-like particles were associated with SAA, a marker of acute inflammation which may...
bind to HDL particles. Critically, CSL112 increased the capacity of STEMI and control plasma to efflux cholesterol to a similar extent (% efflux: 12.1% ± 1.3% in STEMI, 13.2 ± 0.6 % in controls). These observations suggest that CSL112 infusion may acutely restore efflux capacity in ACS patients making it a promising therapy to reduce the risk of recurrent cardiovascular events. This is currently being investigated in a Ph2b trial of post-MI patients.

Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
Potential implications of the new ACC/AHA guidelines in a low-risk Chinese population: the Guangzhou Biobank Cohort

Abstract nr. 294
Author Lin, Jiayan, School of Public Health, The University of Hong Kong, Hong Kong, Hong Kong
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Co-author(s) - Zhang, WS
Co-author(s) - Xu, L
Co-author(s) - Cheng, KK
Co-author(s) - Leung, GM
Co-author(s) - Lam, TH
Co-author(s) - Schooling, CM
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Cardiovascular Disease, Guidelines, Prevention

BACKGROUND: In November 2013, the American College of Cardiology/the American Heart Association (ACC/AHA) published updated guidelines for atherosclerotic cardiovascular risk assessment and treatment for non-Hispanic Caucasian and African Americans, the potential implications of the new guidelines in other settings, such as Chinese, remain unverified. We aimed to determine the potential implications of these new guidelines in a Chinese cohort.

METHODS: In the Guangzhou Biobank Cohort Study recruited from September 2003 to January 2008 (n=30499), followed-up from 2008 to 2012, 24838 participants aged 50 to 79 years (mean age 60.7y, 72.8% women) without atherosclerotic cardiovascular disease (ASCVD) or use of lipid modulating treatment at baseline and with low-density lipoprotein cholesterol (LDL-C) from 70 to 189 mg/d (1.81 to 4.89 mmol/L) were eligible for 5-year CVD risk prediction. Participants were categorized into four groups based on their estimated 10-year ASCVD risk: less than 5%, 5% to less than 7.5%, 7.5% to less than 10% and 10% or above. The observed and predicted 5-year risks of a first hard ASCVD event (coronary heart disease death, nonfatal myocardial infarction or fatal or nonfatal stroke) were calculated by level of predicted 10-year ASCVD risk.

RESULTS: The observed and predicted 5-year ASCVD risks for the group with 10-year predicted ASCVD risk of <5% (n=11297) was 0.1% and 0.8% respectively, for the group with 10-year predicted risk of 5%-<7.5% (n=2817) 0.8% and 2.2%, for the group with 10-year predicted risk of 7.5%-<10% (n=2234) 0.5% to 3.3%, and for the group with risk of ≥10% (n=8490) was 2.9% and 8.4%. Calibration, i.e., prediction, was poor (Hosmer-Lemeshow χ²=330.0, p<0.001), but the C statistic was 0.81 (95%CI, 0.78-0.85) indicating good discriminative ability, i.e., ranking.

CONCLUSIONS: In this large community-based cohort of older Chinese eligible for statin initiation based on the ACC/AHA guidelines, the new risk equation led to substantial overestimation of 5-year risk of ASCVD events. Further validation of the equation is needed to facilitate CVD
prevention for Chinese populations.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
Adverse Events in Patients with LDL-C <25 or <15 mg/dL on ≥2 Consecutive Visits in Fourteen Randomized Trials of Alirocumab

Abstract nr. 295
Author Robinson , Jennifer, University of Iowa, Iowa City, United States of America
Co-author(s) - Farnier , Michel
Co-author(s) - Chaudhari , Umesh
Co-author(s) - Sasiela , William
Co-author(s) - Lorenzato , Christelle
Co-author(s) - Miller , Kathryn
Co-author(s) - Kastelein , John
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, LDL, PCSK9, Pharmacology

Background: Alirocumab added to statin therapy has shown robust reductions in low-density lipoprotein cholesterol (LDL-C) and can reduce LDL-C to very low levels typically not seen with other lipid-lowering therapies. Consequences of achieving very low LDL-C levels are not well understood. Therefore, adverse event rates were examined in patients who achieved 2 consecutive calculated LDL-C values <25 or <15 mg/dL (<0.65 or <0.39 mmol/L) on alirocumab in Phase 2 and 3 trials.

Methods: 14 trials were analyzed; 4 Phase 2 (8-12 weeks and completed) and 10 from the ODYSSEY program (6-24 months with double-blind safety assessment still ongoing in LONG TERM, FH I, FH II, HIGH FH and COMBO II). The pooled group comprises 5234 patients (3340 alirocumab and 1894 control). Treatment-emergent adverse events (TEAEs) were analyzed. In LONG TERM, 2338 pts received alirocumab 150 mg or control every 2 weeks for up to 78 weeks. LONG TERM included laboratory tests for parameters that could potentially be related to very low LDL-C.

Results: In the pooled alirocumab group, 796 (23.8%) patients, including 562 (36.3%) patients from LONG TERM achieved LDL-C <25 mg/dL on ≥2 consecutive visits, and 288 (8.6%) from the pooled alirocumab group, including 223 (14.3%) from LONG TERM, achieved LDL-C <15 mg/dL. TEAEs were generally similar across all groups. There were no cases of hemolytic anemia. In LONG TERM, no clinically meaningful effect was observed in changes to cortisol levels or fat soluble vitamins.
<table>
<thead>
<tr>
<th>Primary system organ class, % (n)</th>
<th>Pooled control (N=1894)</th>
<th>Pooled alirocumab (N=3340)</th>
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<tr>
<td>Infections and infestations</td>
<td>36.3 (687)</td>
<td>38.5 (1286)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>9.3 (176)</td>
<td>9.8 (326)</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>6.7 (126)</td>
<td>6.1 (203)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>4.1 (77)</td>
<td>4.1 (137)</td>
</tr>
<tr>
<td>Influenza</td>
<td>3.9 (73)</td>
<td>5.2 (173)</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>3.3 (63)</td>
<td>3.8 (126)</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>2.7 (51)</td>
<td>2.6 (87)</td>
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<tr>
<td>Lower respiratory tract infection</td>
<td>1.4 (26)</td>
<td>1.6 (53)</td>
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<tr>
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<td>2.3 (43)</td>
<td>1.9 (62)</td>
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<tr>
<td>Musculoskeletal and connective tissue disorders</td>
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<td>24.2 (808)</td>
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<td>Back pain</td>
<td>4.3 (82)</td>
<td>4.0 (133)</td>
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<tr>
<td>Arthralgia</td>
<td>5.0 (95)</td>
<td>4.0 (134)</td>
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</table>
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Postprandial changes in expression of lipid-metabolism related genes in duodenal biopsies after high- vs. low-fat intake in healthy men

Abstract nr. 296
Author Smolders, Lotte, Maastricht University, Maastricht, Netherlands
Co-author(s) - Mensink, Ronald P
Co-author(s) - Boekschoten, Mark Van
Co-author(s) - Ridder, Rogier JJ De
Co-author(s) - Plat, Jogchum
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Intervention, Lipids

Background/aim To further understand intestinal dietary lipid handling, a nutrigenomic approach was used by comparing gene expression in human duodenum biopsies after consumption of a high fat (HF) vs. a low fat (LF) meal.

Method In a randomized, double blind crossover study, 10 healthy men consumed a HF and a LF meal. Both meals contained around 960kcal, 18g protein and 340mg cholesterol. The HF meal contained 61g fat and 86g carbohydrate, while the LF meal contained 11g fat and 194g carbohydrates. During the postprandial period, blood was sampled frequently for analysis of triglycerides, apolipoprotein B48, free fatty acids (FFA), and glucose. Five hours after meal intake duodenal biopsies were taken using duodenoscopy.

Results Postprandial triglyceride and apolipoprotein B48 curves were clearly elevated till 180 min after the HF meal compared to LF meal. Glucose increased more after HF consumption. This increase was seen till 45 min, after which glucose decreased. FFA showed a decrease after meal consumption with a faster return to baseline after consumption of the HF meal. Microarray analysis identified 288 differentially expressed genes (fold change >1.2 or <-1.2, and P<0.05) when comparing the HF and LF meals. We here focus on genes involved in postprandial lipid metabolism. The HF meal increased expression of DHCR7 (cholesterol synthesis), ABCG5 and ABCG8 (apical excretion of cholesterol from enterocytes), SREBF-1 (a transcription factor involved in fatty acid uptake and synthesis), LDLR (basolateral LDL-c uptake) and MTTP (chylomicron synthesis).

Conclusion Based on gene expression, a HF meal may increase duodenal chylomicron and cholesterol synthesis, and surprisingly both LDL-c uptake and cholesterol efflux. Our results therefore suggest that a HF meal increases chylomicron appearance and simultaneously increases basolateral cholesterol uptake and apical secretion.

This research is supported by the Dutch Technology Foundation STW, which is part of the Netherlands Organisation for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs.

Keywords: human, postprandial, high fat, cholesterol, gene expression, lipid metabolism, intestine.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
PBMC Whole genome transcriptional profiling of children with FH

Abstract nr. 297
Author Christensen, Jacob Juel, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
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Co-author(s) - Halvorsen, Bente
Co-author(s) - Olstad, Ole Kristoffer
Co-author(s) - Retterstøl, Kjetil
Co-author(s) - Aukrust, Pål
Co-author(s) - Narverud, Ingunn
Co-author(s) - Ulven, Stine Marie
Co-author(s) - Holven, Kirsten Bjørklund
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Familial Hypercholesterolemia, Genetics, Inflammation

Background and aim: Children with familial hypercholesterolemia (FH) exhibit elevated levels of total and LDL cholesterol and, as a result, elevated risk of atherosclerosis. The aim of the present study was to analyse the transcriptome profile in peripheral blood mononuclear cells (PBMCs) in children with FH.

Methods: Four children with FH (three boys, one girl; mean (SD) age and LDL cholesterol level; 13.3 (3.4) years and 6.8 (0.4) mmol/l) and four healthy control children (three boys, one girl; mean (SD) age and LDL cholesterol level; 14.0 (1.4) years and 2.2 (1.0) mmol/l) were included in the study. PBMCs were isolated using BD Vacutainer Cell Preparation tubes, and total RNA was isolated with RNeasy kit. RNA quantity and quality was assessed, and samples were subjected to a standard microarray pipeline workflow. Samples were labelled and hybridized to a GeneChip® Human Gene 1.0 ST Array and scanned on a GeneChip® Scanner 3000 7G. Principal component and cluster analysis revealed that one PBMC sample (oldest FH child) deviated from the rest, and were consequently excluded from further analysis. Normalization was performed by Robust Multi-array Average. After filtration (2^5 method) the data set consisted of approximately 11 000 genes transcripts. To analyse differences in gene expression between the two groups ANOVA was used and false discovery rate (FDR) was used to correct for multiple testing. No gene transcript was significantly different between FH and control children with FDR q-value < 0.25. In order to identify regulated biological processes, pathways and networks gene transcripts with a nominal p-value < 0.05 were subjected to further gene ontology analyses with the DAVID software tool (http://david.abcc.ncifcrf.gov) and Metacore (GeneGo). In addition Gene Set Enrichment Analyses (GSEA) was performed.

Results: Data from the transcriptomic analyses will be presented at the meeting.
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Statin treatment reduced adverse cardiovascular events in very old patients with acute coronary syndrome

Abstract nr. 298
Author Tie-Hui Xiao, Tie-Hui, BeiJing, China
Co-author(s) - Li Sheng, Li
Co-author(s) - Jin Zheng, Jin
Co-author(s) - Ping Ye, Ping
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords ACS, Elderly, Prevention

BACKGROUND: Accumulating evidence suggests that Statins play a central role in the secondary prevention of coronary heart disease. Despite that very old patients (>80 years old) with acute coronary syndromes have a higher mortality risk, most clinical trials on statins have excluded these patients.

Objectives: The purpose of this study was to determine whether statin treatment may reduce adverse cardiovascular events in very old patients (over 80 years age) with acute coronary syndrome.

Methods: We retrospectively analysed patients over 80 years who were admitted with the diagnosis of acute coronary syndrome between 2006 and 2010 (n = 744). All the patients included 256 patients with no statins treatment and 488 patient with statins treatment. The primary end point included nonfatal myocardial infarction, re-hospitalization for reoccurrence of acute coronary syndrome and death from coronary events.

Results: The primary end point was significantly reduced, which occurred as (55%) in statin-treated patients, compared with 69.14% in non statin-treated patients (P < 0.05). Pooled rates of all cause mortality were 25% in statin-treated patients and 47.2% in non statin-treated patients, which was significantly lower in patients receiving statin treatment (P < 0.01). For the patient receiving statins treatment the main adverse reactions was the elevation of transaminases, but only less than 1% patients had transaminases elevation more than 3 times above the normal range.

Conclusion:
Our results indicate that statins play an important role in treatment of very old patients with acute coronary syndrome, it is safe and effective.

KEY WORDS: statins, adverse cardiovascular events, very old patients, acute coronary syndrome

Subdivision 3. Clinical Studies

Presentation Preference: Electronic poster presentation

Additional information
The relation between HbA1c and vascular events in patients with type 2 diabetes mellitus with and without vascular disease

Abstract nr. 299
Author Kranenburg, Guido, UMC Utrecht, Utrecht, Netherlands
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Co-author(s) - Graaf, Yolanda van der
Co-author(s) - Nathoe, Hendrik
Co-author(s) - De Borst, Gert Jan
Co-author(s) - Kapelle, Jaap
Co-author(s) - Visseren, Frank
Co-author(s) - Westerink, Jan
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Diabetes, Risk Factor

Objective – Poor glycemic control is related with vascular events in patients with type 2 diabetes, however as previous studies have indicated, the presence of vascular disease might influence this relation. Therefore we evaluated the relation between glycemic control (HbA1c) and (new) cardiovascular events and mortality in patients with type 2 diabetes, with and without cardiovascular disease.
Research Design and Methods - In a cohort of 1687 patients with type 2 diabetes enrolled in the SMART (Second Manifestations of ARTerial disease) study, the relation between continuous HbA1c and vascular events (composite of myocardial infarction, stroke and vascular mortality) and all-cause mortality was evaluated using Cox proportional hazard analyses stratified for the presence of manifest vascular disease. Results – During a median follow-up of 6.1 years (IQR 3.1-9.5 years), 293 patients developed a (new) vascular event and 340 patients died. A 1% point higher HbA1c was related to a 27% higher risk for a vascular event in patients with type 2 diabetes without vascular disease (HR 1.27, 95%CI: 1.06-1.51) and not in patients with vascular disease (HR 1.03, 95%CI: 0.93-1.15). In patients with clinical manifest vascular disease at baseline, a 1% point higher HbA1c was related to a 16% higher risk of death (HR 1.16, 95%CI: 1.06-1.28), while in patients without vascular disease a non-significant 13% higher risk for all-cause mortality per 1% point higher HbA1c (HR 1.13, 95%CI 0.97-1.31) was observed. Conclusions – Glycemic control is related to cardiovascular events in patients with diabetes mellitus type 2 without vascular disease, in contrast to patients with type 2 diabetes and preexisting vascular disease, in whom a relation between HbA1c and all cause mortality exists.
Table 2. Relation between HbA1c and (new) cardiovascular events and all cause mortality

<table>
<thead>
<tr>
<th>Model</th>
<th>New cardiovascular events</th>
<th>p-value</th>
<th>All cause mortality</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HR (95%CI)</td>
<td></td>
<td>HR (95%CI)</td>
<td></td>
</tr>
<tr>
<td>DM2 and Vascular disease (n=1156)</td>
<td>New cardiovascular events</td>
<td>All cause mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
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<td>0.642</td>
<td>1.12 (1.02-1.22)</td>
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<tr>
<td>II</td>
<td>1.07 (0.96-1.18)</td>
<td>0.230</td>
<td>1.19 (1.05-1.31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>III</td>
<td>1.03 (0.93-1.15)</td>
<td>0.549</td>
<td>1.16 (1.06-1.28)</td>
<td>0.002</td>
</tr>
<tr>
<td>DM2 without vascular disease (n=53)</td>
<td>New cardiovascular events</td>
<td>All cause mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1.16 (0.99-1.35)</td>
<td>0.074</td>
<td>1.11 (0.96-1.27)</td>
<td>0.154</td>
</tr>
<tr>
<td>II</td>
<td>1.24 (1.03-1.43)</td>
<td>0.023</td>
<td>1.15 (1.00-1.32)</td>
<td>0.057</td>
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<tr>
<td>III</td>
<td>1.27 (1.06-1.51)</td>
<td>0.008</td>
<td>1.13 (0.97-1.31)</td>
<td>0.124</td>
</tr>
</tbody>
</table>

* Model I: crude; Model II: sex and age; Model III: Model II + current smoking, systolic blood pressure, diabetes duration, non-HDL, and MCRD. Hazard ratio per 1% higher HbA1c. For example, in patients with type 2 diabetes without vascular disease a 1% higher HbA1c is associated with a 2.27 fold higher risk of vascular events and a 1.13 fold higher risk of all cause mortality.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Blood lipids and coronary lesions in patients with type 2 diabetes undergoing transfemoral amputation in different age groups

Abstract nr. 300
Author Shalaeva, Evgeniya, Tashkent Medical Academy, Tashkent, Uzbekistan
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Co-author(s) - Janabaev, Bakhtiyar
Co-author(s) - Bobabekov, Azam
Co-author(s) - Shalaeva, Aleksandra
Co-author(s) - Kasimov, Ulugbek
Co-author(s) - Dadabayeva, Nailya
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Cardiovascular Disease, Diabetes, Dyslipidemia

The aim of the study was to evaluate lipid metabolism and atherosclerotic plaques in patients with type 2 diabetes undergoing high lower limb amputation in different age groups.

Material and methods. In the Republican Specialized Center of purulent surgery and complications of diabetes in 2013 we operated 1578 patients with Type 2 diabetes, among them we selected 109 consecutive patients undergoing high lower limb amputation (74 men and 35 women), without myocardial infarction and who previously did not use statins. We divided them into age groups of 40-50 (n=6), 51-60 (n=31), 61-70 (n=55), and ≥71 years old (n=17) (data given in this sequence). All the patients underwent laboratory tests (including blood lipids), ECG, echocardiography, coronary computed tomographic angiography (CCTA).

Results. At the time of admission in patients with Type 2 diabetes we diagnosed coronary heart disease functional class II in 38 (34.9%), class III in 71 (65.1%), congestive heart failure functional class II (NYHA) in 29 (26.6%), III in 65 (59.6%) and IV in 15 (13.8%) patients. All men smoke more 12 cigarettes per day 5 years more. We detected that lipid and glucose metabolism was impaired in all age groups, with a significant increase with age. HbA1c was significantly worse in patients older 71 years (11.2±0.4% / 11.5±0.8% / 12.2±0.3% vs. 14.2±0.3%, p=0.001). Total cholesterol 7.0±0.5 / 7.7±1.1 / 8.1±0.8 / 8.4±0.7 mmol/l (p=0.001); low-density lipoprotein 3.1±0.4 / 3.5±0.3 / 3.6±0.5 / 4.4±0.2 mmol/l (p=0.001); triglyceride 2.9±0.3 / 3.1±0.4 / 3.4±0.2 / 3.8±0.4 mmol/l (p=0.011); fibrinogen 699±125 / 666±99 / 740±145 / 880±136 (p=0.001) respectively. There was no difference in high-density lipoprotein 0.73±0.11 / 0.72±0.09 / 0.72±0.18 / 0.71±0.2 mmol/l.

Among 109 consecutive patients had 5 (4.6%) no coronary artery disease (CAD) by CCTA (mean age 48±1 years), 24 (22.0%) had non-obstructive CAD (56.2±4.4), in 41 (37.6%) there was 1 coronary segment with >70% stenosis (63.9±5.4), 20 (18.3%) had 2-vessel (68.8±5.2), and 19 (17.4%) had 3-vessel obstructive CAD (69.2±5.1).

Conclusion. Lipid metabolism was impaired in all age groups, the number of coronary artery segments with atherosclerotic disease and prevalence of obstructive coronary artery disease...
increased with age.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Atherosclerosis is a lipid driven disease in which activation of the immune system plays a pivotal role. GITR is a receptor belonging to the TNFR superfamily selectively activated by its ligand, GITRL. GITR is expressed on activated T cells, regulatory T cells (Treg), and antigen presenting cells (APCs). GITR is involved in inhibiting the suppressive activity of Treg and thereby extending the survival of effector T cells. On the other hand, GITRL is expressed on APCs and after activation endothelial cells show a high expression of GITRL suggesting it to be an adhesion molecule. The aim of this study was to gain more insight into the potential role of the GITR-GITRL system in atherosclerosis, and the potential therapeutic use of fusion proteins and antibodies modulating the GITR/GITRL system.

Methods: Gitr⁻/⁻ mice were crossed with Apoe⁻/⁻ mice. Male Gitr⁺/+ Apoe⁻/⁻ and Gitr⁻/⁻ Apoe⁻/⁻ littermates were fed a normal chow for a period of 18 and 28 weeks. Ldlr⁻/⁻ mice were irradiated and transplanted with Gitr⁻/⁻, Gitrl or Gitr⁺/+ bone marrow, and subjected to 11 weeks of diet feeding. The extent of atherosclerosis was assessed in aortic roots. Cell suspensions of aorta, lymph nodes (LNs), spleen, and thymus were analyzed using flow cytometry.

Results: Compared with that in Apoe⁻/⁻ control mice, plaque formation in the aortic root was decreased in Gitr⁻/⁻ Apoe⁻/⁻ mice after 18 weeks and even further decreased after 28 weeks of normal chow. Flow cytometry analysis showed a significant increase in CD4⁺ effector T cells as well as Treg cells in lymph node and spleen of Gitr transplanted Ldlr⁻/⁻ mice compared with both Gitrl and/or Gitr⁺/+ transplanted mice. Additionally, total plasma cholesterol levels in Gitr⁻/⁻ transplanted Ldlr⁻/⁻ mice were significantly increased when compared with both Gitrl and/or Gitr⁺/+ transplanted mice.

Conclusion: These findings indicate an atheroprotective as well as an anti-inflammatory role of GITR-GITRL signaling in atherosclerosis.
Non-invasive molecular ultrasound imaging: Approach for diagnosis, monitoring and efficacy testing of thrombolytic drugs

Abstract nr. 302
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Imaging, Therapy, Thrombosis

Introduction: Molecular ultrasound imaging offers a non-invasive technology widely available for rapid clinical diagnosis. We tested whether microbubbles (MBs), which are selectively targeted to activated platelets, provide a high-resolution, real-time imaging of thrombosis and monitoring of thrombolysis. We used this approach to evaluate a platelet targeted urokinase plasminogen activator (targ-scuPA) that is hypothesized to offer anti-thrombolytic potency without bleeding complications.

Methods and Results: Lipid-shell based air-filled MBs were conjugated to a single-chain antibody specific for an epitope called Ligand Induced Binding Site on activated GPIIb/IIIa (LIBS-MB). Flow-chamber adhesion assays demonstrated strong binding of LIBS-MB to immobilized activated platelets or micro-thrombi. Thrombi, induced in carotid arteries of C57Bl6-mice in vivo by ferric chloride injury, were assessed with ultrasound before and 20 minutes after microbubble injection. Analysis of the thrombus area demonstrated a significant increase in decibel after LIBS-MB but not after MB injection (p<0.01). After thrombolysis with high dose of commercial urokinase (commUPA at 500U/g BW), LIBS-MB ultrasound imaging allows monitoring of the reduction in thrombus size (p<0.001). No reduction in thrombus size was observed using a low dose of commUPA (75U/g BW). In addition, a low dose of of targ-scuPA (75U/g BW) is sufficient for thrombolysis, whereas the same dose of commUPA or non-targ-scuPA are not (p<0.01). The high dose (500U/g BW) of commUPA, the concentration required to match the effectiveness of the low dose (75U/g BW) of targ-scuPA, resulted in prolonged tail bleeding time, whereas no increase in bleeding was observed when the equally effective but lower dose of scuPA (p<0.001).

Conclusion: Our targeted LIBS-MBs specifically bind to activated platelets enabling real-time molecular ultrasound imaging of thrombosis and monitoring of thrombolysis in vivo. In an exemplary application a highly promising clot-targeted thrombolytic drug was shown to provide effective thrombolytic potential without compromising haemostasis.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Heparan Sulfate Proteoglycans Only Modestly Affect Postprandial Hepatic Remnant Clearance in Humans

Abstract nr. 303
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Genetics, Metabolism, Triglyceride-Rich Proteins

Introduction and Methods Elevated circulating levels of Triglyceride-rich Remnant Lipoproteins (TRL) are strongly associated with increased risk for CVD. The hepatic clearance of TRL involves lipoprotein receptors i.e. the low-density lipoprotein receptor (LDLr) and heparin sulfate proteoglycans (HSPG). The relevance of each pathway in humans remains to be established. To further dissect the relative contribution of each of these receptors, we studied postprandial TRL metabolism with an oral fat tolerance test using cream supplemented with retinyl palmitate (RP) in 1) patients with a heterozygous loss-of-function (LOF) variant in LDLR stratified for a low (n=10) or high (n=10) HSPG gene score; 2) patients with heterozygous LOF variants in EXT1 or EXT2 (n=13), characterized by decreased HSPG chains length but normal sulfation pattern, and compared to matched healthy controls (n=13) and 3) diabetic patients (n=29) stratified for a functional SNP in SULF2, that predisposes to lower SULF2 expression and increased 6-O-sulfation of HSPG chains.

Results Postprandial TRL clearance was significantly delayed in patients with FH compared to controls (AUC-RP FH: 1971±190 vs Con: 646±110 nmol/l/h; P<0.0001 and iAUC-TG FH 6.9±1.0 vs Con 3.8±1.0 mmol/l/h, P<0.05) supporting the important role of LDLr in TRL clearance. No additional effect was observed if the FH group was stratified for HSPG gene score. Also, in patients with LOF variants in EXT, resulting in shorter HSPG chains, no difference in TRL clearance versus controls could be observed. In contrast, improved 6-O-sulfation due to lower hepatic protein expression of SULF2 resulted in improved fasting and postprandial TG levels and significantly lower iAUC-RP (iAUC-TG AA 6.9±1.1 vs GG 4.1±1.2 mmol/l/h P<0.05; AUC-RP AA 97±15 vs GG 15±2 mg/l/h; P<0.001)

Conclusion Our findings clearly indicate an important role for the LDLr in postprandial TRL clearance in humans. In contrast to murine studies, HSPGs do only modestly contribute to hepatic TRL clearance in humans, and implicate that sulfation of HSPG’s is of more relevance for TRL clearance than HSPG chain length.
Essential role of arginine 123 of apolipoprotein A-I in lecithin:cholesterol acyltransferase activation.

Abstract nr. 304
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, HDL, Reverse Cholesterol Transport

Lecithin:cholesterol acyltransferase (LCAT) catalyzes the esterification of free cholesterol in plasma lipoproteins. Activation of LCAT by apolipoprotein (apo) A-I is crucial for the antiatherogenic process of reverse cholesterol transport and HDL metabolism. Upregulation of LCAT function is one of the therapeutic strategies for reducing atherosclerosis. Yet, the molecular basis for LCAT activation by apoA-I is not understood in detail. The molecular dynamic simulations of HDL particles by Segrest's group (Biochemistry 2009, 48, 11196) suggest that the central domains of apoA-I form an amphipathic "presentation tunnel" for migration of acyl chains and unesterified cholesterol from the lipid bilayer to the active sites of LCAT. Our recent high resolution crystal structure of the truncated apoA-I(Δ185-243) clearly shows a ~5 Å tunnel between the flexible central antiparallel domains of apoA-I, with a single positively charged residue Arg123 projected toward the hydrophobic inside of the tunnel (J. Biol. Chem. 2011, 44, 38570). We hypothesized that the uniquely positioned Arg123 plays an essential role in LCAT activation by interacting with fatty acids produced by the phospholipase activity of LCAT. To test the importance of Arg123 in apoA-I-mediated activation of LCAT, we generated the apoA-I(Arg123Ala) mutant and WT apoA-I and used them to make reconstituted discoidal HDL particles (rHDL) consisting of phosphatidylcholine, cholesterol, and apoA-I. Circular dicroism and fluorescent analysis showed that the Arg123Ala mutation did not affect the secondary structure or destabilize apoA-I. The mutant had lipid-binding properties comparable to those of WT apoA-I. Stokes diameters of the major rHDL particles formed with the mutant were similar to those with WT apoA-I (96-98 Å). LCAT activity was assayed with rHDL containing 3H-cholesterol and recombinant LCAT. Ablation of the basic residue Arg123 led to more than two-fold reduction in the catalytic efficiency $V_{max}/K_m$ of the LCAT reaction resulting from a large reduction in the maximal velocity $V_{max}$ with insignificant change in the dissociation constant $K_m$. These data suggest that the specifically positioned residue Arg123 of apoA-I participates in LCAT activation, and offer new insights into the mechanistic details of the reaction that is central in the reverse cholesterol transport.

Supported by NIH grant 1R01HL116518-01A1.
Presentation Preference Electronic poster presentation
Additional information
Several published reports suggest the association of single nucleotide polymorphisms (SNPs) residing in the interleukin-1 (IL-1) gene cluster with coronary artery disease (CAD). None have however systematically assessed its association status among Asian Indian ethnology. Its overall association status worldwide is also still unclear. Our objectives were thus to prospectively test the association of 11 common IL-1 gene cluster SNPs and CAD (vide a case-control study with 323 cases and 400 healthy, age and sex matched controls) among a cohort of Asian Indian ethnicity and to validate our results using an updated meta-analysis of all relevant published association studies. Significant association of two common IL-1 gene cluster SNPs with CAD, viz. B -511 T>C and RN 86bp VNTR was found in our study cohort. We found that the presence of >1, T (minor) allele IL1B -511 T>C in a genotype provided protection against CAD (OR= 0.62, p= 0.044), while the presence of >1, T (major) allele was associated with an increased risk (OR= 1.36, p= 0.041). On the other hand mutant allele 2 and genotype X/2 of RN 86bp VNTR was found to be associated with increased risk of disease (OR= 2.25, p= 0.031). The rest nine SNPs(residing in IL1A, IL1B and IL1RN genes) showed lack of association with CAD(p >0.05). Several haplotype combinations constructed out of studied SNPs belonging to IL1B and IL1RN genes also showed association CAD. The present meta-analysis was conducted for 8 out of 11 selected IL-1 SNPs, with 53 different studies with a total sample of 25,305 (13,309 cases; 11,996 controls). The results of our meta-analysis somewhat concurred with those presented in our case control study and confirmed the association of B -511 T>C establishing the resulting “TT” (mutant) as a protective for CAD in all pooled population (p range= 0.02-0.03). The corresponding “CC” (wild) genotype automatically suggested being a risk genotype. However, no association of RN 86bp VNTR with CAD was established in the present meta-analysis(p >0.05), which suggested considerable variation in association status among different ethnologies. Our results should be interpreted clinically, and the generated information can be used for early risk stratification.
Presentation Preference Oral presentation
Additional information
An Antisense Inhibitor of Apolipoprotein C-III Significantly Decreases Fasting Apolipoprotein C-III in all Lipoprotein Subfractions in Severe Hypertriglyceridemia

Abstract nr. 306
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Lipoproteins, Triglyceride-Rich Proteins, Triglycerides

Background: Apolipoprotein C-III (apoC-III) plays a pivotal role in regulating plasma triglyceride (TG) levels. Multiple apoC-III protein molecules reside on the surface of apoB-containing lipoproteins (VLDL, LDL) and HDL and exchange rapidly between these particles. ApoC-III concentrations in VLDL and LDL are positively associated with the progression of atherosclerosis or risk of coronary vascular disease (CVD). HDL with apoC-III is also associated with increased CVD. We have previously reported that an antisense inhibitor of apoC-III (ISIS-APOCIIIRx) significantly reduces total TG and apoC-III protein levels in patients with moderate to severe hypertriglyceridemia. The present study assessed how this apoC-III reduction was distributed in various lipoprotein subfractions (VLDL, LDL, and HDL).

Methods: Adult patients with severe hypertriglyceridemia (SHTG) were enrolled in a randomized double-blind Phase 2 study to receive ISIS-APOCIIIRx (up to 300 mg) or placebo as a single agent (monotherapy, n=57) or as an add-on to stable fibrate therapy (n=28) in weekly subcutaneous injections for 13 weeks. Baseline and end-of-treatment ultracentrifuged fasting delipidated serum samples were analyzed for apoC-III levels in lipoprotein subfractions (VLDL, LDL, and HDL).

Results: Results show dose dependent significant reductions in serum apoC-III levels. At the 300 mg/week dose (the Phase 3 dose), ISIS-APOCIIIRx reduced VLDL, LDL, and HDL apoC-III levels in monotherapy patients by 92% (p<0.001), 73% (p<0.001), and 63% (p<0.001) respectively (p values are paired-wised comparison between ISIS-APOCIIIRx and placebo groups). In patients on a stable dose of fibrate, these parameters were reduced by 79% (p=0.021), 49% (p=0.069), and 53% (p=0.006) respectively. Similar reductions (89%, 67%, and 77% respectively) were seen in a small cohort of patients with familial chylomicronemia syndrome (n=3, open label, no placebo comparison). ISIS-APOCIIIRx was generally safe and well tolerated. There were no clinically meaningful changes in liver tests or other laboratory values.

Conclusions: ISIS-APOCIIIRx is effective in lowering apoC-III levels in all serum lipoprotein subfractions in SHTG patients on a stable dose of fibrate or not on background TG-lowering therapy suggesting the potential for improvement in CVD risk in these patients.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Epicardial fat thickness, hsCRP and galectin 3 in patients with metabolic syndrome

Abstract nr. 307
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Inflammation, Metabolism, Risk Factor, Visceral Fat

Objective: Visceral adipose tissue has proinflammatory and profibrogenic effects on cardiovascular system. Epicardial fat is localized near cardiomyocytes and may influence upon remodeling of the heart. Study objective - to evaluate the epicardial fat thickness (EFT) and to reveal the possible relationship of this parameter with high sensitive C-reactive protein (hsCRP) and galectin 3 (Gal 3) as markers of inflammation and fibrosis in patients with metabolic syndrome (MetS).

Design and method: 60 patients 35-65 years old with MetS according to the criteria IDF were examined. The control group was 60 persons without metabolic disorders and cardiovascular disease. Groups did not differ significantly by gender, age, eGFR (p>0,05). The examination includes: medical history, anthropometry, echocardiography, lipids, glucose, blood pressure, serum hs-CRP level (Immunoturbidimetric method) and Gal 3 (Enzyme immunoassay). The patients with acute and chronic inflammation, as well as with hs-CRP levels greater than 10 mg/L were excluded. The EFT was measured with transthoracic echocardiography over the free wall of the right ventricle, in at least two positions of the longitudinal and transverse parasternal.

Results: The epicardial fat thickness was more than 2 fold greater in the MetS compared with the control group (4,7±1,3 and 2,4±0,9 mm; p<0,001). Hs-CRP was more than 3 fold higher in the MetS compared with the control group (2,4±0,2 and 0,8±0,1 mg/L; p<0,001) and median of Gal 3 also was more higher in patients with MetS (0,48 [0,42;1,39] and 0,27 [0,24;0,32] ng/ml; p<0,001). Correlation analysis among all examined persons showed a strong positive correlation between EFT and diameter, volume of the left atrium and left ventricle mass index (r=0,64, 0,61, 0,51; p<0,001). Correlation between EFT, hs-CRP and Gal 3 was also strong positive (r=0,71, 0,61; p<0,001).

Conclusions: Epicardial fat in patients with MetS is thicker than in healthy people. Greater
thickness of epicardial fat is associated with higher levels of markers of inflammation and fibrosis: high sensitive C-reactive protein and galectin 3. Epicardial fat can influence on remodeling of the heart.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Coronary artery calcium (CAC) volume, CAC density, and incident cardiovascular events

Abstract nr. 308
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, Imaging, Risk stratification

Background- We have reported that coronary artery calcium (CAC) volume by computed tomography (CT) was strongly related to incident cardiovascular disease (CVD) in a multi-ethnic cohort, but that at any given CAC volume, CAC density was inversely related (JAMA 2014;311:271-8). We also noted that CAC density was somewhat more protective at lower levels of CAC volume, though the interaction term was not significant. Longer follow-up has now increased the number of hard CVD events by 47%.

Purpose - To evaluate with longer follow-up 1) whether CAC density remains inversely associated with CVD events and 2) whether the effect of CAC density is modified by the level of CAC volume.

Methods - 3398 men and women in the Multi-Ethnic Study of Atherosclerosis with CAC scores >0 at baseline had CAC volume and density calculated from cardiac CT scans. Cox proportional hazards models were adjusted for ethnic group and the new ethnic-specific ASCVD risk score. Hazard ratios (HR) were calculated for CAC volume, CAC density, and for CAC density by quartile of CAC volume.

Results - After an average follow-up of 10.0 years, there were 264 hard coronary heart disease (CHD) events and 126 non-CHD CVD events for a total of 390 hard CVD events. For CHD, the HR was 1.83 for each standard deviation (SD) of CAC volume, and the HR was 0.71 for each SD of CAC density. For CVD, the HR was 1.68 for each SD of CAC volume, and the HR was 0.75 for each SD of CAC density (all p-values <0.001). CAC volume and CAC density each independently and significantly increased the area under the ROC curves for both CHD and CVD. The HRs for CAC density were similar across the 4 quartiles of CAC volume with no evidence of a trend, and multiplicative interaction terms were not significant.

Conclusions - After 10 years of follow-up, 1) CAC density remained strongly inversely related to both incident CHD and CVD, and 2) this inverse association was similar at all levels of CAC volume. CAC scoring systems should include density to improve estimation of CHD and CVD risk.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Concentration of hyaluronic acid in patients with non-alcoholic fatty liver disease (NAFLD) and its correlation to Apolipoprotein E polymorphisms.

Abstract nr. 309
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Introduction: Apolipoprotein E (apoE) plays a crucial role in metabolism of triglyceride-rich lipoproteins. An interaction between apoE and receptors determines the removal of apoE rich lipoproteins and homeostasis of cholesterol and triglycerides. There are three major isoforms of human apoE (apoE2, apoE3 and apoE4). It was reported that the response of plasma lipids was different in subjects with apoE genotypes but still is unknown whether variants of apoE may change plasma components thus predisposing patients to NAFLD progression. ApoE3 variant is most frequently reported and is considered as wild-type allele. Individuals with apoE4 genotype may manifests a numerous symptoms related to metabolic syndrome. It was reported that increased concentration of hyaluronic acid in plasma is related to liver steatosis.

Aim: The aim of the study was to compare hyaluronic acid concentration in plasma in apoE3 and apoE4 genotype variants among patients with NAFLD.

Materials and methods: 22 patients with NAFLD were enrolled in the study. The study group included 11 apoE4 carriers, as well as a control group, also consisting of 11 patients, with apoE3 phenotype. Genotypes were determined by real-time polymerase chain reaction (RealTime-PCR) using Taqman® SNP genotyping assays. Hyaluronic acid, a marker of liver fibrosis in subjects with NAFLD was estimated by ELISA kit (Wuhan EIAab Science)

Results: ApoE4 carriers had significantly increased (p.value<0.005) hyaluronic acid concentration in comparison to patients with apoE3 genotype (Table 1).

Discussion: Patients with ApoE4 genotype showed increased concentration of hyaluronic acid in plasma. It is strongly related to liver fibrosis, which is caused by non-alcoholic fatty liver disease so it seems that ApoE4 genotype favors a tendency to liver fibrosis.

Key words: Apolipoprotein E, apoE3, apoE4, hyaluronic acid, liver steatosis

Table 1. Concentration of hyaluronic acid in study population
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ApoE3 allele</th>
<th>ApoE4 allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>Hyaluronic acid (ng/ml)</td>
<td>40 (6)</td>
<td>59 (20)</td>
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<tr>
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<td>40 ± 6</td>
<td>60 ± 12</td>
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Nanoparticles for target-specific imaging and treatment of atherothrombosis: Platform for physico-chemical characterization and in vitro cytotoxicity testing.

Abstract nr. 310
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Endothelium, Prevention, Therapy

Background: Nanomedicine offers a possibility of improved diagnosis of cardiovascular disorders, along with localized treatment of vulnerable plaque and stroke. However, for the application in humans, a substantial amount of preclinical studies is necessary to analyse the effects of nanoparticles on the vascular cells. Here, we describe the establishment of a platform for systematic physico-chemical characterization of nanosystems, and for the standardized investigation of their effects on endothelial cells, which are the first-contact cells for circulating nanoparticles.

Methods: Solid lipid nanoparticles and liposomes, polymeric and inorganic nanoparticle systems were produced. Z-averaged hydrodynamic diameter, dispersity (PDI) and ζ-potential were determined with a Zetasizer Nano ZS (Malvern). For long-term in vitro toxicity testing, endothelial cell growth and vitality upon treatment with different nanoparticle systems (0-400 mg/mL) was monitored for up to 96h using two complementing methods: real-time cell analysis (impedance measurement, xCELLigence) and live-cell microscopy. Moreover, the effect of circulating nanoparticles on endothelium was assessed in an in vitro model of arterial bifurcations.

Results: We report here the establishment of a platform for systemic characterization and standardized toxicity testing of nanosystems intended for the intravascular applications. In total, 11 nanoparticle systems were tested (3 types of lipidots, 2 types of polymeric nanoparticles, 3 types of iron oxide nanoparticles and 3 types of liposomes). The hydrodynamic diameter, depending on the nanosystems, ranged between 49 and 230 nm, and the ζ-potential between +9.4 and -53.4 mV. All nanosystems were stable over the 6-months period. With regard to toxicity testing in static conditions, the majority of nanosystems were well tolerated up to 100 µg/mL, whereas liposomal nanoparticles showed no toxicity up to the highest tested concentration (400 µg/mL). In the dynamic assay, none of the nanosystems induced endothelial toxicity at 100
Characterization of nanosystems stability and biological compatibility is critical for their application in humans. In the future studies, in vivo effects and biodistribution of nanosystems, as well as complement activation-related pseudoallergy tests are planned. These investigations, constituting an essential part of nanotoxicology and in vivo safety assessment, are necessary before any nanosystem is considered for clinical use. Funding: EU project FP7-NMP-2012-LARGE-6-309820 “NanoAthero”.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
LOWER: a registry evaluating long-term lomitapide use in patients with homozygous familial hypercholesterolemia

Abstract nr. 311
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Familial Hypercholesterolemia, LDL, Prevention

Homozygous familial hypercholesterolemia (HoFH) is a rare genetic disease characterized by high levels of low-density lipoprotein cholesterol (LDL-C) and premature atherosclerosis. Lomitapide has been approved in the U.S., EU and certain other countries as an adjunct to other lipid-lowering therapies to reduce LDL-C levels in adult patients with HoFH. Although lomitapide significantly reduced LDL-C levels in clinical trials, no studies have been performed to evaluate real-world use, particularly long-term safety, effectiveness, or patterns of use. LOWER (Lomitapide Observational Worldwide Evaluation Registry) is a large, long-term, prospective, observational registry conducted within the United States, Canada, Europe, Asia and Latin America. LOWER will enroll at least 300 adult lomitapide-treated patients, with all patients followed for a minimum of 10 years providing a minimum of 3000 patient exposure years. In non-European countries, the registry will continue for 10 years from the date that the 300th patient is enrolled. In European countries, the registry will remain open indefinitely.

The objectives of LOWER are to evaluate the occurrence of events of special interest, including hepatic and gastrointestinal events, tumors, events associated with coagulopathy, cardiovascular outcomes, and the occurrence and outcomes of pregnancy. LOWER will also provide data on long-term lipid control and adherence of prescribers to product label screening and monitoring recommendations. LOWER will provide clinically meaningful, real-world information on the clinical characteristics of patients with HoFH, including baseline characteristics, diagnostic criteria, and previous therapies. The registry was opened to enrollment in March, 2014. As of November 30, 2014, 68 patients were enrolled; enrollment of 300 patients is anticipated by March 2018.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Risk of recurrent vascular events if modifiable risk factors are at guideline target in patients with clinically manifest vascular disease

Abstract nr. 312
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, Guidelines, Risk stratification

Background With the emergence of novel therapeutic options for the secondary prevention of major vascular events, information is needed about the risk that remains if patients are optimally treated. We evaluated the risk of recurrent vascular events and mortality in contemporary patients with various clinical manifestations of vascular disease and estimated the risk that remains if modifiable risk factors would be at target according to secondary prevention guidelines.

Methods In a prospective cohort of 4,584 patients with manifest vascular disease, the 10-year risk of recurrent vascular events was estimated with the SMART risk score. The amount and distribution of risk that remains if modifiable risk factors would be at target (the optimal risk) was estimated based on recommendations from secondary prevention guidelines. These included no smoking, systolic blood pressure <140 mmHg, LDL-cholesterol <2.6 mmol/L, or <1.8 mmol/L for very high risk patients, physical activity at least 30 minutes 5 times a week, BMI <25 kg/m² and use of at least 1 antiplatelet agent or anticoagulant.

Results The 10-year risk of recurrent vascular events ranged from <10% in 43% of the patients, to >20% in 20% of the patients (mean risk 14.7%, SD 11.5%). The 10-year risk was highest in patients with polyvascular disease (mean 27.5%, SD 17.0%) and lowest in patients with coronary artery disease (12.1%, SD 8.5%). Of all patients, 17% had no or only 1 modifiable risk factor not at target. If modifiable risk factors would be at target according to secondary prevention guidelines, the 10-year risk would on average be 2.3% (SD 2.9%) lower, resulting in an average optimal 10-year risk of 12.4% (SD 10.0%) in patients with vascular disease.

Conclusion The 10-year risk of recurrent vascular events ranges from low to very high in contemporary patients with clinically manifest vascular disease. The average 14.7% 10-year risk can be further reduced to an average of 12.4% if modifiable risk factors would be at target for all patients according to current secondary prevention guidelines.
The optimal risk of recurrent vascular events was calculated for all study participants by setting the modifiable risk factors of the SMART risk score

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation

Additional information
A study to evaluate the effect of lomitapide on atheroma in homozygous familial hypercholesterolemia (the "CAPTURE" study)

Abstract nr. 313  
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins  
Keywords Atherosclerosis, Familial Hypercholesterolemia, Imaging, LDL

There are currently no CV outcomes or morbidity data for lomitapide. CAPTURE (Carotid and Aortic atherosclerosis in Patients Treated with Lomitapide in Usual Care) is a sub-study of a prospective, observational registry evaluating the long-term safety and effectiveness of lomitapide in the usual care of patients with homozygous familial hypercholesterolemia (HoFH). CAPTURE will investigate the effects of lomitapide on carotid and aortic atherosclerosis in adult patients treated with lomitapide. The study will enroll 57 adult patients at sites in the EU, USA, and Canada, to provide at least 40 evaluable patients. The primary objective is to assess changes in average carotid vessel wall area on MRI scanning after 2 years of lomitapide treatment. Secondary objectives include an assessment of other indices of atheroma burden in the ascending aortic and carotid arteries after 1 and 5 years of treatment with lomitapide. Changes in LDL-C and other lipoproteins will be correlated with MRI parameters. Exploratory objectives include an evaluation of plaque composition features, as well as markers of inflammatory and cardiovascular processes over 5 years of treatment with lomitapide. High spatial-resolution MRI is a robust and reproducible non-invasive technique able to evaluate atherosclerosis over time. Elevated low-density lipoprotein cholesterol (LDL-C) is implicated in the pathophysiological process underlying atherosclerosis, and lowering LDL-C is the primary goal of treatment for homozygous familial hypercholesterolemia. CAPTURE will evaluate the hypothesis that LDL-C lowering with lomitapide reduces and/or stabilizes atheroma burden, which can be regarded as an intermediate endpoint between LDL-C and cardiovascular outcomes.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Relationship between IGF system and obesity, metabolic syndrome and cardiovascular risk

Abstract nr. 314
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Diabetes, Metabolism, Obesity, Risk Factor

When fat distribution is predominantly abdominal or visceral, it is often associated with a constellation of metabolic features that have now been categorized as the metabolic syndrome (MS). The metabolic syndrome is a combination of metabolic and clinical features that aggregate in individuals and increase cardiovascular disease risk and development of atherosclerotic vascular disease, the commonest cause of death in Western countries. It is believed that the underlying basis for this syndrome is insulin resistance (IR) and accompanying compensatory hyperinsulinaemia. Insulin and insulin-like growth factor 1 (IGF-1) have significant homology and interact with differing affinity with the same receptors. The function of IGF system is primarily to regulate growth and differentiation of cells and tissues, although recently, it has been implicated in tumorigenesis. There is a strong and independent inverse relation between circulating total and free IGF-1 concentrations and metabolic syndrome. Reduced IGF-1 levels are independently associated with glucose intolerance, diabetes, abdominal obesity, and atherogenic dyslipidemia and these data suggest an important and independent role of IGF-1 in protecting against the development of cardiovascular risk. To evaluate a relationship between IGF-1 and metabolic syndrome in children, we arranged a group of 47 children that consisted of 18 children with MS and 29 children without MS. In the group of children with MS were lower levels of IGF-1 than in the group with children without MS, what confirms results of previous studies made in adults. Although further work is needed to define specifically the pathogenetic mechanisms involved, these different interactions are potentially subject to pharmacological manipulation in the treatment of, and protection from, disorders associated with the metabolic syndrome and enhanced cardiovascular risk.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
High-throughput Metabolite Profiling of Cardiovascular Event Risk: A Prospective Study of Three Population-Based Cohorts

Abstract nr. 315
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Epidemiology, Metabolism, Risk Factor, Risk stratification

Aim: To discover circulating biomarkers for cardiovascular event risk by high-throughput metabolomics profiling in large prospective cohorts. This can help to uncover the aetiology of atherosclerosis and improve cardiovascular risk prediction.

Method: We applied quantitative NMR metabolomics to identify biomarkers for incident cardiovascular disease during 12–20 years of follow-up. Metabolite biomarker discovery was conducted in the FINRISK study (n=7256; 800 events). Replication and incremental risk prediction was assessed in the SABRE study (n=2622; 573 events) and British Women’s Health and Heart Study (n=3563; 368 events).

Results: In targeted analyses of 68 lipids and metabolites, 33 measures were associated with incident cardiovascular events at P<0.0007 after adjusting for age, sex, blood pressure, smoking, diabetes and medication. When further adjusting for routine lipids, four metabolites were associated with future cardiovascular events in meta-analyses: higher serum phenylalanine (hazard ratio per standard deviation: 1.18 [95%CI 1.12-1.24]; P=4×10^{-10}) and monounsaturated fatty acid levels (1.17 [1.11-1.24]; P=1×10^{-8}) were associated with increased cardiovascular risk, while higher omega-6 fatty acids (0.89 [0.84-0.94]; P=6×10^{-5}) and docosahexaenoic acid levels (0.90 [0.86-0.95]; P=5×10^{-5}) were associated with lower risk. The metabolite associations were comparable to or stronger than that of LDL-cholesterol. A risk score incorporating these four biomarkers was derived in FINRISK. Risk prediction estimates were more accurate in the two validation cohorts, albeit discrimination was not enhanced. Risk classification was particularly improved for persons in the 5–10% risk range (net reclassification 27% and 15%). Biomarker associations were further corroborated with mass spectrometry in FINRISK (n=671) and the Framingham Offspring Study (n=2289).

Conclusions: This study underscores the value of high-throughput metabolomics for biomarker
discovery and improved cardiovascular risk assessment. As the metabolomics platform quantifies amino acids and fatty acids simultaneously with standard lipids, these novel biomarkers could be implemented to augment risk prediction without additional clinical chemistry. 

Supported by the Academy of Finland.

Biomarkers for cardiovascular risk independent of established risk factors
Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Cost-effective Quantitative Metabolite Profiling in Large-Scale Cardiovascular Epidemiology

Abstract nr. 316
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, Metabolism, Risk Factor

Aim: To quantify circulating lipids and metabolites in large prospective cohorts, and use the metabolite concentration data to uncover biomarkers for future onset of cardiovascular disease and diabetes. The high-throughput metabolomics method can help to uncover the etiology of cardiometabolic diseases and improve risk prediction.

Method: We have developed a serum NMR metabolomics platform optimized for the large sample numbers required in epidemiological studies and biobanks. At costs comparable to routine lipid analyses, our metabolomics platform offers robust quantification of >225 molecular measures, including the lipid concentrations and composition of 14 lipoprotein subclasses (Figure 1), various fatty acids, amino acids, glycolysis metabolites and ketones (Figure 2); (www.computationalmedicine.fi/platform). The absolute metabolite concentrations can be analyzed with the standard medical statistics toolset, i.e., these data can be meta-analysed and combined with other ‘omics and conventional clinical data. This ease interpretation of biological findings and clinical implications.

Results: The high-throughput metabolomics platform has been used in numerous epidemiological studies with over 10,000 individuals, and >230,000 samples have been measured in total. The detailed metabolic profiling has provided insights into the mechanisms of obesity and revealed biomarkers for cardiovascular disease, diabetes, and all-cause mortality. Applications will be exemplified for biomarker discovery, replication and improved risk prediction for incident cardiovascular disease in >15,000 individuals.

Conclusions: Several large-scale studies demonstrate the value of large-scale metabolomics for cardiovascular biomarker discovery and improved risk assessment. As the metabolomics platform quantifies amino acids and fatty acids simultaneously along with the standard lipid measures, the novel biomarkers could be implemented to augment risk prediction without the need for traditional clinical chemistry.
Lipoprotein subclass measures quantified by high-throughput NMR metabolomics

Metabolite measures quantified by high-throughput NMR metabolomics

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Metabolic signatures of adiposity in 12,664 healthy young adults: Causal effects on lipoproteins, fatty acids, inflammation, hormones and amino acids

Abstract nr. 317
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Epidemiology, Metabolism, Obesity, Risk Factor

Aim: Increased adiposity is linked with higher risk for cardiometabolic diseases. We aimed to determine to what extent elevated body mass index (BMI) within the normal weight range has causal effects on the detailed systemic metabolite profile, including lipoprotein subclasses and fatty acid composition, in early adulthood.

Methods: We used Mendelian randomization to estimate causal effects of BMI on 82 metabolic measures in 12,664 adolescents and young adults from four population-based cohorts in Finland (mean age 26 y, range 16–39 y; 51% women). Circulating metabolic risk factors were quantified by high-throughput nuclear magnetic resonance metabolomics and biochemical assays.

Results: In cross-sectional analyses, elevated BMI was adversely associated with cardiometabolic risk markers throughout the systemic metabolite profile, including lipoprotein subclasses, fatty acid composition, amino acids, inflammation and liver function markers, and various hormones (p < 0.0005 for 68 measures). Metabolite associations with BMI were generally stronger for men than for women (median 136%, interquartile range 125%–183%). A gene score for predisposition to elevated BMI, composed of 32 established genetic correlates, was used as the instrument to assess causality. Causal effects of elevated BMI closely matched observational estimates (correspondence 87 ± 3%; $R^2 = 0.89$), suggesting causative influences of adiposity on the levels of numerous metabolites (p < 0.0005 for 24 measures), including lipoprotein lipid subclasses and particle size, branched-chain and aromatic amino acids, and inflammation-related glycoprotein acetyl. These results demonstrate similar risk factor clustering from genetic predisposition to obesity as observed in the metabolic syndrome.

Conclusion: Mendelian randomization indicates causal adverse effects of increased adiposity with multiple cardiovascular risk markers across the metabolite profile in adolescents and young adults.
within the non-obese weight range.

**Funding:** Academy of Finland.
Causal effect estimates of BMI on metabolite risk factors
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
The features of strain characteristics of myocardium in patients with STEMI depending on the efficacy of reperfusion therapy

Abstract nr. 318
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Cardiovascular Disease, Vulnerable Plaque

Objective: To evaluate the features of strain characteristics of myocardium in patients with STEMI after reperfusion therapy by the method of X-Strain.

Methods: The study included group 1 (16 patients with STEMI), 13 men and 3 women (47.5±9.9 years old), and control group 2 of 20 healthy volunteers (12 women and 8 men), mean age 52.2±9.4 years. The inclusion criterion for the group 1 was the absence any cardiovascular pathology. Myocardial infarction was confirmed by ECG, cardiac markers of myocardial necrosis (troponin T, CK-MB) and the results of coronary angiography. Echocardiography was performed with an ultrasonic scanner MyLab 90 (Esaote, Italy) in 6-7 days after the onset of the disease. To evaluate the parameters of the global strain the software X-Strain ™ has been used. The following deformation parameters were identified: global longitudinal strain (GLS), global circumferencial strain (GCS) and global radial strain (GRS).

Results: 1st group was divided into two subgroups based on evaluation of the effectiveness of revascularization on a scale of TIMI: in subgroup 1a (56%) patients with good angiographic result (TIMI 2-3) were included, in the subgroup 1b (44%) - with poor reperfusion effect (TIMI 0-1). GLS in subgroup 1a and 1b was 19.08±4.07 and 9.95±5.29, in the control group – 20.3±2.6. Thus, the reductions in GLS values in subgroup 1a and 1b of 6% and 51% (p=0.01) was observed when compared with healthy subjects. GCS in the study groups was 18.7±5.7, 16.03±5.6 and 25.1±4.01, respectively. In a subgroup 1a, a decrease of GCS values by 15% (p=0.01) was revealed, compared to subgroup 1b - 36%. Parameter of GRS in patients with TIMI 2-3 exceeded on 17% (39.1±12.4) that of control group (33.5±2.2); with TIMI 0-1 were lower (p=0.001) than the control by 23% (25.7±8.2).

Conclusions: the parameters of global myocardial strain in patients with STEMI, who underwent revascularization, depend on its effectiveness on TIMI scale. In patients with TIMI 2-3 deformation characteristics exceed similar indicators in patients with TIMI 0-1. In case of ineffective reperfusion the longitudinal and circumferencial strain of the left ventricle suffer mostly.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Objective: To establish the correlations of mean daily values of the central aortic pressure with parameters of myocardial structure of the left ventricle (LV) in patients with metabolic syndrome (MS) in combination with arterial hypertension (AH) 1-2 degrees.

Methods: 67 subjects with three or more symptoms of MS (ESH guidelines) were examined. Obligatory condition was the presence of hypertension of 1-2 degree. The average age of patients was 49.2 years; height - 168,7±10,6 cm; weight - 90,7±15,4 kg; waist circumference - 102,7±9,9 cm; hip circumference - 112,9±11,3 cm. Office SBP was 148,8±11,7 mmHg and DBP - 97,2 ± 5,1 mm Hg, heart rate - 73,5±6 1 beats/min. Structural and functional properties of the large arteries were assessed by ambulatory blood pressure monitoring device BpLab using the technology Vasotens ("Peter TELEGIN", Russia). The mean daily values of the central aortic pressure and vascular stiffness were evaluated (SBPao, DBPao, MBPao, PPao, Aixao). Echocardiography was performed on a MyLab 90 (Esaote, Italy) with the definition of standard parameters - left ventricular posterior wall thickness (PWLV) and interventricular septum (IVS), the mass and LV myocardial mass index (LVM, LVMI). To determine the rank correlations the Spearman's correlation coefficient was used.

Results: A significant relationship between the mean daily values of SBP in the aorta and IVS thickness (r = 0,44; p <0.01), PWLV (r = 0,24; p <0.05), LVM (r = 0, 37; p <0.01), LVMI (r = 0,36; p <0.01) has been revealed. Central DBP according to ambulatory monitoring correlated with LVM (r = 0,28; p <0.05) and IVS (r = 0,27; p <0.05). There was a significant direct correlation between mean daily MBPao and IVS (r = 0,44; p <0.01), PWLV (r = 0,27; p <0.05), LVM (r = 0,41; p <0, 01), LVMI (r = 0,40; p <0.01). Daily value of PPao correlated with IVS (r = 0,28; p <0.05), and central augmentation index Aixao - with LVMI (r = 0,30; p <0.05).

Conclusions: in patients with metabolic syndrome the diurnal characteristics of the central pressure and aortic stiffness correlated with echocardiographic parameters characterizing the structure of the LV myocardium.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Effect of olmesartan treatment on parameters of local stiffness in patients with coronary artery disease combined with hypertension 1-2 degree

Abstract nr. 320
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis,Blood pressure,Cardiovascular Disease, Therapy

Objective: To evaluate the effect of olmesartan therapy (Kardosal®) on parameters of local vascular stiffness in patients with proven coronary artery disease (CAD) in combination with arterial hypertension (AH) 1-2 degrees.

Methods: The study involved 20 patients with CAD and hypertension: 10 men and 10 women (mean age 56.3±7.5 years). Inclusion criterion was the presence of proven CAD. Confirmation of the diagnosis was a history of myocardial infarction (more than 3 months ago); revascularization intervention on 2 or more major coronary arteries; identification of more than 50% stenosis of at least one coronary artery; positive noninvasive stress test; documented hospitalization for unstable angina. The diagnosis of hypertension was confirmed by the data of history, and three-time measurements of office BP. Local stiffness was studied by ultrasound of the carotid arteries using the echo-tracking technology by device My Lab 90 («Esaote», Italy) on the following parameters: the intima-media thickness (ITM), the coefficient of cross distensibility (DC), compliance coefficient (CC), stiffness index β, augmentation index in the carotid artery (Alx), the local pulse wave velocity in the carotid artery (PWV). The study was conducted before treatment and 24 weeks after the pharmacotherapy. All patients received olmesartan in average dose of 37±7.3 mg daily.

Results: At baseline, in patients with CAD and hypertension the parameters of local arterial stiffness were: DC – 0.01 (0.01; 0.02) 1/kPa, SS - 0.63 (0.46; 1.11) mm²/kPa, index β – 13.6 (11; 18.5), Alx – 11.2±10.6 %, PWV – 9.9 (9.1; 11.5) m/s. After 24 weeks of treatment the reliable regression of index β - to 10.9 (7.3; 11.7) (p<0.05) has been registered, also PWV - to 8.3 (6.6; 8.6) m/s (p <0.01), and increase of CC - up to 0.73 (0.63; 1.48) mm²/kPa (by 13.7%, p<0.05). A significant changes of the Alx and DC have not been identified. The regression of carotid atherosclerosis was revealed: a decrease of IMT from 773.6±155.7 µm to 736.3±124.1 µm (p<0.05).

Conclusion: in patients with CAD and hypertension 1-2 degree the 24-weeks antihypertensive therapy with olmesartan medoksomil has a marked vasoprotective action which is expressed in the improvement of local vascular stiffness.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
complex influence of diabetes and osa on endothelial function in patients with arterial hypertension

Abstract nr. 321
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease,Diabetes,Endothelium

Objective: To evaluate the effect of diabetes and obstructive sleep apnea syndrome on the endothelial function in patients with hypertension.

Methods: The study involved 74 people aged 40 to 65 years with diabetes 2 type and hypertension 1-2 degree, the mean age - 56.4±8.6 years, body mass index (BMI) - 35.3±5.9 kg/m2; systolic blood pressure (SBP) - 148.3 ± 13.6 mm Hg, diastolic blood pressure (DBP) - 88.1±8.9 mmHg. Assessment of the SDB degree was performed using the device for cardiorespiratory monitoring SOMNOcheck2 (Weinmann, Germany). Patients were divided into two groups: the first - 28 patients with apnea-hypopnea index (AHI), according to the CRM of more than 30 events per hour, (which corresponds to clinically significant impairment of breathing), second - 46 patients with AHI<30 events per hour. The patients in both group were matched for age, sex, height, office BP values. Endothelial function was studied by flow-mediated dilation on MyLab 90 ultrasound device (Esaote, Italy). The index of reactivity (IR), flow-mediated dilation index (FMD) were determined. The diameter of common carotid artery (CCA) and the intima-media thickness (IMT) were evaluated.

Results: the patients of group 1 had a higher mean diameter of CCA: 7.5±1.5 mm, in group 2 - 6.3 (5.8; 6.9) mm, respectively (p<0.01), due to significant increase of the neck circumferences in patients with OSAS. In patients with DM in combination with OSAS the mean IMT was 1.02 (0.94, 1.2) mm; in patients without clinical evidence of obstructive breathing disorders during sleep – 0.94±0.2 mm (p>0.05), respectively. The values of IR did not differ: group 1 had 1.2 (1.1, 1.3); group 2 – 1.29±0.32. The prevalence of pathological IR in subjects with diabetes in both groups was 11%. In the group of diabetic patients with symptoms of OSAS the impaired FMD was detected in 85 % of cases, whereas in the comparison group - in 55%. FMD values in 1 group were 6.3±4.6%, in group 2 – 9.8±6.2% (p<0.05).

Conclusions: According to flow-mediated dilation the combination of diabetes and apnea syndrome impairs endothelial function in hypertensive patients compared to patients without SDB.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Roles of an HDL-associated Anti-inflammatory Protein, Progranulin, in Acute Coronary Syndrome

Abstract nr. 323
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords ACS, Vulnerable Plaque

Progranulin (PGRN) is a multifunctional protein. The mutations in the PGRN gene have been reported to cause frontotemporal dementia. PGRN is also known to be involved in inflammation and it has an anti-inflammatory effect. We previously reported that PGRN is secreted from human monocyte-derived macrophages and bound to HDL/ApoA-I. Recently, we demonstrated that deletion of PGRN aggravates atherosclerosis in mice. We hypothesized that PGRN may play some roles in coronary plaque stability in acute coronary syndrome (ACS).

We enrolled consecutive patients (40 males and 11 females; mean age, 65.6±10.3 years) with ACS who underwent emergency coronary angiography. We also enrolled patients without coronary artery disease (79 males and 79 females; mean age, 61.8±13.5 years). There are no significant differences in peripheral blood PGRN concentration between the two groups. We obtained peripheral vein blood samples, arterial blood samples as well as the aspirated samples obtained from the culprit lesion at emergency PCI. Serial blood samples were obtained from on arrival to at discharge from the hospital. Immunostaining of aspirated samples showed PGRN was mainly expressed in macrophages. PGRN concentration in the coronary artery was significantly lower than that in the peripheral vein and arteries (mean: 2.53, 3.00, 2.97 ng/ml, respectively). On the other hand, peripheral vein PGRN concentration gradually increased after PCI and became highest after 48 h (mean, 3.00 vs 3.90 ng/ml). It is well known that MMP-9 concentration in the culprit lesion is increased, therefore we investigated whether PGRN could be a substrate of MMP-9 or not. In in vitro study, PGRN could be cleaved by MMP-9 in a dose dependent manner. Furthermore, we also found that recombinant PGRN suppressed TNF-α-induced expression of MMP-9 in THP1 macrophages, suggesting that higher plasma PGRN concentration might stabilize the vulnerable plaque. Taken together, PGRN may be involved in the stabilization of vulnerable coronary arterial plaques.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Galectin 3, arterial stiffness and carotid intima-media thickness in patients with metabolic syndrome

Abstract nr. 324
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Metabolism, Risk Factor

Objective: Carotid-femoral pulse wave velocity (PWV) and carotid intima-media thickness (cIMT) are surrogate methods of evaluating arterial stiffness and early atherosclerotic lesions, which associated with increased risk of cardiovascular complications. Galectin 3 (Gal 3) - substance with profibrogenic and proinflammatory effects on cardiovascular system. The objective of this study was to compare the level of Gal 3 in patients with metabolic syndrome (MetS) and healthy individuals and to identify the relationship with lipid fractions, PWV and cIMT.

Design and method: 90 persons (47 female and 43 male, 52±8 years old) were examined and divided into 2 groups: MetS (n=50) with 3 or more components (IDF, 2005) and healthy control (n=40) without metabolic disorders and cardiovascular diseases. Groups did not differ significantly by gender, age and eGFR (p>0,05). The examination included: medical history, anthropometry, lipids (Cobas Integra 400/700/800) and Gal 3 (Enzyme Immunoassay) levels in serum. The assessment of carotid intima-media thickness was performed with ultrasound scanning. Carotid-femoral PWV was determined by Sphygmocor.

Results: Level of Gal 3 in patients with MetS was higher compared with the healthy individuals (0,48 [0,42;1,24] and 0,27 [0,24;0,32] ng/ml; p<0,001), also as carotid-femoral PWV (8,82±1,91 and 6,43±0,92 m/s; p<0,001) and cIMT (0,93±0,21 and 0,61±0,12 mm; p<0,001). Level of Gal 3 positive correlated with triglycerides (r=0,50, p<0,001), total cholesterol (r=0,21, p=0,04) and negative correlated with HDL-cholesterol (r=-0,47, p<0,001). Strong positive correlation between Gal 3 and PWV (r=0,76, p<0,001) was revealed.

Conclusions: “Gold standart” of arterial stiffness – carotid-femoral pulse wave velocity, carotid intima-media thickness and level of galectin 3 were higher in metabolic syndrome patients, than in
healthy persons. We propose that profibrogenic and proinflammatory effects of galectin 3 can induce morphological changes in arterial wall, because we revealed strong significant relationship between these parameters.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Therapeutic role of TLR9 agonist in the atherosclerosis

Abstract nr. 325
Author Chen, Yung-Chih, BakerIDI Heart and Diabetes Institute, Melbourne, Australia
Co-author(s) - Koulis, Christine
Co-author(s) - Agrotis, Alex
Co-author(s) - Peter, Karlheinz
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Immunity, Inflammation

Background: Atherosclerosis is driven by inflammatory reactions that are shared with the innate immune system. Toll-like receptor-9 (TLR9) is an intracellular pattern recognition receptor of the innate immune system that is currently under clinical investigation as a therapeutic target in inflammatory diseases. Here we investigated whether TLR9 has a role in the development of atherosclerosis in ApoE-/- mice.

Methods and Results: Newly generated double-knockout ApoE-/-:TLR9-/- mice and control ApoE-/- mice were fed a high-fat diet from 8 weeks of age and effects on lesion size, cellular composition, inflammatory status, and plasma lipids were assessed after 8, 12, 15 and 20 weeks. All four time points demonstrated exacerbated atherosclerotic lesion severity in ApoE-/-:TLR9-/- mice, with a corresponding increase in lipid deposition and accumulation of macro-phages, dendritic cells and CD4+ T cells. Although ApoE-/-:TLR9-/- mice exhibited an increase in plasma VLDL/LDL cholesterol, the VLDL/LDL:HDL ratio was unaltered because of a parallel increase in plasma HDL cholesterol. As a potential mechanism accounting for plaque progression in ApoE-/-:TLR9-/- mice, CD4+ T cell accumulation was further investigated and depletion of these cells in ApoE-/-:TLR9-/- mice significantly reduced lesion severity. As a final translational approach, administration of a TLR9 agonist (type B CpG oligodeoxy-nucleotide ODN-1668) to ApoE-/- mice resulted in a reduction of lesion severity.

Conclusions: Genetic deletion of the innate immune receptor TLR9 exacerbated atherosclerosis in ApoE-/- mice fed a high-fat diet. CD4+ T-cells were identified as potential mediators of this effect. A type B CpG ODN TLR9 agonist reduced lesion severity, thus identifying a novel therapeutic approach in atherosclerosis.

Keywords: Atherosclerosis, TLR9, Oligodeoxynucleotide.

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Alirocumab treatment effect did not differ between patients with/without low HDL-C or high triglyceride baseline levels in Phase 3 trials

Abstract nr. 326
Author Hovingh, Kees, University of Amsterdam, Amsterdam, Netherlands
Co-author(s) - Ceska, Richard
Co-author(s) - Louie, Michael
Co-author(s) - Minini, Pascal
Co-author(s) - Miller, Kathryn
Co-author(s) - Ginsberg, Henry
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords HDL, LDL, PCSK9, Triglycerides

Aims: High triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-C) levels are associated with increased risk of cardiovascular disease (CVD). Data from 10 Phase 3 trials with the PCSK9 inhibitor alirocumab were analysed for potential treatment differences in low-density lipoprotein cholesterol (LDL-C) lowering efficacy and safety between patients with baseline TG below or at/above 150 mg/dL (1.69 mmol/L), or baseline HDL-C below or at/above 40 mg/dL (1.03 mmol/L). The study cohort comprised 4915 patients with heterozygous familial hypercholesterolemia and/or at high CVD risk (those with coronary heart disease or risk equivalents).

Methods: Two trials (LONG TERM, HIGH FH, n=2416) compared alirocumab 150 mg every two weeks (Q2W) versus placebo. Eight trials (n=2499) evaluated alirocumab 75 mg Q2W, increasing to 150 mg Q2W at Week 12 if LDL-C was ≥70 mg/dL (1.81 mmol/L) at Week 8 (or ≥70 or 100 mg/dL [2.59 mmol/L] depending on risk in OPTIONS studies, ALTERNATIVE, MONO); comparator was ezetimibe in five trials and placebo in three. Patients received stable background statin with/without other lipid-lowering therapy except in ALTERNATIVE and MONO (conducted without statin).

Results: Overall, alirocumab reduced LDL-C levels by 38.8–65.5% in patients with baseline HDL-C <40 mg/dL (n=743) and 43.6–59.9% in those ≥40 mg/dL (n=2398), and by 41.9–58.8% in patients with baseline TG <150 mg/dL (n=1963) and 34.2–64.5% in those ≥150 mg/dL (n=1178). Except for two very small subgroups, none of the 95% confidence intervals for treatment effect crossed the no-effect line. LDL-C reductions within individual trials were generally consistent regardless of baseline HDL-C or TG (Figures 1-2). Generally, there was no interaction between baseline HDL-C or TG subgroups and treatment effect of alirocumab versus controls. The rate of treatment-emergent adverse events was similar in alirocumab and control groups irrespective of baseline TG and HDL-C (64.7–80.6%). There was a higher rate of local injection site reactions reported with alirocumab compared with control.

Conclusion: Alirocumab consistently lowers LDL-C levels regardless of baseline TG or HDL-C. This finding holds potential for patients who are at high CVD risk due to low HDL-C and/or high TG
levels.
Analysis funded by Sanofi and Regeneron Pharmaceuticals, Inc.

Figure 1 – Percent change from baseline in calculated LDL-C at Week 24: sub-group analyses according to baseline HDL-C* (ITT analysis)

<table>
<thead>
<tr>
<th>Comparison Study</th>
<th>Subgroup</th>
<th>N</th>
<th>LS means (SE)</th>
<th>LS means difference (95% CI)</th>
<th>Interaction p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alirocumab 150 vs placebo (with statins)</td>
<td>LONG TERM &lt;40 mg/dL</td>
<td>457</td>
<td>0.5 (2.3)</td>
<td>-65.5 (1.6)</td>
<td>0.4989</td>
</tr>
<tr>
<td></td>
<td>≥40 mg/dL</td>
<td>1853</td>
<td>0.9 (1.1)</td>
<td>-59.9 (0.8)</td>
<td>0.2690</td>
</tr>
<tr>
<td></td>
<td>HIGH FH &lt;40 mg/dL</td>
<td>26</td>
<td>4.8 (9.8)</td>
<td>-46.3 (7.2)</td>
<td>0.5693</td>
</tr>
<tr>
<td></td>
<td>≥40 mg/dL</td>
<td>80</td>
<td>-10.4 (5.7)</td>
<td>-45.9 (4.0)</td>
<td>0.5693</td>
</tr>
<tr>
<td>Alirocumab 75/150 vs placebo</td>
<td>COMBO I &lt;40 mg/dL</td>
<td>85</td>
<td>-1.7 (5.6)</td>
<td>-47.2 (3.7)</td>
<td>0.9269</td>
</tr>
<tr>
<td></td>
<td>≥40 mg/dL</td>
<td>226</td>
<td>-2.4 (3.1)</td>
<td>-46.6 (2.3)</td>
<td>0.6052</td>
</tr>
<tr>
<td></td>
<td>FH I &lt;40 mg/dL</td>
<td>138</td>
<td>10.7 (4.0)</td>
<td>-46.4 (3.1)</td>
<td>0.6052</td>
</tr>
<tr>
<td></td>
<td>≥40 mg/dL</td>
<td>347</td>
<td>8.5 (2.7)</td>
<td>-46.6 (1.9)</td>
<td>0.6052</td>
</tr>
<tr>
<td></td>
<td>FH II &lt;40 mg/dL</td>
<td>44</td>
<td>8.6 (7.0)</td>
<td>-47.2 (4.6)</td>
<td>0.6052</td>
</tr>
<tr>
<td></td>
<td>≥40 mg/dL</td>
<td>203</td>
<td>1.7 (3.0)</td>
<td>-40.0 (2.2)</td>
<td>0.6052</td>
</tr>
<tr>
<td>Alirocumab 75/150 vs ezetimibe (without statins)</td>
<td>COMBO II &lt;40 mg/dL</td>
<td>218</td>
<td>-16.1 (3.4)</td>
<td>-52.1 (2.4)</td>
<td>0.0762</td>
</tr>
<tr>
<td></td>
<td>OPTIONS I &lt;40 mg/dL</td>
<td>57</td>
<td>-22.8 (3.3)</td>
<td>-49.9 (1.9)</td>
<td>0.6000</td>
</tr>
<tr>
<td></td>
<td>OPTIONS II &lt;40 mg/dL</td>
<td>143</td>
<td>-21.1 (2.7)</td>
<td>-46.2 (3.9)</td>
<td>0.4217</td>
</tr>
<tr>
<td></td>
<td>≥40 mg/dL</td>
<td>151</td>
<td>-10.5 (4.6)</td>
<td>-43.6 (4.9)</td>
<td>0.4217</td>
</tr>
<tr>
<td>Alirocumab 75/150 vs ezetimibe (without statins)</td>
<td>ALTERNATIVE &lt;40 mg/dL</td>
<td>59</td>
<td>-10.5 (5.2)</td>
<td>-46.3 (4.2)</td>
<td>0.3096</td>
</tr>
<tr>
<td></td>
<td>≥40 mg/dL</td>
<td>189</td>
<td>-15.5 (2.5)</td>
<td>-44.4 (2.6)</td>
<td>0.2970</td>
</tr>
<tr>
<td></td>
<td>MONO &lt;40 mg/dL</td>
<td>13</td>
<td>-11.6 (10.7)</td>
<td>-56.4 (7.2)</td>
<td>0.1138</td>
</tr>
<tr>
<td></td>
<td>≥40 mg/dL</td>
<td>90</td>
<td>-16.0 (3.2)</td>
<td>-45.4 (3.2)</td>
<td>0.1138</td>
</tr>
</tbody>
</table>

CI, confidence interval; ITT, intent-to-treat; LS, least squares; N, number of patients; Q2W, every 2 weeks; SE, standard error

The subgroup baseline levels were: <1.69 mmol/L and ≥1.69 mmol/L

Figure 2 – Percent change from baseline in calculated LDL-C at Week 24: sub-group analyses according to baseline fasting TG† (ITT analysis)

<table>
<thead>
<tr>
<th>Comparison Study</th>
<th>Subgroup</th>
<th>N</th>
<th>LS means (SE)</th>
<th>LS means difference (95% CI)</th>
<th>Interaction p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alirocumab 150 vs placebo (with statins)</td>
<td>LONG TERM &lt;150 mg/dL</td>
<td>1378</td>
<td>2.2 (1.3)</td>
<td>-58.8 (0.8)</td>
<td>0.3431</td>
</tr>
<tr>
<td></td>
<td>≥150 mg/dL</td>
<td>931</td>
<td>-1.1 (1.6)</td>
<td>-45.4 (1.2)</td>
<td>0.9685</td>
</tr>
<tr>
<td>Alirocumab 75/150 vs placebo (without statins)</td>
<td>COMBO I &lt;150 mg/dL</td>
<td>190</td>
<td>0.2 (3.3)</td>
<td>-46.9 (2.5)</td>
<td>0.5073</td>
</tr>
<tr>
<td></td>
<td>≥150 mg/dL</td>
<td>356</td>
<td>-8.8 (2.9)</td>
<td>-48.1 (1.8)</td>
<td>0.4320</td>
</tr>
<tr>
<td></td>
<td>FH I &lt;150 mg/dL</td>
<td>129</td>
<td>10.7 (4.5)</td>
<td>-50.9 (3.1)</td>
<td>0.5904</td>
</tr>
<tr>
<td></td>
<td>≥150 mg/dL</td>
<td>194</td>
<td>2.9 (3.1)</td>
<td>-47.4 (2.2)</td>
<td>0.5904</td>
</tr>
<tr>
<td>Alirocumab 75/150 vs ezetimibe (without statins)</td>
<td>COMBO II &lt;150 mg/dL</td>
<td>409</td>
<td>-22.8 (2.5)</td>
<td>-50.2 (1.8)</td>
<td>0.2200</td>
</tr>
<tr>
<td></td>
<td>≥150 mg/dL</td>
<td>298</td>
<td>-17.9 (2.9)</td>
<td>-51.0 (2.1)</td>
<td>0.6763</td>
</tr>
<tr>
<td></td>
<td>OPTIONS II &lt;150 mg/dL</td>
<td>130</td>
<td>-24.1 (4.0)</td>
<td>-49.6 (4.0)</td>
<td>0.0908</td>
</tr>
<tr>
<td></td>
<td>≥150 mg/dL</td>
<td>70</td>
<td>-16.2 (5.7)</td>
<td>-45.9 (9.4)</td>
<td>0.0908</td>
</tr>
<tr>
<td></td>
<td>ALTERNATIVE &lt;150 mg/dL</td>
<td>119</td>
<td>-18.3 (3.0)</td>
<td>-46.0 (3.4)</td>
<td>0.3073</td>
</tr>
<tr>
<td></td>
<td>≥150 mg/dL</td>
<td>73</td>
<td>-16.4 (3.5)</td>
<td>-41.9 (3.5)</td>
<td>0.0226</td>
</tr>
<tr>
<td></td>
<td>MONO &lt;150 mg/dL</td>
<td>133</td>
<td>-13.3 (5.6)</td>
<td>-60.1 (5.4)</td>
<td>0.0226</td>
</tr>
</tbody>
</table>

CI, confidence interval; ITT, intent-to-treat; LS, least squares; N, number of patients; Q2W, every 2 weeks; SE, standard error

The subgroup baseline levels were: <1.69 mmol/L and ≥1.69 mmol/L

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
**Aims:** Despite standard-of-care statin therapy, many patients incur atherothrombotic events and/or have sub-optimally controlled hypercholesterolemia. Data from nine Phase 3 trials were analysed to determine whether or not treatment differences exist in low-density lipoprotein cholesterol (LDL-C) lowering efficacy of the PCSK9 inhibitor alirocumab in patients with prior myocardial infarction (MI)/ischemic stroke when compared with patients without these prior events.

**Methods:** Eligible patients for the overall ODYSSEY program had heterozygous familial hypercholesterolemia, established coronary heart disease or cardiovascular risk factors, and LDL-C ≥70 or 100 mg/dL (1.81 or 2.59 mmol/L) at screening, depending on risk. Two trials (LONG TERM and HIGH FH; n=2416) evaluated alirocumab 150 mg every 2 weeks (Q2W) versus placebo. In the remaining seven trials (n=2396), patients received alirocumab 75 mg Q2W with increase to 150 mg Q2W at Week 12 if LDL-C was not at target goal by Week 8. Comparators are indicated on the Figure. In all but one trial (ALTERNATIVE was conducted in a statin-intolerant population), patients received stable background statin ± other lipid-lowering therapy.

**Results:** Across studies, between 20% and 65% of patients had experienced previous MI/ischemic stroke. Alirocumab reduced LDL-C levels by 41.7–63.0% in patients with prior MI/ischemic stroke and 35.4–59.2% in patients without such vascular events (Figure). Within 8 trials there was no interaction between prior MI/ischemic stroke status at baseline and treatment effect with respect to LDL-C reductions for alirocumab versus control group. The incidence of treatment-emergent adverse events was generally similar between alirocumab and control group patients regardless of prior MI/ischemic stroke status (65.1–79.6%). There was a higher rate of local injection site reactions reported with alirocumab compared with control group.

**Conclusion:** Patients with or without prior MI/ischemic stroke (a known high risk group) have similar LDL reductions with alirocumab. An ongoing large outcomes study will more fully assess the clinical benefit in this and other high risk populations.

Analysis funded by Sanofi and Regeneron Pharmaceuticals, Inc.
### Subdivision 3. Clinical Studies

#### Presentation Preference Oral presentation

**Additional information**

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**Figure** – Percent change from baseline in calculated LDL-C at Week 24: sub-group analyses according to prior myocardial infarction or ischemic stroke (ITT analysis)

<table>
<thead>
<tr>
<th>Comparison Study</th>
<th>Subgroup</th>
<th>LS means (SE)</th>
<th>LS means difference (95% CI)</th>
<th>Interaction p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alirocumab 150 vs placebo</strong> (with statins)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LONG TERM</strong></td>
<td>Prior MI/stroke</td>
<td>1109</td>
<td>0.3 (1.5)</td>
<td>−63.0 (1.1)</td>
</tr>
<tr>
<td></td>
<td>No prior MI/stroke</td>
<td>1201</td>
<td>1.3 (1.4)</td>
<td>−59.2 (1.0)</td>
</tr>
<tr>
<td><strong>HIGH FH</strong></td>
<td>Prior MI/stroke</td>
<td>28</td>
<td>4.8 (9.7)</td>
<td>−43.4 (7.1)</td>
</tr>
<tr>
<td></td>
<td>No prior MI/stroke</td>
<td>80</td>
<td>−10.4 (5.7)</td>
<td>−46.5 (4.0)</td>
</tr>
<tr>
<td><strong>Alirocumab 75/150 vs placebo</strong> (with statins)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>COMBO I</strong></td>
<td>Prior MI/stroke</td>
<td>178</td>
<td>0.4 (3.6)</td>
<td>−52.3 (2.5)</td>
</tr>
<tr>
<td></td>
<td>No prior MI/stroke</td>
<td>133</td>
<td>−5.7 (4.1)</td>
<td>−42.6 (3.0)</td>
</tr>
<tr>
<td><strong>FH I</strong></td>
<td>Prior MI/stroke</td>
<td>135</td>
<td>4.8 (4.2)</td>
<td>−50.0 (3.1)</td>
</tr>
<tr>
<td></td>
<td>No prior MI/stroke</td>
<td>350</td>
<td>10.9 (2.6)</td>
<td>−48.3 (1.9)</td>
</tr>
<tr>
<td><strong>FH II</strong></td>
<td>Prior MI/stroke</td>
<td>54</td>
<td>1.5 (5.8)</td>
<td>−50.5 (4.2)</td>
</tr>
<tr>
<td></td>
<td>No prior MI/stroke</td>
<td>193</td>
<td>3.0 (3.1)</td>
<td>−48.2 (2.2)</td>
</tr>
<tr>
<td><strong>Alirocumab 75/150 vs ezetimibe</strong> (with statins)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>COMBO II</strong></td>
<td>Prior MI/stroke</td>
<td>480</td>
<td>−21.6 (2.4)</td>
<td>−52.2 (1.7)</td>
</tr>
<tr>
<td></td>
<td>No prior MI/stroke</td>
<td>247</td>
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<td>−47.5 (2.3)</td>
</tr>
<tr>
<td><strong>OPTIONS I</strong></td>
<td>Prior MI/stroke</td>
<td>63</td>
<td>−20.5 (5.6)</td>
<td>−41.7 (5.8)</td>
</tr>
<tr>
<td></td>
<td>No prior MI/stroke</td>
<td>137</td>
<td>−21.8 (4.0)</td>
<td>−51.5 (3.9)</td>
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<td><strong>OPTIONS II</strong></td>
<td>Prior MI/stroke</td>
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<td>−21.2 (7.7)</td>
<td>−56.2 (7.2)</td>
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<td>No prior MI/stroke</td>
<td>130</td>
<td>−8.8 (5.3)</td>
<td>−35.4 (5.4)</td>
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<tr>
<td><strong>Alirocumab 75/150 vs ezetimibe</strong> (without statins)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ALTERNATIVE</strong></td>
<td>Prior MI/stroke</td>
<td>49</td>
<td>−15.5 (5.1)</td>
<td>−51.2 (4.8)</td>
</tr>
<tr>
<td></td>
<td>No prior MI/stroke</td>
<td>199</td>
<td>−14.3 (2.5)</td>
<td>−43.4 (2.5)</td>
</tr>
</tbody>
</table>

CI: confidence interval; ITT: intent-to-treat; LS: least squares; N: number of patients; Q2W: every 2 weeks; SE, standard error
Smooth muscle cell actin in atherosclerotic plaques is associated with restenosis after atherectomy of the femoral superficial artery.

Abstract nr. 328
Author Groha, Philipp, Deutsches Herzzentrum München, München, Germany
Co-author(s) - Fusaro, Massimiliano
Co-author(s) - Böttiger, Corinna
Co-author(s) - Steppich, Birgit
Co-author(s) - Ibrahim, Tareq
Co-author(s) - Straub, Melanie
Co-author(s) - Ott, Ilka Verena
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Functionality

Introduction: The composition of atherosclerotic plaques may influence subsequent restenosis after vessel wall injury. Aim of this study was to analyze the association of femoral plaque composition and subsequent restenosis.

Methods and Results: Sixty-seven consecutive patients who underwent atherectomy of the superficial femoral artery using the SilwerHawk atherectomy device (ev3 Europe SAS, Paris) were included in the study. Microscopic sections of the excised material were analyzed blindly on a semiquantitative scale after the following stainings: hematoxylin eosin, Elastica van Gieson, anti-CD31, anti-CD68, anti-CD3, anti-CD15, anti-SMC actin, anti-CD61 and Sudanred. At twelve months follow-up 38 patients presented with symptomatic restenosis. Colour duplex ultrasound of the target superficial femoral artery lesion revealed a ratio of the maximum intrastenotic PSV and the maximum prestenotic PSV >4 or a complete occlusion. Patients with restenosis were comparable to those without restenosis according to risk factor profile, medication, stent implantation or recanalisation. SMC-actin was significantly increased in plaques from patients with restenosis as compared to those without restenosis. Moreover, lipid content was significantly decreased (p<0.05). Local inflammation, calcification, vessel density, haemorrhage and platelet content were similar in both groups.

Conclusion: Smooth muscle cell actin is associated with restenosis after atherectomy and may serve as a marker for those patients with an increased risk for restenosis.

Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
Oxidized low-density lipoprotein induces autophagy in macrophages via CD36-mediated oxidative stress

Abstract nr. 329
Author QIN, SHUCUN, Taian, China
Co-author(s) - YAO, SHUTONG
Co-author(s) - LI, YANYAN
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Inflammation

To investigate the inductive effect of oxidized low-density lipoprotein (ox-LDL) on autophagy in macrophages and the underlying molecular mechanisms. **Methods:** RAW264.7 macrophages were pretreated with 2 mg/L anti-CD36 monoclonal antibody (anti-CD36 mAb) 5 µmol/L diphenyleneiodonium (DPI) 3 mmol/L 3-methyladenine (3-MA) or 1 µmol/L rapamycin for 1 h and then treated with ox-LDL (100 mg/L) for 12 h. Cell viability and apoptosis were measured by MTT assay and TUNEL detection kits, respectively. The activities of lactic dehydrogenase (LDH) in medium and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, superoxide dismutase (SOD) in cells as well as the levels of intracellular reactive oxygen species (ROS) and malonaldehyde (MDA) were determined to characterize the membrane integrity and the oxidative stress, respectively. The protein levels of Beclin-1 and microtubule-associated protein 1 light chain 3-II (LC3-II), two important molecular markers of autophagy, were examined by Western blot analysis. **Results:** Ox-LDL induced autophagy in RAW264.7 macrophages as assessed by upregulation of Beclin-1 and LC3-II. Similar to 3-MA, an autophagy inhibitor, anti-CD36 mAb inhibited significantly the ox-LDL-induced upregulation of Beclin-1 and LC3-II. Anti-CD36 mAb suppressed the ox-LDL-induced oxidative stress as revealed by decreased NADPH oxidase activation, ROS and MDA generation as well as increased SOD activity. Similar results were observed in the cells pretreated by DPI, a NADPH oxidase inhibitor. Moreover, DPI inhibited significantly the ox-LDL-induced upregulation of Beclin-1 and LC3-II. Additionally, the decrease in cell viability and the increase in LDH release and apoptosis induced by ox-LDL were promoted by 3-MA and blocked by rapamycin (an autophagy inducer). **Conclusion:** Ox-LDL can induce autophagy in RAW264.7 macrophages, which may be involved in CD36-mediated ox-LDL uptake and subsequent activation of oxidative stress, and moderate activation of autophagy may protect macrophages from ox-LDL-induced injury.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Hydrogen activates ABCA1-dependent efflux ex vivo and improves HDL function in patients with hypercholesterolemia: a double-blinded, randomised and placebo-controlled trial

Abstract nr. 330
Author QIN, SHUCUN, Taian, China
Co-author(s) - SONG, GUOHUA
Co-author(s) - LIN, QUANQIANG
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Functionality,HDL,Reverse Cholesterol Transport

**Background**- The objective of this study was to characterize the effects of hydrogen (dihydrogen; H₂)-rich water (0.9 L/day) on the content, composition, and biological activities of plasma lipoproteins on patients with hypercholesterolemia and their underlying mechanisms in a double-blinded, randomized and placebo-controlled trial.

**Methods and Results**- A total of 68 patients with untreated isolated hypercholesterolemia were randomly allocated to either drinking H₂-rich water (n=34) or placebo water (n=34) for 10 weeks. High density lipoprotein (HDL) isolated from H₂ group was showed an increased ability to promote ATP-binding cassette transporter A1 (ABCA1)–mediated cholesterol efflux ex vivo. Plasma pre-β-HDL levels was upregulated although there were no changes in plasma HDL-cholesterol levels. Moreover, other HDL functionality, assessed in protection against low density lipoprotein (LDL) oxidation, inhibition of oxidized-LDL induced inflammation, and protection of endothelial cells from oxidized -LDL induced apoptosis, were all significantly improved by H₂ treatment. In addition, H₂ treatment increased effective rate in H₂ down-regulating plasma total-cholesterol (47.06% versus 17.65%) and LDL-cholesterol (47.06% versus 23.53%) levels. Western blot analysis revealed a marked decrease of apolipoprotein B100 and an increase of apolipoprotein M in plasma of H₂ group. Finally H₂ treatment resulted in significant reductions in levels of several inflammatory and oxidative stress indicators in whole plasma and HDL particles.

**Conclusions**- Hydrogen activates ATP-binding cassette transporter A1-dependent efflux, enhances HDL anti-atherosclerotic functions, and has beneficial lipid-lowering effects. The present finding may highlight the potential role of hydrogen in the regression of hypercholesterolemia and atherosclerosis.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Phospholipid Transfer Protein overexpression decreases plaque stability by promoting macrophage lipid accumulation in apoE null mice

Abstract nr. 331
Author QIN, SHUCUN, Taian, China
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Co-author(s) - LI, BIN
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Vulnerable Plaque

Plasma phospholipid transfer protein (PLTP) activity is elevated in patients with coronary heart disease, whereas the role on atherosclerotic plaque stability remains unclear. We studied the plaque stability with or without elevated PLTP expression in apolipoprotein E null mice. PLTP overexpression significantly increased the size of atherosclerosis (AS) lesion and plaque necrosis core. Moreover, the contents of lipid and macrophage were enlarged in plaque, which suggested that PLTP overexpression destabilized plaque. Consistently, oxidative low density lipoprotein (ox-LDL) induced lipid accumulation was increased in PLTP overexpressed bone marrow derived macrophage (BMDM), which could be due to the up-regulation of ox-LDL receptor CD36 and down-regulation of scavenger receptor BI (SR-BI) and ATP binding cassette transporter A1 (ABCA1). Overall, these results reveal a novel role of PLTP in atherosclerosis that elevated PLTP expression decreased plaque stability by increasing macrophage lipid accumulation in plaque.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Reverse-D-4F increases quantity of endothelial progenitor cells in high fat diet-fed mice and improves endothelial progenitor cell dysfunctions through PI3K/AKT

Abstract nr. 332
Author QIN, SHUCUN, Taian, China
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Co-author(s) - Jiao, Peng
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Endothelium, Prevention

Objective—We aim to examine the effect of ApoAI mimetic peptide Reverse-D-4F (Rev-D4F) on peripheral blood cell subpopulations in C57BL/6J mice treated with high fat diet and the mechanism of Rev-D4F in improving the function of endothelial progenitor cells (EPCs) impaired by tumor necrosis factor α (TNF-α).

Approach and Results—6-week-old C57BL/6J mice were treated with high fat diet or Rev-D4F (1mg/kg/d) for 16 weeks, and used flow cytometry, blood cell counting instrument and ELISA method to quantify peripheral blood cell subpopulations and cytokines. Results indicated that high fat diet significantly decreased the number of endothelial progenitor cells (EPCs) and the percentage of lymphocyte in the white blood cells, and increased the number of white blood cells, the percentage of monocyte in the white blood cells, the level of vascular endothelial growth factor (VEGF) and TNF-α in C57BL/6J mice plasma. Rev-D4F obviously inhibited the effect of high fat diet on quantification of peripheral blood cell subpopulations and cytokines levels, and increased stromal cell derived factor 1 (SDF-1) in mice plasma. We provided in vitro evidence that TNF-α impaired EPCs proliferation, migration and adhesion through inactive AKT and eNOS, which could be restored by Rev-D4F treatment. PI3-kinase inhibitor LY294002 (30 μM) obviously inhibited the restoration of Rev-4F on EPCs impaired by TNF-α.

Conclusions—Rev-D4F increases the quantity of endothelial progenitor cells through increasing SDF-1 levels and decreasing TNF-α levels of peripheral blood in high fat diet induced C57BL/6J mice, and restores TNF-α induced dysfunctions of EPCs through stimulating PI3K/AKT signal pathway in part.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Oxidized low density lipoprotein induces apoptosis of macrophage through p66Shc-mitochondrial signal pathway

Abstract nr. 333
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Pathogenesis

Aim: The apoptosis of macrophage aggravates the vulnerability of atherosclerotic plaque and even results in plaque rupture, which can cause acute complication clinically in the advanced stage of atherosclerosis (AS). Existing research shows that oxidized low density lipoprotein (oxLDL) induces the apoptosis of macrophage by mitochondrial pathway, but the specific molecular mechanism has not yet been elucidated. p66Shc, an adapter protein, can be phosphorylated in cytoplasm and transferred into mitochondria, which causes mitochondrial injury. Recent research reveals oxLDL increases the level of phospho-p66Shc (Ser36) in endothelial cells. This study is to evaluate the role of p66Shc-mitochondrial signal pathway in apoptosis of macrophage.

Methods: LDL was extracted from fresh plasma of healthy people by sequence ultracentrifugation, and then it was oxidized by CuSO4 to obtain oxLDL. RAW264.7 macrophages were cultured in vitro. The effect of oxLDL on the levels of p66Shc and phospho-p66Shc (Ser36) protein in cytoplasm and mitochondria were detected by western blotting. Then the cells were divided into control group oxLDL group and oxLDL+siRNA-p66Shc group. Mitochondrial transmembrane potential (ΔΨm), mitochondria-derived reactive oxygen species (mROS) and apoptosis of RAW264.7 cells in each group were measured by flow cytometry and the expression of the proteins related to apoptosis via mitochondrial pathway was analyzed by western blotting.

Results: After RAW264.7 macrophages were treated with oxLDL (100ug/ml), the levels of phospho-p66Shc (Ser36) in cytoplasm and p66Shc in mitochondria were both promoted significantly. Compared with control group, oxLDL decreased the level of ΔΨm, increased the level of mROS, aggravated cell apoptosis, enhanced the release of cytochrome C and upregulated the protein expression of caspase 9 and caspase 3, which were inhibited by gene-silencing p66Shc.

Conclusion: oxLDL induces apoptosis of macrophage, at least partly, through p66Shc-mitochondrial signal pathway.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Induction of MMP-2 and MMP-9 expressions in aorta vascular smooth muscle cells through MAPK pathway by di(2-ethylhexyl)phthalate (DEHP)

Abstract nr. 334
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Pathogenesis

Proliferation and migration of vascular smooth muscle cells (VSMC) play an important role in the development and progression of many cardiovascular diseases, including atherosclerosis. During the early stages of atherosclerosis or arterial wall injury, VSMC would migrate into the intimal layer of the arterial wall, causing intimal thickening. MMP-2 and MMP-9 have shown to be related to the pathogenesis of atherosclerosis by facilitating migration of VSMC through the internal elastic lamina into the intima of a vessel wall, where they proliferate and contribute to plaque formation. According to documents, the expression of MMP-2 and MMP-9 proteins are involved in atherosclerosis via several pathways, including ERK1/2, p38 MAPK and NF-κB. Di(2-ethylhexyl)phthalate (DEHP) is a plasticizer widely used in PVC appliances. It is known that DEHP can induce TNF-α production and formation of reactive oxygen species (ROS), which were found to cause MMP activations. However, a question on whether DEHP has any effects on expression of MMP-2 and MMP-9 has not yet been answered. In our studies, rat aorta VSMC (A7r5 cell line) was treated with DEHP (3.5 and 7 ppm) at 20 min for p38 MAPK, ERK1/2, and Akt determination; the protein expression of NF-κB and MMPs (-2 and -9) was measured at 12h and 24h, respectively. Results showed that the presence of DEHP can induce higher MMP-2 and MMP-9 expression than the controls. An increase of DEHP concentration produced a higher induction. Similar results on MMP regulating proteins, i.e. p38 MAPK, ERK1/2, Akt, and NF-κB was observed. In summary, our current results have showed that DEHP can be a potent inducer of atherosclerosis by increasing expression of MMP-2 and -9 at least through the regulations of p38 MAPK, ERK1/2, Akt, and NF-κB.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
HSP60 as an autoantigen in obesity

Abstract nr. 337
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Immunity, Inflammation, Metabolism, Obesity

Although the association of a chronic low-grade inflammation with obesity has long been appreciated, its molecular basis is yet to be defined. The proven involvement of adaptive immunity, coupled with a phenotypic switch from autoimmune suppressive tolerogenic T_{reg} to pro-inflammatory CD4^{+} T_h1 and CD8^{+} T cells, during progression of obesity necessitates the presence of a triggering antigen as an activator of T and B cells. Not surprisingly in this context, it is found that visceral adipose tissue-specific T cells show severely biased T cell receptor V_{α} repertoires in diet induced obese mice (Winer, et al. 2009), implying an antigen-specific clonal expansion of T cells during obesity.

Heat Shock Protein 60 (HSP60) is an evolutionary conserved mitochondrial chaperonin that assists the correct folding of other mitochondrial proteins. However, its occurrence is not restricted to mitochondria and it can be located in the cytosol or exposed on the cell membrane also. An increase in cell membrane HSP60, which may be accompanied by HSP60 release into circulation, is especially considered a signal of autoimmunity. HSP60 has been associated with a broad range of diseases so far, particularly those with an autoimmune component. More recently, HSP60 is also linked to obesity as a mediator of adipose tissue inflammation and insulin resistance. Moreover, circulating HSP60 levels are found to be higher in obese individuals than lean controls (Märker et al. 2012).

We observed an adaptive immune response against HSP60 at both T cell and B cell (antibody) levels during continuous high fat feeding of C57Bl6 mice. Hence HSP60 appears to be one of the mystery auto-antigens triggering the early T and B cell responses during obesity. Furthermore, we attempted a peptide therapy in a dose escalation protocol aiming to down-regulate the inflammatory related adverse effects of obesity by achieving tolerance in T cell populations and suppressing the pathogenic antibody response. Using previously defined immuno-dominant murine HSP60 peptide combinations, we observed a significant improvement in insulin resistance (p=0.002, 2-way ANOVA). We also observed some improvement in glucose tolerance at borderline significance (p=0.057), which encourages further research to improve peptide therapy.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Design of a Phase 3, double-blind, randomized, placebo-controlled trial of lomitapide in pediatric patients with homozygous familial hypercholesterolemia

Abstract nr. 338
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Familial Hypercholesterolemia, LDL, Therapy

The vasculature of children with homozygous familial hypercholesterolemia (HoFH) is exposed to high levels of LDL-C, starting in utero. Duration of exposure and magnitude of LDL-C elevation (expressed as ‘cholesterol years’) are thought to be associated with increasing cardiovascular risk. As a result, treatment is focused on early reduction in LDL-C levels. Lomitapide is a microsomal triglyceride transfer protein inhibitor approved in the US, EU and certain other countries as an adjunct therapy for the treatment of adults with HoFH. Lomitapide has been shown to decrease LDL-C levels in adult patients with HoFH. This study is designed to determine the efficacy and safety profile of lomitapide in combination with lipid-lowering therapy in HoFH patients aged 5–17 years. The effect of lomitapide on cardiovascular morbidity and mortality has not been determined, nor will it be determined in this study.

This is a Phase 3 international clinical trial, with an initial 6-month double-blind efficacy phase, followed by an 18-month open-label safety phase. 120 patients with HoFH aged 5–17 years will be randomized 1:1 to receive lomitapide or placebo, in addition to stable lipid-lowering therapy (including LDL-apheresis if applicable) established during a 12-week run-in period. Patients will be stratified according to age. The lomitapide starting dose is determined by age and will be escalated to the maximum age-specific dose. The primary endpoint is mean percent change in LDL-C from baseline to month 6 versus placebo. After 6 months, all patients will be offered lomitapide treatment for 18 months; this open-label period will provide further information on the safety and efficacy profile. Growth, sexual maturation, bone health, and neurocognitive function of lomitapide-treated patients will be compared with placebo-treated patients and with existing matched external controls where available. Exploratory analyses will assess changes in carotid intima-media thickness and effects on supravalvular aortic atheroma and xanthomata.

The aim of this trial is to determine whether lomitapide decreases LDL-C in pediatric HoFH patients.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Nondipping Status, Cardiovascular Remodeling, dyslipidemia and Cognitive Dysfunction in Georgian Obese Hypertensive Subjects

Abstract nr. 339
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease,Dyslipidemia,Hypertension,Obesity

**Purpose:** Taking into consideration prevalence of arterial hypertension (AH), dyslipidemia and cognitive dysfunction in obese patients, we studied influence of “nondipping” status (blunted nocturnal decline in BP) on cardiovascular remodeling, dyslipidemia and cognitive function in Georgian obese nondiabetic “dipper” and “nondipper” hypertensive patients.

**Methods:** We studied 62 patients with mild to moderate AH (35males/27females, mean age 51,4±1,6years, BMI 32,4±1,6kg/m², duration of AH 6,5±1,6years). Examination included 24-hour BP monitoring (ABPM), ultrasound evaluation of left ventricular mass index (LVMMI), carotid artery IMT, blood lipid tests, Mini-Mental State Examination test. 33 “nondipper” patients were assigned to group 1 and 29 “dippers” to group 2.

**Results:** The groups were comparable by the age, BMI, duration of AH, daytime mean BP values. Mean values of nocturnal BP (138±2,9/96±3,3vs126±1,6/90±3,8 mmHg), LVMMI (144,3±10,6vs139,1±9,8gr/m) and carotid artery IMT(1,06±0,02vs1,04±0,03mm) were certainly increased in “nondipper” patients compared with “dipper” ones (p< 0,05). Occurrence of LVH (concentric type: 56vs51%; eccentric type: 13vs9 %) (p< 0,01), lipid disturbances (detected dyslipidemia in 28 patients of group 1 and in 21 patients of group 2 (85 vs72%) (p< 0,01) and cognitive disorders (31vs26%)(p< 0,05), were significantly higher in gr1, of concentric remodeling and normal geometry in gr2 (21vs17% and 19vs14%, respectively) (p< 0,05).

**Conclusions:** Thus, in Georgian obese nondiabetic hypertensive subjects with “nondipper” circadian BP profile we detected more pronounced and frequent target-organ injury (LVH, enhanced IMT), lipid disturbances and cognitive dysfunction comparing with patients with normal nocturnal decline in BP. Data of our study demonstrate importance of more profound examination of cardiovascular system and neurological status in obese hypertensive patients with additional negative impact of circadian blood pressure rhythm, to ensure further more aggressive blood pressure and weight reduction, lipid profile correction.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
MicroRNA-X can identify smokers at risk for atherosclerosis

Abstract nr. 341
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Cardiovascular Disease, Risk Factor

Introduction: Worldwide, tobacco use is the most important avoidable cause of cardiovascular disease (CVD), redoubling the risk of developing a myocardial infarction. However, numerous smokers never develop cardiovascular complaints. MicroRNAs (miRNAs) are small non-coding RNAs that control gene expression and are known to be involved in cardiovascular disease.

Objectives: To find monocyte miRNAs profiles that can identify those smoking individuals that develop cardiovascular disease.

Methods/results: We performed a miRNA microarray on isolated monocytes in 40 subjects with premature atherosclerosis and 40 healthy controls. Evaluation of the microRNA expression levels revealed that miRNA-X had a low expression among non-smokers, whereas surprisingly, among smokers there was much variation in expression of miRNA-X. Some smokers showed equal levels as in non-smokers and other smokers showed high miRNA-X expression levels (figure 1). We reasoned that if these results were to be true, the same results should be observed in an independent second cohort. Indeed, we observed the a similar results in a second cohort consisted of 40 CVD patients and 27 of their healthy family members (figure 2). Since it seems that some smoking subjects have normal miRNA-X levels, while others have high expression levels, this lead to the hypothesis that monocytes of certain smokers, handle smoking differently, possibly leading to high miRNA-X levels and that these smokers might be more prone to develop atherosclerosis. To test this hypothesis, we compared miRNA-X levels in a cohort of 38 smokers with subclinical atherosclerosis, as assessed by coronary CT scan (coronary calcium score >80th percentile) and 32 healthy smoking subjects (coronary calcium score of 0), using RT-qPCR. MiRNA-X expression levels were significantly higher in subjects with subclinical atherosclerosis compared to healthy controls (OR 2.4, 95% CI 1.8-4.6, P=0.007).

Conclusion: This study showed that high miRNA-X expression levels can identify subclinical atherosclerosis in smoking individuals. Therefore, miRNA-X may be a useful biomarker to identify smokers at high risk of developing cardiovascular disease.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
The COMMD protein family forms a large protein complex to regulate the intracellular trafficking of LDLR

Abstract nr. 342
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model,LDL,Lipoproteins

LDLR is the main receptor to clear circulating LDL cholesterol and hereby it reduces the susceptibility to atherosclerosis. Various adaptor proteins have been identified in controlling the internalization of LDLR but the mechanism by which LDLR is recycled back to the plasma membrane is still ill defined. Recently we identified Commd1 as a novel gene in regulating cholesterol homeostasis. Hepatic COMMD1 deficiency in mice and dogs results in increased plasma LDL cholesterol levels. We demonstrated that COMMD1 acts at the endosomes to sort LDLR back to the plasma membrane. The COMMD protein family comprises 9 other COMMD proteins. Except for COMMD1, the biological function of these other COMMD proteins is still unknown. In this study we uncovered that COMMD1 forms a stable protein complex together with several other COMMD proteins. Depletion of Commd1 in mouse hepatocytes results in protein instability of a select group of COMMD proteins. Subcellular localization studies indicated that these COMMD proteins act in concert with the WASH and retromer complexes to sort LDLR at the endosomes. To validate the role of other COMMD proteins in cholesterol homeostasis, we specifically depleted Commd9 in murine hepatocytes. Similar to Commd1 deficiency, hepatic Commd9 ablation causes elevated plasma cholesterol. Altogether, our data strongly indicate that a select group of COMMD proteins form a stable protein complex to mediate LDL cholesterol homeostasis. At this moment additional in vitro and in vivo experiments are being performed to identify the exact composition of the COMMD protein complex, and to validate the role of the WASH complex in LDLR vesicular transport.

Subdivision 1. Basic Science
Presentation Preference Electronic poster presentation
Additional information
E3 ubiquitin-protein ligase Casitas B lineage Lymphoma b deficiency aggravates atherosclerosis

Abstract nr. 343
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis

‘E3 ubiquitin ligase Casitas B lineage Lymphoma b’ (Cbl-b) is a negative regulator of peripheral T cell activation. Cbl-b deficient T cells are hyper-reactive due to CD28-independent activation. Here we studied the effect of Cbl-b deficiency on T cell homeostasis in hypercholesterolemia and on atherogenesis in 20 wk old Cbl-b-/-/ApoE-/- mice. Flow cytometric analysis of lymphoid tissues and aortic arches showed decreased CD4⁺:CD8⁺ T cell ratios in Cbl-b-/-/ApoE-/- mice. Cbl-b deficiency induced the expansion of central memory CD8⁺ T cells, whereas the proportion of naïve T cells decreased. We found that Cbl-b deficient CD8⁺ T cells are less apoptotic as indicated by decreased AnnexinV positivity and elevated expression of anti-apoptosis markers, such as Bcl-2. Pro-inflammatory, TNFα and IFNγ, and cytotoxicity markers, granzyme B, are increased in Cbl-b deficient CD8⁺ T cells. As expected from the increase of CD8⁺ T-cells in the aortic arch, Cbl-b-/-/ApoE-/- mice showed significantly more plaque development in the aortic root. The plaques contained higher leukocyte and T cell counts, but contained surprisingly less macrophages. The latter is caused by decreased monocyte recruitment resulting from lower MCP-1 levels. Moreover, the excess of CD8⁺ T cells induced enhanced cell death of macrophages. In vitro co-culture of Cbl-b deficient and wildtype CD8⁺ T cells with bone marrow derived macrophages revealed enhanced macrophage apoptosis in increased CD8⁺:macrophage ratios, irrespective of the CD8⁺ T cell genotype. Expression of the M1, pro-inflammatory, macrophage markers, CD115 and CD64 was upregulated in Cbl-b deficient aortic arches, whereas M2 markers, CD206 and Arg-1, were decreased. In conclusion, we show that Cbl-b deficiency decreases CD4⁺:CD8⁺ T cells ratio during hypercholesterolemia, through reduced apoptosis and possibly less susceptibility of CD8⁺ T cells to regulatory T cell suppression. This contributes to exacerbated atherosclerosis in Cbl-b deficient mice. Although plaques contained an excess of lymphocytes and T-cells, and macrophages where of an M1 phenotype, macrophage counts were decreased. This was caused by low MCP-1 levels due to CD8⁺ T cell induced macrophage apoptosis. These results reveal that Cbl-b balances immune reactions in atherosclerosis.
Presentation Preference Mini-oral presentation
Additional information
An association detected between APOC1 rs4420638 polymorphism and myocardial infarction in two populations of the Volga-Ural region of Russia

Abstract nr. 344
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Atherosclerosis, Genetics, Risk Factor

Impaired lipid metabolism is one of the key mechanisms in the development of coronary artery disease. Genome-wide association studies have identified several genetic loci associated with lipid traits in European, Asian, and African American population. The aim of our study was to replicate these associations in the native populations of the Volga-Ural region (Russian Federation).

Genotyping of rs1042034 (APOB) and rs4420638 (APOC1, intergenic region) polymorphic loci was performed in the study group consisting of 528 Tatars (225 patients with myocardial infarction, 303 healthy individuals) and 631 Russians (344 patients with myocardial infarction, 287 healthy individuals) originating from the Republic of Bashkortostan (Russian Federation). Statistical analysis was performed using IBM SPSS software.

The results of our study have not demonstrated an association between APOB rs1042034 polymorphism and the risk of myocardial infarction in either of the study groups; however, we found that APOC1 rs4420638 was associated with the risk of myocardial infarction both in Tatars and in Russians. APOC1*A/A genotype frequency was decreased in patients with myocardial infarction compared to the control group (65.79% vs. 73.87% in the Russian ethnic group, OR=0.68, CI 0.48 – 0.96, P=0.03; and 56.00% vs. 67.33% in Tatars, OR=0.62, CI 0.43 – 0.89, P=0.008). APOC1*G allele carriers were at an increased risk of myocardial infarction (OR=1.41, CI 1.04 – 1.94, P=0.03 in Russian ethnic group; and OR=1.62, CI 1.2 – 2.18, P=0.002 in Tatars). In the Tatar ethnic group, APOC1*G/G genotype carrier status also indicated an increased risk of myocardial infarction (OR=3.24, CI: 1.31 – 8.01; P=0.009).

APOC1 rs4420638*G allele has been shown to increase LDL cholesterol level and decrease HDL cholesterol level, and to indicate higher risk of Alzheimer’s disease, rapid cognitive decline, and lower chances of survival beyond 90 years of age. Our results suggest that APOC1 rs4420638 polymorphism may influence the development of myocardial infarction in Tatars and Russians from the Volga-Ural region of Russia.

The study was supported by the RFBR grant No. 13-04-01561.

Subdivision 1. Basic Science
Presentation Preference Oral presentation
Additional information
Chronic pravastatin treatment induces Ca2 mediated mitochondrial dysfunction and increased antioxidant activity in muscle of LDLr-/- mice

Abstract nr. 345
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Familial Hypercholesterolemia, Metabolism, Therapy

The safety and efficacy of statins treatment in hypercholesterolemic patients is well established. However, about 10% of patients develop side effects in skeletal muscle such as myalgia, myopathy and, rarely, rhabdomyolysis. Previous studies indicate that myotoxicity caused by statins may be linked to impairment of mitochondrial function associated with alterations in calcium homeostasis, inhibition of β-oxidation followed by oxidative stress.

Thus, our aim is to better understand the mechanisms underlying skeletal muscle toxicity caused by statins in LDL receptor knockout mice (LDLr-/-), a model of familial hypercholesterolemia. For this purpose, LDLr-/- mice were treated with pravastatin (40 mg/kg/day) for 3 months. Muscles with distinct fiber type composition were harvested and evaluated for respiration rates and antioxidant enzymes activities.

Our results show that pravastatin treatment slowed down the rates of ADP-, oligomycin- and FCCP-stimulated respiration (up to 40%) supported by glutamate/malate in permeabilized LDLr-/- plantar muscle fiber bundles in the presence of Ca2+, compared to non-treated LDLr-/- mice. In contrast, no alterations were observed in soleus muscle. In addition, respiratory parameters were not altered in the presence of EGTA, indicating a possible Ca2+-mediated respiratory impairment in plantar muscle.

Pravastatin treatment also increased catalase activity (48%) in plantaris muscle homogenates with no alterations in superoxide dismutase, glutathione reductase and peroxidase as well as glucose-6-phosphate dehydrogenase activities. No enzymatic alterations were observed in soleus muscle. Taken together, our results indicate that distinct muscle fiber present distinct sensitivity to pravastatin as occur with other statins. Furthermore, Ca2+-mediated mitochondrial dysfunction induced by pravastatin might act as a signal that leads to a higher activity of cellular antioxidant system in plantaris muscle of LDLr-/- mice under this treatment.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Responses of primary hepatocytes (gold standard for evaluating hepatic metabolism) and HepG2 cells to components influencing apoA-I production are comparable.

Abstract nr. 346
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins

Background. Apolipoprotein-AI (apoA-I), the predominant protein of HDL, is produced in the human liver. Accumulating evidence shows that elevated apoA-I production is athero-protective. Thus, there is a need for a human-relevant hepatic cellular model to discover compounds that increase apoA-I production. Currently, primary hepatocytes (PH) are considered the gold standard, whereas HepG2 are frequently used. Therefore, we compared side-by-side the apoA-I producing capacity of human PH and HepG2 in response to a series of compounds.

Methods. HepG2 and PH (two donors) were plated into 48-well plates. In addition to several natural compounds, thapsigargin, FeAc, or cytokines were used to decrease, whereas JQ1(+), GW7647, or FB-OM31 were used to increase apoA-I production. Cells medium was analysed for human apoA-I protein concentrations (ELISA), and cell lysates were analysed for mRNA expression (qPCR).

Results. JQ1(+) and FB-OM-31 both increased apoA-I protein levels and mRNA production in HepG2 and PH. Theobromine, linoleic acid and DHA showed similar response patterns in HepG2 and PH. Thapsigargin and cytokines decreased apoA-I protein levels and mRNA production in HepG2. In PH, however, these compounds did not alter apoA-I protein levels, while thapsigargin even increased apoA-I mRNA production.

Conclusion. Although PH has become the gold standard for evaluating hepatic lipid metabolism, the size and the direction of the effects of compounds on apoA-I production were comparable between PH and HepG2. Therefore, HepG2 are a suitable model to evaluate compounds that increase apoA-I production in vitro.

This research is supported by the Dutch Technology Foundation STW, which is part of the Netherlands Organisation for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
ER-stress players and their effects on attenuated apolipoprotein A-I production during ER-stress and inflammation in HEPG2 cells.

Abstract nr. 347
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Background. Increased apoA-I production is thought to be anti-atherogenic. Thus, there is an urgent need to identify factors involved in apoA-I production. Transcriptional regulation of apoA-I involves PPAR-α activation, but factors like ER-stress and inflammation also influence apoA-I production.

Methods. Gene expression profiles of FeAc- and GW7647-treated HepG2 cells were compared using microarrays. HepG2 cells were treated with thapsigargin or cytokines to induce ER-stress or inflammation with or without isoform-specific C/EBP-β overexpression, C/EBP-β silencing, or ATF3 overexpression. ApoA-I production was determined with ELISA and qPCR. ER-stress markers (C/EBP-β, CHOP, XBP1s) and apoA-I levels were analysed by western blot.

Results. mRNA expression of PPAR-α, C/EBP-β, ATF3, DDIT4, GDF15 and NF-IL3 was strongly up-regulated by FeAc as compared to GW7647. This ER-stress signature associated with lower apoA-I secretion. Direct ER-stress induction via thapsigargin also decreased intracellular apoA-I concentrations, while those of ER-stress markers (CHOP, XBP1s, C/EBP-β) increased. Also, addition of cytokines increased intracellular C/EBP-β levels and lowered apoA-I concentrations. The presence of C/EBP binding-sites in the apoA-I promoter further suggested a role for C/EBP-β in apoA-I production. However, isoform-specific overexpression of C/EBP-β or C/EBP-β silencing did not affect apoA-I production. Likewise, overexpression of ATF3, a second ER-stress target derived from the gene expression profile, did not influence apoA-I production.

Conclusions. Although ER-stress and inflammation increase C/EBP-β expression and simultaneously lower apoA-I production, overexpression or inhibition of C/EBP-β or overexpression of ER-stress related protein ATF3, did not influence hepatic apoA-I production. Therefore, C/EBP-β or ATF3 are not a target to increase apoA-I synthesis.

This research is supported by the Dutch Technology Foundation STW, which is part of the Netherlands Organisation for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
A systematic review on the impact of age on carotid artery intima-medial thickness in healthy humans

Abstract nr. 348
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis,Cardiovascular Disease,Epidemiology,Risk stratification

Aims:
Assessment of carotid artery intima-medial thickness (cIMT) represents a popular measure of atherosclerosis and is predictive of future cardio- and cerebrovascular events. Nevertheless, the relationship between the occurrence of cardiovascular events and age is not linear, and the increase in event rate starts earlier in life in men than women. However, relatively little is known about the precise relationship between age and the progression in cIMT. Therefore, the principal aim of this review was to describe the relationship between age and carotid artery IMT, and to elucidate whether this relationship is consistent across the age range examined in previously published studies on healthy individuals.

Methods: A systematic review of published studies that examined cIMT in human populations free from cardiovascular diseases was undertaken. The literature search was conducted using Pubmed and cross-checked with Scopus and WebofScience. Twenty publications with 38 individual studies were included in the final analysis, involving a total of 11,441 individuals (6,148 men and 5,293 women).

Results: When all data were pooled a strong positive association was evident between age and cIMT, with an annual cIMT progression of 0.00863 mm/year (r=0.90). Analysis of men and women demonstrated a comparable age-related increase in cIMT (0.00875 versus 0.00874 mm/year respectively). However, men demonstrated a larger cIMT across all age groups (y-axis intercept 0.246 mm in men versus 0.206 mm in women).

Conclusion: Advanced age is associated with a gradual, linear increase in cIMT. Although cIMT in men is consistently larger than in women, the age-related increase in cIMT is comparable. This data suggests that, in healthy asymptomatic individuals, cIMT is closely related to the (chronological) age of vessels. Importantly, this observation questions the potential independent predictive capacity of cIMT for future cardiovascular events in healthy individuals.
Figure 1 – Relation between age and cIMT in asymptomatic men and women

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
H3K27 methylation in macrophages and atherosclerosis

Abstract nr. 349
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Inflammation, Pathogenesis

Atherosclerosis is a lipid-driven chronic inflammatory disorder. Monocytes and macrophages are key immune cells in the development of disease and clinical outcome. It is becoming increasingly clear that epigenetic pathways govern many aspects of monocyte and macrophage differentiation and activation. This is regulated by a large number of histone modifying enzymes (HMEs) that regulate activating- (e.g. H3K4me3, H3K27Ac) or repressive- (e.g. H3K27Me3) histone marks. The H3K27 demethylase Jumonji-domain 3 (Jmjd3) is induced upon LPS activation in macrophages and was shown to be involved in both M1/M2 polarization. The role of its counteracting partner, the methyltransferase Enhancer of the zeste homolog 2 (Ezh2) however remains unknown. Using bone marrow derived macrophages we found that where Jmjd3 is induced upon LPS stimulation, Ezh2 is down regulated, both at the mRNA and protein level. Also oxidized LDL induced foam cell formation resulted in decreased Ezh2 expression. Moreover, human unstable plaques show an up regulation of Ezh2 compared to stable advanced plaques. Hereby we show that Ezh2 is regulated in macrophages and atherosclerotic lesions, however its exact function remains unknown. Hereto we generated a myeloid specific Ezh2 deficient mice (LysMCre fl/fl). To validate the role of macrophage Ezh2 in atherosclerosis we performed a bone marrow transplantation to LDLr^{-/-} mice and compared WT (Cre-) to Ezh2 deficient (Cre+) transplanted mice after 9 weeks of high fat diet. We observe a 20% reduction in plaque size in the Ezh2 deficient transplanted mice and are currently characterizing its plaque phenotype. We also observe reduced foam cell formation in peritoneal macrophages of these mice. Currently, we are investigating the mechanisms by which macrophage Ezh2 regulates macrophage activation, foam cell formation and atherosclerotic plaque development. Supported by the GENIUS project (CVON2011-19).

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Cardiovascular risk profile in 40-year old men and 50-year old women in the Czech Republic: results of a cross-sectional survey

Abstract nr. 350
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease,Dyslipidemia,Hypertension,Prevention

Background: Early identification of CVD risk factors may help decrease cardiovascular morbidity and mortality.

Methods: 961 males and 851 females were included in the survey. Family and personal medical history were recorded as well as smoking, alcohol consumption, physical activity patterns, basic anthropometrical parameters and laboratory measures including blood lipids and glycaemia were recorded. Newly identified risk factors were noted and the global cardiovascular CV risk was determined using the SCORE charts. Percentage of those already treated for any of the main CV risk factors and, among these, also attaining the treatment goals were calculated as well as the percentage of patients with newly identified CV risk factors.

Results: 49% of males and 31% of females were overweight and 32% of both genders were obese. There were 36% of smokers among men and 22% among women. The prevalence of type 2 diabetes (T2DM) was 11% in both genders. Arterial hypertension (AH) was present in 43% of males and 45% of females, dyslipidaemia in 39% of males and 45% of females. Pharmacological treatment of any of the above mentioned diseases was used in 48% of the probands, however, only 8% of them attained treatment goals. T2DM was newly identified in 3% of both genders, AH in 8% of males and 3% of females and dyslipidaemia in 20% of both genders. Pharmacological treatment was recommended to 62% males and to 65% females. Pharmacological treatment was initiated in 53% of males and 51% of females, mostly antihypertensive treatment with ACE inhibitors and lipid lowering therapy with a statin.

Conclusion: The survey showed high prevalence of CV risk factors in the selected population. The high frequency of modifiable risk factors and the often need to initiate pharmacological treatment documents the necessity of early detection of CV risk among others genetic determination.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Transcription factor Runx2 promotes aortic fibrosis and stiffness in type 2 diabetes

Abstract nr. 351
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Diabetes

Cardiovascular disease is the most common cause of death in type 2 diabetes mellitus. One proposed mechanism linking type 2 diabetes to increased cardiovascular risk is accelerated arterial stiffening. Arterial stiffening results from extensive extracellular matrix remodeling (elastin breakdown, collagen accumulation). The present study investigates the role of the osteogenic transcription factor Runx2 as a potential mediator of aortic fibrosis and stiffening in diabetes. Serial ex vivo mechanical testing of the thoracic aorta and volume-pressure recording (VPR) based tail-cuff blood pressure measurements revealed that aortic stiffening precedes blood (pulse) pressure elevations in diabetic db/db mice. Vascular stiffening was accompanied by enhanced Runx2 expression in the medial layer of db/db aortae as well as increased expression of downstream target genes, Col1a1, Col1a2, Fn1 and Spp1. Similar findings were observed in thoracic aortic samples from diabetic patients confirming translational relevance of our preclinical results. Moreover, vascular smooth muscle-specific overexpression of Runx2 in transgenic mice increases expression of Col1a1 and Col1a2, and leads to medial fibrosis and aortic stiffening. Interestingly, increased Runx2 expression per se is not sufficient to induce aortic calcification. Treatment of db/db mice with the antioxidant, TEMPOL, attenuated Runx2, ECM target gene expression and functional vascular stiffness, implicating an important role played by local oxidative stress. Through further in vitro studies, we demonstrate that increased nuclear translocation/activation of NF-κB in human aortic VSMCs under high glucose stimulation was reversed by TEMPOL. In addition, siRNA knockdown of the NF-κB subunits RelA (p65) and NfkB1 (p50) completely inhibits the high glucose-stimulated up-regulation of RUNX2, COL1A1 and COL1A2. In silico analysis identified and mutational studies confirmed a functional RelA (p65) recognition motif (5'-GGAAAGGCCT-3') 198bp upstream of the Runx2 transcription start site. Taken together, our results suggest that oxidant stress induced Runx2 is a critical regulator of diabetic aortic stiffening, and therefore a potential therapeutic target.
This work was supported by research grants from the NIH (1R01HL105299 to P.S. Tsao), the Deutsche Forschungsgemeinschaft (RA 2179/1-1 to U. Raaz), the Boehringer Ingelheim Fonds and the Medical School of the University Erlangen, Germany (to I. N. Schellinger) and the Stanford Cardiovascular Institute (to J.M. Spin).

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
HIGH ROSUVASTATIN DOSES GIVEN ALONG WITH A CHOLESTEROL-RICH DIET INDUCE EARLY DAMAGE ON HEPATOCYTES OF CD-1 MICE

Abstract nr. 352
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model,Dyslipidemia

3-hydroxy-3-methylglutaryl CoA-reductase inhibitors are widely used to control plasma cholesterol levels. Previous studies from our group have shown that high doses of statins given to mice, along with a cholesterol-rich diet, are harmful for hepatocytes, with alteration of their mitochondrial structure and function.

The aim of this report was to study the initial effects of the co-administration of rosuvastatin, 25 mg/Kg/day and a 2% cholesterol diet. Animals were sacrificed daily from day 0 to 5. A control normal and a 5 day cholesterol-treated groups were also included. Serum was obtained for the measurement of biochemical parameters. The liver morphology was studied by optical and electron microscopy.

Mice with rosuvastatin/cholesterol treatment showed an increment in serum cholesterol levels through the time from 102 mg/dL to 237 mg/dL, HDL-C decreased from 43.2 mg/dL to 25.2 mg/dL, triacylglycerol also decreased from 138 to 66 mg/dL, AST raised from 165.3 to 864.9 IU/L and ALT from 64.5 to 635.1 IU/L. Microscopic studies showed a progressive hepatic steatosis, higher in the central vein zone, with inflammatory cells at the portal area, stasis in the sinusoids, acidophylic cells that suggest an apoptotic process due to treatment. The cholesterol-rich diet or the rosuvastatin treatment alone did not induce any damage on the liver.

The co-administration of high dose of rosuvastatin and a cholesterol-rich diet induce early steatosis and probable apoptotic response in the liver.

Acknowledgement: this study was supported in part by PAPIIT IN221914.
Phytoestrogens and future of soy cultivation

Abstract nr. 353
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Hypolipidemic Drugs, Nutraceuticals, Nutrition

Among edible plants, phytoestrogens are most abundant in soy. Soy products are widely used in infant food and other foodstuffs; and, at the same time, phytoestrogens have been applied for compensation of hormone deficiency in menopause. Soy is used as livestock fodder, and residual phytoestrogens and their active metabolites such as equol can remain in meats. There are few reports on modified gender-related behavior or feminization in humans as a result of soy consumption. In animals, the intake of phytoestrogens was reported to impact fertility, sexual development and behavior. Feminizing effects in humans can be subtle and identifiable only statistically in large populations, and may be of particular significance for children and adolescents. Given that the biological action of estrogens is mediated by receptors, it should be questioned, why must incidental plant analogues be used for replacement therapy instead of the natural or synthetic hormones that are complimentary to the receptors. It has been argued that phytoestrogens are selective receptor modulators thus acting differently from the natural estrogens, not necessarily feminizing. Yet the question remains whether such modulations are desirable for consumers of soy products, including adolescents and infants receiving soy nutrition. Ger Med Sci. 2014;12:Doc18. This matter should be clarified by independent research, which can have implications for the future of soy in the agriculture.

Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
Combining kidney function and size and its relation to kidney function decline, cardiovascular events and all-cause mortality

Abstract nr. 355
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, Renal function, Risk Factor

**Background and objectives** Estimated glomerular filtration rate (eGFR) and kidney size are known to be related. Combining eGFR and kidney size may be seen as a measure of kidney functionality. Therefore the relations between the ratio of eGFR and kidney size and kidney function decline, cardiovascular events and all-cause mortality were evaluated in patients with clinical manifest vascular disease.

**Design, setting, patients & measurements** A prospective cohort study in 6904 patients enrolled in the Second Manifestations of ARTerial disease (SMART) study with symptomatic arterial disease (cerebrovascular disease, coronary artery disease, peripheral arterial disease or abdominal aortic aneurysm) was performed. The CKDEPI equation was used to estimate GFR. The ratio of eGFR to kidney size was calculated by dividing eGFR by mean kidney volume and length (eGFR/cm$^3$ and eGFR/cm) as measured by ultrasonography. In a subgroup of 1469 patients with follow up measurements of kidney function linear regression analysis was used to quantify the relation between eGFR/cm$^3$, eGFR/cm and annual kidney function decline. Cox proportional hazard models were used to determine the relation between eGFR/cm$^3$, eGFR/cm and cardiovascular events and all-cause mortality.

**Results** After a median follow up of 8.9 years (IQR 4.1-10.8) mean annual change in eGFR was -0.79 mL/min/1.73m$^2$ per year, indicating a yearly decline. The ratios eGFR/cm$^3$ and eGFR/cm were significantly related to annual kidney function decline, -0.55 (CI:-0.52 - -0.34) and -0.78 (CI -0.89 - -0.68) mL/min/1.73m$^2$ per year respectively. Showing the greater the ratio the stronger the decline. A total of 1012 cardiovascular events occurred and 1119 patients died. Overall, eGFR/cm$^3$, eGFR/cm and all-cause mortality followed a U-shaped curve (Figures 1 and 2). Low eGFR/cm$^3$ and eGFR/cm were related to the occurrence of subsequent cardiovascular events.

**Conclusions** High eGFR/cm$^3$ and eGFR/cm is related to annual kidney function decline and all-cause mortality in patients with clinical manifest vascular disease. The combination of eGFR and kidney size may be useful as an early marker of kidney deterioration.
Relation between eGFR/cm kidney and all-cause mortality

Relation between eGFR/cm³ kidney and all-cause mortality

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation

Additional information
Distinct associations of alternative pathway complement factors Bb, D and properdin with human fatty liver disease: the CODAM study

Abstract nr. 356
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Epidemiology, Inflammation, Metabolism, Risk Factor

Objective: Non-alcoholic fatty liver disease (FLD) a liver manifestation of the metabolic syndrome and alcoholic FLD are the most common causes of liver disease in Western society. We previously showed (Wlazlo et al Eur J Clin Invest. 2013; 43: 679-88) a potential role for complement C3 activation in human FLD. The alternative pathway (AP) is an important amplifier of complement activation. Therefore we here investigated whether (activation of) the alternative pathway (AP) is also association with FLD.

Methods: In a cross-sectional evaluation of the Cohort of Diabetes and Atherosclerosis Maastricht (CODAM) study (n=511, 61% men; 59±7 years) associations of several AP components with FLD were investigated. We measured circulating concentrations of the factor B activation product (Bb) as marker of alternative pathway activation, of factor D (FD), which is the rate-limiting protease of B activation, and of properdin which is a positive regulator of complement activation. As markers of FLD, we used a validated equation to estimate liver fat percentage (eLF%) and a liver enzyme score (LE-score) – a compilation of averaged standardized (std) alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT). Multiple linear regression models were (when appropriate) adjusted for age, sex, impaired glucose metabolism (IGM), type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), smoking, alcohol consumption, medication use, physical activity and waist circumference.

Results: Factor Bb was not significantly associated with eLF% (stdβ= -0.01 [95%CI: -0.07; 0.05] or LE-score (stdβ=0.02 [-0.06; 0.10]). In crude analyses, FD was positively and significantly associated with eLF% and LE-score, but this association largely disappeared after adjustment for waist circumference (eLF%: stdβ= -0.04 [-0.11; 0.04], LE-score: stdβ= 0.07 [-0.02; 0.17]). Properdin was positively and significantly associated with eLF% and LE-score and remained so after adjustment for all potential confounders (stdβ= 0.13 [0.07; 0.19] and stdβ= 0.12 [0.05; 0.20], respectively).

Conclusion: These data suggest a potential involvement of the AP in human FLD, although distinct results were obtained for individual components. Longitudinal data, including direct measurements...
of steatosis and/or steatohepatitis, are desirable to further investigate the role of the AP in the development and progression of FLD.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Cardiovascular risk assessment and carotid intima-media thickness in young adults, survivors of Hodgkin's lymphoma

Abstract nr. 357
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Cardiovascular Disease, Dyslipidemia, Endothelium

Cardiovascular disease is the most common non-malignant cause of death in Hodgkin lymphoma (HL) survivors, particularly following mediastinal irradiation. We aimed to perform an extensive cardiovascular risk assessment in survivors of childhood and adolescence HL. We hypothesized that survivors will have an increased rate of impaired endothelial function and increased carotid intima-media thickness (IMT) compared with healthy peers.

Twenty-seven patients (females=14; aged 25±5y, 8.6±4 years after diagnosis) were recruited. Seventeen were treated with chemotherapy and chest irradiation, nine with chemotherapy alone, and one with radiation only. All patients were euthyroid at the time of evaluation (six with replacement treatment). Cardiovascular risk assessment included brachial artery flow-mediated dilation (FMD), IMT measurement with echo-color Doppler, an extended lipid profile, liver function tests, glucose and insulin levels, HS-CRP and lifestyle assessment. Control group for IMT measurements comprised 55 (33 females) healthy normo-cholesterolemic patients aged 18-30 years.

Regarding traditional risk factors, five patients were smokers, 11 patients had a family history of coronary heart disease, and none had hypertension or diabetes. Mean BMI was 23.9±4.2kg/m², waist circumference of males 84.9±9.4cm and of females=79.1±8.7cm. Liver function tests and glucose were normal in all patients. 6/27 had increased insulin resistance as calculated in the Homeostasis Model Assessment (HOMA). Mean cholesterol levels were 179.8±37 mg/dl; mean LDL-c was 111±35.8mg/dl, mean TG were 107±52.8 mg/dl, and mean HDL-c levels 53.2±15.2mg/dl. Lp (a) levels were 19.7±17.9mg/dl and were elevated in 6 patients. Apo A1 levels were 134.3±25.2 mg/dl and Apo B levels were 88.3 ±23.7, mean HS-CRP levels were 4.61±6.9. Vitamin D levels were 28.3±12.2 ng/ml. One participant fulfilled the criteria for the metabolic syndrome and four others fulfilled 2 out of 5 criteria. Five patients had abnormal endothelial function.

Average bilateral IMT was 0.537±0.11mm, compared to 0.49±0.056mm in healthy controls. Four patients had increased carotid IMT according to current normal range (>0.65mm for <40 years old).

Conclusions: Our preliminary results suggest an increased rate of pro-atherogenic markers and
metabolic abnormalities in these young patients with the presence of at least one feature of "dysmetabolism" in most patients, which may enhance their future risk for premature atherosclerosis.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Mini-oral presentation
Additional information
The relationship between cardio-ankle vascular index and carotid atherosclerosis severity

Abstract nr. 358
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Prevention, Risk stratification

Introduction. Cardio-ankle vascular index (CAVI) is an arterial stiffness parameter based on pulse wave velocity (PWV). CAVI has been shown as useful screening method which correlates with SCORE risk chart index. The aim of this study was to reveal if the CAVI could predict the severity of atherosclerosis process.

Materials. 66 patients at age 52,26 ± 11,2 (13 males and 53 females) was underwent CAVI estimation and carotid ultrasound examination.

Results. Because of there wasn't significant difference between right and left CAVI (Spearman R 0,96; p < 0,001) for further analyze had been used right measured CAVI (R-CAVI). There was established moderate correlations between CAVI and intima-media thickness (Spearman R 0,70, p < 0,05), CAVI and the number of carotid plaques (Spearman R 0,74, p < 0,05), CAVI and carotid plaque diameter (Spearman R 0,65, p < 0,05).

Discussion. It was shown moderate but significant correlations between CAVI and carotid atherosclerosis severity. The further large population studies may provide an important data for new risk charts development.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
The personalized assessment of severity of myocardial infarction: clinical application of genetic polymorphisms, associated with lipid disorders and hypertension

Abstract nr. 359
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords ACS,Apolipoproteins,Atherosclerosis,Genetics

Aim: To investigate the clinical significance of single-nucleotide polymorphisms (SNPs) APOA1 (rs670), APOA5 (rs662799), ACE (rs4646994) in patients with ST-segment elevation myocardial infarction.

Materials and Methods: 179 patients (114 males (63.7%), 65 females (36.3%), the mean age 61.8 ± 11.1 years) admitted with a diagnosis of STEMI were included in the study. Blood samples were collected on days 2-14 for genotyping. DNA was isolated from peripheral blood lymphocytes by phenol-chloroform extraction followed by ethanol precipitation. Amplification of SNPs APOA1 G-75A (rs670), APOA5 T-1131C (rs662799), ACE (rs4646994) was detected with the real-time polymerase chain reaction (PCR). Results: The apolipoprotein A1 (APOA1) C allele reported a statistically significant association with the presence of multivesel coronary artery disease (OR = 1.59; 95% CI = 1.09-2.33; p = 0.02). The apoA1-GG genotype demonstrated high genetic risk of developing diabetes (OR = 2.74; 95% CI = 1.31-5.73; p = 0.02), and was associated with a history of severe chronic heart failure (OR = 3.43; 95% CI = 1.37-8.55; p = 0.02). The GG-genotype is also associated with less frequent primary PCI, usually because of severe multivesel coronary artery disease (OR = 0.61; 95% CI = 0.38-0.96; p = 0.03).

Patients with the rs662799(T) polymorphism variant of apolipoprotein A5 (AROA5) gene demonstrated more severe course of myocardial infarction, assessed in the TIMI score, than patients without this allele. The AROA5 T allele in MI patients is associated with the presence of obesity (OR = 2.64; 95% CI = 1.04-6.67; p = 0.03), previous myocardial infarction (OR = 1.75; 95% CI = 1.05-2.92; p = 0.03) and the intima-media complex thickening (OR = 1.58; 95% CI = 1.09-2.30; p = 0.02). The I allele of rs4646994 polymorphism variant of ACE gene in post-MI patients was associated with a three-year unfavorable prognosis.

Conclusion: The studied polymorphism variants of genes, associated with lipid metabolism disorders and the onset of arterial hypertension (APOA1, AROA5, ACE genes), may be used to clarify the clinical severity of myocardial infarction patients.
Subdivision 2. Translational Research

Presentation Preference Mini-oral presentation
Additional information
Strong correlation between serum level of uric acid and malondialdehyde-modified low-density lipoprotein in patients with acute non-cardioembolic ischemic stroke.

Abstract nr. 360
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Dyslipidemia, Lipoproteins

Objective: Recent experimental findings showed uric acid (UA) is not just a risk factor for atherosclerosis, but one of the important key markers to reflect oxidative stress. Several reports revealed strong correlation between UA and oxidized low-density lipoproteins in healthy subjects, but few report studied in patients with stroke. Our aim of this study is to clarify the relation between oxidative stress and other atherosclerosis risk factors in acute phase of non-cardioembolic ischemic stroke (NCIS), especially focusing on UA.

Design and Method: We defined NCIS as ischemic strokes including large artery atherosclerosis (LAA), small vessel disease (SVD) and branch atheromatous disease (BAD) in this study. From consecutive 225 ischemic stroke patients, 140 NCIS patients (52 LAA, 33 BAD and 55 SVD) were enrolled. Malondialdehyde-modified low-density lipoprotein (MDA-LDL), the most prevalent modification found in oxidized LDL, and classical atherosclerosis risk factors including UA are examined on their admission. We employed Pearson bivariate correlation analysis to examine the relation between MDA-LDL and those risk factors.

Results and Conclusions: In the Pearson analysis, serum UA showed significant correlation with MDA-LDL (r=0.295; P<0.001). MDA-LDL was also significantly related to triglyceride (r=0.350; P<0.001), total cholesterol (r=0.526; P<0.001) and low-density lipoprotein cholesterol (r=0.540; P<0.001). Body mass index, which is still controversial risk factor for cardiovascular event, was correlated with MDA-LDL (r=0.180; P=0.03). Other risk factors, such as systolic/diastolic blood pressure, hemoglobin A1c and estimated glomerular filtration rate did not show significant correlation with MDA-LDL. These result suggested hyperlipidemia associated with hyperuricemia strongly indicate the existence of elevated oxidized-LDL, which would reflect existing unstable plaque. In conclusion, hyperuricemia in acute phase of NCIS indicate existing oxidative stress and vulnerable plaque in cerebral artery.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Cardiovascular risk profile in midge age patients with severe dyslipidemia

Abstract nr. 361
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Cardiovascular Disease, Dyslipidemia, Risk Factor

Objective: The aim of this study was to determine the prevalence of severe dyslipidemia (SD) in Lithuania population, associated variables and how strongly they are related to cardiovascular risk (CVR).

Methods: Cross-sectional investigation of cardiovascular risk factors of 26,707 subjects was conducted. The study recruited men and women aged 40–64 without overt cardiovascular disease. SD definition used was: total cholesterol (TC) ≥ 7.5 mmol/l, low-density lipoprotein cholesterol (LDL-C) ≥ 6 mmol/l or triglycerides (TG) ≥ 4.5 mmol/l. Subjects with SD were further categorized into groups having main types of familial dyslipidemia. CVR was classified using the SCORE system.

Results: 12.3% of study population had SD. The prevalence of familial dyslipidemia was 5.44%: hypercholesterolemia – 3.47%, hypertriglyceridemia – 1.87% and mixed dyslipidemia – 0.1%. The subjects with SD had significantly higher rates of arterial hypertension (65.4% vs. 56.1%, p<0.001), diabetes mellitus (13% vs. 9.7%, p<0.001), abdominal obesity (53.6% vs. 45.5%, p<0.001), body mass index higher than 30 kg/m² (40.9% vs. 37%, p<0.001), metabolic syndrome (46.9% vs. 29%, p<0.001), unbalanced diet (69% vs. 62%, p<0.001), insufficient physical activity (50.5% vs. 44.7%, p<0.001), family history of cardiovascular disease (27.1% vs. 23.6%, p<0.001) and 3 or more cardiovascular risk factors (84.1% vs. 73.8%, p<0.001). However, differences in rates of smoking (20.5% vs. 21.1%, p=0.405) and familial diabetes mellitus (13.5% vs. 12.4%, p=0.073) were not of statistical importance. SCORE index (2.91±2.24 vs. 1.87±1.59, p<0.001) was also significantly higher in the presence of SD (p<0.001).
Conclusions: About 12% of study subjects satisfy SD criteria. High prevalence of severe dyslipidemia in Lithuania is associated with high frequency of other cardiovascular risk factors, such as arterial hypertension, diabetes mellitus, abdominal obesity and metabolic syndrome. SD presence shows a possibility of increased SCORE index.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
HIGH SYSTEMIC LDL CHOLESTEROL LEVELS DURING EXPERIMENTAL OSTEOARTHRITIS LEAD TO INCREASED SYNOVIAL ACTIVATION AND ECTOPIC BONE FORMATION AT END-STAGE OSTEOARTHRITIS.

Abstract nr. 362
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Inflammation, LDL, Pathogenesis

Objective: A relation between osteoarthritis (OA) and increased cholesterol levels is apparent. In the present study we investigate OA pathology in ApoE\(^{-/-}\) mice with and without a cholesterol-rich diet, a model for high systemic LDL cholesterol levels independently of weight.

Methods: Wild type (WT) and ApoE\(^{-/-}\) mice received a standard or cholesterol-rich diet. Experimental OA was induced by intra-articular injection of collagenase and animals were sacrificed at day 10 (early OA) and 36 (end-stage OA).

Results: ApoE\(^{-/-}\) mice on a normal diet showed markedly higher LDL levels than WT mice (8.90 mmol/L and 0.40 mmol/L, respectively; p<0.001). At end-stage OA, ApoE\(^{-/-}\) mice showed a strong increase of ectopic bone formation, mainly at the medial collateral ligament (fold increase 5.4; p<0.001) compared to WT mice. No significant differences in cartilage damage were found between the two groups; a slight increase in synovial thickening, however, was found in ApoE\(^{-/-}\) mice (arbitrary score 1.9 versus 1.1 in WT mice; p<0.05). Furthermore, synovial gene expression of both S100A8 and S100A9 (fold increase 1.8 and 1.4, respectively; p<0.05) and S100A8/S100A9 protein levels of synovial wash-outs were increased in ApoE\(^{-/-}\) mice (fold increase 5.8; p<0.05), suggesting an activated status of synovial lining cells. In addition, we investigated whether a cholesterol-rich diet could increase joint pathology after induction of OA. The diet increased LDL levels even more in ApoE\(^{-/-}\) mice (fold increase 2.1, compared to ApoE\(^{-/-}\) mice on a normal diet; p<0.001). In both ApoE\(^{-/-}\) and WT mice on a cholesterol-rich diet, excessive bone formation was found in the medial collateral ligament at day 36, however, no significant difference was found between the two groups. Interestingly, at the early time point, histological differences between the two groups were observed. Synovial thickening was four times increased (p<0.001) in ApoE\(^{-/-}\) mice on a cholesterol-rich diet and also ectopic cartilage formation in the medial collateral ligament was strongly increased (fold increase 2.7; p<0.01) compared to WT mice on a cholesterol-rich diet.

Conclusion: Increased cholesterol levels result in synovial activation and ectopic bone formation in experimental OA. Excessive LDL levels strongly elevate synovial activation and ectopic bone
formation in early-stage collagenase-induced OA.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Treatment of atherosclerosis by the chemotherapeutic agent carmustine associated to a lipid nanoemulsion in rabbits

Abstract nr. 363
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Lipoproteins, Therapy

After injection in bloodstream, a lipid nanoemulsion (LDE) resembling low-density lipoprotein (LDL) concentrate in atherosclerotic lesions of cholesterol-fed rabbits. Here, rabbits with atherosclerosis were treated with carmustine, an antiproliferative agent used in cancer chemotherapy, associated to LDE to investigate the effects on the lesions.

Eighteen male New Zealand rabbits were fed a 1% cholesterol diet for 8 weeks. After 4 weeks, nine animals were treated intravenous saline solution and nine with intravenous LDE-carmustine (4 mg/kg, weekly for 4 weeks).

LDE-carmustine inhibited atherosclerotic lesions by 90%, compared to controls. LDE-carmustine reduced presence of macrophages, smooth muscle cells, and regulatory T-cells in the arterial intima as well as the expression of matrix metallopeptidase-9, interleukin-1β and TNF-α and lipoprotein receptors (LDL receptor, LDL-related protein-1 and scavenger receptor class B member 1).

In conclusion, LDE-carmustine treatment resulted in marked reduction of lesion area, invasion by macrophages and intimal smooth muscle cells and pro-inflammatory factors. Therefore, this new formulation shows great potential for therapy of atherosclerotic cardiovascular disease.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Relation between cardiovascular disease and risk factors in offspring and occurrence of events in
their parents already at high risk

Abstract nr. 364
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Genetics, Risk Factor

Aims To determine whether the presence of cardiovascular disease or risk factors in offspring as
an indicator of a patient’s genetic load or exposure to (unknown) risk factors is related to the
development of new or recurrent events in patients at high risk of cardiovascular events.
Methods The presence of coronary artery disease, cerebrovascular disease, peripheral artery
disease, AAA, hypertension, diabetes and hypercholesterolemia was assessed in 10,564 children
of 4,267 patients with at least one of these conditions enrolled in the SMART cohort. The relation
between presence of cardiovascular events or presence of vascular risk factors in their offspring,
and new or recurrent vascular events was determined by Cox-proportional hazard analyses.
Results Of the patients, 506 (12%) had offspring with cardiovascular disease or risk factor. During
a median follow-up of 7.0 years (IQR3.7–10.4), the composite outcome of myocardial infarction
(MI), stroke or vascular mortality occurred in 251 patients. Patients with offspring with vascular
disease or with a major risk factor had an increased risk of vascular mortality
(HR2.9; 95%CI1.2–7.1), MI (HR1.6; 95%CI1.1–2.5) and the composite outcome
(HR1.5; 95%CI1.1–2.2) (figure 1). Presence of diabetes in offspring was related to an increased
risk of the composite outcome (HR2.7; 95%CI1.5–5.0), MI (HR3.3; 95%CI1.7–6.6) and probably to
vascular mortality (HR3.4; 95%CI0.8–14.8) (figure 2).
Conclusion Presence of cardiovascular disease and vascular risk factors in offspring, with
diabetes mellitus being the most contributing vascular risk factor, is related to an increased risk of
developing new or subsequent vascular events in patients already at high risk for developing
vascular events.
Relation between presence vascular disease or risk factors in offspring and risk of vascular events in patients

Relation between diabetes, hypertension or hypercholesterolemia in offspring and risk of vascular events in patients
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
High depressive symptoms, antidepressant use and central autonomic dysfunction. The Paris Prospective Study III

Abstract nr. 365
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Epidemiology,Imaging,Prevention

Aims: To assess the relationship between high depressive symptoms and antidepressant use with baroreflex sensitivity in initially healthy men and women.

Methods: In the Paris Prospective Study III, 10157 subjects aged 50-75 underwent an echotracking of their right carotid arteries. Low neural baroreflex sensitivity (nBRS) corresponded to a ratio between the distension rate in the low frequency (sympathetic tone) and the high frequency (parasympathetic tone) below the median. The presence of high depressive symptoms was defined by a 13-item standardized depressive symptoms questionnaire. Information on antidepressants use was obtained on a face-to-face interview with a medical doctor combining the most recent medical prescriptions and/or medical package together with answers to a self-report questionnaire on medications use. The respective associations between high depressive symptoms, antidepressant use and low nBRS were quantified by logistic regression after adjustment for age, sex, hypertension, heart rate, body mass index and diabetes.

Results: Among the 9213 studied participants, 38.6% were women. Respectively, 5.6% and 5.2% had high-depressive symptoms and used antidepressants. There was no significant association between high depressive symptoms and low nBRS even in unadjusted analysis (OR=1.09; 95%CI: 0.91-1.30). However, antidepressant use was related to lower nBRS (OR=1.32; 1.06-1.65), even after adjustment for confounding factors. Association with antidepressant use remains consistent after matching on propensity score. The multivariable association with antidepressant existed for serotonin and norepinephrine reuptake inhibitors (SNRI) (OR=1.94; 1.16-3.22).

Conclusions: Robust analyses suggest that antidepressant use and particularly SNRI was associated with low nBRS in apparently healthy subjects.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
CAD patients are more sensitive to MDCO-216 induced cholesterol efflux than healthy volunteers with similar pharmacokinetics: A comparative PK/PD study

Abstract nr. 366
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Atherosclerosis, HDL, Reverse Cholesterol Transport

Introduction: MDCO-216, a complex of dimeric recombinant apolipoprotein A-1 Milano (ApoA-1 M) and a phospholipid (POPC), is currently under development to improve cardiovascular outcomes by reducing plaque burden in patients with atherosclerotic disease. The purpose of this study was to assess and compare the pharmacokinetics (PK) and pharmacodynamics (PD) of newly manufactured MDCO-216 in healthy volunteers (HVs) and coronary artery disease (CAD) patients.

Methods: 24 HVs received a single dose of MDCO-216 (5, 10, 20, 30 or 40 mg/kg) or placebo (in 2:1 ratio) as a 2 hour IV infusion in a double-blind, randomized design. In second phase of the study 24 CAD patients received MDCO-216 in the same manner except for the lowest dose. An ex-vivo cholesterol efflux assay was used as an exploratory PD biomarker.

Results: Plasma mean (SD) T1/2 of MDCO-216 was almost identical, 56.6 (14.2) h in HVs and 52.5 (15.7) h in patients (p=0.44, non-significant). Tmax in both groups ranged similarly, between 2 to 4 hours. No difference in CL was observed with increases in dose and ranged from 0.62 to 0.98 mL/hr/kg in HVs and from 0.7 to 0.8 mL/hr/kg in patients. Exposure parameters of Cmax and AUC increased with dose in a dose-proportional or nearly dose-proportional manner. No anti-drug Ab was detected with any dose in both groups.

Dose-dependent increases in ABCA1-mediated efflux capacity of up to 4-fold above baseline and smaller increases of SRB1 and ABCG1-mediated efflux capacity occurred at all doses. The dose-response analysis for ABCA1-mediated efflux best fitted into a sigmoid Emax (maximum effect) model and predicts an Emax of 15.6% which saturates around a 30 mg/kg dose in HVs vs. an Emax of 12.7% in CAD patients which saturates after 15 mg/kg dose. ED50 parameters (dose that achieves 50% of Emax) were estimated as 15.2 and 9.4 mg/kg in HVs and CAD patients, respectively.

Conclusions: This data demonstrate that MDCO-216 can profoundly stimulate the first step of reverse cholesterol transport at clinically achievable and well tolerated doses with similar PK profiles both in HVs and CAD patients; but with higher potency in the latter group.

Subdivision 3. Clinical Studies
Presentation Preference Oral presentation
Additional information
Pilot study of safety and feasibility of treatment of paclitaxel associated to a nanoemulsion in patients with aortic atherosclerotic disease

Abstract nr. 367
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Lipoproteins, Therapy

Previously, we showed that the toxicity of chemotherapeutic agents used in cancer treatment can be drastically reduced by association to nanoemulsions (LDE) that mimic the lipid composition of low-density lipoprotein (LDL), without loss of pharmacological action. Injected into the circulation, LDE concentrates associated drugs, such as the anti-proliferative agent paclitaxel, in neoplastic tissues and in atherosclerotic lesions. In rabbits with atherosclerosis lesions were reduced by 65% by LDE-paclitaxel treatment. Tolerability and safety of high dose LDE-paclitaxel was shown in several patients with advanced cancers.

Based on those findings, this pilot study was designed to test, in 9 aged patients with extensive aortic atherosclerosis, LDE-paclitaxel at 175 mg/m2 body surface dose (I.V., 3/3 weeks for 6 cycles). All were under statin treatment that was not discontinued during the 18-week treatment period.

No observable clinical or laboratorial toxicity was observed. One death occurred but was unrelated with treatment toxicity. Images acquired by Multiple Detector Computer Tomography Angiography (64-slice scanner) taken before and at 2-3 month after the treatment end showed that the mean volume of the aortic artery wall was reduced in 4 of the 8 patients, while in 3 it remained unchanged and in one it increased.

Therefore, the treatment was tolerable for patients with cardiovascular disease and these results encourage large, placebo-controlled clinical studies to ascertain the ability of LDE-paclitaxel to reduce atherosclerotic lesions.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Concerns About the Use of Non-HDL Cholesterol as a Lipid Predictor

Abstract nr. 368
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Dyslipidemia, Guidelines, Risk Factor

Introduction:
The National Lipid Association (NLA) has advocated the use of Non-HDL (high-density lipoprotein) Cholesterol as its favored lipid predictor. Cut points are based on low-density lipoprotein (LDL) values, with a lower end at 100 mg/dl (2.50 mmoles/L) and a high end at 190 mg/dl (4.75 mmoles/L), adding 30 mg/dl (0.75 mmoles/L) to keep triglyceride levels below 150 mg/dl (1.70 mmoles/L). The NLA has no randomized controlled trials on which to base these guidelines.

Objectives:
The author will show that the use of Non-HDL cholesterol has not been fully considered.

Methods:
The author will examine his general population lipid database to demonstrate the non-HDL cholesterol frequency distribution in the part of the population that developed some form of atherothrombotic disease (ATD) and in the part that did not. He will demonstrate the incidence of ATD in each of the five Non-HDL Cholesterol quintiles and will stratify each Non-HDL Cholesterol quintile in terms of another lipid predictor (the Cholesterol Retention Fraction, or CRF, defined as [LDL-HDL]/LDL) and cigarette smoking.

Findings:
All Non-HDL Cholesterol quintiles above the lowest quintile in men and above the next to lowest quintile in women have higher frequencies in the ATD population than in the non-ATD population. In the lowest and highest quintiles, the ATD risks are low and high respectively, but in the middle quintiles, the ATD risk varies considerably.
At any Non-HDL Cholesterol quintile, the average age of ATD onset depends on cigarette smoking and the CRF. The higher the CRF, the earlier is the average age of ATD onset; and the lower, the later. Current cigarette smoking portends an earlier average age of ATD onset, whereas non-smoking portends a later average age. A 75 year old hypertensive diabetic non-smoking male patient, not on statin because of low lipid levels had clean arteries on angiography, whereas a 45 year old normotensive nonsmoking with severe dyslipidemia (obtained at first encounter) had a massive stroke due to carotid stenosis. Both had the same Non-HDL Cholesterol quintiles.

Conclusions:
The use of Non-HDL Cholesterol should be reconsidered.

Subdivision 4. Not applicable. Abstract matches with track c
Presentation Preference Oral presentation
Additional information
Low- and High-Density Lipoprotein Subclasses in Subjects with Simple Steatosis and Non-Alcoholic Steatohepatitis

Abstract nr. 369
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords HDL, LDL, Lipoproteins

Non-alcoholic fatty liver disease (NAFLD) is associated with increased cardio-metabolic risk. Although dyslipidemia represents a key factor in this disease, the role of distinct lipoproteins is largely unknown. The full low-density lipoprotein (LDL) and high-density lipoprotein (HDL) profiles (7 LDL and 10 HDL subfractions) were assessed by gel electrophoresis (Lipoprint, Quantimetrix Corporation, USA) in men with biopsy proven NAFLD (simple steatosis (SS) (n=17, age: 34±7 years) and non-alcoholic steatohepatitis (NASH) (n=24, age: 32±6 years). Exclusion criteria included robust alcohol consumption, hepatitis B or C virus, body mass index (BMI) ≥40 kg/m², diabetes mellitus and hypertension. Compared to SS, NASH patients had similar BMI, HOMA-IR index and plasma lipids, with increased levels of both AST and ALT. NASH subjects had lower levels of larger LDL1 (10±4 vs. 13±4%, p=0.010) and increased smaller LDL3 and LDL4 particles (9±5 vs. 5±5%, p=0.017 and 3±3 vs. 1±2%, p=0.012, respectively). No changes were found in the HDL subclass profile. Sd LDL correlated significantly with triglycerides and HDL-cholesterol (see figure). By multiple regression analysis we found a predictive role only for the presence of smaller LDL3 (p=0.0470). In conclusion, the increased levels of small, dense LDL3 and LDL4 in NASH may help to explain the greater cardio-metabolic risk associated with the progression of fatty liver disease.
Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation

Additional information
Cardiovascular risk factors are associated to self reported stress

Abstract nr. 371
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Risk Factor

Purpose
Psychosocial stress may cause increased sympathetic activity and is considered a risk factor for cardiovascular disease. Previous case-controls studies suggested a possible role for psychosocial stress on the occurrence of myocardial infarction and sudden death. However, there are limited data on the relationship between psychosocial stress and cardiovascular risk factors. The objective of our study was to analyze the association of psychosocial stress with cardiovascular risk factors.

Methods
We included 1844 subjects (74.9% men, 43±8 years old) participating in a check-up health program. All subjects were submitted to clinical (personal and family history of cardiovascular disease and risk factors, blood pressure, body mass index, rest heart rate), laboratorial (lipid profile, glucose, C reactive protein, uric acid, creatinine) and abdominal ultrasound (liver steatosis) evaluation. Metabolic syndrome was defined according to International Diabetes Federation definition. Psychosocial stress was self-reported.

Results
Two hundred and thirty (12.4%) subjects referred stress. Stressed group have higher blood pressure, heart rate, body mass index and LDL-cholesterol. They also have higher prevalence of diabetes mellitus, metabolic syndrome and liver steatosis. After multivariate logistic regression analysis, we found that diabetes mellitus (odds ratio: 3.47; confidence interval 95%: 1.24-9.53; p=0.017), liver steatosis (odds ratio: 1.55; confidence interval 95%: 1.55-2.08; p=0.003) and LDL-cholesterol (odds ratio: 1.005; confidence interval 95%: 1.000-1.009; p=0.031) were independently associated with self reported stress.

Conclusions
Markers of insulin resistance like diabetes mellitus and liver steatosis are associated with self reported stress. Further studies are needed to determine the pathophysiological association
between stress and insulin resistance. Also if these findings are associated to higher cardiovascular risk.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
TRAF-STOP-HDL-nanoparticles reduce atherosclerosis

Abstract nr. 372
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Immunity, Inflammation, Pharmacology

Inhibition of the co-stimulatory CD40-CD40L receptor/ligand dyad drastically reduces atherosclerosis. However, its long-term blockage results in immune suppression. Inhibition of the CD40-CD40L dyad further downstream in the signaling pathway is therefore required. The interaction between CD40 and its signaling intermediate TNF receptor associated factor 6 (TRAF6) plays a pivotal role in atherosclerosis. To identify drug like molecules that inhibit the CD40-TRAF6 interaction, an in silico structure-based virtual ligand screening approach was used. Several small molecule inhibitors (SMI) blocking CD40-TRAF6 interactions were identified. Surface plasmon resonance experiments confirmed direct binding of the compounds to the TRAF6 C-domain. Two of these SMIs, the TRAF-STOPs, reduced atherosclerosis by >40% in Apoe-/mice, when they were treated from 12-18 wks of age, by hampering monocyte and neutrophil recruitment. In accordance, expression of chemokines and cytokines was remarkably reduced in compound treated macrophages. Interestingly, when the TRAF-STOPs were administered to mice with existing atherosclerosis (from 22-30 wks of age), TRAF-STOPS were able to halt atherosclerosis, resulting in a >45% decrease in atherosclerotic plaque area.

However, these SMIs had a low solubility, and had a half-life of only 8 hrs, and had to be injected daily. To improve the therapeutic applicability of our TRAF-STOPs, TRAF-STOP 6877002 was packed in HDL-based nanoparticles, and administered twice a week for 6 wks (wk 12-18) to ApoE-/mice. The HDL-TRAF-STOP nanoparticles preferentially homed to macrophages, and the expression level in plaque macrophages was high. HDL-TRAF-STOP nanoparticle treatment reduced atherosclerosis by 42.6%.

These newly developed, nanoparticle based CD40-TRAF6 inhibiting SMIs (TRAF-STOPs) are a promising lead for the development of therapeutics for the treatment of atherosclerosis.

Subdivision 1. Basic Science
Presentation Preference  Electronic poster presentation
Additional information
Role of gap junctions in atherosclerosis

Abstract nr. 373
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Immunity, Inflammation, LDL

In subendothelial intima of adult human aorta cells form a common three-dimensional network contacting each other with their processes. This network consists mainly of pericyte-like stellate cells and smooth muscle cells. Cellular network disintegrated in early atherosclerotic lesions, in advanced atherosclerotic plaque cells lost all contacts. We believe that integration of smooth muscle cells in the form of a common cellular network is necessary for the normal physiology of the tissue while disintegration of this network play significant role in atherosclerosis. In present study, using immunocytochemistry we have found that connexin43 (Cx43), the major protein of gap junctions, drops in atherosclerotic lesions as compared with uninvolved intima. In atherosclerotic lesions, we observed that the number of Cx43 plaques is lower on lipid-laden cells than on cells free from lipid inclusions. In primary culture, subendothelial intimal cells tend to create multicellular structures in the form of clusters. Cluster creation is accompanied by the formation of gap junctions between cells; the degree of gap junctional communication depends on the density of cells in culture. We showed that such important atherosclerosis-related processes as proliferation (DNA synthesis), fibrosis (protein synthesis) and lipidosis (accumulation of intracellular cholesterol) depend on the degree of cell-to-cell communication. Dependence of proliferation and fibrosis was bell-shaped. We have incubated cells cultured from uninvolved subendothelial intima with various forms of modified LDL causing intracellular cholesterol accumulation. After incubated with modified LDL intercellular communication dropped considerably. This suggests that intracellular lipid accumulation is a reason for decrease of gap junctions. However, latex beads decrease of gap junctions, too. Thus, not cholesterol accumulation but stimulation of phagocytosis reduces intercellular contact communication. We believe that stimulation of phagocytosis accompanied by accumulation of modified LDL in intimal cells is the cause of reduction and disruption of cell-to-cell contacts. Supported by Russian Scientific Foundation (Grant # 14-15-00112).

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Desialylation of LDL particle

Abstract nr. 374
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, LDL, Lipids, Metabolism

Electronegative LDL, small, dense LDL, and desialylated LDL circulating in the blood of patients were obtained by different methods. Naturally, the question arises what are the similarities and differences between these forms of LDL modification. We believe that multiple modified LDL particle (small, dense, electronegative, desialylated, etc.) occurs in blood. Ex vivo experiments have revealed mechanisms of multiple modification of LDL in the blood. Fraction of native LDL was isolated from blood plasma of healthy subjects. Blood serum of patients with assessed atherosclerosis was also obtained. LDL and serum were mixed and incubated for various periods at 37°C. After 1 hour incubation of native LDL with atherosclerotic serum desialylated LDL appears. After 3 hours, LDL becomes able to cause accumulation of cholesterol in cultured cells. After 6 hours, LDL demonstrates reduction of neutral lipids and phospholipids as well as reduction in its size. After 36 hours, an increase in the electronegativity of the lipoprotein particle is detected. After 48-72 hours, loss of α-tocopherol, increase of susceptibility to oxidation, and accumulation of lipid peroxidation products in LDL are observed. Thus, multiple modification of LDL is a cascade of sequential changes in lipoprotein particle, namely: desialylation, loss of lipids, size reduction, increase of electronegative charge, lipid peroxidation in LDL. Desialylation of LDL particle is one of the first or primary events of atherogenic modification. We have established that the reason of LDL desialylation is trans-sialidase. We found trans-sialidase activity in the blood of patients with atherosclerosis and other cardiovascular diseases. We have shown that human neuraminidases 2 and 4 possess trans-sialidase activity. On the other hand, selective inhibitors of viral sialidases suppress trans-sialidase activity in the blood of atherosclerotic patients. Thus, trans-sialidase causing atherogenic desialylation of LDL may be of both endogenous and exogenous origin. Supported by Russian Ministry of Education and Science (Project RFMEFI61614X0010).

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Activation of Human Monocytes in Asymptomatic Atherosclerosis

Monocytes were isolated from blood of three groups of patients, namely those with normal carotid intima-media thickness (CIMT), patients with increased CIMT and patients with plaques in carotid arteries. To assess susceptibility of isolated monocytes to M1 and M2 activation, cells were placed in primary culture with the appropriate stimulator for activation. The degree of M1 or M2 activations was determined by the culture medium level of TNFα and CCL18, respectively. When comparing the average values of TNFα and CCL18 in patients of three groups we were faced with dramatic individual differences. These individual differences have been found both within each group of patients and in the pool (groups 1+2+3). Monocytes in early atherosclerotic lesions may migrate back into the circulation, possibly serving as a lipid clearance system. Circulating cells laden with lipid have been found in blood. We attempted to find the relationship between cholesterol in monocytes and their susceptibility to activation. There was an obvious trend towards a reverse correlation between intracellular cholesterol level and susceptibility to activation. To reveal the reason of relationship between intracellular cholesterol level and monocyte susceptibility to activation, the experiments on cells cultured with atherogenic modified LDL were carried out. LDL isolated from blood of patients with documented atherosclerosis induces cholesterol accumulation in cultured cells, while LDL from healthy subjects does not affect intracellular cholesterol level. Although modified LDL induced cholesterol accumulation in cultured monocyte-derived cells neither cytokine secretion nor cytokine genes expression were affected. Therefore no LDL but other sources of lipids accumulated in circulating monocytes determine their susceptibility to activation. We consider that difference in monocyte activation is very important because it may determine functional capacity of innate immunity in different patients. Cellular test cell used in this study may serve as a basis for diagnostic method for determining the individual response of innate immunity. This model may be used for the search of new immune correctors. Supported by Russian Scientific Foundation (Grant # 14-15-00112).
Cardiovascular disease prevention among African-Americans by FAITH!: An application of the American Heart Association’s Life’s Simple 7(TM)

Abstract nr. 377
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease,Lifestyle,Prevention,Risk Factor

Introduction: Cardiovascular disease (CVD) disproportionately affects African-Americans who have higher morbidity and mortality. Lack of knowledge, awareness, and perception of CVD impacts health disparities. Strategies using national guidelines as frameworks to target racial and ethnic minorities may increase awareness of CVD risk factors.

Hypothesis: We present the design and baseline findings of a yearlong, church-based, CVD community-engaged program to evaluate the impact on knowledge of modifiable CVD risk factors.

Methods: Thirty-seven participants from three predominately African-American churches in Rochester, MN enrolled in the health education program, Fostering African-American Improvement in Total Health (FAITH!). A community-based participatory approach utilizing the American Heart Association’s (AHA)Life’s Simple 7™ framework guided development of a 16-week educational program. Education sessions delivered by health professionals were held twice monthly at participating churches and local facilities. We report baseline assessments: demographics, prevalent health concerns, perceptions and knowledge of CVD prevention and health disparities, dietary and physical activity patterns, anthropometrics and laboratory data.

Results: Mean age was 52.3 years (SD, 13.9); 70% were women. Education attainment of some college or above was present in 73% and 43% reported annual household income of less than $35,000. Participants reported the following health-professional diagnoses: overweight/obese (59.5%), hypertension (27%), high cholesterol (21.6%) and diabetes (5.4%). The top five CVD concerns for self/family were high cholesterol (38%), obesity (32%), diabetes (30%), hypertension (30%) and coronary artery disease (22%). Correct responses on CVD prevention and health disparities knowledge questions were 48% and 61% on average, respectively. Mean daily fruit and vegetable consumption was 3 (SD, 1.5) and mean days/week of moderate-intensity physical activity was 2.0 (SD, 2.1). Overall, 64% were obese (mean BMI (33 kg/m² [SD, 8.5]) and 28% were hypertensive (mean 145/88 mmHg [SD, 23/9.9]). Mean waist circumference was 102.1 cm (SD, 20.6). Median total cholesterol was 190 mg/dL (interquartile range [IQR], 156-216) and hemoglobin A1c, 5.8% (IQR, 5.4-6.3).

Conclusions: The use of community interventions promoting ideal CVD health behaviors has the potential to increase knowledge and promote behavioral change towards alleviating CVD
disparities among African-Americans. This program may fulfill this need by addressing CVD risk factors within this high-risk group.

Subdivision 3. Clinical Studies

Presentation Preference  Electronic poster presentation

Additional information
Platelet adhesion to fibrillar collagen via the membrane glycoprotein a2b1 integrin are the initial events in vascular thrombosis. Genetic variants in the gene encoding a2 integrin gene (ITGA2) have been reported to be implicated in the risk for ischemic stroke. The purpose of this study was to investigate the relationship between the Bgl II polymorphism of α2 integrin gene and risk of ischemic stroke in Tunisian patients.

Methods: We have genotyped 34 patients with stroke and 35 healthy controls by polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP). Alleles were separated on agarose gels stained with ethidium.

Results: The genotype distributions for Bgl II in patients with stroke (+/+ =16.7%, +/- = 40%, -/- =43.3%) and controls (+/+ =2.9%, +/- =43.9% and -/- =54.2%) were not significantly different. The odds ratio (95% CI) of stroke was not significant 0.16 [0.01-1.56] (p=0.11) for +/- heterozygous and 0.13 [0.01-1.31] (p=0.08) for -/- homozygous.

Conclusion: Our preliminary study suggested that the Bgl II polymorphism in the a2 integrin gene is not associated with stroke in Tunisian patients.
Coupling factor 6 is an endogenous stimulator for atherosclerosis through salt-sensitive hypertension and type 2 diabetes

Abstract nr. 379
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Diabetes,Hypertension,Pathogenesis

**Background:** Atherosclerosis due to diabetes and hypertension is a major health problem with high morbidity and mortality. Proton regulates cellular function by modulating the charge and structure of macromolecules, but the role of proton in the pathogenesis of atherosclerosis still remains unclear. We recently identified that a circulating peptide coupling factor 6 (CF6) binds to the plasma membrane-bound ATP synthase and stimulates proton import, resulting in chronic intracellular acidosis. We investigated whether chronic intracellular acidosis contributes to the pathogenesis of hypertension and diabetes by means of overexpression of CF6.

**Methods and Results:** We generated two-fold CF6-overexpressing transgenic mouse (TG). Intracellular pH measured by $^{31}$P-magnetic resonance spectroscopy was decreased by 0.1 to 0.15 pH unit in the skeletal muscle and the liver (both p<0.05) in TG compared with wild type mice (WT). TG mice manifested both salt-sensitive hypertension by the increase in 21±3 mmHg under a high salt diet (p<0.05) and type 2 diabetes (serum glucose level;133±10 vs 108±1 mg/dl in WT, serum insulin level;698±87 vs 295±55 pg/ml in WT, both p<0.05) under a high sucrose diet. Since Rac1, a member of the Rho-GTPase, was identified as an activator of mineralocorticoid receptor, the Rac1 activity in the TG kidney was measured by pull-down assay. The ratio of Rac1-GTP to total Rac1 was increased in the TG kidney compared with the WT kidney. Phospho-insulin receptor b (Tyr1150/1151) in the skeletal muscle and liver was decreased in TG compared with WT. Insulin receptor substrate (IRS)-1 in the skeletal muscle was decreased by 64±9% in TG compared with WT (n=5, p<0.05), and phospho-IRS-2 (panTyr) was decreased in the TG liver. Phosphoinositide 3-kinase activity and phospho-Akt1 (ser 473) in the skeletal muscle and liver were both decreased in TG compared with WT. Intraperitoneal administration of amiloride, an inhibitor of proton extruder, at 50 mg/kg for 2 days exacerbated insulin resistance, while anti-CF6 antibody ameliorated insulin resistance.

**Conclusions:** CF6 regulates cytosolic pH by interacting with ecto-F$_1$F$_0$ complex and functions as an endogenous stimulator for atherosclerosis by linking hypertension with diabetes through Rac1 activation and insulin receptor inactivation.
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Clustering of circulating tumor cells on vessel wall during metastasis

Abstract nr. 380
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Imaging

In most solid cancers, thromboembolism is a risk factor to affect survival outcome of patients. In addition to fibrinogen and platelet, it is recently reported that macrophages and circulating tumor cells (CTC) also have an important role in formation of clamps. Thus, the presence of clamp seems to be related to metastatic process of cancer cells. Previous studies from other research groups revealed metastasis is reduced in fibrinogen-knockout mice. Expression of fibronectin showed positive correlation with an invasive and metastatic phenotype. Further, patients treated with (LMW) heparin had a significant survival benefit. Those evidences above suggest that embolism induced by fibrin (ogen) promote tumorigenesis and metastasis. However, it is still not conclusive for how the clamps support metastasis and whether cluster of CTCs is directly associated to thromboembolism.

To address the clinically important questions, we utilized in vivo confocal endoscopy and directly observed vasculature and blood flow around primary tumors in mice. Although CTCs existed only between 5~10% according to disease progression in circulation, the number was increased as primary tumor grew. Our imaging-based experiment also indicated that flow rate of CTC cluster is noticeably slower due to their adhesive property compared with single CTCs. Further they covered more than 20% of lung metastases. Based on these newly revealed behaviors of CTCs, we suggest suppressing CTC cluster formation or dissociating CTC cluster may ameliorate metastasis and be a target of novel chemical intervention.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Effects of Bisphenol A exposure on blood lipids in female rats and their offspring

Abstract nr. 382
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Apolipoproteins, Lipids

Bisphenol A (BPA), is a high volume chemical with estrogenic properties, widely used in polycarbonate plastics and epoxy resins due to its cross linking properties. BPA is widespread in the general population and more than 90% of the US population has detectable levels in their urine. It has been suggested that BPA exposure, besides having effects on reproduction and the nervous system, may be involved in cardiovascular complications.

Three groups of pregnant female rats were, via their drinking water, exposed to only water or low doses of BPA (0.0025mg/L BPA, n=9; 0.25mg/L BPA, n=9), corresponding to an exposure of 0.5 and 50 µg/kg bodyweight/day, respectively. The primary aim was to investigate if perinatal (in utero and during lactation) low dose exposure of BPA disrupts the balance between adipose and bone tissue development. Blood lipids were analyzed in mothers and offspring (five weeks old; females n=27, males n=27). PON-1 arylesterase activity was analyzed in mothers. Apo A-I and apo B intensities were measured using SDS-PAGE/WB and HDL-C, LDL-C and TGs were analyzed by standard procedures.

The BPA exposed mothers showed a significant increase in apo A-I compared to the controls while TGs, apoB, Cholesterol and PON-1 activity were not significantly changed. Offspring showed no significant alterations in apo A-I, apo B, LDL-C or HDL-C. Interestingly, TGs were significantly increased at the lowest BPA dose in both genders, but at 50 µg/kg bodyweight/day BPA TGs in the female offspring were further increased while male pups showed a decrease, indicating a non-monotonic dose response.

The biological response to BPA differ comparing direct and indirect “in utero” exposure. Estrogenic effects of BPA exposure on plasma apo A-I levels in adult female rats were found while increased TGs in offspring at the lowest dose of BPA further emphasizes the present concern for metabolic alterations increasing CVD risk.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation

Additional information
Relation between CETP lowering SNP rs3764261 and risk of developing diabetes mellitus and vascular events in patients with vascular disease.

Abstract nr. 383
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Diabetes, Genetics, HDL

Background
Genetic variants of the Cholesteryl Ester Transfer Protein (CETP) are associated with elevated high-density lipoprotein cholesterol (HDL-C). We investigated the relation between a single nucleotide polymorphism (SNP) located in the CETP gene (rs3764261) and the risk of incident type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) and the level of insulin resistance in patients with vascular disease.

Method
The Secondary Manifestations of ARTerial disease (SMART) study is an ongoing prospective cohort study performed in patients with CVD (n=5601). CETP genotype was divided in three groups: no SNP (CC), one SNP (Cc) and two SNPs (cc). Insulin resistance was expressed as HOMA-IR. During a median follow-up of 6.9 (IQR 4.3-9.9) years, 427 patients developed T2DM and 985 CVD events occurred.

Results
Presence of rs3764261 was associated with higher plasma HDL-C levels (cc 1.33±0.40 mmol/L; Cc 1.24±0.37 mmol/L; CC 1.18±0.35 mmol/L (P <0.001)), but did not affect the risk of T2DM (Cc HR 0.92, 95%CI 0.75-1.13; cc HR 0.97, 95%CI 0.70-1.36) or CVD (Cc HR 0.94, 95%CI 0.82-1.07; cc HR 0.83, 95%CI 0.66-1.05). HOMA-IR was not affected by presence of a CETP SNP in patients with T2DM.

Conclusion
Although presence of the CETP SNP rs3764261 is associated with higher HDL-C, there was no relation with the risk of T2DM or CVD in patients with clinical manifest vascular disease.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Single infusions of MDCO-216 (ApoA1 Milano/POPC) were well tolerated in healthy volunteers and in patients with stable CAD

Abstract nr. 384
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Apolipoproteins, Atherosclerosis, Pharmacology, Prevention

Introduction: MDCO-216, a complex of dimeric recombinant apolipoprotein A-1 Milano (ApoA-1 M) and a phospholipid (POPC), is currently under development to improve cardiovascular outcomes by reducing plaque burden in patients with atherosclerotic disease. Development of a predecessor agent was halted due to safety concerns. The purpose of this study was to assess the safety, pharmacokinetics and pharmacodynamics of newly manufactured MDCO-216 in healthy volunteers (HVs) and coronary artery disease (CAD) patients.

Methods: 24 HVs and 24 CAD patients received a single 2 hour IV infusion of MDCO-216 (5, 10, 20, 30 or 40 mg/kg in HVs and 10, 20, 30, 40 mg/kg in CAD patients) or placebo (2:1 ratio) in a double-blind, randomized design.

Results: MDCO-216 was well-tolerated with no serious AE or death. There were no clinically relevant findings in other safety parameters including laboratory parameters, vital signs and ECGs. The most common adverse events were headache and fatigue. One case of deep vein thrombosis (DVT) was reported in a healthy volunteer with a previous history of DVT following 30 mg/kg MDCO-216. This was considered to be unlikely to be related to MDCO-216. No other AEs or laboratory changes consistent with coagulopathy were reported in the study.

No clinically relevant changes in laboratory parameters were observed after infusion of MDCO216. Isolated, transient increases were seen in serum amylase and pancreatic lipase in two CAD subjects following 10 and 20 mg/kg MDCO-216 and two placebo-treated subjects. Given the absence of other clinical signs and symptoms these findings were not suggestive of pancreatic damage.

No pro-inflammatory effect, as measured by cytokine elevations, was observed following infusion of MDCO-216 in vivo or after incubation of whole blood with MDCO-216 ex vivo. No induction of antidrug antibody (ADA) was observed following infusion of MDCO-216.

Conclusions: MDCO-216 was well tolerated with no serious adverse events and no significant adverse safety findings. The most common adverse events were headache and fatigue. No dose dependent effect on any safety parameter was noted and there was no difference in safety parameters between healthy volunteers and patients with CAD. These data support the continued development of MDCO-216.
Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Clearance of LDL cholesterol is dependent on COMMD1 stability, which is controlled by COMMD6

Abstract nr. 385
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, LDL, Lipids, Lipoproteins

The COMMD proteins form the family of proteins that consists of 10 members. The exact biological function of the COMMD family is still unknown, but recently we identified COMMD1, the founder of this family, as a novel gene in regulating cholesterol homeostasis. Hepatic Commd1 deficient mice have increased plasma LDL cholesterol levels. Interestingly, dogs homozygous for a COMMD1 loss-of-function mutation also show increased circulating LDL cholesterol. We demonstrated that COMMD1 mediates the endosomal sorting of LDLR, but the mechanism by which the function of COMMD1 is regulated remains unclear.

In this study we show that COMMD1 is in complex with COMMD6, and loss of COMMD6 results in COMMD1 protein instability in various cell lines. Our data indicate that COMMD6 is not in complex with the endosomal sorting machinery to sort LDLR back to the membrane but prevents COMMD1 proteolysis. To evaluate the role of COMMD6 in COMMD1-mediated LDLR trafficking we generated liver specific Commd6 knock out mice. Commd6 ablation in hepatocytes results in reduced Commd1 levels similar as we found in our in vitro systems. In line with hepatic Commd1 depletion, Commd6 deficiency in hepatocytes causes elevated circulating LDL cholesterol. Altogether, our data suggest that COMMD6 tightly regulates COMMD1 function to mediate LDL cholesterol homeostasis indirectly. Currently the mechanism by which COMMD6 prevents COMMD1 degradation is being investigated.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation

Additional information
Metabolic syndrome increases intima-media thickness in non-alcoholic fatty liver disease: interaction with patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene variant

Abstract nr. 386
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Topic: The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords: Atherosclerosis, Genetics, Metabolism

Background. Non-Alcoholic Fatty Liver Disease (NAFLD) has been proposed to be an independent risk factors for atherosclerosis. NAFLD is strongly associated with several metabolic abnormalities, in particular with those clustering in the metabolic syndrome (MetS) phenotype. Beside metabolic abnormalities, also genetic factors have been reported to contribute to the development of NAFLD. The most well-validated genetic variant is the missense mutation in PNPLA3 gene (I148M), which has a minor allele frequency of 0.26. How MetS and the I148M variant interact in increasing vascular damage has been only partially investigated.

Aims and Methods. To understand if there is an interaction between PNPLA3 genotype and Mets in affecting carotid intima media thickness (CIMT) in subject with fatty liver, we compared 3 groups of NAFLD subjects: 1) I148I carriers with metabolic disorders (metabolic NAFLD) (n= 82), 2) M148M carriers without metabolic disorders (genetic NAFLD) (n=28) and 3) M148M carriers with metabolic disorders (metabolic and genetic NAFLD) (n= 32). Fatty liver grade was evaluated by ultrasounds. CIMT was measured as marker of subclinical atherosclerosis. Mets was diagnosed according to ATP III definition. Comparisons were adjusted for age, gender and smoke by generalized linear model (GLM).

Results. Gender and smoke were different among groups but not age. As expected, subjects with metabolic disorders had significantly higher values of anthropometric variables, lipids, glucose, HOMA IR and ALT as compared to healthy M148M carriers (p<0.05). In metabolic M148M NAFLD, AST were higher in comparison with the other two groups. Triglycerides levels were significantly lower in M148M carriers compared to non-carriers (p<0.05). Frequency of Mets was 58% in metabolic NAFLD I148I, 50% in metabolic and genetic NAFLD and 0% in genetic NAFLD. In Mets population, M148M genotype seems to worsen CIMT according to the degree of liver
steatosis. Indeed, a significant interaction between Mets and PNPLA3 genotype was found to affect CIMT (p=0.016), independently from other predictors.

Conclusions. In our study we observed that PNPLA3 genotype seems to increase the progression of subclinical atherosclerosis in subject with NAFLD through interaction with Mets phenotype.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
The effect of the dietary fiber inulin on atherosclerosis in APOE*3-Leiden.CETP mice

Abstract nr. 387
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Inflammation, Nutrition

The microorganisms that inhabit the gastrointestinal tract (the microbiota) have a direct role in metabolism and clearly impact human health. Disadvantageous microbiota (dysbiosis) have been linked to hyperlipidaemia, inflammation and the risk for cardiovascular disease. Dietary fibers such as inulin, are digested by specific intestinal bacteria and have the potential to modulate the composition of the microbiota. Dietary fiber interventions have been shown to lower plasma cholesterol levels and modulate inflammation in both mice and human. We hypothesize that inulin will beneficially modify atherosclerosis development by reducing inflammation independently of lowering plasma cholesterol.

Previous experiments have shown that a Western type diet (WTD) with >0.5% cholesterol will induce both hypercholesterolemia and inflammation in APOE*3-Leiden.CETP mice. Female APOE*3-Leiden.CETP mice were fed a WTD with 0.5% cholesterol without inulin (control group) or with 10% inulin (inulin groups) for 11 weeks. No significant differences were found on plasma cholesterol exposure (total cholesterol, LDL-c, HDL-c, and non-HDL) between the groups. Furthermore, no differences in body weight, food intake, and body composition were found. However, treatment with dietary inulin resulted in increased liver- and cecum weight. Liver lipid analysis revealed no significant difference on liver triglycerides or phospholipids between the groups. White adipose tissue (WAT) weight was significantly increased in the inulin group. DNA extraction from the cecum yielded a significant increase in total DNA, suggesting changes in microbiota content. No differences were found on atherosclerotic lesion area between the groups. Currently, real-time qPCR analysis of inflammatory markers is performed, and the intestinal microbiota will be sequenced for their composition and diversity.

Our preliminary data showed no effect of dietary inulin on plasma cholesterol when added to a WTD containing 0.5% cholesterol. However dietary inulin seems to increase liver weight, which is not explained by changes in liver triglycerides. WAT weight was significantly increased, suggesting a role for dietary inulin in lipogenesis, most likely via SCFA production. No effect on atherosclerotic lesion area was found. However, the gastrointestinal tract was significantly affected by inulin treatment, which indicates an effect on the gut microbiota. Whether inflammation plays a role in
this process remains to be determined.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
(+) -episesamin interferes with PDGF-BB-induced mitogenic response of VSMC via anti-oxidative activity and diminished activation of NF-κB, ERK1/2 and AKT signaling

Abstract nr. 388
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Nutrition, Pharmacology, Prevention

**Aims:** Activation of vascular smooth muscle cells (VSMC) represents a key event in the pathogenesis of atherosclerosis and is triggered by cytokines and growth factors such as tumor necrosis factor alpha (TNF-α) or platelet-derived growth factor (PDGF)-BB. The polyphenol (+)-episesamin (ES) has recently been shown to counteract TNF-α-induced effects e.g. in macrophages and in VSMC which resulted in a reduced mitogenic VSMC response. Aiming to establish a basis for further research on novel therapeutic options, we here investigated whether ES protects VSMC from PDGF-BB-induced growth and migration, which both contribute to the onset and progression of atherosclerosis.

**Methods:** Murine, rat and human VSMC were treated with combinations of ES and PDGF-BB. Gene and protein expressions were analyzed by RT-PCR and western blot, respectively. Proliferation was determined by colorimetric substrate assays. Cell signaling was analyzed by western-blotting and reporter gene assays. Migration was assessed by wound healing assays and effects of ES on PDGF-BB-induced intracellular free calcium concentrations ([Ca]i) were detected using Fluo-4-loaded VSMC.

**Results:** Experiments with specific inhibitors revealed the ES at 5 µM reduced basal and PDGF-BB-induced VSMC proliferation and migration due to impaired activation of the transcription factor nuclear factor-kappa B (NF-κB), of protein kinase B (AKT) and of extracellular signal-regulated kinases (ERK)1/2. This was accompanied by reduced expression of the vascular cell adhesion molecule (VCAM)-1. Further, ES reduced the PDGF-BB-induced levels of reactive oxidative species and concomitantly induced an upregulation of anti-inflammatory heme oxygenase (HO)-1 expression. Moreover, ES prevented VSMC from PDGF-BB-induced elevations of [Ca]i.

**Conclusion:** ES interferes with PDGF-induced activation of VSMC due to anti-mitogenic and anti-oxidative properties which involves interference with pro-atherogenic signaling pathways. In conjunction with preceding studies, our results provide a rationale for further animal studies with ES as a complemental treatment of VSMC specific vascular diseases such as atherosclerosis.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Homozygous familial hypercholesterolemia features a wide range of LDL-C levels

Abstract nr. 389
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia, Genetics, Hypolipidemic Drugs, LDL

Objectives: Homozygous familial hypercholesterolemia (HoFH) is a rare genetic disease characterized by highly elevated LDL-C levels, limited response to conventional lipid-lowering therapies, and increased risk of premature CVD. Severe atherosclerosis may develop before the age of 30. Traditionally, HoFH has been diagnosed according to untreated plasma LDL-C levels >500mg/dL; or treated levels ≥300mg/dL. More recently, studies suggest that the range of LDL-C levels in HoFH may be more variable. To explore this, we analyzed baseline LDL-C levels in a group of treated clinical trial subjects with genetically confirmed HoFH.

Methods: Baseline characteristics of 29 HoFH patients from a multinational Phase 3 study were collected. All subjects were ≥18 years and met the diagnosis of HoFH based on: untreated TC >500 mg/dL and both parents with documented untreated TC >250mg/dL; documented genetic mutations of genes known to affect LDLR functionality; or skin fibroblast LDLR activity <20% normal.

Results: All patients had confirmed mutations in both alleles of LDLR (n=28) or LDLRAP1 (n=1) gene. Age range was 18-55 years. Nearly all patients (93%) had a history of CVD: CABG, 35%; coronary angioplasty, 10%; aortic valve replacement, 10%; mitral valve replacement/repair, 10%. Therapy included statins (93%; 76% with ezetimibe) and apheresis (62%). Treated LDL-C levels were in the range 152-564mg/dL; 38% had LDL-C <300mg/dL and 14% <200mg/dL (Table). There were no differences in LDL-C levels between subjects who were receiving apheresis treatment and those who were not.
**Conclusion:** Consistent with other recent studies, this analysis provides additional evidence of the heterogeneity of on-treatment LDL-C values in patients with genetically defined HoFH. Diagnosis of HoFH should not rely solely on treated LDL-C >300 mg/dL, but should also include other clinical or genetic factors.

<table>
<thead>
<tr>
<th>Overall</th>
<th>Number (%)</th>
<th>Mean age, years (range)</th>
<th>Apheresis, n (%)</th>
<th>Mean baseline LDL-C, mg/dL (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>29 (100)</td>
<td>31 (18-55)</td>
<td>18 (62)</td>
<td>336 (152-564)</td>
</tr>
<tr>
<td>Apheresis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18 (62)</td>
<td>30 (18-54)</td>
<td>N/A</td>
<td>325 (152-500)</td>
</tr>
<tr>
<td>No</td>
<td>11 (38)</td>
<td>31 (19-55)</td>
<td>N/A</td>
<td>355 (166-565)</td>
</tr>
</tbody>
</table>

**Table of baseline characteristics**

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Increased lycopene intake influences HDL-associated CETP, LCAT and PON-1

Abstract nr. 390
Author Henry, Sarah-Louise, Queen's University Belfast, Belfast, United Kingdom
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Co-author(s) - Young, Ian
Co-author(s) - Woodside, Jayne
Co-author(s) - Mceneny, Jane
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords HDL, Nutrition

High density lipoproteins (HDL) possess a wide range of anti-atherogenic properties that lower cardiovascular disease (CVD)-risk. Such functions are related, at least in part, to several of HDL’s associated enzymes, including cholesteryl ester transfer protein (CETP), lecithin-cholesterol acyltransferase (LCAT), and paraoxonase-1 (PON-1). Diets rich in lycopene are associated with decreased CVD-risk and may enhance the anti-atherogenic properties of HDL. Therefore, the purpose of this study was to investigate the influence of lycopene, via diet or supplementation, on the functional properties of HDL-associated CETP, LCAT and PON-1 in a large cohort of subjects at increased CVD-risk.

Methods: Following a 4-week wash out period, serum was collected pre and 12-weeks post intervention from 225-moderately-overweight (26.7 kg/m²), middle-aged (40-60 years) individuals at increased CVD-risk. Subjects were randomised to one of three groups, control-diet (<10 mg-lycopene/week); lycopene-rich diet (224-350 mg-lycopene/week); lycopene-supplement (70 mg-lycopene/week). HDL₂ and HDL₃ were isolated from serum by rapid ultracentrifugation. The activity of HDL₂ and HDL₃ associated CETP and LCAT were measured by fluorometric assays, and PON-1-activity was measured by a spectrophotometric assay.

Results: CETP-activity was significantly different between the groups for HDL₂ and HDL₃ (p=0.005 and p=0.002, respectively), which was due to lower CETP-activity following the lycopene-rich diet and lycopene-supplement (p<0.05 for all comparisons). LCAT activity was also significantly different between groups, although only in HDL₃ (p=0.022), which was mainly driven by higher LCAT-activity following the lycopene-supplement (p=0.018). Similarly to LCAT, the activity of PON-1 was significantly different between groups in HDL₃ (p=0.036), although unlike LCAT, this was mainly due to higher PON-1-activity following the lycopene-rich diet (p=0.005).

Conclusion: In a group of subjects at increased CVD-risk, increased lycopene intake, via diet or supplementation, produced positive effects on the HDL-associated enzymes, CETP, LCAT and PON-1. We suggest that these changes may augment the anti-atherogenic functions of HDL, which may help lower CVD-risk in these subjects.
Presentation Preference Mini-oral presentation
Additional information
Leukocyte TLR5 Deficiency Inhibits Atherosclerosis By Reduced Macrophage Recruitment and Defective T cell Responsiveness

Abstract nr. 391
Author Duijvenvoorde, Amerik van, UMC Utrecht, Utrecht, Netherlands
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Co-author(s) - Hoefer, Imo E
Co-author(s) - Poll, Tom van der
Co-author(s) - Kuiper, Johan
Co-author(s) - Jager, Saskia C A de
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Immunity, Inflammation

Background: Toll-like-receptors (TLRs) provide a critical link between innate and adaptive immune responses. It has been shown that TLR5 ligand Flagellin can enhance the suppressive capacity of regulatory T-cells, but can also function as an immune adjuvant. The immune response in atherosclerosis is a lipid storage disease with key inflammatory features and the latter is characterized by an imbalance of pro- and anti-atherogenic T-cells. We aimed to establish if the TLR5/Flagellin axis is involved in the immune response of atherosclerosis.

Methods: First, we studied the in vitro effect of Flagellin exposure on primary macrophage maturation and activation. In addition, we assessed the expression of TLR5 on T cells by flow cytometry and determined the T helper cell polarization capacity of Flagellin. Next, we created TLR5^-/-/LDLr^-/- chimeras to assess the role of the TLR5 axis in atherosclerotic lesion formation.

Results: Flagellin exposure did not induce a clear polarization of macrophages, while TLR5 deficient macrophages did display a less migratory phenotype, as shown by decreased MCP-1 and CCR2 expression compared to wildtype macrophages (-66%, p=0.006). Interestingly, we observed TLR 5 expression on CD4^+ T cells (13.9%) with a higher prevalence in activated (44.0%) and regulatory T cells (56.9%). Apart from that, Flagellin exposure induces expression of the T-cell polarizing cytokine Interleukin 6, which was not observed in TLR5 deficient macrophages. Flagellin can induce expansion of regulatory T-cells under non-inflammatory conditions, however this induction is overruled under inflammatory conditions. Atherosclerotic lesion formation in the aortic arch was markedly reduced in TLR5^-/- bone marrow chimeras (1.03±0.06 x10^6 μm^2 vs 0.79±0.06 x10^6 μm^2 in TLR5^-/-, p = 0.01). This reduction was accompanied by decreased macrophage area (-46%, p=0.01) and necrotic core size (-32%, p<0.05), while collagen content was similar between groups. There was less inflammation in TLR5
chimeras, as observed by lower levels of IL-6 in plasma (40.2±6.3 in WT vs. 15.1±2.1 pg/ml in TLR5−/−, p=0.003). Similarly, proliferation of splenic T cells upon ex vivo Flagellin exposure was impaired in TLR5−/− chimeras.

**Conclusion:** Absence of TLR5 on circulating leukocytes inhibits development of atherosclerosis in mice with a marked reduction in macrophage recruitment and T cell signaling.
Poor glycaemic control augments SAA’s associated with HDL in subjects with type 1 diabetes mellitus

Abstract nr. 392
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Co-author(s) - Moore , Kevin
Co-author(s) - Stevenson , Michael
Co-author(s) - Young , Ian
Co-author(s) - Gibney , James
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Diabetes, HDL, Inflammation

Inflammatory atherosclerosis is increased in subjects with type-1 diabetes mellitus (T1DM), which is augmented by poor glycaemic control. Normally high density lipoproteins (HDL) protect against atherosclerosis, however, in the presence of serum amyloid-A (SAA)-related inflammation this property may be reduced.

The aim of this study was to examine if poor glycaemic control augmented SAA’s association with HDL in subjects with T1DM.

Methods: Fasting blood was obtained from 25 T1DM subjects with poor glycaemic control (≥8.34% HbA1c), matched for age, gender and BMI with 25 T1DM subjects with better glycaemic control (<8.34% HbA1c). HDL was subfractionated into HDL$_2$ and HDL$_3$ by rapid ultracentrifugation. Serum-high sensitive C-reactive protein (hsCRP) and serum-, HDL$_2$- and HDL$_3$-SAA were measured by ELISA methodologies.

Results: Compared to the better controlled T1DM subjects, although hsCRP was not different (p=0.355), SAA was significantly increased in serum and HDL$_3$ (serum: p=0.031; HDL$_3$: p=0.019) in subjects with poorly control T1DM. We also identified a positive relationship between HbA1c and serum-SAA and HDL$_3$-SAA (serum r=0.231, p=0.042; HDL$_3$: r=0.452, p=0.027). This cross-sectional study has illustrated that poor glycaemic control augments SAA levels in the circulation and associated with HDL, which we suggest may contribute to increased atherosclerosis-risk in such subjects. This study also highlights SAA was a more useful biomarker to identify increased inflammation under conditions of poor glycaemia than hsCRP.

Subdivision 2. Translational Research

Presentation Preference Mini-oral presentation
Additional information
Cuban prospective study of tobacco, alcohol and mortality.

Abstract nr. 393
Author Armás Rojas, Nurys Bárbbara, National Institute of Cardiology, Havana, Cuba
Co-author(s) - Dueñas Herrera, Alfredo
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology

In Cuba, 26% of men and 18% of women die between ages 35-69 years, mainly from vascular, cancer and respiratory causes. Objective: To determine mortality related with tobacco in Cuba. 1999-2011. Methods: 140,000 adults recruited 1997-2000 from Cuba; 50,000 men and 60,000 women with no reported cancer or CVD at entry. Mean age: 53 years. Mean follow-up for mortality: 11 years. Specific causes of death were obtained from mortality statistics of the Ministry of Health. Data matched by name and ID number. Variables: socioeconomics, demographic, tobacco smoking, alcohol consumption and medical history. Physical measurements: height, weight, blood pressure. Statistic analysis: Cox regression models, adjusted for age and alcohol “Current” cigarette includes stopped within 5 years of baseline survey and “Ex”: stopped any previous smoking habit for at least 5 years. Results: In both men and women, there was a strong positive association between all-cause mortality and the number of cigarettes smoked, mortality was strongly and inversely associated with age started smoking so that male smokers who smoked 20 cigs/day and started before 15 years of age were at 50% higher risk than never smokers, and female smokers who smoked 20 cigs/day who started at 10 years of age were at twice the risk of never smokers, stopping young works. For those men who had given up before 45 years old there was no excess risk. After 5 years the risks approach those of never smokers. Current smoking was associated with 4-fold risk of lung cancer, upper aero-digestive cancer and COPD and 50% increased risk of IHD and stroke. There was almost 50% increased risk of all-cause mortality among men drinking 2 or more bottles of rum/day compared with those drinking less than one bottle/week. Conclusions Smoking: Lower RRs than in US/UK studies (RR=4 for COPD, lung cancer, upper aero-digestive cancer and RR=1.5 for vascular disease), starting young causes bigger risk; stopping works (Risk diminishes >5 years after stopping smoking and stopping before age 45 avoids most of the risk. Alcohol minimal total mortality in light drinkers (RR=1.15 in never-drinkers and RR=1.42 in heavy drinkers)
Cuba: all-cause mortality by years stopped smoking

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>No. of events</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>4301</td>
<td>1.00 (0.97 - 1.03)</td>
</tr>
<tr>
<td>Stopped 40 years ago</td>
<td>238</td>
<td>1.13 (1.00 - 1.27)</td>
</tr>
<tr>
<td>Stopped 5-9 years ago</td>
<td>67</td>
<td>1.09 (0.95 - 1.26)</td>
</tr>
<tr>
<td>Stopped 10+ years ago</td>
<td>128</td>
<td>1.60 (1.45 - 1.78)</td>
</tr>
<tr>
<td>Currently smoking</td>
<td>3472</td>
<td>1.41 (1.31 - 1.51)</td>
</tr>
</tbody>
</table>

Cuba: Alcohol & all-cause mortality, men aged 30-79

Alcohol (500ml rum bottles/week)

Cuba: All-cause mortality by cigarettes/day

Males (3028 deaths) | Females (3815 deaths)

Cuba: All-cause mortality by age started smoking

Males (3028 deaths) | Females (3815 deaths)

All ages 30-79 adjusted for age, alcohol and cigarettes/day
Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
Antioxidant and antiatherogenic properties of nitroaliphatic compounds

Abstract nr. 394
Author Thomson, Leonor, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay
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Co-author(s) - Celano, Laura
Co-author(s) - Cerecetto, Hugo
Co-author(s) - Gonzalez, Mercedes
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Apolipoproteins, Atherosclerosis, Therapy

Inflammation and oxidative stress have a relevant role in cardiovascular diseases, being the oxidation of low density lipoprotein (LDL) a key event. Previous reports of our group, pointed out interesting antioxidant properties from a series of aromatic nitrocompounds (Celano et al 2014). In this study, a series of nitroalkenes and their corresponding nitroalkanes were synthesized and fully characterized by $^1$H and $^{13}$C NMR. The antioxidant capacity of the compounds was also determined using the ORAC assay. Methylthio derivatives were ~50% more effective than Trolox (1.5 ± 0.2 and 1.6 ± 0.3 Trolox Eq/mol) to prevent 2,2’-Azobis(2-amidinopropane) dihydrochloride (AAPH)-mediated fluoresceine oxidation, whereas the effect of the dimethylamine derivatives was more than 5 times higher (8 ± 1 and 13 ± 2 Trolox Eq/mol). The rate constant for the reaction with peroxyl radicals was $~10^2\text{ M}^{-1}\text{s}^{-1}$. Finally, the ability to prevent LDL oxidation mediated by AAPH-derived peroxyl radicals was evaluated by HPLC and oxygen consumption techniques. Lipid peroxidation induced by the exposure to 30 mM AAPH for 30 min at 37°C generated 13.6 µM malondialdehyde equivalents, whereas in the presence of nitroalkenes those values were reduced in a 50%. Also a decrease in the oxygen consuming rates during the propagation phase of lipid peroxidation was observed in the presence of the dimethylamine derivatives. We are now analyzing the capability of the molecules to prevent foam cell formation and their interaction with molecular targets involved in macrophage activation.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Andrographolide, a compound isolated from Andrographis paniculata, reduces the cholesterol levels by targeting on SR-B1

Abstract nr. 395
Author Tengku Muhammad, Tengku Sifizul, Universiti Malaysia Terengganu, Kuala Terengganu, Malaysia
Co-author(s) - Chong, Shu-Chien
Co-author(s) - Nadarasan, Karthiyayini
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis,HDL,Intervention,Reverse Cholesterol Transport

Cardiovascular diseases (CVD) still remain the leading cause of death worldwide. The underlying cause of CVD is atherosclerosis. It is normally characterised by the formation of foam cells along the lining of blood vessels resulted from macrophages that engulf large amount of lipids. The accumulation of foam cells leads to the thickening of arterial walls and the reduction of lumen diameter of blood vessels, which in turn, lead to the occlusion of the blood flow to vital organs such as heart. One of the major risk factors of atherosclerosis is hypercholesterolaemia. Recently, understanding of the fundamental mechanisms of atherosclerosis and its association with cholesterol transport has progressed significantly. Reverse cholesterol transport (RCT) is a pathway that transports cholesterol from extrahepatic cells such as foam cells to the liver for excretion. By reducing the accumulation of cholesterol in foam cells via the efflux mechanism as well as in blood serum, RCT may prevent the development of atherosclerosis. High-density lipoprotein (HDL) is an important molecule that is responsible in reverse-transporting cholesterol to the liver. The cholesterol-rich HDL delivers and deposits cholesterol to the liver via scavenger receptor class B type I (SR-B1) located on the surface of liver cells which allows cholesterol to be metabolised in the liver and secreted into bile. Therefore, SR-B1 forms an interesting target for therapeutic intervention against atherosclerosis. Thus, the main aim of the study was to identify natural products with potential activity in inducing the translocation of SR-B1 to the surface of liver cells. Our group demonstrated a compound, Andrographolide, isolated from a local plant Andrographis paniculata, increased the transcriptional activity of SR-B1 promoter, the mRNA levels and translocation of SR-B1 to the surface of liver cells. The compound also induced the uptake of cholesterol component of HDL due to an increase number of SR-B1 present on the surface of human liver, HepG2 cells. In conclusion, Andrographolide may form a potential candidate in reducing the cholesterol levels and subsequently atherosclerosis via SR-B1 in reverse cholesterol transport mechanism.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
TNF-α, Foam Cells and Abdominal Aortic Wall Thickness in Rats with Subchronical Inhalation Exposure of Transfluthrin

Abstract nr. 396
Author Prameswari, Asri, Brawijaya University, Malang, Indonesia, Malang, Indonesia
Co-author(s) - Soeatmadji, Djoko Wahono
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Inflammation, Intervention

Introduction: Transfluthrin is one that is widely used insecticide in South East Asia. Transfluthrin with subchronic exposure, known as endocrine-disrupting chemicals which affect the endocrine system through a variety of disorders of various genes, nuclear receptors and cell's morphology, so it can increased the risk of cardiovascular by increasing levels of pro-inflammatory cytokines and histopathological changes.

Aim: To determine TNF-α level, the number of foam cells and abdominal aortic wall thickness in Rats with subchronical inhalation of transfluthrin.

Methods: Experimental in vivo, post-test only, in 35 male Rattus norvegicus strain Wistar, divided into five groups, namely negative control (without exposure), positive control (inhalation with n-hexane/solvent of transfluthrin), group 1 (inhalation with transfluthrin 0.1mg/dl), group 2 (inhalation with transfluthrin 0.2mg/dl), and group 3 (inhalation with transfluthrin 0.4mg/dl). On the 56th days, the rats was terminated. Serum level of TNF-α was measured with ELISA (Biologend, USA kit) and histopathological examination to calculate the number of foam cells and abdominal aortic wall thickness.

Results: Means and standard deviation of serum TNF-α in negative control was 15.93±12.76 ng/mL, positive control was 16.64±14.91 ng/mL, group 1 was 42.42±9.99 ng/mL, group 2 was 97.86±17.76 ng/mL, group 3 was 135.56±35.80 ng/mL. Means of foam cells in negative control was 5/hpf, positive control was 15/hpf, group 1 was 24/hpf, group 2 was 34/hpf, group 3 was 70/hpf. Means and standard deviation of abdominal aortic wall thickness in negative control was 2173.99±305.56 m, positive control was 2206±269.75 m, group 1 was 2297.22±301.52 m, group 2 was 2494.30±399.34 m, and group 3 was 2459.74±319.02 m. One way ANOVA shows differences between groups. Meanwhile, there was correlation between transfluthrin’s concentration with TNF-α (r=0.853, p=0.05) and foam cells (r=0.865, p=0.05), but not in abdominal aortic wall thickness (r=0.317, p=0.173). Presumably due to its effects, oxidative stress with increase of inflammatory cytokines, and the cells changes, including macrophage and smooth muscle cells, but not in whole tissue changes.

Conclusion: Subchronical inhalation exposure of transfluthrin in Rats increase the TNF-α level and foam cells but not in abdominal aortic wall thickness.

Keywords: Transfluthrin, subchronical inhalation, endocrine-disrupting chemicals, foam cells, abdominal aortic wall thickness
Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
N-linked glycosylation disorders cause hypobetalipoproteinemia

Abstract nr. 397
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Co-author(s) - Wevers , R.
Co-author(s) - Stroes , Erik
Co-author(s) - Lefeber , D.J.
Co-author(s) - Holleboom , A.G.
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia,LDL,Lipoproteins,Metabolism

Rationale
We identified an entirely new group of dyslipidemias: caused by defective glycosylation of proteins involved in lipid metabolism. Congenital disorders of glycosylation (CDG) are phenotypically diverse inborn errors of metabolism, affecting multiple systems including the central nervous system and muscle function.

Based on the link between plasma lipids and protein glycosylation and incidental reports of hypocholesterolemia in CDG patients with defects in N-linked glycosylation, we studied plasma lipids in the two most common subtypes of these CDGs and the mechanism underlying the lipid abnormalities.

Methods & Results
Compared to 30 age- and gender-matched controls,20 PMM2- and ALG6-CDG patients had hypobetalipoproteinemina (HBL: total cholesterol, LDL-cholesterol and apolipoprotein B (apoB) below the 5th percentile.

As a cell model of these defects, siRNA knockdown of PMM2 and ALG6 in HepG2 cells was used. ApoB secretion was stimulated with oleic acid. Knockdown in serum free conditions caused significant ER stress, increased lipogenesis and apoB hypersecretion (184% in siPMM2 and 132% in siALG6 treated cells). ER stress in siPMM2 cells was independent of medium conditions; there is still significant ER stress and apoB hypersecretion in FBS or LPDS medium (for latter: 130% and 166% of control, respectively). Contrastingly, ALG6 knockdown in LPDS or FBS medium causes little to no ER stress and trended towards hyposecretion of apoB (90% of control).

Proteasomal inhibition increases apoB secretion in siALG6 cells by 150% yet had no effect on controls; suggesting a role for proteasomal degradation in the apoB hyposecretion in ALG6 knockdown.

Conclusion
Patients with CDG1 have HBL, only the third known genetic cause of HBL and the first form of HBL caused by enzymes not principally involved in lipid metabolism. Our data of ALG6 knockdown suggests a role for proteasomal degradation and consequently hyposecretion of apoB as a cause of the lipid phenotype. PMM2 knockdown in HepG2 is not a proper model to study HBL in CDG1-patients as it causes severe ER stress.

**Clinical Relevance**

Our findings demonstrate that N-glycosylation of proteins is crucial for LDL metabolism; further elucidating the mechanism could reveal novel targets in the treatment of dyslipidemia and cardiovascular disease.

**Subdivision 2. Translational Research**

**Presentation Preference** Oral presentation

**Additional information**
Monocytes show Increased M1 and Decreased M2 Markers with Atherosclerotic Risk and in CVD

Abstract nr. 398
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Co-author(s) - Pertsoulis, Nicholas
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Co-author(s) - Hitos, Kerry
Co-author(s) - Li, Stephen
Co-author(s) - John, Fletcher
Co-author(s) - Medbury, Heather

Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Immunity, Inflammation, Lipids

Macrophages play a major role in atherosclerotic plaque stability with M1 macrophages thought to promote instability and M2 macrophages stability. Their precursors, the monocytes, are heterogenous consisting of classical (~85%), intermediate and nonclassical monocytes. Expansion of the intermediate subset has been reported cardiovascular disease (CVD) which, accompanied with a proposed inflammatory role, has led to this subset being implicated in atherosclerosis. However, studies examining alterations in monocyte profile, rather than counts, are scarce. As such, we sought to determine whether, and how, specific monocyte subsets differ in inflammatory profile with atherorosclerotic risk, and between patients and controls.

Blood was collected from patients and controls. Monocyte subset expression of M1 and M2 markers was determined by whole blood flow cytometry. Luminex assays were used to determine serum M1 chemokine concentrations and lipid profile was measured.

A proportional increase in the intermediate subset of patients compared to controls was observed, in line with the reports in the literature. Of note, when total counts were examined, all subsets were shown to increase in the patients. The intermediate subset was more M1-like than the classical subset with greater expression of M1 markers (CD319, CD282), lesser expression of M2 markers (CD93, CD163) and a higher CD86/CD163 ratio. Interestingly, the classical subset displayed positive correlations of CD282 with ApoB/A1 ratio and CD319 with Apo B ratio as well as negative correlations of CD86/CD163 with HDL and Apo A1. Conversely, CD163 and CD93 correlated positively with both HDL and Apo A1, suggesting increased atherosclerotic risk may skew the major monocyte population to an M1 phenotype. In patients, the classical subset was further skewed as seen by a higher CD86/CD163 ratio compared to controls. In addition, patients had higher serum M1 chemokines (CCL19, CXCL9) than controls.

The classical monocyte, despite not showing a proportional expansion in atherosclerosis, shows a shift towards a more M1-like phenotype with atherosclerotic risk and presence of disease. As this is the major monocyte subset, the M1 shift may have an additive, or greater, impact on
atherosclerosis than increases in intermediate or nonclassical cell number

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Adherence to the Mediterranean diet is inversely associated to vascular events and subclinical atherosclerosis progression. Results from the IMPROVE study.

Abstract nr. 399
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Co-author(s) - Castelnuovo, Samuela
Co-author(s) - Sansaro, Daniela
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Co-author(s) - Bonomi, Alice
Co-author(s) - Amato, Mauro
Co-author(s) - Tremoli, Elena

Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Cardiovascular Disease, Epidemiology, Lifestyle

Background: Observational and experimental studies have shown that Mediterranean Diet (MD), a dietary pattern more diffused in Southern than in Northern Europe, is associated with a reduction of cardiovascular (CV) events and subclinical atherosclerosis. Moreover, our group recently documented a strong negative trend from North to South of the average intima-media thickness (IMT), an established index of subclinical atherosclerosis, in Western Europe (1). MD might be one of the potential determinants of this geographical trend.

Objectives: To investigate whether a very simplified MD adherence score (MD-score) is sensitive enough to detect the association with incident cardiovascular events and with IMT progression, in a cohort of subjects enrolled in different European countries.

Methods: The dataset of the IMPROVE Study (3703 individuals from Italy, France, the Netherland, Sweden and Finland, followed for 3 years) was interrogated. Using a basic 7 items dietary questionnaire, we computed a simplified version of the MD adherence score that Trichopulou devised by using the very inclusive EPIC questionnaire (2). Data were analysed by Cox proportional hazard and linear regression models.

Results: Our MD-score was inversely and significantly associated with CV events, independently from classical CV risk factors, pharmacological treatments, and demographic, geographic, behavioural and socio-economic variables. The adjusted hazard ratio was 0.76 (95% confidence interval 0.67, 0.86) for one point increment of MD-score, and was similar in men and women and for cardio and cerebrovascular events. In Northern countries the protection associated with MD was even stronger (P interaction = 0.03) than in Southern countries. Moreover, the MD-score was inversely associated with the “Fastest-IMT max progression”, an index of carotid IMT progression that we have shown to be predictive of future CV events.

Conclusions: A very simplified dietary questionnaire is sufficient to compute a MD score which is
predictive of lower rates of vascular events and slower IMT progression. This effect was more
evident in the Northern countries, where the incidence of CV disease is higher and the MD diet is
less widespread.

References:

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Novel Intranasal vaccine composition of micellar lipid/peptide nanoparticles against the development of atherosclerotic and fatty liver disease.

Abstract nr. 401
Author Mas-Oliva, Jaime, Instituto de Fisiología Celular, UNAM. Hamol Biosolutions LLC., México D.F., Mexico
Co-author(s) - García-González, Victor
Co-author(s) - Delgado-Coello, Blanca
Co-author(s) - Pérez-Torres, Armando
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Pathogenesis, Prevention, Therapy

For years now, the search for new and useful alternative pharmacological interventions in the prevention of atherosclerosis and its associated complications has taken place with limited success. The present study presents a novel vaccine composition of micellar lipid/peptide nanoparticles to be administered intranasally to prevent the process of atherogenesis and associated pathologies such as fatty liver development. Peptides employed correspond to the carboxy-end sequence of the cholesteryl-ester transfer protein (CETP), importantly implicated in atherogenesis (Biochem. Biophys. Res. Commun. 434:54-59, 2013; J. Struct. Biol. 186:19-27, 2014). The novelty of the vaccine compound of the present study includes the use of membrane lipids isolated from the archaeabacteria *Thermus aquaticus* and lysophospholipids, which give peptides their adequate secondary structural conformation, paramount for autoimmune activity. The vaccine composition has been shown to be useful raising an autoimmune response against CETP and therefore increasing HDL and lowering LDL-cholesterol plasma concentrations in two experimental animal models. The preclinical *IFC* study carried out in rabbits and the *Barcelona* study performed with pigs have shown that vaccination prevents the formation of plaque and vascular lesion in the aorta and drastically reduces the development of fibrosis and fatty liver in animals long term fed with a high cholesterol diet. The successful disease-preventive results obtained with our non-invasive intranasal vaccine composition during the preclinical stages of our study, has allowed us to advance and begin with clinical trials phase 1 and phase 2 in humans starting on the spring and summer of 2015. Patent coverage: EP 1242446 B1; US 7749721 B2; MX 246945; PCT/MX2013/00078. Funding support: Conacyt grants 083673; 0141588; 0219327. Hamol Biosolutions development grant HB08.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
A Xanthine Oxidase Inhibitor Febuxostat Improves Endothelial Function as Evaluated by Flow-Mediated Vasodilatation in Hyperuricemic Patients

Abstract nr. 402
Author Ayaori, Makoto, Tokorozawa Heart Center, Tokorozawa, Japan
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Co-author(s) - Masumi, Kamiyama
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Co-author(s) - Masami, Sakurada
Co-author(s) - Katsunori, Ikewaki
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Endothelium, Risk Factor, Therapy

Patients with hyperuricemia reportedly have endothelial dysfunction, which could be restored by reducing serum urate levels with a xanthine oxidase (XO) inhibitor, allopurinol (Allo). However, it remains unclear whether this favorable property is a class-effect of XO inhibitors. We therefore performed a prospective, randomized crossover trial comparing Allo and another XO inhibitor, febuxostat (Febu).

Twenty-two hyperuricemic patients were randomized to 12 weeks treatment using Allo or Febu, which were started at half-dose then increased to full-dose after 4 weeks. On-treatment urate levels at 4/12 weeks were significantly lower in Febu phase as compared to Allo phase. Evaluation of endothelial function revealed that Febu, but not Allo, increased flow-mediated vasodilation. Our results suggest that Febu is more effective on urate levels and endothelial function as compared to Allo.
Subdivision 3. Clinical Studies

Presentation Preference: Electronic poster presentation
Additional information
CETP-HDL interaction is one fundamental step in the reverse cholesterol transport process during which cholesterol ester lipids are transferred to the cholesterol ester transfer protein (CETP) and delivered to the liver for further being metabolized. Inhibition of CETP, a protein mediating transfer of neutral lipids between lipoproteins, has been proposed as a means to elevate atheroprotective HDL subpopulations and thereby reduce atherosclerosis. However, off-target and adverse effects of the inhibition proved that informations on CETP and or/HDL are still missing and hampered the understanding of the molecular mechanism of CETP–HDL interaction. Although structural consideration of both CETP and HDL would suggest simple key-lock interaction through a match-to-match curvature approach, recent experimental findings have demonstrated the penetration of CETP into HDL. However, the atomic level resolution was not enough to clarify the mechanism of CETP penetration into HDL [1]. In the present study we constructed an HDL particle mimicking the actual human HDL mass composition and investigated for the first time by large-scale atomistic molecular dynamics the interaction of an upright CETP with a human HDL mimicking model. The results demonstrate that CETP can penetrate the phospholipid surface layer of HDL particle with the formation of an opening in the N barrel domain end of CETP. A tryptophan-rich region of this domain seems to play the major anchoring role of CETP with HDL. Furthermore we reveal the presence of a phenylalanine barrier which controls access of HDL-derived lipids to the tunnel area of CETP molecule. In conclusion atomistic MD demonstrated how CETP can penetrate HDL particle surface and reveal novel atomistic details of CETP–HDL interaction mechanism that could provide new insight into therapeutic strategies [2].

References:
Cilpa-Karhu, G., M. Jauhiainen, and M.-L. Riekkola. 2015. Atomistic molecular dynamics simulation reveals the mechanism by which CETP penetrates into HDL enabling lipid transfer from HDL to CETP. J. Lipid Res. 56:98-108.
Additional information
Vascular risk factors, vascular disease, lipids and lipid targets in patients with Familial Dysbetalipoproteinemia: a European cross-sectional study

Abstract nr. 405
Author Koopal, Charlotte, UMC Utrecht, Utrecht, Netherlands
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Co-author(s) - Bertolini, Stefano
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Dyslipidemia, Genetics, Hypolipidemic Drugs

Background Familial dysbetalipoproteinemia (FD), also known as type III hyperlipoproteinemia, is a genetic dyslipidemia characterized by elevated very low density lipoprotein (VLDL) and chylomicron remnant particles that confers increased risk of cardiovascular disease (CVD). The objective of this study was to evaluate the prevalence of vascular risk factors, CVD, lipid values, treatment and lipid targets in patients with FD across Europe.

Methods This cross-sectional study was performed in 305 patients with FD from seven academic hospitals in four European countries. Information was collected from clinical records.

Results Patients mean (±standard deviation (sd)) age was 60.9±14.4 years, 201 (66%) were male, 69 (23%) had diabetes mellitus (DM) and 87 (29%) had a prior history of CVD. Mean body mass index was 28.5±5.0 kg/m². Lipid-lowering medication was used by 227 (74%) patients (27% usual dose (theoretical low-density lipoprotein cholesterol (LDL-C) reduction ≤40%) and 46% intensive dose (theoretical LDL-C reduction >40%). Non high-density lipoprotein cholesterol (non-HDL-C) levels below treatment target (<3.3 mmol/L) were present in 123 (40%) patients and 163 patients (53%) had LDL-C levels below target (<2.5 mmol/L). No significant determinants were found for having non-HDL-C levels below target, while a prior history of CVD (OR 1.90, 95%CI 1.05-3.47) and presence of DM (OR 2.00, 95%CI 1.08-3.70) were associated with having LDL-C levels below treatment target.

Conclusion The majority of FD patients had non-HDL-C levels above the treatment target of 3.3 mmol/L. Intensive dose lipid-lowering medication was used by only half of the patients, leaving them at increased cardiovascular risk.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Medium-term effect of sublingual L-glutathione supplementation on flow-mediated dilation and oxidative stress markers in male subjects with cardiovascular risk factors.

Abstract nr. 406
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Co-author(s) - Giannattasio, Cristina
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Endothelium, Hypertension, Nutraceuticals, Risk stratification

Cardiovascular risk factors (CVRF) alter endothelial function through direct endothelium oxidative damage, and by decreasing nitric oxide availability. Supplementation of GSH, an important cellular scavenger, may improve endogenous antioxidant defence and contribute to decrease the oxidative tissue alteration. However, the efficacy of GSH treatment seems related to the degree of its absorption. We tested a new sublingual formulation of L-GSH able to bypasses gastro-enteric tract entering directly the systemic circulation. We would assess whether medium-term administration of this new compound to male subjects with CVRF for 4 weeks may result in improved vascular function and/or reduced oxidative stress markers when compared to placebo. Sixteen healthy subjects with CVRF such as smoking habit, hypertension or not treated dyslipidemia were enrolled in a double-blind randomized placebo-controlled cross-over study. At each visit, blood samples were collected for routine biochemistry and oxidative stress markers assessment and peripheral arterial function (RHI) and stiffness (AI@75) were measured by means of Endo-PAT2000.

Although in the population as a whole no difference was observed in RHI and in oxidative stress markers between L-GSH and placebo, the seven subjects with abnormal RHI (≤ 1.67) at baseline showed a significant reduction of vascular stiffness and serum urea after L-GSH, with respect to those with normal endothelial function (P=0.007 and P=0.037, respectively). Interestingly, in the overall population a decrease in total and LDL cholesterol was highlighted after L-GSH compared to placebo (P=0.023 and P=0.04, respectively).

Medium-term L-GSH administration improves vascular elasticity and renal function in subjects with abnormal vasodilating capability. Supplementation of L-GSH compared to placebo influences the lipid profile of subjects with CVRF. L-GSH, in the investigated formulation, may represent a valid
prevention of vascular damage in CVRF subjects with endothelial dysfunction.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
BMI correlates with atherogenic lipoprotein profile even in non-obese, normoglycemic, normolipidemic healthy men

Abstract nr. 407
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Co-author(s) - Ribalta, Josep
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia

Aim
Our aim was to demonstrate that BMI has an impact on lipid and lipoprotein parameters even among non-obese, normoglycemic, normolipidemic healthy men carefully selected for not having any cardiovascular, metabolic and chronic diseases.

Methods
297 healthy, non-smoking males, aged 20-75 years, were recruited. Exclusion criteria were familial hypercholesterolemia, any chronic diseases and BMI≥30 kg/m².

Lipid and lipoprotein particles were determined by standard methods, using ultracentrifugation and NMR. Cholesterol in remnant-like particles (RLPc) was also determined.

Results
Participants were separated in two groups: normoweight [BMI<25 kg/m² (n=143)] and overweight [BMI≥25 kg/m² (n=154)]. Overweight participants were older (p<0.001) compared to normoweight. Both groups had normal total and LDLc levels, and while both groups had plasma TG levels within the normal range, overweight participants presented 30% higher TG levels (p<0.001) and accordingly lower HDLc compared to those normoweight.

Lipoproteins isolated by sequential ultracentrifugation showed that cholesterol, TG and apoB100 in VLDL and IDL particles were increased, and cholesterol and apoAI in HDL were decreased in overweight subjects. While LDL was comparable between groups, NMR analysis uncovered alterations in the number of LDL particles. Overweight participants had 27% more total LDL particles (p<0.001) as a result of 16% lower levels of the large LDL (p<0.001) and 70% increased levels of the smaller subclasses (p<0.001).

NMR also showed 20% higher number of Chylomicrons and VLDL particles (p=0.016), 2-fold
larger VL DL (p=0.001), and 30% more medium VL DL particles (p=0.020). Overweight participants also had 70% more IDL particles (p=0.010), 30% decrease in large HDL particles (p<0.001) and 39% higher RLPc levels (p=0.005).

Among subjects with normal weight, BMI positively correlated with large VL DL and chylomicron particles (r=0.246, p=0.018), the smallest LDL particles [for small LDL (r=0.239, p=0.022); for medium small LDL (r=0.237, p=0.023); and for very small LDL (r=0.238, p=0.023)] and circulating RLPc levels (r=0.245, p=0.019).

Results were adjusted for age and fat intake.

Conclusion

BMI is correlated with a shift towards a more proatherogenic lipoprotein profile even in the situation of normoglycemia and normolipidemia in non-obese healthy men. This shift is most evident when particle size and number are assessed by NMR.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
HDL proteins as predictors of cardiovascular risk and mortality in diabetic patients on hemodialysis

Abstract nr. 408
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Co-author(s) - Wanner, Christoph
Co-author(s) - Mäź, Winfried
Co-author(s) - Säemann, Marcus D
Co-author(s) - Weichhart, Thomas
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Chronic Kidney Disease, HDL, Risk stratification

**Aim:** The protein composition of HDLs are altered in several chronic diseases with elevated cardiovascular morbidity and mortality resulting in enrichment of specific proteins in HDLs. Here we investigated if two proteins found enriched on HDL of end-stage renal disease patients, serum amyloid A (SAA) and surfactant-protein B (SP-B), can predict cardiovascular events and mortality in the German Diabetes and Dialysis study (4D study).

**Methods:** We developed an easy applicable, laboratory assay based on an ELISA principle to measure the amount of HDL-associated SAA and SP-B without the need for a preliminary isolation of HDLs. In a post-hoc analysis of 1152 patients with type II diabetes on hemodialysis which were randomly assigned to atorvastatin treatment (20 mg/day) or placebo, we assessed the association of SAA(HDL) and SP-B(HDL) with cardiovascular outcomes in adjusted multivariate regression models. We further investigated if SAA(HDL) or SP-B(HDL) concentrations affect the efficacy of statin treatment.

**Results:** We established a simple and reproducible test for quantification of HDL-bound proteins allowing detection of SAA and SP-B on HDL directly from serum. High concentrations of SAA(HDL) were significantly associated with increased risk of cardiac events in the 4D cohort, whereas SP-B(HDL) was a significant predictor of overall mortality. Importantly, both associations were independent of HDL cholesterol levels. SAA(HDL) also modified the effect of atorvastatin treatment on the incident rate of cardiac events in the second and third quartile.

**Conclusion:** In diabetic patients on hemodialysis, SAA(HDL) and SP-B(HDL) were significant predictors for cardiovascular outcomes and death. Our findings indicate that specific disease-associated proteins may serve as novel biomarkers to predict cardioprotective effects of HDLs and could provide a meaningful stratification model to identify individuals who benefit from therapeutic interventions. Our newly established assay may be applicable to evaluate further HDL-associated proteins for predicting clinical events in several high-risk populations.

Subdivision 2. Translational Research
Presentation Preference Oral presentation
Additional information
Proteomic analysis of human atherosclerotic plaque reveals gender specific alterations in protein expression between different lesion sites.

Abstract nr. 409
Author Ward, Liam J., Linköping University, Linköping, Sweden
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Co-author(s) - Liang, Wenzhao
Co-author(s) - Ljunggren, Stefan A.
Co-author(s) - Li, Wei
Co-author(s) - Yuan, Xi-Ming
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Pathogenesis

Different sites within atherosclerotic plaque have distinctive proteomics profiles, which have functional implications in plaque growth and vulnerability. We aimed to identify if gender has a profound effect on the protein profiles of individual sites, and as a whole, of human carotid plaque. Six carotid endarterectomy tissues (equal gender distribution) were sampled and biopsies of five distinct sites analysed; internal control, fatty streak, plaque shoulder, plaque centre and fibrous cap. Two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionisation-time of flight mass spectrometry were used for protein quantification and identification. Additionally, carotid plaque samples (39 men/23 women) were immuno-stained for ferritin using rabbit anti-human ferritin and visualised using the DAKO EnVision™+/HRP method. Predominantly, significant increases in expression of ferritin light chain were seen in lesions from men but not in women, these results were supported by ferritin immuno-staining. Other gender specific alterations include: in women overexpression of apolipoprotein A-IV and transthyretin in plaque shoulder and fatty streak, respectively, and in plaque centre; in men reduced biglycan expression in all plaque regions. Proteomic analysis of different lesion sites in men and women have revealed a number of gender specific alterations; ferritin and apolipoprotein A-IV expressions, as well as a number of non-gender specific alterations. These gender and/or site-specific differences in protein expression may have functional implication in the understanding plaque growth and vulnerability. Funding: Provided via grants from Swedish Heart and Lung Foundation and Linköping University.

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Proteomic analysis of oxysterol treated human macrophages reveals significant alterations in lipid- and apoptosis-related proteins

Abstract nr. 410
Author Ward, Liam J., Linköping University, Linköping, Sweden
Co-author(s) - Ljunggren, Stefan A.
Co-author(s) - Karlsson, Helen
Co-author(s) - Li, Wei
Co-author(s) - Yuan, Xi-Ming
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Immunity, Pathogenesis

Oxysterols are a cytotoxic constituent of oxidised-LDL (oxLDL), which when taken up by macrophages exacerbates functions of foamy macrophages. Macrophages loaded with atheroma relevant oxysterols present an established cell model with pathologic similarities to dysfunctional macrophages in atherosclerotic process. The aim of this study was to investigate the effect of the oxysterol loading on the macrophage proteome. Human THP-1 macrophages were exposed to an atheroma relevant mixture of 7β-hydroxycholesterol and 7-ketocholesterol. Two-dimensional electrophoresis (2-DE) and mass spectrometry techniques were used to analyse the alterations in macrophage proteome. The quantitative analysis resulted in the identification of 19 proteins with significant differential expression upon oxysterol loading; 8 upregulated and 11 downregulated. Two groups of proteins were highlighted based on functional similarities, lipid- and apoptosis-related proteins. Alterations in these protein groups provide an atheroma relevant cell model to further study the mechanisms behind lipid metabolism and macrophage longevity in atherosclerosis. This is the first study aimed at the proteomic analysis of oxysterol loading on macrophages, and results presented further suggest that lipid retention and alteration of apoptosis related proteins in macrophages induced by atheroma relevant oxysterols is critical in the development of foam cells and the progression of atherosclerosis.

Funding: Provided via grants from Swedish Heart and Lung Foundation and Linköping University.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Cleavage of apoA-I and HDL is closely associated with glycation to lost antioxidant and anti-inflammatory activity in zebrafish embryo

Abstract nr. 411
Author Park, Ki-Hoon, Yeungnam University, Gyeongsan, South-Korea
Co-author(s) - Yoo, Jeong-Ah
Co-author(s) - Park, Jeong-Euy
Co-author(s) - Cho, Kyung-Hyun
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Functionality, Inflammation

HDL is a strong antioxidant and anti-inflammatory molecule in plasma and native apoA-I (nA-I) is major protein constituent of HDL. Glycation of apoA-I is a main feature of diabetes mellitus under hyperglycemia with senescence. Glycated apoA-I (gA-I) showed severe multimerization and lost antioxidant ability with increase of conjugated diene level in lipid-free and rHDL state. The gA-I and native apoA-I (nA-I) was fractionated by fast protein liquid chromatography in according to multimerized and fragmented size. The gA-I showed slower elution profile than that of nA-I, even though gA-I had more multimerized apoA-I. In gA-I chromatogram, the earlier peaks contained multimerized apoA-I up to pentamer and the last peak showed protein cleavage. Western blot analysis revealed that the cleaved gA-I could not make the multimerization as much as uncleaved gA-I or nA-I, indicates that the mechanism of multimerization is different between nA-I and gA-I. Furthermore, treatment of protease inhibitor cocktail (EDTA free) could not inhibit the proteolysis. However, EDTA treatment partially attenuated the cleavage of apoA-I under presence of fructose, indicating that the proteolysis might be occurred via metal ion dependent process. Under co-injection with oxLDL, the cleaved gA-I (F#7) caused the lowest survivability, while multimerized gA-I (F#3) caused 41% survivability. Multimerized nA-I caused 45% survivability (F#11) and monomeric nA-I showed 34% survivability, while PBS injection caused 21% survivability.

In conclusion, gA-I-rHDL lost the beneficial anti-inflammatory and anti-oxidant activity by cleavage and multimerization of apoA-I.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Cardiovascular risk factors in the staff of Public Hospital

Abstract nr. 412
Author Don , Gracia Luz, Hospital San Martín, Paraná, Argentina
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease,Prevention,Risk Factor

INTRODUCTION
Smoking, sedentarism, hypertension, dyslipidemia, diabetes, overweight and obesity are key factors to develop cardiovascular diseases.
The aim of this study was to analyse the impact of the National Strategy for Prevention and control of non-communicable chronic diseases (NCDs) in the incidence of cardiovascular risk factors in the staff of San Martin Public Hospital.
Smokers were asked to complete the Modified Fagerstrom test for assessing the degree of nicotine dependence.

METHODS AND MATERIAL
448 including health professionals, nursery, administrative and general service staff were asked to complete an anonymous and self-administered survey.
Data required comprised age, sex, hypertension (HBP) or antihypertensive treatment, overweight or obesity, sedentarism, diabetes, dyslipidemia and smoking.

RESULTS
283 female, 157 male and 8 non-gender-mentioned people were studied. The ages were grouped as follows: 56% from 20 to 40; 24% from 41 to 50, 18% from 51 to 60 while 2% did not reply.
Overweight, obesity, HBP, dyslipidemia and diabetes increase with age.
Physical inactivity appears in all age ranges.
Smoking, present in all ranges, lowers in the middle range.
Comparisons with the National Risk Factors Survey (2014) were performed.
The addicted to cigarettes showed lower level of nicotine dependence.

Conclusions

Females showed a higher sedentarism and smoking incidence, with low dependence on nicotine.
Males showed higher tendency to metabolic syndrome; HBP, dyslipidemia, Diabetes and nicotine dependence.
Overweight, obesity and Diabetes levels were similar to the national ones. A significant lower sedentarism, HBP, dyslipidemia incidence was found, related to better health care and information from the Health National Promotion Program.
### Subdivision 3. Clinical Studies

**Presentation Preference** Electronic poster presentation

**Additional information**

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An egg protein hydrolysate improves arterial stiffness and metabolic parameters in overweight/moderately obese subjects with impaired glucose tolerance/diabetes type 2

Abstract nr. 413
Author Severins, Nadine, Maastricht University, Maastricht, Netherlands
Co-author(s) - Mensink, Ronald P.
Co-author(s) - Plat, Jogchum
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Diabetes, Intervention, Nutrition

Background The egg protein-hydrolysate NWT-03 has recently been shown to inhibit angiotensin-converting enzyme (ACE), to reduce blood pressure (BP), and to improve endothelial function in Zucker diabetic fatty rats and spontaneously hypertensive rats. Moreover, in vitro NWT-03 inhibited dipeptidyl peptidase-IV (DPP-IV) activity. Therefore, this egg protein-hydrolysate is an interesting ingredient to improve vascular dysfunction and consequently lower future cardiovascular disease (CVD) risk. We therefore designed the first study in humans using NWT-03 to assess the acute and short-term effects (2 hours and 2 days respectively) on arterial stiffness and metabolic parameters in overweight / moderately obese subjects with impaired glucose tolerance (IGT) or type 2 diabetes mellitus (T2DM).

Methods The study had a crossover design with two treatment arms (5 gr/d NWT-03 or placebo) separated by a 2 weeks wash out period and included 40 subjects. The hydrolysate or placebo was provided in capsules consumed in the morning. During both periods, pulse wave velocity (PWV), augmentation index corrected for heart rate (AI_HR75), blood pressure (BP) and heart rate (HR) were measured at day 1 fasting (t0) and 120 minutes (t120) following consumption of the capsules. The same parameters were measured at day 3 again at t120. Blood was sampled on day 1 at t0, at 60 minutes (t60) following consumption of the capsules and at t120, and at day 3 at t120.

Results The egg protein-hydrolysate was well tolerated without reported side effects. Carotid-radial PWV (cr-PWV) (acute and short-term), AI_HR75 (short-term), triacylglycerol (TAG) (short-term), apolipoprotein B48 (apoB48) (short-term) and HOMA-index (acute) all decreased, while high-density lipoprotein cholesterol (short-term) increased significantly during NWT-03 consumption compared to placebo consumption. Interestingly, effects on vascular function apparently occurred without a significant reduction in blood pressure and the question is whether the improvements in glucose and lipid metabolism are the cause.

Conclusion Based on the results of animal studies this first placebo controlled intervention study in humans, we conclude that the egg protein-hydrolysate NWT-03 is an interesting dietary ingredient to examine its longer-term effects on unfavourable metabolic profiles and vascular dysfunction associated with T2DM and IGT.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Liraglutide prevents aortic plaque progression, but does not affect plaque regression in Apo e knock-out mice

Abstract nr. 414
Author Rolin, Bidda, Maaloev, Denmark
Co-author(s) - Kirk, Rikke K.
Co-author(s) - Rasmussen, Salka E.
Co-author(s) - Knudsen, Lotte B.
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Animal model, Atherosclerosis, Diabetes, Pharmacology

Introduction and Aim
Atherosclerosis leading to coronary artery disease is a huge medical problem, particularly in connection with diabetes. Glucagon-Like Peptide 1 (GLP-1) based therapy effectively lowers blood glucose and body weight in type 2 diabetes. In this study, we investigated whether liraglutide, a once-daily GLP-1 analogue, could either prevent plaque progression or induce plaque regression in Apo e KO mice.

Methods
Study 1 (prevention): Female Apo e mice on Western Diet were treated with either liraglutide (1 mg/kg) or vehicle s.c. for 12 weeks. A blood sample was taken at termination for lipid measurements. Body weight was monitored throughout the study. Plaque burden was estimated by the "en face" method.

Study 2 (regression): Female Apo e mice on Western Diet for 10 weeks were changed to standard chow and then treated with either liraglutide (2 x 0.3 mg/kg) or vehicle s.c. for 6 weeks. A blood sample was taken at termination for lipid measurements. Body weight was monitored throughout the study. Plaque burden was estimated by the "en face" method.

Results
Study 1: Liraglutide prevented aortic plaque progression resulting in a plaque area of 12.9±0.8 % compared to a plaque area in the vehicle control group of 20.5±1.0 % ( p<0.0001). Triglyceride levels were significantly lower in the liraglutide treated group (1.9±0.1 vs 0.9±01 mmol/l, p<0.0001), whereas total cholesterol, HDL, LDL and VLDL cholesterol fractions were not affected. End body weight was significantly lower in the liraglutide treated group (23±0.4 g) compared to vehicle (33±1 g), p<0.0001.

Study 2: Aortic plaque area, when already established, was not affected by liraglutide treatment (22.0 ± 1.5%) compared to vehicle (23.7 ± 1.5 %) ns. Triglyceride levels and total cholesterol were significantly lower in the liraglutide treated group. Body weight was slightly lower in the liraglutide treated group (23.7±0.4 g) compared to vehicle (26.2±0.6 g), p<0.01.

Conclusion
Liraglutide at a dose of 1 mg/kg was able to significantly inhibit plaque progression in this animal
model of atherosclerosis. Using a dose of 0.6 mg/kg, there was no effect of liraglutide on already established plaque in this study.

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Prevention of Vascular Calcification in Uremic Rats by Pharmacological Inhibition of Matrix Metalloproteinases (MMP)

Abstract nr. 415
Author Querfeld, Uwe, Charité Universitätsmedizin Berlin, Berlin, Germany
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Co-author(s) - V.Websky, Karoline
Co-author(s) - Nasser, Hamoud
Co-author(s) - Hocher, Berthold
Co-author(s) - Freise, Christian

Keywords Cardiovascular Disease,Chronic Kidney Disease,Pathogenesis,Prevention

Background: The manifestations of cardiovascular disease (CVD) limit survival in adult and pediatric patients with chronic kidney disease (CKD). The presence of vascular calcifications is a marker for advanced CVD in patients with CKD. Transition of vascular smooth muscle cells (VSMC) from a contractile to a chondro/ostoblastic phenotype is a key event in the pathogenesis of media calcifications in uremic subjects.

Objective: We investigated whether pharmacological inhibition of MMPs can decrease chondro/osteoblastic transition of VSMC and the development of media calcifications in an aggressive model of uremic vascular calcifications induced by treatment of uremic rats with calcitriol and a high phosphate (HP) diet.

Methods: Uremia was created by 5/6-nephrectomy and all animals were fed a HP diet (1,2% phosphate). Arterial calcifications were induced by treatment with calcitriol (250ng/kg/d). MMP inhibition was achieved with doxycycline (100mg/kg/d), an unspecific MMP inhibitor. Five groups of rats were studied: uremic, shamp-operated, uremic treated with doxycyclin, uremic treated with calcitriol, and uremic treated with calcitriol and doxycycline. After 5 weeks, animals were sacrificed and the extent of media calcifications was quantified in aortic sections by von Kossa staining. Osteoblastic transition of VSMC was assessed by RT-PCR for osteogenic proteins. Aortic content of MMP-2 and MMP-9 was quantified by staining and RT-PCR.

Results: Creatinine and phosphate levels were significantly higher in groups receiving calcitriol, and not significantly different in the doxycycline-treated group. Massive calcifications were found in 5/6 nephrectomized rats treated with calcitriol and HP diet. MMP inhibition with doxycycline resulted in almost complete absence of media calcification in uremic rats treated with calcitriol and HP diet. Uremic rats treated with calcitriol and a HP diet, but not rats treated with doxycycline, showed significantly increased gene expression levels of MMP-2 MMP-9, osteopontin, osteocalcin, Runx2, and osterix.

Conclusions: Phenotypic transition of VSMC and the development of vascular calcification were almost completely prevented by MMP inhibition. This effect was not due to differences in the degree of uremia, calcium or phosphorus levels or systolic blood pressure in experimental
animals. MMP inhibition seems a promising strategy in the prevention of uremic vascular calcifications.

Subdivision 2. Translational Research

Presentation Preference Mini-oral presentation
Additional information
Anthocyanins-rich chokeberry fruit extract restored endothelial progenitor cells functions impaired by angiotensin II through Nrf2/HO-1 pathway activation

Abstract nr. 416
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Co-author(s) - Naruszewicz, Marek
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Angiogenesis,Endothelium,Pharmacology

In hypertension, plaque microvessels are immature and fragile and the distorted integrity of microvessels endothelium likely leads to intraplaque haemorrhage and plaques at increased risk for rupture. Endothelial progenitor cells (EPC) may provide protection against atherosclerosis and plaque rupture by their innate ability to replace dysfunctional or damaged endothelial cells in plaque microvessels. There is evidence that angiotensin II may impair functions of EPCs by senescence accelerating and inhibition of their proliferation through oxidative stress induction. In this study, we examined whether chokeberry (Aronia melanocarpa) fruit extract, containing mainly anthocyanins with potent antioxidative properties, could protect EPC against angiotensin-induced impairment.

EPCs were isolated from peripheral blood of young healthy volunteers and cultivated on fibronectin-coated plates in the presence or absence of angiotensin II (1μM) and chokeberry extract (1-50 μg/mL). Cell senescence was measured using a β-galactosidase staining kit; ROS generation was measured using a ROS/Superoxide detection kit; proliferative activity was assayed using colorimetric BrdU ELISA kit; Nrf2 activation and heme oxygenase-1 expression was measured using ELISA kits; adhesion to fibronectin and migration were also assessed. Angiogenic potential was measured by tube formation in Matrigel™.

EPC exposed to angiotensin II showed an increase in the number of β-galactosidase positive cells. These effects were significantly reduced by chokeberry extract in a concentration-dependent manner. Incubation of EPC with chokeberry extract reduced ROS formation and increased proliferative activity of EPC. Furthermore, extract increased migration ability, adhesion to fibronectin and the angiogenic potential of EPC in vitro impaired by angiotensin II. This activity of chokeberry extract was correlated with the increase of heme oxygenase-1 expression and Nrf2 activation.

Our results suggested that chokeberry extract may protect EPC against angiotensin II-induced oxidative stress. Moreover, induction of Nrf2 transcription factor in EPCs by extract and increase of HO-1 level in these cells, not only protect EPCs against oxidative stress, but also increase their ability to re-endothelization of injured arterial wall or neovascularization of ischemic tissue.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Hydrogen sulfide donors exert anti-inflammatory effect by reducing monocyte cell adhesion to endothelial monolayer in an in vitro atherosclerotic model

Abstract nr. 417
Author Giannakopoulou, Efstathia, Democritus University of Thrace, Alexandroupolis, Greece
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Co-author(s) - Topouzis, Stavros
Co-author(s) - Manolopoulos, Vangelis
Co-author(s) - Tavridou, Anna
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Endothelium, Inflammation, Pathogenesis

Inflammation and monocyte cell adhesion to endothelium are two important processes in the early stages of atherogenesis. We sought to examine the possible vascular anti-atherosclerotic effect of three hydrogen sulfide (H$_2$S) donors, GYY4137, thioglycine and thiovaline in an in vitro model.

EAhy926 endothelial cells ($3 \times 10^6$) were grown to confluence in 24-well plates. Cells were serum-starved for 12h and then incubated with several concentrations of GYY4137, thioglycine and thiovaline for various time points. In some experiments, various concentrations (500μM, 1mM, 2mM, 3mM) of Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) were added with the H$_2$S donors in order to block nitric oxide (NO) production. After treatment with H$_2$S donors, cells were incubated with TNF-α (10ng/ml) for 6h. Medium was then removed, THP-1 monocytes were counted and seeded on TNF-α activated EAhy926 monolayers and incubated for 1h at 37°C. Non-adherent THP-1 cells were removed and EAhy926 were fixed with 4% paraformaldehyde.

Adhesion was counted on three randomly selected 40x magnification microscopic fields/well in four independent experiments.

Control confluent EAhy926 showed minimal binding for THP-1 cells, while the adhesion of THP-1 was significantly increased to TNF-α-stimulated EAhy926. Pre-treatment of EAhy926 with increasing GYY4137 (300μM & 500μM) or thioglycine (100μM, 300μM & 500μM) concentrations for 1h before TNF-α activation caused a concentration dependent inhibition of THP-1 adhesion up to 69.3% ± 2.2 % and 48.3% ± 2.8 %, respectively. Pre-treatment of EAhy926 with thiovaline or glycine under similar conditions did not attenuate the adhesion of THP-1 to EAhy926 cells.

Inhibition of NO production did not affect the inhibitory effect of H$_2$S donors.

H$_2$S exerts a potentially protective role in atherogenesis by inhibiting monocyte cell adhesion to inflamed endothelial cells. Utilization of H$_2$S donors might, thus, be a new therapeutic strategy against this pathological process.

Acknowledgement: This research has been co-financed by the European Union (European Social Fund –ESF) and Greek national funds through the Operational Program “Education and Lifelong Learning” of the National Strategic Reference Framework (NSRF) - Research Funding Program:
Thales: “Hydrogen sulfide a new endogenous regulator of angiogenesis: signaling, physiology/pathophysiology and development of pharmacological inhibitors” (MIS 380259)

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
**Icosabutate for the treatment of very high triglycerides: A placebo-controlled, randomized, double-blind, 12-week proof-of-concept study**

Abstract nr. 419
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Co-author(s) - Vige, Runar
Co-author(s) - Zhou, Rong
Co-author(s) - Fraser, David
Co-author(s) - Hustvedt, Svein Olaf
Co-author(s) - Orloff, David G.
Co-author(s) - Kastelein, John
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Hypolipidemic Drugs, Intervention, Triglycerides

**Introduction**
Icosabutate is a structurally enhanced omega-3 fatty acid (SEFA) having a number of potential biologic properties that may help lower triglyceride (TG) and apolipoprotein C3 (APOC3) levels. Prior rodent models of dyslipidemia and a small phase Ib human study support icocabutate as efficacious in lowering TG and APOC3 levels.

**Purpose**
The purpose of this trial was to evaluate the TG-lowering efficacy and safety of icosabutate 600 mg once daily orally in patients with very high TG levels, as well as its effects upon other lipid parameters.

**Methods**
After an initial screening period that included diet and lifestyle stabilization, this multicenter, double-blind, placebo-controlled trial randomized men and women between 18 and 79 years with TG ≥ 500 and ≤ 1500 mg/dL to icosabutate 600 mg once daily or placebo. The primary endpoint was change in TG from baseline to week 12. Other efficacy endpoints included effects upon APOC3, and other lipid parameters. Safety was assessed by adverse events (AEs). Efficacy analyses were derived from the intention to treat population; lipid parameter changes were reported as medians.

**Results**
87 subjects were randomized and 79 subjects completed the study. Baseline demographics were comparable among the groups; 21 % were treated with stable statin doses. At week 12, icosabutate significantly reduced fasting TG vs baseline (-51% [-66.8,-32.4]) and placebo (-33% [-46.7,-20.0]). Efficacy results are shown in table 1. Treatment-emergent adverse events (TEAE) occurred in 28 (65%) of the icosabutate group vs. 32 (74%) in the placebo group. No serious AEs were reported; 1 patient in the icosabutate group discontinued treatment due to a drug-related
TEAE ("feeling jittery") versus 0 in the placebo arm.

**Conclusion**

In this 12-week proof-of-concept study, icosabutate 600 mg once daily significantly reduced TG levels. Icosabutate also reduced APOC3 and other lipid markers of atherosclerotic risk. Icosabutate was well tolerated, with a safety profile generally comparable to placebo.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (n=43)</th>
<th>Icosabutate 600 mg once daily (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (IQR)</td>
<td>688 (266, 892)</td>
<td>611 (543, 873)</td>
</tr>
<tr>
<td>12-week (IQR)</td>
<td>590 (445, 774)</td>
<td>514 (304, 456)</td>
</tr>
<tr>
<td>% change vs baseline (IQR)</td>
<td>-17.1 (-30.3, 8.4)</td>
<td>-51.4 (-66.8, 32.4)</td>
</tr>
<tr>
<td>% change vs placebo (95% CI)*</td>
<td>-32.8 (-46.7, 20.6)</td>
<td></td>
</tr>
<tr>
<td>VLDL-C (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (IQR)</td>
<td>118 (102, 148)</td>
<td>106 (95, 153)</td>
</tr>
<tr>
<td>12-week (IQR)</td>
<td>100 (74, 145)</td>
<td>51 (50, 58)</td>
</tr>
<tr>
<td>% change vs baseline (IQR)</td>
<td>-19.7 (-42.7, 20.0)</td>
<td>-50.9 (-68.2, 34.0)</td>
</tr>
<tr>
<td>% change vs placebo (95% CI)*</td>
<td>-35.7 (-51.5, 22.4)</td>
<td></td>
</tr>
<tr>
<td>RLP-C (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (IQR)</td>
<td>40 (35, 46)</td>
<td>38 (31, 73)</td>
</tr>
<tr>
<td>12-week (IQR)</td>
<td>39 (24, 41)</td>
<td>19 (16, 20)</td>
</tr>
<tr>
<td>% change vs baseline (IQR)</td>
<td>-18.8 (-30.8, 46.6)</td>
<td>-19.9 (-68.9, 22.1)</td>
</tr>
<tr>
<td>% change vs placebo (95% CI)*</td>
<td>-33.5 (-51.1, 13.6)</td>
<td></td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (IQR)</td>
<td>70 (55, 97)</td>
<td>92 (75, 134)</td>
</tr>
<tr>
<td>12-week (IQR)</td>
<td>62 (51, 109)</td>
<td>141 (120, 199)</td>
</tr>
<tr>
<td>% change vs baseline (IQR)</td>
<td>-14.0 (-6.0, 24.8)</td>
<td>42.6 (11.7, 71.2)</td>
</tr>
<tr>
<td>% change vs placebo (95% CI)*</td>
<td>-33.5 (-51.1, 13.6)</td>
<td></td>
</tr>
<tr>
<td>Non-HDL-C (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (IQR)</td>
<td>297 (110, 249)</td>
<td>220 (190, 267)</td>
</tr>
<tr>
<td>12-week (IQR)</td>
<td>189 (165, 244)</td>
<td>195 (168, 221)</td>
</tr>
<tr>
<td>% change vs baseline (IQR)</td>
<td>-1.6 (-13.1, 9.1)</td>
<td>-8.1 (-19.0, 3.6)</td>
</tr>
<tr>
<td>% change vs placebo (95% CI)*</td>
<td>-7.1 (16.2, 0.3)</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (IQR)</td>
<td>31 (26, 33)</td>
<td>32 (29, 37)</td>
</tr>
<tr>
<td>12-week (IQR)</td>
<td>30 (27, 34)</td>
<td>38 (24, 41)</td>
</tr>
<tr>
<td>% change vs baseline (IQR)</td>
<td>0.0 (-5.0, 11.5)</td>
<td>23.8 (9.7, 32.4)</td>
</tr>
<tr>
<td>% change vs placebo (95% CI)*</td>
<td>10.3 (11.0, 25.0)</td>
<td></td>
</tr>
<tr>
<td>APOC3 (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (IQR)</td>
<td>38 (23, 33)</td>
<td>39 (24, 34)</td>
</tr>
<tr>
<td>12-week (IQR)</td>
<td>37 (26, 34)</td>
<td>16 (13, 39)</td>
</tr>
<tr>
<td>% change vs baseline (IQR)</td>
<td>-4.7 (-23.6, 23.1)</td>
<td>-41.3 (-53.9, -28.9)</td>
</tr>
<tr>
<td>% change vs placebo (95% CI)*</td>
<td>-24.0 (-46.8, 22.2)</td>
<td></td>
</tr>
</tbody>
</table>

APOC3, apolipoprotein C3; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; RLP-C, remnant lipoprotein cholesterol; TG, triglycerides; VLDL-C, very-low-density lipoprotein cholesterol.

*Asymptotic 95% Hodges-Lehmann CI

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Icosabutate, a novel structurally enhanced fatty acid (SEFA), increases hepatic uptake of cholesterol, triglycerides and hepatic LDL receptor expression

Abstract nr. 420
Author Fraser, David, Pronova BioPharma- part of BASF, Oslo, Norway
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Co-author(s) - Larsen, Laila N.
Co-author(s) - Husberg, Cathrine
Co-author(s) - Hovland, Ragnar
Co-author(s) - Pieterman, Elsbeth J.
Co-author(s) - Hoek, Anita M van den
Co-author(s) - Princen, Hans MG
Co-author(s) - Hustvedt, Svein Olaf
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Hypolipidemic Drugs, LDL, Lipids, Triglycerides

Background:
At a tenth of the dose of unmodified omega-3 fatty acids, icosabutate, a structurally enhanced fatty acid (SEFA) achieves potent lowering of both plasma triglycerides (TG) and non-HDL cholesterol (C) in APOE*3Leiden.CETP transgenic mice. The mechanism/s by which Icosabutate exerts its hypolipidemic effect was investigated.

Methods:
Male APOE*3Leiden.CETP mice were fed a semi-synthetic Western-type diet (WTD, 15% cocoa butter, 40% sucrose and 0.25% cholesterol; all w/w) without or with icosabutate (112 mg/kg bw/day). Hepatic production and clearance of lipids/lipoproteins, lipolytic activity, hepatic lipids and expression of LDL receptor protein (LDLR) were assessed. Predicted transcription factor activation was determined by Ingenuity upstream regulator analysis.

Results:
After 4 to 6 weeks of treatment icosabutate lowered both plasma TG and C, confined to the non-HDL particles, by 68% (p<0.001 vs. control). No significant effects were seen in lipoprotein lipase and hepatic lipase activity. However, hepatic uptake of VLDL-like $^{14}$C-cholesteryl oleate particles (as marker for VLDL-CE) and $^{3}$H-triolein (as marker for VLDL-TG) were significantly increased vs. control by 72% and 87%, respectively (both p<0.001). Icosabutate tended to decrease hepatic TG (38%, p=0.083) and significantly decreased CE (25%, p<0.05). Hepatic expression of LDLR protein was increased 1.9 fold (p<0.05) without change in LRP protein. In contrast to fenofibrate, icosabutate (1) activated sterol regulatory element binding factor 2 (SREBF2) and (2) did not increase in hepatic VLDL-TG production rate.

Conclusion:
Icosabutate lowers plasma lipids primarily via a markedly increased hepatic uptake and is
associated with a significant increase in hepatic LDLR expression (which may occur secondary to
*SREBF2* activation). Despite the increased hepatic TG uptake, no compensatory increase in
hepatic lipid storage or TG production was observed. Icosabutate thus offers a promising new
approach to lowering plasma TG and non-HDL cholesterol.

**Subdivision 1. Basic Science**

**Presentation Preference** Oral presentation

**Additional information**
Elucidation of the finer structural changes of ApoA1 upon addition of cholesterol by solution based small-angle X-ray scattering

Abstract nr. 421
Author Midtgaard, Søren, University of Copenhagen, Copenhagen E, Denmark
Co-author(s) - Pedersen, Martin
Co-author(s) - Arleth, Lise
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, HDL, Lipoproteins, Reverse Cholesterol Transport

Structural and functional aspects of high density lipoproteins have been studied for over half a century. Due to the plasticity of this highly complex system, new aspects continue to be investigated. Here, we present a structural study of the human Apolipoprotein A1 (ApoA1) and the role of its N-terminal domain, the so-called globular domain of ApoA1, in discoidal complexes with phospholipids and increasing amounts of cholesterol. Using a powerful approach of solution-based small-angle X-ray scattering (SAXS) and rigorous data modeling, we show that the ApoA1-based particles are disc-shaped with an elliptical cross-section and composed by a central lipid bilayer surrounded by two stabilizing ApoA1 proteins. This structure is very similar to the particles formed in the so-called Nanodisc system, which is based on a N-terminal truncated ApoA1 protein. The N-terminal domain of ApoA1 is found not to be globular but instead to be an integrated part of the “protein belt” stabilizing the particles. Upon incorporation of increasing amounts of cholesterol into the particles, the presence of the N-terminal domain allows the bilayer thickness to increase while an overall flat bilayer structure is maintained. This is contrasted by the energetically more strained and less favorable lens-shape observed when the N-terminal domain is not present. Based on this it is concluded that the N-terminal domain of ApoA1 actively participates in the stabilization of the discoidal particle and allows for a more optimal lipid packing upon cholesterol uptake.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Case report of successful reintroduction of statin therapy following statin-associated rhabdomyolysis

Abstract nr. 422
Author Don-Wauchope, Andrew, McMaster University, Hamilton, Canada
Co-author(s) - Simons, Janet
Co-author(s) - Holbrook, Anne
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Hypolipidemic Drugs

Introduction
HMG-CoA reductase inhibitors (statins) are discontinued following the serious adverse event of rhabdomyolysis. There is no recommendation for ongoing lipid lowering therapy in patients who have had a statin related serious adverse event. Typically alternative agents to HMG-CoA reductase inhibitors such as bile acid sequestering resins, nicotinic acid and ezetemibe will be prescribed in an attempt lower LDL-cholesterol.

Case Report
The case report demonstrates a successful use of an alternative statin following a statin related episode of rhabdomyolysis. The CK peaked at 47595 U/L and the patients creatinine rose from 75 mmol/L to 107 mmol/L. With fluid management the patient recovered in hospital. He was discharged home without any lipid lowering medication. One year later, the patient suffered a myocardial infarct and as part of his cardiac rehabilitation was started on non-statin lipid lowering medication. He was subsequently referred to the lipid clinic for specialist management. He used alternative lipid lowering therapy for 2 years. His LDL-cholesterol at 3.05 mmol/L was not meeting typical secondary prevention targets on a combination of ezetemibe and cholestyramine. After careful discussion with the patient an alternative statin was reintroduced at a low dose. The patient was followed closely for changes in CK and his results ranged from 65-141 U/L. He has been followed for 4 years without recurrence of the rhabdomyolysis nor has had he had any symptoms from his coronary artery disease. His LDL-cholesterol of 1.91 mmol/L is now at Canadian targets for secondary prevention.

Conclusion
Rhabdomyolysis is a rare side effect of statin therapy and this case suggests it may be time to reconsider if this event requires permanent avoidance of statin therapy.
Table 1 Patient lipid profiles before and after re-challenge with rosvastatin

<table>
<thead>
<tr>
<th>Event</th>
<th>Total Cholesterol (mmol/L)</th>
<th>HDL-Cholesterol (mmol/L)</th>
<th>LDL-Cholesterol (mmol/L)</th>
<th>Triglycerides (mmol/L)</th>
<th>CK (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial presentation post MI</td>
<td>4.58</td>
<td>0.76</td>
<td>2.48</td>
<td>2.92</td>
<td>77</td>
</tr>
<tr>
<td>Initiation of Rosuvastatin (2 years after initial presentation)</td>
<td>5.38</td>
<td>0.78</td>
<td>3.05</td>
<td>3.40</td>
<td>139</td>
</tr>
<tr>
<td>6 weeks post statin</td>
<td>4.65</td>
<td>0.84</td>
<td>2.59</td>
<td>2.68</td>
<td>106</td>
</tr>
<tr>
<td>One year post statin</td>
<td>3.45</td>
<td>0.89</td>
<td>1.81</td>
<td>1.64</td>
<td>65</td>
</tr>
<tr>
<td>Four years post statin</td>
<td>3.31</td>
<td>0.71</td>
<td>1.91</td>
<td>1.52</td>
<td>141</td>
</tr>
</tbody>
</table>

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
A new fatty acid profile score: a predictor of coronary heart disease

Abstract nr. 423
Author Giroli, Monica G., Centro Cardiologico Monzino, IRCCS, Milan, Italy
Co-author(s) - Veglia, Fabrizio
Co-author(s) - Laguzzi, Federica
Co-author(s) - Risé, Patrizia
Co-author(s) - Werba, José P.
Co-author(s) - Tremoli, Elena
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Lipids, Nutrition

Background and aim:
Fatty acids influence lipoprotein levels and take part in metabolic pathways of inflammation and thrombosis, potentially representing a cardiovascular risk factor. Aim of this study was to compare the blood fatty acid (FA) profile between Coronary Heart Disease (CHD) patients and healthy controls, in order to find a predictor of CHD more accurate than single FAs or FA classes (saturated SFA, monounsaturated MUFA or polyunsaturated fatty acids PUFA).

Methods: In a case-control study, we evaluated 179 patients with CHD and 155 age-and gender matched healthy controls, mean age 62.5±7 for both groups. The FA profile was determined in fasting blood by gas-chromatography. The association of FAs with CHD was assessed by logistic regression, with adjustment for clinical variables (anthropometric data, traditional risk factors and current drug therapies) and life habits (diet by EPIC food frequency questionnaire, smoke and level of physical activity). The most predictive FAs were combined in a numeric Fatty Acid Profile Score (FAPS) providing the predicted risk of CHD. The reproducibility of the results was assessed by an internal cross-validation procedure.

Results: Five FAs were selected by the logistic regression to be included in the FAPS: three with a positive association with CHD [Palmitic acid (C16:0), Erucic acid (C22:1 n-9) and Arachidonic acid (C20:4 n-6)] and two with a negative association [Palmitoleic acid (C16:1 n-7) and Ecosatrienoic acid (C20:3 n-9)]. The FAPS was strongly associated with CHD status [OR for 0.1 point increment = 1.78 (95% c.i. 1.44, 2.21)] adjusting for diet, education, LDL and HDL Cholesterol, triglycerides waist circumference, DBP, pack-years and statin use. In ROC curve analysis the AUC was 0.83. Moreover, FAPS was significantly correlated with several dietary items usually considered to be unhealthy.

Conclusion: The FA score was a significantly better predictor of CHD status than single FA or FA classes (SFA, MUFA or PUFA). These data suggest that the former may be a useful index of CHD risk, and prompt for future studies aimed at assessing whether dietary interventions favorably change an adverse FAPS in patients with CHD.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Mini-oral presentation
Additional information
HDL Particles and HDL Functions

Abstract nr. 424
Author Asztalos, Bela, Tufts University, Boston, United States of America
Co-author(s) - Horvath, Katalin
Co-author(s) - Goldfine, Allison
Co-author(s) - Schaefer, Ernst
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, HDL, Lipoproteins, Reverse Cholesterol Transport

Introduction: The role of HDL in CVD risk is not fully understood.
Hypothesis: We tested the hypothesis that HDL functions are significantly associated with HDL subpopulation profile.
Methods: Lipoproteins, including apoA-I-containing HDL particles, cell-cholesterol efflux (Global, ABCA1- and SRB1-mediated) and HDL Inflammatory Index were measured in a total of 289 subjects. These included 60 CHD patients, 109 subjects with either abnormal lipids (high triglycerides/low HDL or elevated β-sitosterol) or increased levels of the inflammatory markers C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, or myeloperoxidase (MPO), as well as 120 controls.
Results: Global efflux was highly correlated with ABCA1-mediated efflux (r=0.921). Cholesterol efflux via the ABCA1 pathway was significantly correlated with small prebeta-1 HDL particles (r=0.762). CHD patients had significantly higher preβ-1 level than controls; however, per-particle prebeta-1 HDL effluxed 41% less cholesterol in CHD patients as compared to controls, resulting in no significant difference in overall efflux capacity between cases and controls. Cholesterol efflux via the SRB1 pathway was significantly correlated with large α-1 (r=0.784) and α-2 (r=0.460) HDL particles. These two large HDL particles effluxed about 82% of cell cholesterol via the SRB1 pathway. There was no significant difference in SRB1-mediated cholesterol efflux between CHD cases and controls. The HDL Inflammatory Index (HII) was significantly higher (unfavorable) in CHD cases than in controls and was significantly (inversely) associated with α-2 HDL particles (r=454). Increased levels of β-sitosterol significantly altered the HDL subpopulation profile and increased HII. Moreover, HII was not correlated with the concentrations of CRP, SAA, fibrinogen and MPO.
Conclusion: HDL subpopulation profile is a major determinant of HDL’s overall cholesterol-efflux capacity. Efflux capacity of preβ-1 particles are compromised in CHD patients by yet unknown factor(s). HDL Inflammatory Index correlates with HDL subpopulation profile, but not with markers of inflammation in plasma.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Leukadherin Prevents Neointimal Hyperplasia after Vascular Injury and Atherosclerosis Diseases Severity in Hypercholesterolemic Mice

Abstract nr. 425
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation

Background: Leukadherins (LA) is novel family of Mac-1 agonists that increase leukocyte adhesion while preventing inflammatory cell mobilization and inflammation. This study demonstrates that leukadherin LA1 protects hypercholesterolemic ApoE-null mice from excessive atherosclerosis development and rat injured vessels from pathological development of neointimal hyperplasia (NIH). Methods and Results: LA1 caused no toxicity or abnormality in body weight and blood serum chemistry values in mice and rats. The daily administration of LA1 significantly reduced atherosclerosis in high fat diet fed ApoE-null mice as determined by Sudan IV en face analysis of the entire aorta. Interestingly, LA1 impaired monocyte emigration from medullary and extramedullary centers. LA1-treated mice showed less circulating but more BM resident monocytes (Lin2− cd11c− cd11b+) with respect to control animals. The increased number of monocytes occurred without notable changes in myeloid progenitor cells, which suggests that the impaired mobilization of leukocytes was due to the chronic activation of Mac-1. LA1 didn’t modify in-vitro foam cell formation. Finally, we determined if the anti-inflammatory effects of LA1 could accelerate vascular re-endothelialization (healing) thereby decreasing post injury neointimal formation in LA-treated versus control (vehicle) rats. Only minimal blue staining was observed in injured arteries of LA1-treated rats while control animals showed minimal re-endothelialization. LA1 reduced also inflammation and neointimal formation after vascular injury. Conclusions: Mac-1 activation with LA1 significantly protects vasculature from vascular proliferative diseases most likely by compromising monocyte mobilization from medullary and extramedullary centers.
INFLUENCE OF STEROLS ON CHOLESTEROL CONCENTRATIONS IN PATIENTS WITH MUTATIONS IN THE LDL-RECEPTOR GENE

Abstract nr. 426
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Familial Hypercholesterolemia, LDL, Nutrition, Therapy

Familial hypercholesterolemia (FH) caused by mutations in the LDL receptor gene belongs to the most common genetic defects leading to premature coronary heart disease. In Poland heterozygous FH has recognized in about 76,000 subjects. The cornerstones of treatment for FH are diet and lifestyle modifications and pharmacotherapy. The effective dietary regimen is an important issue in the management of this life-long disease. Therefore, the aim of the present study was to assess the effectiveness of dietary intervention and plant sterols intake for lowering serum and LDL-cholesterol in patients with genetic diagnosis of FH. The group of 39 patients (aged 18-60 years) with defined mutations in the LDL-receptor gene took part in the study. A 14 day menus were prepared for total energy intakes of 1500 and 1800 kcal and used for four weeks by study participants (baseline), and in addition to dietary recommendations all patients were taken 2g plant sterols/day for additional 6 weeks. All patients were under supervision of a dietitian. The 6 weeks intervention was associated with a significant (p< 0.05) decrease of serum LDL-cholesterol from 180 (±63) mg/dl (range: 75 – 369 mg/dl) to 144(± 49) mg/dl (range: 60 - 274 mg/dl) and total serum cholesterol concentrations from 263(±64) mg/dl (range: 185 – 439 mg/dl) to 222(±48) mg/dl (range: 174 - 346 mg/dl). No significant changes in HDL-cholesterol were observed (before: 55(± 13) mg/dl (range: 25 – 70 mg/dl) and after the intervention: 54,9 (± 11,7) mg/dl (range: 32 – 78 mg/dl)). The proposed dietary intervention decreased both LDL-cholesterol and total serum cholesterol concentrations in all study participants indicating that plant sterols can independently on pharmacological therapy significantly decrease LDL-cholesterol levels in patients with different mutations in the LDL-receptor gene.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Accumulation of Remnant Lipoproteins in Patients with Type II Diabetes Mellitus is ameliorated by the DPP4-Inhibitor, Sitagliptin

Abstract nr. 427
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins,Diabetes,Triglyceride-Rich Proteins,Triglycerides

In patients with type II diabetes mellitus, fasting hypertriglyceridermia and low HDL cholesterol level are often complicated. Fasting hypertriglyceridermia is caused by the accumulation of TG-rich lipoproteins (TRL) and remnant lipoproteins, which are highly atherogenic. Therefore, impaired lipoprotein metabolism in combination with diabetes may enhance the risk status for atherosclerotic cardiovascular diseases. Recently, it was shown that a DPP4 inhibitor improved both fasting and postprandial hypertriglyceridermia in diabetic patients, however, its effectiveness for lipoprotein profile was unknown. In this study, we investigated whether the sitagliptin might ameliorate the impaired lipoprotein metabolism in patients with type II diabetes mellitus. We enrolled 38 patients with type II diabetes mellitus whose HbA1c levels were less than 8.4% and all patients gave written informed consents. The oral administration of sitagliptin (50mg/day) was started in addition to the current anti-diabetic treatments. The daily dose of sitagliptin was allowed to increase up to 100 mg/day in order to achieve their HbA1c levels less than 7.4%. We compared biomarkers for glucose metabolism (fasting plasma glucose, HbA1c, insulin and glucagon), lipoprotein metabolism (LDL-C, HDL-C, TG, and apolipoproteins such as apo AI, AI, B, CII, CIII and E) and remnants (RemL-C and apoB-48). Cholesterol and TG concentrations of lipoprotein fractions in the size of CM, VLDL, LDL and HDL were compared by the high performance liquid chromatography (HPLC) analysis. There were significant decreases in fasting glucose levels (150±47 vs 129±27 mg/dl, p<0.01) and HbA1c levels (7.1±0.6 vs 6.6±0.7 mg/dl, p<0.001) as well as fasting TG levels (161±90 vs 130±66 mg/dl, p<0.01) and non HDL-C levels (129±29 vs 116±20 mg/dl, p<0.01). Fasting apoB-48 and RemL-C levels were significantly decreased by sitagliptin (7.8±6.7 vs 5.6±4.0 μg/ml, p<0.01, 15.3±9.5 vs 12.0±7.9 mg/dl, p<0.05, respectively) as well as apoB, CII, CIII and E. Cholesterol and TG concentrations of lipoprotein fractions in the size of VLDL and LDL were significantly decreased by sitagliptin. These findings indicated that the
sitagliptin treatment improved the lipid and lipoprotein profile in patients with type II diabetes mellitus, which might be due to the decrease in atherogenic remnant lipoproteins.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
The effect of dyslipidemia and weight by physical activity and dietary measures in children

Abstract nr. 428
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Dyslipidemia, Lifestyle, Metabolism, Obesity

Objectives: The aim of the study was to monitor the importance of laboratory, anthropometric and genetic determination of the presence of risk factors for atherosclerosis, obesity, dyslipidemia and components of the metabolic syndrome in obese children and the response to dietary and regimen interventions in obese children.

Design and Methods: Within the project, 353 pediatric patients (46% boys, 54% girls) aged 8-16 years, who took part in a one-month lifestyle intervention program (comprising a reduction of energy intake and a supervised exercise program consisting of 5 exercise units per day, 50 min each) were examined with obesity and dyslipidemia. Standard biochemical methods, including Lp-PLA2 were applied, anthropometric measurements as well as genetic analyzes were performed.

Results: During the reduction program of children, there was a statistically significant decrease in all anthropometric indicators of overweight (<0.001) and in lipid parameters, as well as LpLPA2. Carriers of the FTO GG genotype and/or MC4R CC genotype lost significantly more body weight in comparison to the non-carriers(<0.0009 for BMI and P<0.002 for body weight).

Conclusion: Child obesity is an important social issue. After regimen interventions, there is weight loss, as well as improvement in biochemical parameters. There are individuals with obesity predispositions, as well as individuals with a better response to regimen interventions e.g. carriers of the FTO GG genotype and the MC4R CC genotype.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
CO-induced protein S-glutathionylation modulates STAT3 activation via heme-oxygenase-1 expression

Abstract nr. 429
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Endothelium, Inflammation

Background: IL-6/STAT3 pathway is involved in a variety of biological responses, including cell proliferation, differentiation, apoptosis, and inflammation. Protein glutathionylation is a protective mechanism that functions in response to mild oxidative stress. Carbon monoxide (CO) has been found to increase a low level ROS and which may induce protein glutathionylation. We hypothesized that CO increases protein glutathionylation and inhibits STAT3 activation.

Methods: Bovine aortic endothelial cells (BAECs) from whole bovine aortas were exposed to CO releasing molecules (CORMs). The treated cells were observed the redox state by investigation of the GSSG and ROS level. Protein S-glutathionylation was detected by Bio-GEE and immunoprecipitation. The inhibition mechanism of STAT3 activity was investigated in nuclear translocation and reporter assay. The siRNA of heme-oxygenase-1 (HO-1) were used to examine the regulatory mechanism.

Results: CORMs suppress IL-6-induced STAT3 phosphorylation, nuclear translocation and transactivity in ECs. CO is a bi-product of heme degradation mediated by HO-1. However, CORMs can induce HO-1 expression and then inhibit STAT3 phosphorylation. We found that CORMs increase the intracellular GSSG level and induce the glutathionylation of multiple proteins including STAT3. GSSG can inhibit STAT3 phosphorylation and increase STAT3 glutathionylation whereas the antioxidant enzyme catalase can suppress the glutathionylation. Furthermore, catalase blocks the inhibition of STAT3 phosphorylation by CORMs treatment. We further found that HO-1 increases STAT3 glutathionylation and that HO-1 siRNA attenuates CORM-induced STAT3 glutathionylation.

Conclusions: Our current results thus indicate that the inhibition of STAT3 activation is likely to occur via a CO-mediated increase in the GSSG level, which augments protein glutathionylation, and CO-induced HO-1 expression, which may enhance and maintain its effects in IL-6-treated ECs.
Proposed model
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Effects of increased potassium and sodium on endothelial and vascular function

Abstract nr. 430
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Endothelium, Nutrition

Background: Increased potassium intake has been related to improved endothelial function and a high sodium intake is known to impair endothelial function. The effect of increasing potassium in the presence of high sodium in the postprandial state is not known.

Objective: The aim was to determine the effect of increased potassium and increased sodium on post prandial endothelial function (as assessed by flow mediated dilatation (FMD)) and arterial compliance as assessed by pulse wave velocity (PWV) and augmentation index (AIx).

Methods: Thirty nine healthy, normotensive volunteers (age 37±15 and BMI 23.0±2.8) received a meal with 3.1mmol potassium and 65mmol sodium (LKHN), a meal with 38mmol potassium and 65mmol sodium (HKHN) and a control meal (LKLN) with 5.5mmol sodium and 3.1mmol potassium on three separate occasions in a randomized order. FMD, PWV, Alx and BP were measured while participants were fasting and at 30, 60, 90 and 120 minutes after the meal. Repeated-measures ANOVA was used to assess the effects of the meal type on the dependent variables over time.

Results: The addition of potassium (HKHN meal) significantly attenuated the post meal decrease in FMD when compared to the high sodium meal (p<0.05 meal by time) (Figure 1). FMD was significantly lower following the LKHN meal when compared to the HKHN meal at 30 minutes (p<0.05). Alx decreased after all meals (p<0.05). There were no significant differences in Alx, PWV or BP between treatments over time.

Conclusion: The addition of potassium to a high sodium meal attenuates the post meal reduction in endothelial function as assessed by FMD. There were no between meal differences on PWV and Alx.
Figure 1: (a) Mean (±SEM) brachial artery FMD at baseline and in response to consumption of test meals containing 3 mmol of potassium and 65 mmol of sodium (HHKN) versus 3 mmol of potassium and 65 mmol of sodium (LRHN). n = 39 (18 men and 21 women). Repeated measures ANOVA: p<0.01 for meal effect; p=0.07 for time; p=0.02 for meal x time interaction. (b) Mean (±SEM) brachial artery FMD at baseline and in response to consumption of test meals containing 3 mmol of potassium and 6 mmol of sodium (HHKN) versus 3 mmol of potassium and 6 mmol of sodium (LRHN). n = 39 (18 men and 21 women). Repeated measures ANOVA: p<0.05 for meal effect; p=0.07 for time; p=0.07 for meal x time interaction.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation

Additional information
Long-term consumption of NaCl resulted severe degradation of lipoprotein associated with hyperlipidemia, hyperglycemia, and infertility via impairment of testicular spermatogenesis

Abstract nr. 431
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Lipoproteins, Risk Factor

Background: Although the effect of NaCl on serum lipid levels and hypertension has been well known, the detail mechanism on lipoprotein metabolism is still remained unclear.

Objective: To study physiological effects of high salt consumption in lipoprotein metabolism, NaCl was treated to human cells and zebrafish.

Methods: Wildtype zebrafish (10-week old) were fed NaCl (final 5%, wt/wt) in tetrabit diet with or without 4% cholesterol (wt/wt) for 21 weeks.

Results: Treatment with NaCl accelerated oxidation and glycation of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) as well as induced proteolytic degradation and aggregation. NaCl treatment also exacerbated phagocytosis of oxLDL into macrophage as well as cytotoxicity. Consumption of high salt diet (HSD, final 5% diet, wt/wt) containing with or without 4% cholesterol for 21 weeks resulted remarkable elevation of serum cholesterol, triglyceride, glucose, and hepatic inflammation levels in zebrafish with significant weight loss. Fertility based on egg production was reduced by up to 45% in the HSD group. However, embryonic survivability after hatching was significantly lowered to less than 55%, whereas the control group showed 87% survival. HSD group showed abnormal testicular histology as well as spermatogenic defects, especially upon consumption of HCD.

Conclusion: These results suggest that hyperlipidemia and high salt consumption have an additive effect on male fertility impairment. High salt consumption exacerbates hyperlipidemia, inflammation, spermatogenic defects, and infertility via modification of lipoproteins.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
IMPAIRED SYNTHESIS OF SPECIALISED PRORESOLVING LIPID MEDIATORS IN THE METABOLIC SYNDROME AFTER n-3 FATTY ACID SUPPLEMENTATION AND ASPIRIN

Abstract nr. 432
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Inflammation, Intervention, Nutrition, Obesity

**Background:** The metabolic syndrome (MetS) is associated with a chronic low-grade inflammatory state. The ability to resolve inflammation can affect immune responses and is an active process driven by specialised proresolving lipid mediators (SPM) derived from n-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). **Aim:** To examine the effect of n-3 fatty acid supplements and aspirin on plasma SPM in a case control study of volunteers with the MetS. **Methods:** 21 controls and 22 MetS volunteers entered a 4 week study taking n-3 fatty acids (2.4g/day, 35% EPA + 25% DHA) with the addition of aspirin (300mg/day) during the last 7 days. Blood was collected at baseline, after 3 weeks of n-3 fatty acids and after 4 weeks (7 days of aspirin) for measurement of plasma SPM including 18-hydroxyeicosapentaenoic acid (18-HEPE), and E-series resolvins, 17-hydroxydocosahexaenoic acid (17-HDHA) and D-series resolvins and protectins, and 14-hydroxydocosahexaenoic acid (14-HDHA) and maresin-1 (MaR-1), using liquid chromatography tandem mass spectrometry. **Results:** Volunteers were aged 57-59 years and those with MetS had significantly higher BMI, waist circumference, blood pressure, triglycerides and glucose. Baseline plasma phospholipid EPA and DHA were not different between the groups and were significantly increased in both groups following n-3 fatty acids. At baseline concentrations of all SPM were not different between the groups. There was a significant attenuation of the increase in 18-HEPE, 17-HDHA and 14-HDHA after 3 weeks of n-3 fatty acids in the MetS group (P<0.05). Plasma E series resolvins were increased (P<0.05) after n-3 fatty acids but the levels did not significantly differ between the MetS and control groups. The D-series resolvins, protectins and MaR-1 were not significantly altered by n-3 fatty acids in either group. The addition of aspirin to n-3 fatty acid supplements during the last 7 days did not significantly alter SPM in either group. **Conclusion:** Reduced levels SPM precursors in the MetS after n-3 fatty acid supplementation suggests that resolution of inflammation may be impaired affecting the ability to mount an appropriate immune response to infection.

Funding: National Heart Foundation of Australia

Subdivision 3. Clinical Studies
Presentation Preference Oral presentation
Additional information
Rs6922269 marker at the MTHFD1L gene predict cardiovascular mortality in males after acute coronary syndrome

Abstract nr. 433
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords ACS, Genetics, Risk stratification

Introduction: Myocardial infarction (MI) is the leading cause of death in industrialized countries. Attention therefore has recently focused on genetic variants that are not associated with conventional risk factors. One of them is the marker rs6922269, which has been suggested as a risk factor for development of MI in Western populations.

Methods: We analyzed the relationship between rs6922269 variant on MTHFD1L gene and i/ risk of the acute coronary syndrome (ACS) in the Czech population and ii/ mortality in 7 years follow up.

Results: Rs6922269 (G>A) variant was analyzed (CR = 99.3% for patients and 98.0% for controls) in consecutively examined 1,614 men and 503 women with ACS (age below 65 years) and in population-based controls – 1,191 men and 1,368 women (aged up to 65 years). ANOVA and chi-square were used for statistical analysis. The genotype frequencies were almost identical (P = 0.87) in the ACS patients and in controls and no differences were observed, if males (P = 0.73) and females (P = 0.93) were analysed separately. In addition, rs6922269 polymorphism was not associated with the classical risk factors (dyslipidemia, hypertension, obesity, smoking, diabetes) in control population. Cardiovascular mortality was significantly higher in males, carriers of the AA genotype (P < 0.001, OR = 2.52, 95%CI 1.40–4.55, for AA vs. +G).

Conclusion: We conclude, that rs6922269 variant at MTHFD1L gene could be an important prognostic factor for cardiovascular mortality in patients after ACS. This work was supported by project NT12217-5 (Internal Grant Agency, Ministry of Health, Czech Republic)
**Thrombin generation indices and subclinical atherosclerosis**

Abstract nr. 434  
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Topic Epidemiology of CVD; The Risk Factor Concept  
Keywords Atherosclerosis, Cardiovascular Disease, Imaging, Thrombosis

**Introduction:** In addition to thrombus formation, thrombin generation has been suggested to have a pro-atherogenic role and may be involved in the development of subclinical atherosclerosis. We tested the association between indices of thrombin generation (TG) with subclinical atherosclerosis and clinical cardiovascular disease (CVD).

**Methods:** Presence of atherosclerotic plaques in any of the four main bifurcations (both common carotid and femoral arteries) was assessed with B-mode ultrasound in community-dwellers from the “Cyprus study” cohort. Prevalent cardiovascular disease (myocardial infarct, stroke, transient ischemic attack, angina and lower limb ischemia) was also assessed. In a cohort of 539 individuals thrombin generation was assessed using the Calibrated Automated Thrombogram assay (PPP-Reagent-5pM Diagnostica Stago, France). The DecayIndex, a new parameter of thrombogram calculated by the formula \( DI = \frac{\text{Peak}}{\text{StartTail} - \text{ttPeak}} \), was investigated. The profile of TG was compared between control subjects (no prevalent CVD, no atherosclerotic plaques and no anti-hyperlipidemic, anti-hypertensive or anti-platelet therapy), subjects with subclinical atherosclerosis (no prevalent CVD but having any atherosclerotic plaque present) and subjects with prevalent CVD, using t-test and logistic regression analysis. Subject taking anticoagulants were excluded.

**Results:** Subjects with both subclinical atherosclerosis and prevalent CVD had significantly longer LagTime (\( p=0.02 \) and 0.005 respectively) and startTail (\( p=0.035 \) and 0.0003 respectively) compared to controls and lower levels of Decay Index (\( p=0.03 \) and 0.002 respectively). After adjustment for age and sex, LagTime, startTail and DecayIndex were no longer associated with prevalent CVD compared to controls (\( p=0.32, \) 0.08 and 0.41 respectively). However, subjects with atherosclerotic plaques had significantly prolonged startTail and lower levels of DecayIndex compared to controls, with a one standard deviation (SD) increase in startTail levels being associated with a 1.35 times the odds for having any plaque present (\( OR_{\text{adjusted}} = 1.35; \) 95%CI=1.007 to 1.81; \( p=0.045 \)) and a one SD increase in DecayIndex being associated with 0.76 times the odds for plaques (\( OR_{\text{adjusted}} = 0.76; \) 95%CI=0.59 to 0.98; \( p=0.032 \)). LagTime was not associated with subclinical atherosclerosis after adjustment.

**Conclusions:** Our results show that prolongation of the startTail and a decreased Decay Index are
associated with subclinical atherosclerosis (i.e. plaques) in a community dwelling population of Cypriot origin, supporting a pro-atherogenic role for thrombin generation.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Mini-oral presentation
Additional information
Intermediate-density lipoprotein net charge is an independent risk factor to atherosclerosis in patients with systemic lupus erythematosus

Abstract nr. 435
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Lipoproteins

Background: Systemic lupus erythematosus (SLE) is a chronic, multisystemic, inflammatory and autoimmune disease which mainly affects women. Patients with SLE have an accelerated atherosclerosis and cardiovascular disease (CVD) is the commonest cause of death, despite presenting rather normal lipid profile and cardiovascular risk scores. We seek to identify additional factors that can help explain residual risk in these patients.

Objectives: The aim of this study is to investigate whether the net charge of each individual lipoprotein has a significant contribution to atherosclerosis in SLE patients.

Material and methods: Lipoproteins of eighty-two SLE patients were isolated by sequential ultracentrifugation and net charge of VLDL, IDL, LDL and HDL were measured with Zetasizer Nano-Zs. Net charge was correlated to carotid intima-media thickness (IMT) which is considered as a subclinical atherosclerosis marker.

Results: All lipoproteins showed a negative net charge. VLDL and HDL particles were significantly more electronegative (-21.72 mV and -22.28 mV, respectively) than IDL and LDL (-17.61 mV and -16.61 mV, respectively) (p< 0.001). IDL net charge was negatively correlated with IMT (R= -0.260; p= 0.021) in SLE patients adjusting for age, BMI and gender. IDL net charge explained 8.7% of the IMT variability in these patients, and this contribution was independent of age, BMI, gender, LDL cholesterol and number of IDL particles.

Conclusion: IDL net charge could be considered as a new biomarker of subclinical atherosclerosis (IMT) in SLE patients. The observed association was independent of age and lipid concentrations and reinforces the prominent role that IDL has for cardiovascular risk in SLE.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Attenuation of vascular calcification by inhibition of matrix metalloproteinases involves impaired Wnt signaling

Abstract nr. 436
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Chronic Kidney Disease, Pharmacology, Prevention

**Aims:** The matrix metalloproteinases (MMP) MMP-2 and MMP-9 are physiological regulators of vascular remodeling. Their dysregulation could contribute to vascular calcification. Although previous studies have shown preventive effects of MMP inhibition on vascular calcification, potential mechanisms underlying these effects have not been described. We therefore aimed to investigate the individual contribution of MMP-2 and MMP-9 to vascular calcification with a special focus on the Wnt pathway which is known to promote the expression of certain calcification and osteoblast marker genes.

**Methods:** The impact of pharmacological MMP inhibition on the development of media calcifications was studied in an in vitro model of vascular calcification using murine vascular smooth muscle cells (VSMC). VSMC phenotypic transition and calcification were induced by a calcification medium (CM) containing elevated levels of Ca^{2+} (2.7 vs. 1.8 mM) and PO_{4}^{3-} (2.8 vs. 1.0 mM) and detected by alizarin red staining and measurements of calcium contents and release of alkaline phosphatase. Gene expressions were analysed by RT-PCR. MMP activities were determined by specific substrate assays and gelatin zymographies and were modulated by recombinant MMPs, specific inhibitors and siRNA. Wnt signalling was analysed by luciferase reporter gene assays.

**Results:** Treatment with CM resulted in marked calcifications which were further increased by the presence of recombinant MMP-2 or MMP-9. This was accompanied by enhanced secretion of gelatinases by VSMC. Vice versa, specific inhibitors of MMP-2 or -9, of both gelatinases (Ro28-2653) and a selective knockdown of MMP-2/-9 mRNA expression blocked CM-induced calcifications of murine VSMC. The induction of calcifications by CM involved activation of the Wnt-pathway, which was attenuated by MMP-inhibitors.

**Conclusions:** These data indicate that both gelatinases provide essential signals for phenotypic VSMC conversion and the initiation of vascular calcification. Their inhibition seems a promising strategy in the prevention of vascular calcifications.

Subdivision 1. Basic Science
Presentation Preference
Electronic poster presentation
Additional information
PCSK9 inhibition for Autosomal Recessive Hypercholesterolemia

Abstract nr. 437
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia,Hypolipidemic Drugs,LDL,PCSK9

**Rationale:** Proprotein Convertase Subtilisin Kexin Type 9 (PCSK9) inhibitors lower Low-Density Lipoprotein Cholesterol (LDL-C) by more than 50% in Heterozygous Familial Hypercholesterolemia (FH) and by 30% in receptor defective Homozygous FH patients.

**Objectives:** Will PCSK9 inhibition with monoclonal antibodies, particularly alirocumab, be of therapeutic value for patients with Autosomal Recessive Hypercholesterolemia (ARH)?

**Methods and Results:** Fasting plasma and primary lymphocytes were obtained from 28 ARH patients genetically characterized for the presence of mutations in the LDL receptor adaptor protein 1 (LDLRAP1) and 14 normolipemic controls. Circulating PCSK9 levels were significantly higher in ARH patients than in non-ARH/non-FH dyslipidemic individuals treated with similar doses of statins (526±32 vs. 365±23ng/mL; p<0.01). ARH and control CD3+ lymphocytes were maintained in 0.5% serum and 10μg/mL mevastatin and subsequently incubated with increasing doses of recombinant PCSK9 (up to 600ng/mL) with or without alirocumab. Cell surface LDL receptor (LDLR) expression measured by flow cytometry was higher in ARH than in control lymphocytes [1228±167 vs. 617±60 mean fluorescence intensity (MFI), p<0.001]. PCSK9 significantly reduced LDLR expression in ARH lymphocytes [-22% (min+25% to max-55%, p<0.001 vs. no PCSK9) albeit to a lower extent than in control lymphocytes [-79% (min-59% to max-92%), p<0.001 vs. no PCSK9]. As anticipated, fluorescent LDL cellular uptake was reduced in ARH lymphocytes compared with control lymphocytes (357±29 vs. 567±82 MFI, p<0.001). PCSK9 significantly reduced LDL cellular uptake in ARH lymphocytes, on average by 19% [[min-4% to max-31%], p<0.01 vs. no PCSK9], compared with a 52% reduction of LDL uptake observed in PCSK9 treated control lymphocytes [min-38% to max-67%, p<0.01 vs. no PCSK9]. The effects of recombinant PCSK9 on LDLR cell surface expression and function were significantly less pronounced in ARH than in control cells (Figure). Saturating concentrations of alirocumab...
significantly reversed the effects of PCSK9 on LDLR expression and function in control and ARH lymphocytes.

**Conclusion:** These observations indicate that PCSK9 inhibition with alirocumab on top of statins could potentially lower LDL-C in Autosomal Recessive Hypercholesterolemia.

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**Subdivision 2. Translational Research**

**Presentation Preference** Oral presentation

**Additional information**
Effect of Cardiotonic Pills on Common Carotid Arterial elasticity in Normal subject

Abstract nr. 438
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Co-author(s) - Moon, Sang-Kwan
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Cardiovascular Disease

Objectives: The progress of atherosclerosis is closely correlated with cardiovascular disease, but the area of weakness of these field is difficulty of diagnosis in early stage of atherosclerosis. This study was conducted to verify the anti-atherogenic effect of Cardiotonic pill®(Tasley, China), widely used to care Angina pectoris, Hypertension, Hyperlipidemia, by using Two dimensional speckled tracing system, which is useful to assess the degree of carotid arterial elasticity in early stage.

Methods: The improvement of carotid elasticity after taking Cardiotonic pill® was studied in 15 healthy male volunteers (mean age, 27.1±0.7 years). The segmental circumferential strains of Rt. common carotid artery and Intima-Media thickness(IMT) by using ultrasound imaging system(Vivid S5, GE medical system, Milwaukee, WI, USA), systolic and diastolic blood pressure, and pulse rate were measured at rest as a baseline and 2 hours, 4 hours after medication. The mean value of six segmental strain levels was corrected to pulse pressure(systolic blood pressure minus diastolic blood pressure), and was expressed as 'Corrected Carotid Strain'.

Results: The Corrected carotid strain of right carotid artery increased significantly after taking Cardiotonic pill®, whereas the both systolic and diastolic blood pressure, pulse rate, and common carotid IMT did not change significantly.

Conclusion: Cardiotonic pill® can increase strain value of common carotid artery, which indicates that it can increase vasodilatory potential of the carotid artery and expressed as advance of arterial wall elasticity. Further, it suggests that we could diagnose and treat early stage of atherosclerosis before structural deterioration of vascular motility.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Genetic diagnosis improves lipid control in Familial Hypercholesterolemia. The Spanish Atherosclerosis Society (SEA) Dyslipemia Registry

Abstract nr. 439
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia, LDL

The Spanish Atherosclerosis Society (SEA) Dyslipemia Registry is a national database created in 2013 incorporating cases attending lipid clinics around Spain. Most patients with Familial hypercholesterolemia (FH) are referred to the lipid clinics, although their clinical management, including genetic diagnosis, is not uniformly implemented. The clinical value of genetic testing in FH is debatable and its potential effect on lipid control has not been established.

**Objective**
The aim of this study was to study the effect on LDL cholesterol reduction of a positive genetic test in FH heterozygous

**Material and Methods**
Retrospective and observational analysis of subjects with the clinical diagnosis of heterozygous FH with LDL cholesterol > 220 mg/dL from lipid clinics with and without genetic testing. Lipid values before and after lipid-lowering treatment, and percentage on LDL cholesterol reduction from baseline were recorded. Results are expressed in median (percentile 25-percentile 75) or percentage as applicable.

**Results**
The results are showed in the table. 1254 patients were included in this analysis: 180 cases without genetic diagnosis; 904 cases with a positive FH genetic diagnosis (FH+); and 170 cases with a negative genetic diagnosis (FH-). There were statistically significant differences in the baseline LDL cholesterol among groups (p<0.001). LDL cholesterol reductions were significantly higher in the FH+ in comparison with FH- and FH without genetic diagnosis (p<0.001).

**Conclusion**
FH heterozygous subjects with a positive genetic test get greater LDL cholesterol reductions than those with a negative test or without genetic diagnosis. Our results suggest that a positive genetic diagnosis improves the management of FH patients, and that genetic diagnosis is a useful tool to maximize treatment.

<table>
<thead>
<tr>
<th></th>
<th>FH+</th>
<th>FH-</th>
<th>FH without genetic diagnosis</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>44.0 (32.0-55.0)</td>
<td>53.0 (44.0-60.0)</td>
<td>46.0 (36.0-57.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender, % males</td>
<td>48.2</td>
<td>37.8</td>
<td>43.2</td>
<td>0.166</td>
</tr>
<tr>
<td>Baseline LDLc, mg/dL</td>
<td>281.4 (248.9-331.0)</td>
<td>231.7 (235.8-288.2)</td>
<td>272.1 (240.8-320.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDLc with treatment, mg/dL</td>
<td>133.0 (110.2-162.0)</td>
<td>127.5 (109.5-157.80)</td>
<td>137.8 (108.6-196.9)</td>
<td>0.349</td>
</tr>
<tr>
<td>LDLc variation, %</td>
<td>-54.0* [-62.7-(-42.6)]</td>
<td>-45.4 [-56.8-(-34.1)]</td>
<td>-51.9 [-59.4-(-37.7)]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* FH+ vs FH- p<0.001; FH+ vs FH without genetic diagnosis p = 0.019

Table 1
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Mutations in LPL, APOA5 and LMF1 genes in subject with primary hypertriglyceridemia

Abstract nr. 440
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Genetics, Triglycerides

The LPL, LMF1 and APOA5 genes have been described as cause of severe hypertriglyceridemia, (HTG), a heterogeneous group of genetic disorders. The Lipoprotein lipase (LpL) hydrolyzes triglycerides (TG) in VLDL and QM releasing free fatty acids (FFA). The complete function of ApoA-V is not clear but it has been shown that affects the intracellular assembly of VLDL. The Lipase Maturing Factor 1 (Lmf1) is involved in dimerization and maturation of Hepatic Lipase (HL) and LpL.

We selected 73 unrelated subjects affected of severe HTG defined as TG > 500 mg/dL, excluding secondary causes: uncontrolled diabetes, BMI> 30Kg/m2, alcohol abuse, renal or liver disease, hypothyroidism, hemochromatosis and hypolipemic drugs. We sequenced the promoters, exons and exon-intron boundaries of LPL, LMF1 and APOA5 genes.

Eight patients (8.2%) were carriers of 5 rare variants: p.Leu69Leu and p.Pro562Arg in LMF1, c.(1488+1G>A) in LPL, p.(Leu173Pro) and p.Pro97* in APOA5. Two of them were described for the first time in this work: c.(1488+1G>A) and p.(Leu173Pro). We observed common variants associated with HTG that showed differences with statistical significance (p<0.05) in allele frequencies when comparing with 1000 genomes: c.281T> G, p.Asn36Asn in LPL, c.288+298C>T and p. Leu69Leu in LMF1, and c.-3A>G, p.Ser19Trp, p.Ile44Ile and c.162-43A>G in APOA5.


Our results suggest that primary HTG with TG ≥ 500-1000 mg/dL has an accumulation of genetic variants compared to the general population, putting together the moderate-aggressive effect of rare mutations with polymorphisms classically associated with this disease.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Asian specific definition for metabolic syndrome and relationship with alanine aminotransferase in an Indonesian population: The Jakheart Study

Abstract nr. 441
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Co-author(s) - Valk, Harold de
Co-author(s) - Heine, Robert
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, Metabolism, Risk Factor

Objective:
To assess the relationship of various measures of adiposity with the metabolic syndrome (MetS) in an Indonesian population. The World Health Organization suggested a new definition of central adiposity for Asians. We investigated if these new cut-off points identify more adequately subjects with MetS (using ATPIII criteria). In addition, we determined the associations between anthropometrics parameters, features of MetS and alanine aminotranferase (ALT), a surrogate marker of non alcoholic fatty liver disease (NAFLD)

Methods:
The Jakheart study is a cross-sectional observational study of 167 healthy inhabitants of Jakarta, as well as fasting blood glucose, triglycerides, HDL-cholesterol and ALT were measured. The WHO WC cut-off points for Asians were applied in the ATPIII criteria.

Results:
Sixteen percent of the subjects fulfilled the ATPIII criteria of MetS. Adaptation of WC cut-off points to Asian specific values resulted in an increase of MetS to 25%. The prevalence and severity of individual components of MetS in the people, were identified with the adapted definition (N=16), were similar to people with the MetS according to the original ATPIII criteria. ALT was significantly associated with measures of obesity.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Mini-oral presentation
Additional information
Light at night promotes adiposity: crucial role for brown adipose tissue

Abstract nr. 442
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Co-author(s) - Rensen, Patrick
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Lipids, Metabolism, Obesity

Background: Artificial light exposure and the obesity epidemic have advanced together. Recently, an epidemiological cohort of over 100,000 women showed that light exposure in the bedroom associates with obesity (McFadden 2014). Strikingly, we have shown that constant light exposure in mice causes obesity without increasing food intake (Coomans 2013). We now aimed to investigate the mechanism underlying the association between light and obesity in mice.

Methods and Results: Male C57Bl/6J mice were subjected to 8h, 12h, 16h and 24h of light per day for 5 weeks. Prolonged light exposure correlated with increased fat mass without increasing food intake, suggesting a reduction in energy expenditure. Since brown adipose tissue (BAT) is an important contributor to energy expenditure, we assessed BAT activity by injecting mice with glycerol tri[3H]oleate-labeled lipoprotein-mimicking particles. Prolonged light exposure reduced the uptake of [3H]oleate selectively by BAT, which was accompanied by decreased staining in BAT of tyrosine hydroxylase (TH), the key enzyme in noradrenalin production. A significant correlation was observed between [3H]oleate uptake and TH content. Also, prolonged light exposure reduced downstream adrenergic signaling, evidenced by reduced phosphorylation of AMPK, CREB and HSL. Selective surgical denervation of BAT completely abolished effects of light exposure on the uptake of [3H]oleate by BAT. These data support a major role for the sympathetic nervous system in the relation between light exposure and BAT. Since light exposure is the most important cue for biological clock function, we further investigated the role of the biological clock in BAT. Mice were exposed to 8h, 12h and 16h day length for 5 weeks and the diurnal pattern in [3H]oleate uptake by BAT was assessed. Strikingly, compared to 12h day length, the rhythmicity of BAT was highest at 8h day length, accompanied by an increased overall uptake of [3H]oleate by BAT while 16h day length markedly flattened the rhythm and lowered overall uptake of [3H]oleate by BAT.

Conclusion: Prolonging light exposure from 12h increases body adiposity through reduction of BAT activity, while shortening the light exposure enhances BAT activity. These studies suggest that BAT may mediate the observed association between light exposure and obesity in humans.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
ProSalute: a New Community Program for Cardiovascular Health

Abstract nr. 443
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Lifestyle, Prevention, Risk Factor

Cardiovascular primary prevention programs in the healthcare place and community-based interventions have a variable impact in reducing CV risk factors at the population level. The largest benefit may be obtained by addressing high-risk, disadvantaged and traditionally hard-to-reach groups. Effective actions include health promotion, timely screening of modifiable risk factors, application of evidence-based targets and interventions, broad access to heart-friendly environments/facilities and dissemination of favorable social norms. Thus, community prevention is a multifaceted task that requires multidisciplinary collaboration. A suitable program should be tailored to the specific social context and make the most of local resources to improve access, adherence and continuity, as well as sustainability.

ProSalute (ProHealth) is an experimental model of primary CV prevention for the prevalently low-income and multiethnic community of Ponte Lambro (n=3600 adults), the neighborhood where the coordinating hospital (Centro Cardiologico Monzino, Milan, Italy) is located. CV risk domains assessed through an assisted questionnaire will include family and personal history, life-style and psychological and socioeconomic status. Study organization started in 2014 and a 2-years pilot phase (n=600, age 45-65y) will start on March 2015.

Original program features
1) Personal invitation to foster participation of hard-to-reach groups.
2) Coordination and running by a staff of specialists in CV prevention (nurse, internist, nutritionist, psychologist) from an academic local hospital.
3) Involvement of a trained nurse as motivational-interviewer and administrator of predefined interventions, which are tailored (content and intensity) according to the individual global risk and specific risk factors.
4) Short term modules of specialist care for specific problems (smoking cessation, control of depression, nutritional counseling, pharmacological control of risk factors in agreement with primary physicians).
5) Development of a collaborative network with representatives of the Community to plan
communication and community preventive actions (healthy-cooking course, walking groups, etc).
6) Nudge of participants to utilize environmental, organizational and professional local resources (parks, gyms, social services, etc) that may help to sustain a healthy life-style. Outcomes will be assessed at 6 and 12 months using indexes of adherence, life-style improvement, risk reduction and costs. According to the results, this model could be transferred and adapted to other social contexts.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Mini-oral presentation
Additional information
Plasma microRNAs potentially associated with the increase risk for cardiovascular disease in rheumatoid arthritis patients

Abstract nr. 444
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Co-author(s) - Ibarretxe, Daiana
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Co-author(s) - Castro, Antoni
Co-author(s) - Masana, Luis
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Cardiovascular Disease, Inflammation, Pathogenesis

Introduction: Rheumatoid arthritis (RA) patients are at increased risk for cardiovascular diseases and an accelerated atherogenesis is considered as the cause of this increase. MicroRNAs (miRs) are small, non-coding RNA molecules which negatively regulate gene expression. Plasmatic miRs have been identified as biomarkers in a wide range of cardiovascular diseases (CVD).

Objective: To identify plasmatic miRs in RA patients that can facilitate earlier diagnosis of CVD and provide insight regarding the increase risk for CVD in these patients.

Design and methods: We compared plasmatic profiles of 754 miRs in 7 RA patients without CVD, in 7 patients with acute myocardial infarction (AMI) but without RA, and in 7 healthy controls with the objective to find miRs commonly expressed in the two groups of patients but at different levels from the controls. All participants were male and were matched for age and for classical cardiovascular risk factors. Plasma profile of miRs were analyzed by real time PCR using validated TaqMan® OpenArray® MicroRNA panels which enables the quantification of 754 human miRNAs. Differential expression was analyzed with Expression Suite software. Selected miRs were further analyzed with 2-ΔΔCt method. Kruskal-Wallis test and Dunns post-test were used for the statistical analyses.

Results: We were able to measure in the three groups, 50% (379) of the miRs represented in the array. Plasma profile of circulating miRs in RA and AMI patients were different from the controls. Plasma miRs that were commonly expressed in AR and AMI and expressed significantly different versus controls were: mir-Let-7a, miR-96, miR-381, miR-451, miR-518d, miR-425-5p, miR-572, miR-190b, miR-708, and miR1180. Interestingly, all 10 miRs were down-regulated compared with controls. Furthermore, 9 miRs were differentially expressed in AMI patients versus controls and 16 miRs in RA patients versus controls.

Conclusions: In the present study we have identified a group of 10 miRs that potentially can be involved in the increase risk for CVD seen in RA patients. Among all these miRs, miR-451 and Let-7a have been previously associated with CVD and it might be the best candidates for future
validation studies.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Objective. Genetic predisposition plays an important role among other risk factors in multifactorial socially significant diseases such as atherosclerosis and its clinical manifestations. This study was aimed to identify the relationship between mtDNA variants and the risk of subclinical atherosclerosis in humans.

Design and method. A total of 77 persons from Moscow region (Russia) were included in the study. 45 study participants had ultrasonographically detected atherosclerotic lesions of the carotid arteries, others were controls without atherosclerosis. DNA was isolated from blood and the enrichment of mitochondrial DNA was performed. For accurate detection of mtDNA mutations, high-throughput sequencing of the mitochondrial genome using "shotgun" DNA libraries and Roche 454 technology with GS Junior Titanium system was carried out. Statistical analysis was performed using IBM SPSS Statistics v.21.0 software. Cambridge reference sequence of human mtDNA (NC_012920.1) was used for mapping.

Results. Sequencing allowed to obtain about 70-fold average coverage of the mitochondrial genome. Total frequency of homoplasmic mutations was significantly higher in controls in comparison with atherosclerotic patients (0.153 vs 0.116%, p=0.022), as the frequencies of all mutations in coding and non-coding regions and frequency of missense+tRNA+rRNA mutations. We distinguished 70 mutations that were characterized by the presence over 5% of the total sample, and half of these mutations were characterized by 1.5-fold up to 7-fold higher occurrence in controls compared with atherosclerotic patients. 25 of them were mutations in the coding region of mtDNA. For 5 mutations characterized by the increased occurrence in controls, significant differences in the occurrence between the compared groups were found by ANOVA analysis (p<0.05): m.73A>G, m.16296C>T, m.7028C>T, m.11251A>G, m.14233A>G.

Conclusions. Taking rCRS as a reference, we found that healthy individuals were characterized by higher variability of mitochondrial DNA compared to atherosclerotic patients. 2706A and 7028C variants, that are markers of mitochondrial haplogroup H, were more common in atherosclerotic
patients, which proofs the role of this haplogroup as a marker for suspectability to atherosclerotic-related diseases. In general, 12 single-nucleotide mtDNA variants were characterized as associated with atherosclerosis in Russian population.

**Acknowledgements.** This work was carried out in part by Russian Ministry of Education and Science, agreement N14.613.21.0006.
Isolation of genomic region involved in nutrigenetic and pharmacogenetic interactions affecting lipoprotein fractions and metabolic profile in congenic rat strain

Abstract nr. 446
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Co-author(s) - Šeda, Ondřej
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model,Lipoproteins,Pharmacology

In a process of positional cloning of metabolic-syndrome related locus we derived new congenic strain by introgressing a limited rat chromosome 4 (RNO4) region comprising A2m gene from spontaneously hypertensive rat (SHR) into BN-Lx (Brown Norway) genomic background. We tested its effect on susceptibility to diet and medication-induced dyslipidemia and insulin resistance.

Methods: We mapped the genomic extent of the differential segment by using >140 microsatellite markers on RNO4. Male rats (n =6/strain/diet) of BN-Lx and BN-Lx.SHR4(A2m) were fed standard diet for 15 weeks (STD, control groups) or STD followed by 10 days of high-sucrose diet (HSD) or dexamethasone (DEX) for 3 days. We assessed comprehensively the morphometric and metabolic profiles of all groups, including glucose tolerance tests, levels of insulin, adiponectin, pancreatic polypeptide, PYY, free fatty acids, concentrations of triglycerides and cholesterol in 20 lipoprotein fractions. All statistical analyses were performed using STATISTICA 12. Two-way ANOVA with STRAIN and DIET as major factors was used.

Results: The differential segment of RNO4 spans about 9Mb between markers D4Mgh30-D4Mit26. The in silico sequence comparison showed several distinct genes with non-synonymous amino acid changes including Ankrd26, A2m and Pex5.

Reaction to stimuli (HSD, DEX) varied strains on level of adipose tissue distribution to different depots. The congenic strain was more sensitive to both HSD-induced increase and DEX-induced decrease of the fat mass. Detailed assessment of lipid profile revealed significant pharmacogenetic interactions of dexamethasone and the RNO4 segment distinguishing the two strains. DEX induced significant increase in free glycerol and TG concentrations in major lipoprotein fractions in BN-Lx (e.g. 46% increase in VLDL-TG, post-hoc p = 0.011) but no effect was evident for BN.Lx-SHR4(A2m). Also, the global insulin resistance indices showed more robust deterioration in BN-Lx.SHR4(A2m) (e.g. area under the glycemic curve rose by 88% compared to 13% in BN-Lx in response to dexamethasone). BN-Lx.SHR4(A2m) exhibited also significantly lower levels of PP and PYY in serum.
Conclusion: We have identified nutrigenetic and pharmacogenetic interactions involving HSD / DEX and RNO4 segment of spontaneously hypertensive rat origin affecting lipid partitioning into lipoprotein fractions, glucose tolerance and fat deposition. Supported by MSMT LK11217.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
ApoA-I/HDL-C levels are inversely associated with abdominal aortic aneurysm progression.

Abstract nr. 447
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords HDL

Background: Abdominal aortic aneurysm (AAA) evolution is unpredictable and there is no therapy except surgery for patients with an aortic size>5cm (large AAA). We aimed to identify new potential biomarkers that could facilitate prognosis and treatment of patients with AAA.

Methods and results: A differential quantitative proteomic analysis of plasma proteins was performed in AAA patients at different stages of evolution [small AAA (aortic size=3-5cm) vs large AAA] using iTRAQ labelling, high-throughput nano-LC-MS/MS and a novel multi-layered statistical model. Among the proteins identified, ApoA-I was decreased in patients with large AAA compared to those with small AAA. These results were validated by ELISA on plasma samples from small (n=90) and large AAA (n=26) patients (150±3 vs 133±5 mg/dl, respectively, p<0.001). ApoA-I levels strongly correlated with HDL-Cholesterol (HDL-C) concentration (r=0.9, p<0.001) and showed a negative correlation with aortic size (r=-0.4, p<0.01) and thrombus volume (r=-0.3, p<0.01), which remained significant after adjusting for traditional risk factors. In a prospective study, HDL-C independently predicted aneurysmal growth rate in multiple linear regression analysis (n=122, p=0.008) and was inversely associated with need for surgical repair (Adjusted HR: 0.18, 95% C.I.: 0.04-0.74, p=0.018). In a nation-wide Danish registry, we found lower mean HDL-C concentration in large AAA patients (n=6,560) compared with patients with aorto-iliac occlusive disease (n=23,496) (0.89±2.99 vs 1.59±5.74 mmol/l, p<0.001). Finally, reduced mean aortic AAA diameter was observed in AngII-infused mice treated with ApoA-I mimetic peptide compared with saline-injected controls.

Conclusions: ApoA-I/HDL-C systemic levels are negatively associated with AAA evolution. Therapies targeting HDL functionality could halt AAA formation.

Subdivision 2. Translational Research
Presentation Preference
Electronic poster presentation
Additional information
Detection of intracranial vessel wall lesions in an elderly asymptomatic population using 7.0T MRI

Abstract nr. 448
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Imaging,Pathogenesis

Introduction
Intracranial atherosclerotic disease (ICAD) is one of the main causes of ischemic stroke and transient ischemic attack. Development of atherosclerotic lesions occurs silently over a long period, before they become symptomatic. Most studies have attempted to target ICAD when it is already symptomatic. Additional information regarding the prevalence of ICAD in the asymptomatic population would provide us with better insight in its development. The aim of this study was to assess the presence of intracranial vessel wall lesions and enhancement in an asymptomatic population using intracranial vessel wall MR imaging at 7.0 tesla (T).

Methods
Twenty healthy volunteers (12 male; age 66 ± 4 years), without a history of cerebrovascular or ischemic heart disease, were included in this study. Imaging was performed on a 7.0T whole-body system. The imaging protocol included a high-resolution T1-weighted intracranial vessel wall sequence [Van der Kolk et al. Stroke 2011] before and after contrast administration, and a time-of-flight (TOF) MRA sequence. Two observers independently scored the vessel wall abnormalities and enhancement in all the major artery segments of the circle of Willis on the vessel wall images. The presence of stenosis was scored on the TOF-MRA images.

Results
Figure 1 shows different examples of vessel wall lesions with and without enhancement or the presence of a stenosis identified in the study population. Table 1 shows an overview of the number, location and thickening pattern of the identified vessel wall lesions in the main arteries of the circle of Willis, the number of lesions that showed enhancement, and the number of scored stenoses in the TOF-MRA scans. In total 100 intracranial vessel wall lesions were identified in all subjects (median per subject, 5; range, 2-11), of which 36% enhanced after contrast administration. Only 10% of the vessel wall lesions showed a corresponding luminal stenosis on the TOF-MRA images.

Conclusion
We hypothesize high-resolution intracranial vessel wall imaging at 7.0T enables detection of small atherosclerotic lesions at an early stage without causing luminal stenosis in asymptomatic patients. Correlation of high resolution *in vivo* and *ex vivo* MRI with histopathology will be needed to validate these findings.

![Figure 1](image1)

**Table 1**

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Thickening (#)</th>
<th>Enhancement</th>
<th>MRA-Stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>eccentric</td>
<td>concentric</td>
<td></td>
</tr>
<tr>
<td>Anterior circulation</td>
<td>38</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>MCA</td>
<td>14</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>ICA</td>
<td>17</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
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<tr>
<td>PCA</td>
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</tr>
<tr>
<td>BA</td>
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<td>VA</td>
<td>38</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>51</td>
<td>43</td>
</tr>
</tbody>
</table>


**Table 1**

**Subdivision 2. Translational Research**

**Presentation Preference** Oral presentation

**Additional information**
The risk of bodyweight in the presence or absence of metabolic dysfunction on the development of type 2 diabetes.

Abstract nr. 450
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Co-author(s) - Visseren, Frank
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Diabetes, Metabolism, Obesity

Objective: To quantify the relation between bodyweight in the presence or absence of metabolic dysfunction and the development of type 2 diabetes in patients with manifest vascular disease or high risk for cardiovascular events.

Design: Prospective cohort study.

Setting: Secondary Manifestations of ARterial disease (SMART) study, the Netherlands.

Participants: 6997 patients with manifest vascular disease or at high risk for cardiovascular events classified according to body-mass index and metabolic dysfunction, defined as ≥3 of the modified NCEP metabolic syndrome criteria (waist circumference replaced by hsCRP≥2).

Main outcome measure: Incident type 2 diabetes assessed with biannually questionnaires.

Results: During a median follow-up of 6.0 years (interquartile range: 3.1 to 9.1 years) and 44216 person-years, 519 patients developed type 2 diabetes. In the absence of metabolic dysfunction (≤2 NCEP criteria) adiposity increased the risk on developing type 2 diabetes compared to normal weight patients; hazard ratio 2.5 (95% confidence interval 1.5 to 4.2) for overweight and hazard ratio 4.3 (95% confidence interval 2.2 to 8.6) for obese patients. In the presence of metabolic dysfunction, an increased risk on type 2 diabetes was observed in patients with normal weight; hazard ratio 4.7 (95% confidence interval 2.8 to 7.8), overweight; hazard ratio 8.5 (95% confidence interval 5.5 to 13.4) and obesity; hazard ratio 16.3 (95% confidence interval 10.4 to 25.6) compared to normal weight patients without metabolic dysfunction.

Conclusion: Overweight and obese patients without metabolic dysfunction and with manifest vascular disease or at high risk for cardiovascular events, are at increased risk for developing type 2 diabetes compared with normal weight patients without metabolic dysfunction. This risk becomes increasingly higher with the presence of metabolic dysfunction and increases with BMI. These findings support adequate assessment and treatment of both adiposity and metabolic dysfunction.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
LOSARTAN CONTRIBUTES TO PREVENT THE EFFECTS OF ADVANCED GLYCATED ALBUMIN IN THE AORTA OF DYSLIPIDEMIC MICE

Abstract nr. 451

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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium

Keywords Atherosclerosis, Diabetes, Inflammation, Metabolism

Advanced glycation end products (AGE) bind to AGE receptor (RAGE) promoting inflammation, oxidative stress and positively feeding back RAGE expression. Angiotensin (ANGII) receptor 1 (AT-1) antagonists diminish RAGE expression and atherosclerotic lesion progression in animal models of diabetes or hypertension. We aimed at evaluating the effects of chronic treatment of advanced-glycated albumin (AGE-albumin) on the expression of mRNA involved in the AGE/RAGE and ANGII/AT-1 axes in the aorta of dyslipidemic mice and the effect of the AT-1 inhibitor, losartan (LOS).

12-week old ApoE knockout mice kept on chow diet were divided into four groups: Control (C), C+LOS, AGE-albumin (AGE), and AGE+LOS. Animals received daily intraperitoneal injection of 2mg of C or AGE-albumin, during 30 days. LOS was administered in water (100mg/dL). AGE-albumin was prepared by incubation with 10 mM glycolaldehyde and C-albumin with PBS alone, during 4 days at 37°C. Biochemical analyses were performed using enzymatic kits. mRNA expression was performed in the aortic arch by qPCR and Actb was used as housekeeping. Results were compared by one-way ANOVA and Newman Keuls post test (mean±SE).

No difference among groups (mg/dL) was observed in baseline and final period regarding plasma total cholesterol [C (n=20)= 471.5±24.2; C+LOS (n=20)= 459.2±19.1; AGE (n=20)= 490.8±22; AGE+LOS (n=20)= 518±22], tryglicerydes [(C= 115±5; C+LOS= 121±12; AGE= 116±10; AGE+LOS= 135.5±8)], glucose [(C= 111±5; C+LOS= 102±3; AGE= 100±3.5; AGE+LOS= 111±6] and body weight (g) [C= 26±0.3; C+LOS= 25±0.4; AGE= 27±0.4; AGE+LOS= 26±0.4]). Systolic blood pressure (mmHg) [C (n=15) = 76±0.7; C+LOS (n=12) = 73±0.8; AGE (n=13) = 78±1; AGE+LOS (n=15) = 74±1)] was reduced both LOS groups. Agrp1 mRNA was similar between C and AGE, however it was 38% and 39% lesser in C+LOS and AGE+LOS groups (p<0.0001). Cybb expression was 85% greater in AGE compared to C (p<0.04) and was not affected by LOS. Ager and Tnf mRNA expression were 1.5-fold and 2.5-fold in AGE compared to C (p<0.01),
respectively, which were prevented by LOS. Besides not changing NOX2 expression, LOS reduces AT-1, and diminishes RAGE and TNF expression induced by AGE. This may contribute to prevent atherosclerosis development related to AGE.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
LIPID SEGREGATION IN NON-FH FAMILIES WITH AUTOSOMAL DOMINANT HYPERCHOLESTEROLEMIA

Abstract nr. 452
Author Perez, Maria Rosario, INSTITUTO DE INVESTIGACIÓN SANITARIA ARAGON, Zaragoza, Spain
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Familial Hypercholesterolemia, Genetics

Introduction- Autosomal dominant hypercholesterolemia (ADH) is a condition associated to increased values of total cholesterol (TC) and early cardiovascular disease. Up to 60% of ADH patients are not carriers of any defect in candidate genes (ADH-). The familial segregation and hypercholesterolemia heritability in this subgroup of patients has been poorly studied.

Objective- To determinate the hypercholesterolemia inheritance pattern in ADH families without any functional mutation in the genes LDLR, APOB, and PCSK9.

Material and Methods- 54 ADH- probands and 256 first-degree relatives were recruited at the Lipid Unit of Hospital Universitario Miguel Servet of Zaragoza, Spain. Basal lipid profile and anthropometric variables were obtained in all participants. Lipid profile was analyzed by HelixTree software and genetic score compared by SPSS were used considering the possibility of the existence of an allele (A) with dominant inheritance pattern. Age, sex, body mass index, apo E genotype and Lipoprotein(a) were introduced as covariates.

Results- 169 subjects showed any type of dyslipidemia. In 31 HAD- families (N=173), TC and LDL cholesterol showed a significant association with the “A” allele (p=5.19 x 10^{-9}; and p=8.73 x 10^{-9}, respectively). Heritability was 0.38 for TC, 0.27 for LDL cholesterol. There was not any association to triglycerides (TG) (p=0.49), HDL cholesterol (p=0.88). A total of 23 families were classified as familial combined hyperlipidemia (FCH) families (N=137 subjects). The putative dominant allele was associated with TC (p=1.70 x 10^{-7}, heritability=0.30) and LDL cholesterol (p=3.33 x 10^{-8}, heritability=0.25). A recessive allele also contributed slightly to TC (p=0.0013, heritability=0.13). HDL cholesterol and TG were associated with a presumed recessive allele (p=0.021, heritability=0.006; and p=1.28 x 10^{-6}, heritability=0.23, respectively). According to the genetic score, 37.5% of TC, 15% of TG, and 33.9% of LDL cholesterol variations in this group of patients were explained by this genetic model.

Conclusions: In HAD- families, TC and LDL cholesterol variation was explained in a high proportion by an autosomal dominant inheritance pattern. These findings support the existence of
genetic loci, other than *LDLR*, *APOB* and *PCSK9*, as contributors to primary hypercholesterolemias.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Models based on the Framingham Offspring Study predict diabetes and cardiovascular disease

Abstract nr. 453
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Co-author(s) - Asztalos, Bela F.
Co-author(s) - Wilson, Peter W. F.
Co-author(s) - Cupples, L. Adrienne
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Diabetes, Dyslipidemia

Our goals were to develop models for diabetes and cardiovascular disease (CVD) risk using data from 3,188 men and women from the Framingham Offspring Study (cycle 6, mean age 58 years), who had been followed for a median of 12.3 years, and were sampled at baseline after an overnight fast.

We measured plasma or serum glucose, insulin, adiponectin, glycated albumin, C reactive protein (CRP), standard lipids, small dense low density lipoprotein (sdLDL) cholesterol (C), lipoprotein (a) or Lp(a), and apoA-I in HDL particles. We had information on body mass index, parental history of diabetes, smoking, hypertension, use of hypertension medications, and use of statins, as well as follow-up information on the development of diabetes and CVD. All subjects with disease at baseline were excluded. In both prediction models only those variables that provided significant information in a stepwise logistic regression analysis were used to generate prediction models based on calculation of areas under the curve and C statistics.

For the diabetes prediction model seven variables entered the model with p values < 0.05, and provided an overall C statistic of 0.924: fasting serum glucose, body mass index, log adiponectin, % log glycated albumin, parental history of diabetes, triglycerides, and use of cholesterol lowering medications. All subjects had a 10 year of developing diabetes of 7.1%, while those with prediabetes (fasting glucose 100 - 125 mg/dL) had a 21% risk. Follow-up data on CVD status for 1,083 men was used, of whom 207 (19.1%) developed CVD. For the CVD prediction model for men the following seven variables entered the model with a p value of < 0.05 and provided an overall C statistic of 0.73: age, blood pressure treatment, Lp(a), sdLDL-C, ApoA-I in large α-1 HDL, smoking, and diabetes. This model was significantly superior to a model using the standard risk factors or one also using CRP (C statistic 0.68).

Our data indicate that fasting glucose, BMI, adiponectin, glycated albumin, parental history, triglycerides, and statin use are all useful for diabetes risk, and that the measurement of sdLDL-C, Lp(a), and apoA-I in HDL particles adds significant information about CVD risk.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
A comprehensive analysis of antigen presenting cell phenotype and kinetic of antigen presentation in the aorta of atherosclerotic mice

Abstract nr. 454
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Co-author(s) - Maffia, Pasquale

Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model,Atherosclerosis,Immunity,Inflammation

Aims: The adaptive immune system plays a crucial role in atherosclerosis. Aortic antigen presenting cells (APCs) are able to present antigen locally to antigen specific T cells but little is known about the kinetic of this antigen presentation and the contribution of the different APC subtypes. We have therefore performed a quantitative assessment of antigen presentation in atherosclerotic vs wild type mice.

Methods: Flow cytometry in combination with the Ealpha (Eα)-GFP/Y-Ae system was employed to profile APCs and to assess their antigen presentation capacity in vivo. The Eα-GFP/Y-Ae system can identify antigen presentation in vivo through the ability of the Y-Ae antibody to recognize the Eα peptide in the context of MHC (I-A^b). We studied the kinetic of antigen presentation in the aorta, para-aortic lymph nodes and secondary lymphoid organs in 6 months old hyperlipidemic apolipoprotein-E (apoE)^/-^ mice fed a chow or a high fat diet (HFD) vs control wild type C57B6L mice.

Results: Y-Ae positive APCs were detectable in the spleen and aorta of both atherosclerotic and control mice starting from 1h after Eα i.v. injection. The number of APCs was greatly increased in the aorta of apoE^-/-^ mice fed HFD. Plasmacytoid dendritic cells (pDCs) as well as monocyte-derived DCs (moDCs) subsets were the main subsets to expand in established atherosclerosis showing enhanced Ag presentation capacity (23 and 20-fold increase, respectively).

Conclusions: This data demonstrate that the aorta is immunologically active and that aortic pDCs as well as moDCs may play key role as APCs in vascular pathology.

Supported by the British Heart Foundation (PG/12/81/29897).

Subdivision 1. Basic Science
Presentation Preference
Electronic poster presentation
Additional information
MAJOR LIPOPROTEINS AND ACUTE ISCHEMIC STROKE SEVERITY AND IN-HOSPITAL OUTCOME

Abstract nr. 455
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Co-author(s) - Savopoulos, Christos
Co-author(s) - Hatzitolios, Apostolos
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Cardiovascular Disease, HDL, Triglycerides

Background-aims: Previous studies reported controversial data regarding the relationship between low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and non-HDL-C levels and acute ischemic stroke severity and in-hospital outcome. We aimed to evaluate these associations.

Patients and methods: We prospectively studied 608 consecutive patients hospitalized for acute ischemic stroke (39.5% males, age 79.1±6.6 years). Stroke severity was evaluated at admission with the National Institutes of Health Score Scale (NIHSS). The outcome was evaluated with the presence of functional dependency at discharge (i.e. modified Rankin scale 2-5) and with in-hospital mortality. Fasting serum total cholesterol, TG and HDL-C levels were measured at the second day of hospitalization and LDL-C levels were calculated using Friedewald’s formula.

Results: Patients with moderate/severe stroke (i.e. NIHSS≥5) had lower HDL-C levels than patients with mild stroke (44±15 and 49±14 mg/dl, respectively; p<0.005) whereas other lipid parameters did not differ between the 2 groups. In binary logistic regression analysis, independent predictors of moderate/severe stroke were older age (odds ratio (OR) 1.045, 95% confidence interval (CI) 1.008-1.084, p=0.017), higher glucose levels at admission (OR 1.006, 95% CI 1.001-1.011, p=0.022) and lower HDL-C levels (OR 0.979, 95% CI 0.964-0.994, p=0.008). Patients with dependency at discharge had lower TG levels than patients who were independent (115±59 and 128±56 mg/dl, respectively; p=0.037) whereas other lipid parameters did not differ between the 2 groups. Independent predictors of dependency were older age (OR 1.153, 95% CI 1.061-1.253, p<0.001) and higher NIHSS at admission (OR 1.674, 95% CI 1.440-1.946, p<0.001). Lipid parameters did not differ between patients who were discharged and those who died during hospitalization. Independent predictors of in-hospital mortality were the presence of atrial
fibrillation (OR 3.147, 95% CI 1.106-8.958, p=0.032), higher diastolic blood pressure at admission (OR 1.055, 95% CI 1.016-1.095, p<0.005) and higher NIHSS at admission (OR 1.158, 95% CI 1.105-1.214, p<0.001).

Conclusions: In elderly patients with acute ischemic stroke, low HDL-C levels at admission are independently associated with more severe stroke whereas low TG levels appear to be related with worse functional outcome.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Redox Regulation of MKP-1 and the Functional Reprogramming of Monocyte-Derived Macrophages by Metabolic Stress

Abstract nr. 456
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Immunity, Inflammation, Metabolism

Rationale: Diabetes promotes the S-glutathionylation, inactivation and subsequent degradation of mitogen activated protein kinase phosphatase 1 (MKP-1) in blood monocytes, and hematopoietic MKP-1-deficiency in atherosclerosis-prone mice accelerates atherosclerotic lesion formation (Kim et al. PNAS 2012), but the underlying mechanisms were not known

Objective: To determine the mechanisms through which MKP-1 deficiency in monocytes and macrophages promotes atherogenesis.

Methods and Results: Transplantation of MKP-1-deficient (MKP-KO) bone marrow into LDL-R^-/- (MKP-1-LeuKO) mice accelerated high fat diet (HFD)-induced atherosclerotic lesion formation. After 12 weeks of HFD feeding, MKP-1-LeuKO mice showed increases in lesion size in both the aortic root and the aorta, despite reduced plasma cholesterol levels. Macrophage content was increased in lesions of MKP-1-LeuKO mice compared to mice that received wt bone marrow. After only 6 weeks on a HFD, the in vivo chemotactic activity of monocytes was already significantly increased in MKP-1-LeuKO mice. Macrophages isolated from MKP-KO mice showed impaired autophagy, increased sensitivity to 7-ketocholesterol-induced apoptosis, and IL-4-induced M2 polarization of these macrophages was blocked. Importantly, macrophages in atherosclerotic lesion of MKP-1-LeuKO mice showed increased macrophage apoptosis and decreased autophagy. Metabolic stress in vitro mimicked the functional phenotype of MKP-1 deficiency, and overexpression of MKP-1 protected macrophages from metabolic stress-induced dysfunction and reprogramming.

Conclusions: MKP-1 deficiency in monocytes promotes the functional reprogramming of monocyte-derived macrophages. Loss of MKP-1 activity accelerates atherosclerotic lesion formation by hyper-sensitizing monocytes to chemokine-induced recruitment, predisposing macrophages to M1 polarization, impaired autophagy and oxysterol-induced cell death. Reduced MKP-1 levels and activity in blood monocytes may therefore be a sensitive biomarker of increased risk of atherosclerosis.

Subdivision 2. Translational Research
Presentation Preference Oral presentation
Additional information
Expression profile of adhesion and chemokine markers on monocyte subsets and their association with atherosclerosis

Abstract nr. 457
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Lipids, Risk Factor

Background and Aims: Adherence of monocytes to the endothelium, their transmigration into the arterial wall and differentiation into macrophages is an early, and ongoing, process in atherosclerosis. Monocytes are heterogeneous, with three subsets recognised: classical, intermediate and non-classical. Their individual contribution to plaque progression in humans is unclear. Here we examine monocyte subset adhesion and migration profile in atherosclerosis.

Method: Blood samples were collected from controls and atherosclerotic patients. Lipid profiles were measured and the expression of different adhesion and migration markers was assessed by whole blood flow cytometry.

Results: Selectins, CD62L and CD44, were expressed highest on classical monocytes whereas the integrins, CD11c and CD18, were highest on intermediate monocytes, except for CD11b which was highest on the classical subset and CD49d which was equally expressed on both intermediate and non-classical monocytes. The expression of CD11a and CD29 was similar for all three subsets. The chemokine receptors, CCR2, CXCR2 and CX3CR2 were expressed greatest on the classical monocytes. Though CX3CR1 expression was highest on the intermediates, and CXCR3 and CCR5 on classics, they were minimally expressed by monocytes. For control donors, CD18 correlated with cholesterol levels, while CD49d correlated positively with ApoA1, and inversely with ApoB/A, on intermediate monocytes. CD44 and CXCR2 both inversely correlated with HDL levels on classical and intermediate monocytes. CX3CR1 correlated with ApoA1 on non-classicals while CCR5 inversely correlated with HDL on classical monocytes. Compared with patients, CD49d and CXCR2 expression was higher on all monocyte subsets of the controls. CCR2 and CCR5, though minimally expressed, were higher on patient than control monocytes. CX3CR1 was similarly expressed between control and patient monocytes.

Conclusion: While there are inherent differences in the ability of the individual subsets to adhere to the endothelium, our results suggest that with increased cardiovascular risk, both the classical and intermediate monocyte subsets have an increased capacity to enter the arterial wall. Given that classical monocytes account for 80-90% of circulating monocytes and if, as in the mouse, CCR2 plays a key role in monocyte entry into the plaque, then classical monocytes may be the key contributors to human atherosclerosis progression.
This work was supported by WMRF.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Effect of long-acting nicotinic acid on apolipoprotein (a) turnover in hypertriglyceridemic patients

Abstract nr. 458
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Co-author(s) - Krempf, Michel
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Cardiovascular Disease, Dyslipidemia, Lp(a)

Introduction: Lipoprotein (a) [Lp(a)] is an LDL particle covalently linked to an apolipoprotein (a) [Apo(a)] secreted by the liver. Elevated Lp(a) plasma levels are associated with an increased risk of coronary heart disease. Long-acting nicotinic acid (Niacin) is known to reduce circulating Lp(a) levels but the exact mechanism(s) underlying this reduction remains unclear. We applied a novel liquid chromatography tandem mass spectrometry (LC-MS/MS) methodology to study Apo(a) kinetics with stable isotopes in hypertriglyceridemic patients treated with Niacin.

Methods: Eight male subjects (Age 48±12 years; Fasting TG>2 g/L; BMI 31.2±1.8 kg/m²) were treated with Niacin in a 8-week crossover study. They received a primed infusion of [5,5,5-2H3]-L-Leucine (10 µM/kg/h) for 14h. Blood samples were collected every hour and lipoproteins fractions were separated by ultracentrifugation. Lipoprotein samples were reduced, alkylated, and trypsin digested to generate peptide fragments. One peptide only found in the protease domain of Apo(a) was selected for quantitation. Another peptide found in the repeated kringle IV2 domains of Apo(a) was selected for apo(a) polymorphism assessment. The fractional catabolic rate (FCR) of Apo(a) was calculated using a monocompartmental model using the VLDL-ApoB100 leucine maximal asymptotic enrichment as precursor pool and FCR was considered equivalent to the fractional synthetic rate (FSR). Statistical analyses were performed using the nonparametric Wilcoxon signed-rank test.

Results: As anticipated, Niacin decreased plasma TG (-46%, p = 0.025) and raised HDL-cholesterol (+19.5%, p=0.05), whereas Apo(a) plasma levels were decreased by 20% (54±14 vs. 43±14 nM, p=0.012). Apo(a) FCR and production rates were decreased by 37% (0.57±0.28 vs. 0.36±0.19 pool/day, p=0.012) and 50% (1.42±0.76 vs. 0.70±0.43 nmol/kg/day, p=0.012), respectively. Noteworthy, the biggest changes in FCR were observed in patients carrying the largest isoforms of Apo(a).

Conclusion: Our kinetic analyses indicate that Niacin had a strong impact on Apo(a) turnover acting both on catabolism and synthesis.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Exercise: Is more, better? Cardiorespiratory fitness and coronary artery calcification in a primary prevention population

Abstract nr. 459
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Cardiovascular Disease, Lifestyle, Prevention

Introduction:
Cardiorespiratory fitness is thought to be associated with lower cardiovascular disease and all-cause mortality. Consequently, prevention advice has focused on "more is better." Yet increased atherosclerosis has been observed in endurance athletes. As such, we sought to further investigate the relationship between exercise fitness capacity, as assessed by functional aerobic capacity (FAC), and coronary calcification since little evidence exists.

Methods:
We retrospectively identified 2947 males from an executive health population without coronary artery disease or beta-blocker use who underwent cardiorespiratory fitness and coronary artery calcium (CAC) testing during preventive health assessment between 1995-2008. A log(CAC+1) model normalized outcome distribution. FAC was based on indexed age- and sex-specific metabolic equivalents on exercise testing and stratified into fitness quartiles: deconditioned (FAC<=69%), below average (70-99%), above average (100-129%), and super conditioned (>=130%). Data was analyzed using different age groups. Two-sample t-test, one-way ANOVA, and Pearson Chi-Square tests analyzed interactions.

Results:
The mean age of the study population was 52.1 years. In all age groups, CAC was consistently lowest in the "above average" FAC group, the reference group for comparison.
In age ≥60, there were no significant differences in log(CAC+1) values in all FAC groups. In those <60, the deconditioned (p<0.0001) and super conditioned (p=0.0013) groups had significantly higher log(CAC+1) values. Respectively, CAC scores were 95 and 133 (reference 54).
In age ≥50, all FAC groups of deconditioned (p=0.0051), below average (p=0.0161), and super conditioned (p=0.0186), had significantly higher log(CAC+1) values. CAC scores respectively were 188, 140, and 203 (reference 125).
In age <50, only the deconditioned group had significantly higher log(CAC+1) with a value of 0.985 (p=0.0004). CAC score was 55 (reference 22).
Upon further age stratification, irrespective of FAC, those between 50-60 years had significantly
higher log(CAC+1) values as compared to the reference.
Figures show CAC and coronary calcification as a function of FAC by age groups.

Conclusions:
Our results suggest maximal exercise capacity may portend increased risk of atherosclerotic burden in a reverse J-shaped relationship. Moderate conditioning appears most cardioprotective. This merits further study to clarify and improve upon delivery of accurate health care advice.

Funding support:
Mayo Clinic

![Figure 1: CAC score as a function of FAC in different age groups.](image1)

![Figure 2: Coronary calcification as a function of FAC in different age groups.](image2)

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation

Additional information
**Objective:** Brown adipose tissue (BAT) has emerged as a novel player in lipoprotein metabolism. In mice and men, BAT is activated via the β3-adrenergic receptor (β3-AR). Activated BAT combuts high amounts of lipoprotein-derived triglycerides into heat, thereby lowering plasma triglycerides and reducing obesity. However, the precise role of BAT in cholesterol metabolism and atherosclerosis development remains unclear. The aim of this study was to investigate the effects of β3-AR-mediated BAT activation on cholesterol metabolism and atherosclerosis.

**Methods and results:** Female APOE*3-Leiden.CETP mice, a well-established model for human-like lipoprotein metabolism and atherosclerosis that unlike Apoe−/− and Ldlr−/− mice expresses functional apoE and LDLR, were fed a Western-type diet and treated with the selective β3-AR agonist CL316243 (3x 20 µg/week; subcutaneous) or vehicle for 10 weeks. β3-AR agonism prevented total fat mass gain (-81%), without affecting lean mass, and increased energy expenditure (+17%). These effects were accompanied by increased activation of existing BAT and increased browning of white adipose tissue. BAT activation markedly decreased plasma triglyceride (-54%) and total cholesterol (-23%) levels, both confined to a reduction in the (V)LDL fraction. A similar lipid-lowering effect was observed after cold exposure (triglycerides -33%; total cholesterol -28%). As a consequence, BAT activation markedly reduced atherosclerotic lesion area (-43%) and the number of severe lesions (-39%) in the aortic root. Mechanistically, we demonstrate, using glycerol tri[3H]oleate and [14C]cholesteryl oleate-double-labeled VLDL-mimicking particles, that BAT activation enhances the selective uptake of fatty acids from triglyceride-rich lipoproteins into BAT depots (+105-214%), subsequently accelerating the hepatic clearance of the cholesterol-enriched remnants (+25%). These effects were dependent on a functional hepatic apoE-LDLR clearance pathway as BAT activation in Apoe−/− and Ldlr−/− mice...
reduced plasma triglyceride levels, but did not attenuate hypercholesterolemia and atherosclerosis.

Conclusions: We conclude that activation of BAT is a powerful therapeutic avenue to ameliorate hyperlipidemia and protect from atherosclerosis development. Moreover, we show the importance of a functional hepatic apoE-LDLR clearance pathway, rendering Apoe\(^{-/-}\) and Ldlr\(^{-/-}\) mice less appropriate models for studying the beneficial effects of BAT modulation on plasma cholesterol metabolism and atherosclerosis development.

Funding: Netherlands Heart Foundation (2009T087), CardioVascular Research Netherlands (CVON-GENIUS, CVON-ENERGISE).

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
M2 macrophages are found in pathological intimal thickened strips and advanced plaques with lipid uptake evident except in Perls regions

Abstract nr. 461
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation

Background: While M1 macrophages are pro-inflammatory and associated with atherosclerotic plaque instability, M2 macrophages are considered athero-protective, found to reside distant to the core, and take up minimal amounts of lipid. In particular, in an area of intraplaque haemorrhage, M2 markers are found on Mhem macrophages which take up iron rather than lipid. As promotion of an M2/Mhem form is considered a potential avenue to stabilize atherosclerotic plaques, a clearer understanding of their distribution and function within the micro-regions of the plaque needs to be acquired.

Aim: To determine the distribution of M2/Mhem macrophage markers within defined plaque (sub)regions of the atherosclerotic plaque.

Method: Sections of human carotid plaques were stained by histochemical and immunohistochemical techniques to define regions and identify cell subsets. Qualitative image analysis was performed on high resolution scanned slides.

Results: The M2 markers (CD163 and CD206) and Mhem marker (ATF) were found in pathological intimal thickened plaque sections where they could also be seen taking up lipid (as evident by ADRP co-staining). They were also present in advanced plaques not only in the cap but, contrary to the current consensus, in the core. Here they were present on foam cells (ADRP+), including being co-located with cholesterol crystals. CD163+, CD206+ and ATF+ foam cells (ADRP+) could be found in regions of intra plaque hemorrhage that were positive for glycophorin A, but not Perls - where CD163+, CD206+ and ATF+ cells were instead laden with iron. ATF, which is proposed to be specific for Mhem macrophages as it directs their differentiation, was not restricted to iron laden cells but was also found in foam cells and in macrophage sparse areas, such as the cap, co-locating with SMC. Neither M2 marker was associated with plaque calcification.

Conclusion: The finding of M2 macrophages in the pathological intimal thickened sections, with some staining for ADRP, suggests that M2 macrophages form early in the plaque, contributing to formation of the core. The uptake of either lipid or iron by M2 marker+ macrophages suggests that they may have diverse roles in the plaque, differentially impacting plaque stability.

This work was supported by WMRF.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Effects of Selective Down-regulation of Apolipoprotein C-III by Antisense Oligonucleotides.

Abstract nr. 462
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins,Pharmacology,Triglycerides

Overproduction of Apolipoprotein C-III (APOC3) is the hallmark of hypertriglyceridemia. APOC3 is related to the serum accumulation of remnant lipoproteins by secreting triglyceride (TG) rich lipoprotein (TRL) and inhibiting lipoprotein lipase (LPL). Furthermore it is recently reported that loss-of-function mutation in APOC3 reduces risk of ischemic cardiovascular disease. Repression of APOC3 expression can therefore be a reliable strategy for lowering plasma triglycerides levels and prevention of atherosclerosis.

We have demonstrated that synthetic antisense oligonucleotides (ASOs) with unique modified nucleic acids, 2’,4’-Bridged Nucleic Acids (2’,4’-BNAs), targeting APOC3 mRNA work as potential inhibitors of APOC3. We thus here screened a number of ASOs targeting APOC3 mRNA to identify more effective candidates. To further investigate the potential of these candidates that showed attractive properties in vitro and in vivo, we directly compared them with conventional fibrates. We weekly administered ASO subcutaneously or fenofibrate (FF) orally to mice fed on western diet.

Through in vitro global screening, we found that ASOs shows high activity by targeting 3’UTR of APOC3 mRNA. This region is important for mRNA to keep its stability and the result may be attributed to this. A single administration (15 mg/kg) of ASOs targeting this site reduced hepatic APOC3 mRNA significantly and enhanced post-heparin lipase activity in mice. One week after the first administration, hepatic APOC3 mRNA was reduced by 60% in mice treated repeatedly with an excess of FF (100 mg/kg/day) and PPARα-related genes also changed, while a single dose (15 mg/kg) of ASO selectively repressed APOC3 mRNA by 95% (Figure. 1). However, it is only in ASO-treated animals that serum APOC3 levels were significantly suppressed. By HPLC analysis, TRL subclasses decreased in ASO administrated group and its lipoprotein profile was similar to that of FF administrated group.

This time, we demonstrated the potential of our ASO as a lipid-lowering drug. We are currently conducting a longer-term study and working additional studies to find advantages of selective Apoc3 inhibition on atherosclerosis formation.
Figure. 1 Hepatic Apoc3 mRNA levels
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Nanoparticle-mediated delivery of Pioglitazone ameliorates inflammation and inhibits atherosclerotic plaque rupture in Apolipoprotein-E deficient mice

Abstract nr. 463
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis

Background—Inflammatory monocytes/macrophages produce various proteinases, including matrix metalloproteinases, and degradation of extracellular matrix by these activated proteinases weaken mechanical strength of atherosclerotic plaques, which results in a rupture of the plaque. Peroxisome proliferator-activated receptor-gamma (PPARg) induce polarity shift of monocytes/macrophages toward less-inflammatory phenotypes and has a potential to prevent atherosclerotic plaque ruptures. Its clinical use, however, is hampered especially in cardiovascular fields due to the adverse effects including heart failure. Therefore, we hypothesized that nanoparticle-mediated delivery of low dose pioglitazone inhibits plaque ruptures without undesired adverse effects.

Methods and Results—We used bioabsorbable poly-lactic-glycolic-acid (PLGA) as a carrier of PPARg agonist, pioglitazone. Pioglitazone incorporated in PLGA were effectively delivered to both circulating monocytes and aortic macrophages. Flow cytometric analysis of peripheral leukocytes performed 2 days after a single intravenous administration of pioglitazone-NPs showed significant change in peripheral monocyte polarity defined as Ly-6C<sup>high</sup> “inflammatory” monocyte count/Ly-6C<sup>low</sup> “non-inflammatory” monocyte count toward less-inflammatory direction. To examine whether pioglitazone-NPs inhibit plaque rupture, we used apolipoprotein E-deficient (ApoE<sup>−/−</sup>) mice fed with high-fat diet and infused with angiotensin II. Weekly injection of pioglitazone-NPs (7 mg/kg/week) for 4 weeks decreased buried fibrous caps, a surrogate marker of plaque rupture, and increased fibrous cap thickness in brachiocephalic arteries (figure A). Plaque size and macrophage accumulation were not affected. In contrast, control-NPs or daily oral pioglitazone treatment (1mg/kg/day) had no significant effects. Nanoparticle-mediated delivery of pioglitazone suppressed local MMP and cathepsin activities in the brachiocephalic arteries (figure B). Pioglitazone-NPs also suppressed the expression of MMP inducer, extracellular metalloproteinase inducer (EMMPRIN), in bone marrow-derived macrophages. Similarly, pretreatment with pioglitazone-NPs resulted in significantly lower EMMPRIN expression in thioglycollate-elicited peritoneal macrophages at both mRNA and protein levels. Oral treatment with pioglitazone increased expression of the epithelial Na channel-gamma (ENaC-g), a key molecule causing
water retention by pioglitazone, in the kidney, whereas pioglitazone-NPs did not.

**Conclusion**—Nanoparticle-mediated delivery of pioglitazone inhibited macrophage activation and atherosclerotic plaque ruptures in hyperlipidemic ApoE−/− mice. These results suggest a promising strategy to prevent atherosclerotic plaque ruptures with a favorable safety profile.

**Subdivision 1. Basic Science**

**Presentation Preference** Oral presentation

**Additional information**
The effect of improving dietary quality on measures of vascular structure and function in a well-controlled population with diabetes

Abstract nr. 464
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis,Diabetes,Intervention,Nutrition

The aim was to determine if increasing fruit (+1 serve; 150g/day), vegetable (+2 serves; 150g/day) and dairy (+1 serve; 200-250g/day) intake slows carotid intima media thickness (cIMT) progression, compared to a control group continuing on their usual diet, in people with type 1 and type 2 diabetes after 12 months. Secondary outcome measures were peripheral and central blood pressure, augmentation index (AI) and pulse wave velocity (PWV).

A 12 month randomised controlled trial was conducted. The primary outcome was mean cIMT, measured at baseline and 12 months using B mode ultrasound. Secondary outcomes were peripheral and central blood pressure, AI and PWV, measured at baseline, 3, 6, 9 and 12 months. Participants in the intervention group received dietary counselling from a dietitian at baseline, 1, 3, 6, 9 and 12 months and compliance was measured using a food frequency questionnaire administered at baseline, 3 and 12 months. Data were analysed using mixed effect modelling. Ninety-five participants completed the study. At baseline mean HbA1c was 7.2±1.4% and blood pressure (127/71mmHg), total (3.5±0.9mmol/L) and LDL cholesterol (1.7±0.7mmol/L) were within the recommendations as defined by the 2013 ESC/EASD guidelines and approximately 60% of the cohort was taking an anti-hypertensive or lipid lowering medication. Vegetable (59g; 95% CI 25, 94g; p=0.001) and fruit (197g; 95% CI 130, 264g; p=0.0001) intake were increased at 3 months in the intervention group, compared to the control group. This increase was not maintained at 12 months but intake increased overall in the cohort (fruit 53g/day; vegetables 17g/day; p<0.01). An increase in dairy consumption was not achieved but yoghurt intake was higher in the intervention group at 3 months (36g; 95% CI 6, 66g; p=0.02); this was not maintained at 12 months. At 12 months cIMT regressed (-0.01±0.04mm; p<0.001) with a greater effect in the treatment group (-0.02±0.04mm vs. -0.005±0.04mm; p=0.045). Baseline HbA1c was an independent predictor of cIMT change. There was no time by treatment effect for peripheral and central blood pressure, AI or PWV.

Improving dietary quality in well-controlled people with diabetes may slow cIMT progression.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Thread like structures in the vascular triad by ultrasound.

Abstract nr. 465
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Imaging,Pathogenesis

Vascular triad(Composite of internal juglar vein, brachio-cephalic vein, and subclavian vein) has complex structure and is not easy to verify all the composite structures. Thoracic duct and external juglar vein also drains into the subclavian vein. These veins are not given much attention during the vascular study. We have observed multiple thread like structures in the vascular triad. The thread like structures are thin and have thickness of about 100-200micron. These are observed in the similar spots in the different patients. One found in the distal left subclavian vein is most prominent. It is long thread like structure. It is at least longer than 1.3cm and about 100 to 300 micron in thickness. Echo reflection shows cord like structure with intermittent less echo dense parts. Some portion shows dual line and looks echolucent and swollen. It is seen in the left subclavian vein at the distal part before it drains into the brachiocephalic vein. The middle part is severely oscillating with both ends not much moving. The main differentiation is valves in the veins. Each of these veins and ducts are known to have valves. Opening and closing of these valves are quite different from the cardiac valves. Valve motion must be closely related to the vascular flow which depends on the driving force of venous or lymph flow. Some thin thread like structure is still, while some others show marked oscillation and dancing movement. It may give us a valuable informations about the anatomy and functional characteristics of venous and lymph channels in the vascular triad. It is intriguing to see whether there is difference in the specific cardiovascular problems such as heart failure or pulmonary hypertension. It is also intriguing to know whether some of these structures may have relationship to primo vascular system(J of Accupuncture and Meridian Studies. 5(5):201-5,2012).

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Calcific aortic valve disease: is congenitally bicuspid calcification related to atherosclerosis?

Abstract nr. 466
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Elderly, Pathogenesis

(Background) Recently, the number of adult patients with calcific aortic valve stenosis (AS) is increasing in Japan. The etiology of the aortic valve calcification in the elderly is considered the association with atherosclerosis. On the other hand, the cause of calcifying progression to AS among congenitally bicuspid valve is unknown. This study aimed to clarify the factors of progression of calcific AS comparing the morphology differences between age-related AS and congenitally bicuspid AS. (Materials and Methods) The serial 625 aortic valves obtained by aortic valve replacement for AS during recent 15 years in a single center were reviewed macroscopically and histopathologically. (Results) AS by rheumatic origin were 294 valves. Cases of suspected congenitally bicuspid AS valve were 126; 63 (50%) males, and age-related AS valve were 205; 95 (46%) males (p=0.520). Besides rheumatic AS valves, we compared AS valve morphology between valves of congenitally bicuspid origin and of acquired AS. Mean age of congenitally bicuspid AS: 63.0 ± 11.67 years, age-related AS: 75.7 ± 6.11 years (p0.0001). Age-related AS valves showed macroscopic nodular calcification with mostly tricuspid cusps and cholesterol crystal deposit microscopically. Calcification of age-related AS was more severe than congenitally bicuspid. Congenitally bicuspid AS showed fibrous thickening of cusps with nodular calcification. There was no significant difference of neovascularization and inflammatory cell infiltrations in valves between age-related AS and congenitally bicuspid AS. Immunohistochemistry pattern for osteopontin revealed no difference between age-related AS and congenitally bicuspid. Complication of ischemic heart disease was seen in 84 cases of age-related AS (41.0%) and in 8 (0.6%) of congenitally bicuspid AS (p0.0001). (Conclusion) Patients with congenitally bicuspid AS underwent surgery at a younger age than those with age-related AS because of valve deformity, but not amount of calcification. Age-related AS showed more severe calcification with cholesterol deposit and complication of coronary heart disease with high tendency. Neovascularization with inflammatory change was not directly related to the amount of calcification of aortic valve. These data showed no obvious relation of atherosclerosis to congenitally bicuspid AS.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Regulation of activation of G6PD by SM22a ubiquitination modulates GSH homeostasis and cell survival in VSMCs

Abstract nr. 467
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease

Apoptosis induced by oxidative stress is closely related to metabolic response during vascular remodeling and atherosclerosis. The glutathione system removes excess hydrogen peroxide in an NADPH-dependent, which reduces apoptosis. The main source of NADPH is the pentose phosphate pathway (PPP), contributing to the maintenance of cardiovascular cell redox state and contractility. Here, we showed that the expression and activity of Glucose-6-phosphate dehydrogenase (G6PD), as a key enzyme in PPP, is promoted in PDGF-BB-induced proliferative VSMC. PDGF-BB induced G6PD membrane translocation and activation in a SM22α ubiquitination-dependent manner. The ubiquitinated SM22α interacted with G6PD, and mediated G6PD membrane translocation. The activation of G6PD was verified by increased NADPH production, contributing to GSH homeostasis and VSMC survival. The knockdown of SM22α or mutation of SM22α K21R abolished the PDGF-BB-induced activation of G6PD. Furthermore, we found that TRAF6 mediated SM22α K21 ubiquitination in a K63-linked manner upon PDGF-BB stimulation. Knockdown of TRAF6 decreased the membrane translocation and activity of G6PD induced by PDGF-BB, parallel with reduced SM22α K21 ubiquitination. Increased G6PD activity facilitated VSMC viability via reduction of apoptosis through NADPH generation and GSH homeostasis. In vivo study, we showed that the generation of NADPH induced by injury was decreased in SM22α−/− mice carotid arteries, accompanied with reduction of G6PD activity, resulting in increased cell apoptosis, which was reversed by transduction of SM22α WT but not SM22α K21R mutant. In summary, we provide evidence that TRAF6-induced SM22α ubiquitination maintained VSMC survival through increases in G6PD activity and NADPH production. TRAF6-SM22α-G6PD pathway is a novel mechanism by which SM22α provides a link between glucose metabolism and VSMC survival, and plays an important role for vascular impair after injury and plaque stability during development of atherosclerosis.
The variability of expression of apoptosis-related genes in atherosclerotic lesions of human aortic intima

Abstract nr. 468
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Pathogenesis

The expression of apoptosis-related genes in arterial wall tissue may be used as molecular characteristics of cellular senescence during atherogenesis. This study was undertaken to estimate the cellular composition and the expression of several apoptosis-related genes, namely, CASP3, CASP9, ENO1, NTN1, NTN4, and UNC5A in unaffected intima of human aorta in comparison with that in different types of atherosclerotic lesions.

Twenty-five autopsy samples of thoracic aorta were examined. This study demonstrated that the expression of several apoptosis-related genes is changed in different types of atherosclerotic lesions, but not in a unanimous way. The mRNA expression of CASP3 is steadily decreasing with atherosclerosis progression, and that of ENO1 demonstrates the inverse trend. The expression of CASP9, NTN1, and UNC5A demonstrated, in general, similar pattern, namely, the bell-shaped relationship between gene mRNA expression and the type of atherosclerotic lesion with the maximum observed in fatty streaks. Only one of tested genes, NTN-4, did not show any relationship of its mRNA expression with atherosclerosis.

It is supposed that the most significant changes in cell composition of intima, namely, the increase of proportion of cells of hematogenic origin in atherosclerotic plaques, and activation of pericyte-like cells are accompanied by the corresponding changes in expression of CASP3, CASP9, ENO1, NTN1, and UNC5A genes. The fall in CASP3, NTN1, and UNC5A expression may be associated with the domination of necrotic processes and cell death in atherosclerotic plaques. So, the changes in expression of apoptosis-related genes in human atherosclerotic lesions are rather ambiguous. The introduction of modern technologies of next generation sequencing allowing transcriptomic approach will provide a room for further comprehensive assessment of the role of apoptosis in atherogenesis.

This study was supported by Russian Scientific Foundation, Grant no. 14-14-01038.

Subdivision 1. Basic Science
Presentation Preference Oral presentation
Additional information
Natural human apoA-I mutations L141RPisa and L159RFin alter HDL structure and functionality and promote the development of atherosclerosis in mice

Abstract nr. 469
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Atherosclerosis, Functionality, HDL

Plasma levels of high-density lipoprotein (HDL) cholesterol in humans are inversely correlated with risk for developing atherosclerosis and coronary artery disease. Apolipoprotein A-I (apoA-I) is the main protein component of HDL and has been implicated in the majority of its atheroprotective functions. Mutations in human apoA-I leading to low HDL cholesterol levels have been associated with pathological phenotypes including premature atherosclerosis, corneal opacity and amyloidosis. In the present study we investigated the effect of two naturally occurring apoA-I mutations, L141R and L159R, in vivo by generating wild-type (WT) and mutant human apoA-I transgenic mice in a mouse apoA-I deficient (apoA-I−/−) background. These mice, as well as control mice (apoA-I−/−), were analyzed for abnormalities in their lipid and lipoprotein profiles, HDL particle size, and functionality. Furthermore, the effect of these mutations on the development of atherosclerosis was assessed following a 14-week high fat diet (n=14-25/group). The expression of either apoA-I mutation resulted in markedly reduced serum apoA-I (less than 10% of WT apoA-I), total and HDL cholesterol levels (~20% and ~15% of WT apoA-I, respectively). Moreover, both mutants were characterized by the production of fewer and smaller spherical HDL particles with preβ2 and α2, α3 electrophoretic mobility. Interestingly, HDL particles bearing the mutant apoA-I forms exhibited attenuated anti-oxidative/anti-inflammatory properties as indicated by their inability to prevent LDL oxidation, and by decreased activities of PON-1 and PAF-AH enzymes in vitro. However, both apoA-I mutants demonstrated increased capacity to promote ABCA1-mediated cholesterol efflux from macrophages and to induce endothelial cell migration. Finally, expression of the human apoA-I L141R or L159R mutations in mice was associated with 3-fold increased diet-induced atherosclerosis compared to either WT apoA-I transgenic or apoA-I−/− mice. Overall, our findings suggest that natural apoA-I mutations L141R and L159R have a strong impact on HDL structure and functionality in vivo as well as on the predisposition to diet-induced atherosclerosis in the absence of any other genetic defect.

Funding: The work was funded by a grant from the General Secretariat for Research and Technology of Greece (ARISTEIA II Grant No 4220) to DK.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Triglyceride-rich lipoproteins in chronic kidney disease patients with maintenance hemodialysis treatment

Abstract nr. 470
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Chronic Kidney Disease, Lipids

Objective; Plasma triglyceride (TG) levels are high in chronic kidney disease (CKD) patients with hemodialysis (HD) treatment. One of the atherogenic cause of hypertriglyceridemia is the increase in TG-rich lipoprotein remnants, equivalent to remnant-like particle (RLP). We compared the plasma levels of TG, a representative indicator of TG-rich lipoproteins and RLP-cholesterol (C) and TG / RLP-cholesterol (C) ratio between control subjects and CKD patients with HD to speculate the atherogenesity of TG-rich lipoproteins in CKD patients with HD treatment. Materials and Methods; Plasma lipid and apoprotein levels and TG / RLP-C ratio were compared between 49 CKD patients with HD and 627 control subjects. Control subjects were separated into 4 subgroups from highest TG group to lowest TG group. RLP-C and apolipoproteins were measured with immunoprecipitation method and turbidimetric immunoassay, respectively. Comparison was also made between HD patients and age-, sex-, and plasma TG level- matched control subjects.

Results; Plasma TG levels were 107±70 (mean±S.D.) mg/dL in HD patients and 115±72 mg/dL in control subjects. Plasma RLP-C levels were 6.7±4.5 mg/dL in HD patients and 4.6±3.5 mg/dL in control group (p<0.0001). RLP-C levels decreased in descending order from highest TG group to lowest TG group in control subjects. RLP-C levels were higher in HD patients than those in control subjects with plasma TG levels lower than 150 mg/dL (p<0.0001). TG / RLP-C ratios were 19.0 ± 12.0 in HD patients and 25.9±9.5 in control subjects (p<0.0001). These ratios were significantly lower in HD patients than in all 4 TG subgroups. The comparison between HD patients and age-, sex-, plasma TG-matched control subjects showed the same results. Conclusions; Plasma RLP-C levels were high and TG / RLP-C ratio was low in CKD patients with HD treatment. These findings indicated that total plasma TG-rich lipoproteins levels were not increased but the distribution of plasma TG-rich lipoproteins skewed to remnant fractions in CKD patients with HD treatment. Therefore, plasma TG-rich lipoproteins seemed to be more atherogenic in those patients.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
RELATIONSHIP HORMONES OF ADIPOSE TISSUE IN LIPID AND CARBOHYDRATE METABOLISM FOR MEN WITH CORONARY ATHEROSCLEROSIS

Abstract nr. 471
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis,Lipids,Lipoproteins,Metabolism

In recent time there have been studies about the effects of the hormones of adipose tissue on the cardiovascular system. Research in this area are numerous, but the results are inconsistent.

**Aim:** to study relationship hormones of adipose tissue in lipid and carbohydrate metabolism in patients with atherosclerosis and coronary heart disease (CHD).

**Materials and methods:** the study included 104 men, average age 60.74±8.1 years, who were divided into control (no CHD) and the main group with angiographically verified coronary atherosclerosis and CHD. All serum samples were determined by the concentration of adiponectin, leptin, resistin, malondialdehyde (MDA); the resistance of low density lipoprotein (LDL) to oxidation, total cholesterol (TC); cholesterol high-density lipoprotein (HDL), cholesterol low-density lipoprotein (LDL ), triglycerides (TG); apolipoprotein A1 (apoA1), apolipoprotein B (apoB), lipoprotein(a) (LP(a)), glucose (GLU); levels of C-peptide and insulin.

Statistical analysis was performed in licensed version of the program SPSS (13.0).

**Results:** in men with coronary atherosclerosis, compared with the control group, from the study of complex hormones of adipose tissue, lipid-of lipoprotein and carbohydrate biomarkers in the blood was the elevated resistin, LDL cholesterol, TG, apoB, LP(a), C-peptide and reduced HDL and LDL resistance to oxidation. When studying the relationship between hormones of adipose tissue with lipid and carbohydrate metabolism were identified relationship between levels of adiponectin with such indicators as leptin, cholesterol HDL, apoA, insulin and C-peptide; resistin with apoE, with UPS; leptin with TG and glucose (p<0.01). When studying the relationship of lipid and carbohydrate metabolism were identified communication glucose with such indicators as HDL cholesterol, LDL and TG (p<0.01) and ApoB (p<0.05). The relationship with body mass index (BMI) was determined for C-peptide, insulin, leptin (p<0.01), LP(a) and HDL (p<0.05).

**Conclusion:** our data suggest a link between hormones of adipose tissue with lipid, carbohydrate metabolism and the development of coronary atherosclerosis.
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Mast cell mediated neutrophil influx enhances plaque progression

Abstract nr. 472

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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Inflammation, Pathogenesis

Activated mast cells have been identified in atherosclerotic plaques and have previously been established to promote plaque progression and destabilization. As mast cells have the ability to release chemokines that mediate leukocyte fluxes, we propose that activated mast cells play a pivotal role in leukocyte recruitment during atherosclerosis.

Western-typed diet fed B cell deficient apoE−/−muChain mice, which lack endogenous IgE, were systemically challenged with IgE or PBS to induce mast cell activation during atherosclerotic lesion development. Mast cell activation in the aortic root was indeed significantly enhanced after IgE treatment (control: 35.2 ± 3.9% versus IgE: 48.2 ± 3.4%, *P<0.05) and we observed a concomitant increase in plaque size (control: 2.0 ± 0.2*10^5 µm^2 versus IgE: 2.8 ± 0.3*10^5 µm^2, P=0.05). Intriguingly, a striking increase in the amount of perivascular neutrophils was observed in the IgE treated mice (control: 57.6 ± 10.6 neutrophils/mm^2 tissue; IgE: 183.0 ± 38.7 neutrophils/mm^2 tissue; *P<0.05). In order to investigate whether activated mast cells can directly attract neutrophils, we injected C57Bl/6 or mast cell deficient Kit(W−/−sh/W−/−sh) mice intra-peritoneally with the mast cell activator compound 48/80. Mast cell activation led to a massive neutrophil influx into the peritoneal cavity (controls: 1.0 ± 0.6 versus compound 48/80: 5.1 ± 0.7, in fold change, **P<0.01), while neutrophil numbers in mast cell deficient mice remained unaffected (controls: 1.0 ± 0.3 versus compound 48/80: 1.0 ± 0.2, P=NS). Furthermore, the newly recruited neutrophils were particularly CXCR2+ and/or CXCR4+. Interestingly, mast cells have been shown to secrete the CXCR2 and CXCR4 ligands CXCL1 and CXCL12, respectively. In vitro, supernatant of activated mast cells caused a 3-fold increase in neutrophil migration (32.3 ± 4.7*10^3 versus 11.6 ± 2.5*10^3 neutrophils after stimulation with control supernatant, **P<0.01), which was seen to be inhibited by anti-CXCR2 (18.8 ± 2.2*10^3 neutrophils, *P<0.05), but not by the CXCR4 receptor antagonist AMD3100 (24.8 ± 6.9*10^3 neutrophils, P=NS).

In this study we demonstrate that chemokines, in particular CXCL1, released from activated perivascular mast cells induce neutrophil recruitment to the atherosclerotic plaque, thereby aggravating the inflammatory response which may further enhance atherosclerotic lesion progression.
progression and destabilization.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Predicting the Presence of a Mutation Resulting in Familial Hypercholesterolemia - Development and Validation of a Prediction-model in 64,000 Subjects

Abstract nr. 473
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Familial Hypercholesterolemia,Prevention,Risk Factor

Introduction
Familial hypercholesterolemia (FH) is a hereditary disease that warrants early diagnosis to prevent premature cardiovascular disease (CVD). A definitive diagnosis is made by identification of a causal mutation. However, DNA analysis is costly and careful selection of subjects for molecular testing is important. Unfortunately, the accuracy of current selection criteria is poor, especially in young subjects. We therefore developed and validated a model to predict the presence of an FH causing mutation in persons referred by general practitioners.

Methods
All participants in the Dutch FH screening program from 1994-2014 were included in the development cohort. The validation cohort consisted of consecutive patients, suspected for FH, attending the outpatient lipid clinic in Saguenay (Quebec) from 1993-2014. Cross-sectional data was available on medical history, lipid profile and DNA analysis. Multivariable logistic regression analysis was used for model development. The primary outcome was the presence of a deleterious FH mutation.

Results
The development cohort comprised 25,809 FH patients and 38,297 unaffected relatives. Our final model included age, gender, levels of LDL-cholesterol, HDL-cholesterol and triglycerides, history and age of CVD, use of statins, smoking, alcohol, and presence of hypertension. The area under the receiver operating characteristic curve (AUC) was 85.2% (95% CI: 84.9 - 85.5). The calibration slope was 1.02 (1.00 is optimal). In the validation cohort (718 FH patients and 881 unaffected persons), the AUC was 97.3% (95%CI: 96.7 - 97.8%) and the calibration slope 1.00. The performance of the model can be illustrated by selecting subjects with a predicted probability of 30% or lower. This would identify 87.0% of all unaffected subjects, and avoid testing in 46.1% of the population.

Conclusion
Our model showed good discrimination and calibration upon internal and external validation. We
expect our model to be specifically of added value for young persons, since LDL-C and age are used as continuous predictors. The equation will be available for smartphone and on internet, which might aid physicians to decide which patients to be referred for molecular testing.

**Calibration plot of the final model in the derivation cohort (left) and validation cohort (right)**

*Receiver under the operating characteristic curve of the final model in the development cohort (blue) and validation cohort (red)*

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation

Additional information
Palmitate drives HCASMC to increase intracellular fat, oxidative stress and inflammation

Abstract nr. 474
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Lipids, Pathogenesis

Background: Palmitate is a major circulating saturated free fatty acid (FFA) that accounts for ≈30% to 40% of serum FFAs. In vitro studies show that palmitate induce atherogenesis by promoting endothelial dysfunction. Although the effects of palmitate on vascular smooth muscle cells have been evaluated in a few studies, their direct effect on migration, proliferation, oxidative stress and inflammation are not fully understood. This study examined the effect of palmitate on human coronary artery smooth muscle cells (HCASMC) dysfunction.

Methods and results: An in vitro wound-healing assay indicated that palmitate decreased HCASMC migration. Dose-response (2.5 μM -100 μM) studies showed a significantly decrease of cell migration from 25 μM to 100 μM at 8h (-20% to -35%; p<0.05) and time-course experiments (0-24h) showed a significantly decrease from 2h at 50 μM (p<0.05). The BrdU incorporation indicated that palmitate decreased HCASMC proliferation in a dose-response from 25 μM to 200 μM (p<0.05). Intracellular reactive oxygen species (ROS) was examined after incubation with 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), previous treatment of the cells with palmitate (0.5 μM to 100 μM) for 24 h. Results showed that palmitate increased ROS levels in a dose-dependent manner from 10 μM to 100 μM (p<0.05). In addition, palmitate increased intracellular lipid droplets in HCASMC by means of nile red-stained cells in a dose-dependent manner from 25 μM to 100 μM (p<0.05). Real-time PCR examination demonstrated that palmitate leaded to a significant increased of the proinflammatory MCP1 gene, with the maximum increased at 100 μM (p<0.05) at 24h. Finally, we showed by western blot that palmitate increased the active form of the proinflammatory nuclear transcription factor NFkB and the oxidative stress sensor Nrf2.

Conclusion: These findings indicate that palmitate might be critically related to HCASMC dysfunction by slowing cell migration and proliferation and inducing lipid-laden cells, oxidative stress and inflammation through activation of NFkB and Nrf2 transcription factors. This could represent another way by which to explain how palmitate exerts their proatherogenic properties.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
An epigenome-wide association study of prenatal famine identifies early gestation as critical period

Abstract nr. 475
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Epidemiology, Genetics, Metabolism, Nutrition

Adverse exposures during critical periods of prenatal life have been linked to an increased cardiovascular risk in adulthood. Animal studies implicated that epigenetic mechanisms including DNA methylation mediate this relationship. In human studies, differential DNA methylation had been reported for various adverse prenatal conditions, but studies systematically investigating the whole pregnancy and its critical periods to nutritional exposures are currently lacking. Therefore, we undertook an epigenome-wide association study (EWAS) of prenatal exposure to the Dutch Famine.

We used the quasi-experimental setting of the Dutch Famine of 1944-45 to evaluate the impact of famine exposure during specific 10-week gestation periods or during any time in gestation on genome-wide DNA methylation levels at age ~59 years. DNA methylation was assessed using the Illumina 450K array in whole blood among 348 individuals with prenatal famine exposure and 463 time- or sibling-controls without prenatal famine exposure. Famine exposure during gestation weeks 1-10, but not weeks 11-20, 21-30 or weeks 31 to delivery, was associated with an increase in DNA methylation of CpG dinucleotides cg20823026 (FAM150B), cg10354880 (SLC38A2) and cg27370573 (PPAP2C) and a decrease of cg11496778 (OSBPL5/MRGPRG) (P<5.9x10^-7, P_{FDR}<0.031). There was an increase in methylation of TACC1 and ZNF385A after exposure during any time in gestation (P<2.0x10^-7, P_{FDR}=0.034). These changes represent a shift of 0.3-0.6 standard deviations and persisted after adjustment for SES, smoking, and diet. The CpG dinucleotides are linked to genes involved in growth, development, and metabolism.

Our EWAS point to early gestation and not mid or late gestation as the critical time-period for adult DNA methylation changes in whole blood after prenatal exposure to severe under nutrition. Prevention strategies should consider early gestation to reduce the burden of cardiovascular disease.

Funding: U.S. National Institutes of Health and EU 7th Framework Program.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
ENDOTHELIAL ACTIVATION DURING CARDIAC SURGERY IN ATHEROSCLEROTIC PATIENTS

Abstract nr. 476
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Endothelium

**Background:** The endothelium is a recognized key player in vascular homeostasis and its dysfunction is one of the main feature characterizing atherosclerosis. Different mechanisms can induce endothelial dysfunction and, between them, oxidative stress plays a pivotal role. During coronary artery bypass surgery a considerable damage affects vascular endothelium. In endothelial cells the predominant cyclooxygenase (COX) dependent prostanoid is prostacyclin (PGI$_2$), which is a vasodilating agent, antagonist of the vasoconstrictor thromboxane (TXA$_2$), mainly produced by platelets.

**Aim:** to assess in vivo prostacyclin production by vascular endothelium in patients undergoing coronary bypass surgery in relation to oxidative stress.

**Material and methods:** In this study 28 consecutive patients treated with enteric coated 100 mg aspirin once-daily (od) undergoing elective coronary artery bypass graft surgery (CABG) were enrolled. After surgery they were randomized to different enteric coated aspirin regimens: 100 mg od (n=9), 100 mg twice a day (bid) (n=10), 200 mg (n=7) od. Endothelial activation (2,3-dinor-6-keto-PGF$_{1\alpha}$ (PGI-M), the urinary PGI$_2$ metabolite), oxidative stress status (urinary 8-iso prostaglandin F$_{2\alpha}$ (8-iso PGF$_{2\alpha}$)) and systemic TXA$_2$ biosynthesis (urinary 11-dehydro-thromboxane B2 (11-DH-TXB$_2$)) were assessed before and 5 days after cardiac surgery.

**Results:** After cardiac surgery we evidenced increased levels of both 8-iso PGF$_{2\alpha}$ (+67.9 pg/mg creatinine [32.8-103.1] mean [interquartile range]) and 11-DH-TXB$_2$ (+84.4 pg/mg creatinine [8.7-126.1]) and a burst of PGI$_2$ (+210.7 pg/mg creatinine [108.4-313.0]); p<0.001 for all. Even if the increment of PGI-M was lesser in the 200 mg od group than in the other groups considered (Figure 1), no significant effect of the type of treatment was evidenced (p=0.3925). Furthermore, positive correlations between the increase of PGI-M levels during surgery and 8-iso PGF$_{2\alpha}$ (r=0.474, p=0.01) and 11-DH-TXB$_2$ (r=0.417, p=0.03) were found.

**Conclusions:** Our data show that cardiovascular surgery induces an increase of oxidative stress
and of systemic $\text{TXA}_2$ production which are associated with endothelial activation and prostacyclin synthesis. The increase of prostacyclin, aimed to counteract the vascular damage, should be taken into account during the treatment with COX inhibitor agents.

Figure 1. Increment in PGI-M concentrations in the three studied groups after surgery

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Identification of a Novel Mutation in the PCSK9 Gene in an Omani Arab Subject with Familial Hypercholesterolemia

Abstract nr. 477
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia, Genetics, LDL, PCSK9

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a crucial protein in LDL (LDL) metabolism by virtue of its pivotal role in the degradation of the LDL receptor. Mutations in the PCSK9 gene have previously been found to segregate with autosomal dominant familial hypercholesterolemia (ADFH). In this study, DNA sequencing of the 12 exons of the PCSK9 gene has been performed for a Omani Arab subject with a clinical diagnosis of familial hypercholesterolemia where mutations in the LDL-receptor (LDLR) and APOB genes (screened for the common Arg3527Gln and Arg3558Cys mutations) have been excluded. One novel missense mutation was detected in the exon 7 of the PCSK9 gene in the subject. The subject was found to be heterozygote for Val333Asp. Using an array of in silico tools, we have investigated the effect of the above mutation on different structural levels of the PCSK9 protein to show that the mutation possibly destabilizes the hydrophobic core of the region of PCSK9 involved in binding to EGFa domain of LDLR, thereby increasing the affinity of the convertase towards LDLR. Mutations occurring in the exon 7 of PCSK9 are rare; therefore, this study not only reports a novel rare mutation in the PCSK9 gene but also delineates a step by step strategy, employing which is the effect of a new mutation that can be appraised on protein structure specifically pertaining to PCSK9 and similar proteins.

Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
Oleacein. Translation from Mediterranean diet to protection of atherosclerotic plaque.

Abstract nr. 478
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Nutrition,Pharmacology,Prevention

Oleacein is one the most abundant compound of phenolic fraction of extra virgin olive oil. Taking into account anti-oxidative and anti-inflammatory effects of oleacein, we examined its potential influence on the stabilisation process of carotid plaque ex vivo. A direct effect of oleacein on the macrophage phenotype were also evaluated.

The effect of oleacein on MMP-9, MMP-9/NGAL, IL-10, HO-1 and HMGB1 secretion from human carotid atherosclerotic plaques obtained from TIA patients were measured by ELISA assays. The expression of CD 163 and IL-10 in macrophage cells was determined by Flow Cytometry. The expression of CD163 receptor was confirmed by Real-time quantitative RT-PCR.

We have shown that oleacein in dose-dependent manner (from 5 to 20μmol/L) significantly decreases secretion of proteases, such as MMP-9 and complex MMP-9/NGAL as well as HMG1 and TF by the plaque stimulated by LPS. At the same time we observed increase IL-10 and HO-1 secretion. Complexes of oleacein with hemoglobin and haptoglobin 1-1 and 2-2 stimulate the expression of CD163 macrophage scavenger receptor and IL-10. This process can lead to changes macrophage phenotype cells from pro-inflammatory M1 to anti-inflammatory M2. Oleacein may play significant role in the stabilization of human carotid plaque and could be potentially useful in the reduction of the risk of ischemic stroke.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
The risk factors for essential hypertension development in adult Caucasians of Polish origin

Abstract nr. 479
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Elderly, Genetics, Hypertension, Obesity

Essential hypertension (EH) is a major risk factor for cardiovascular diseases. EH is a multifactorial disease caused by environmental, metabolic and genetic factors. It is also considered as a polygenic syndrome and various genetic variants have been reported to be significantly associated with hypertension in different populations, indicating that gene-gene and gene-environment interaction may be of great importance. The aim of the present study was to assess the impact of obesity and polymorphisms in ACE and eNOS genes on essential hypertension development in adult Caucasians of Polish origin. For the study 1 027 unrelated adults, aged 45.5 ± 13.6 years, were consecutively recruited on the basis of clinical investigation. Exclusion criteria from the study were as follows: acute endocrine dysfunction, chronic kidney or liver diseases, alcoholism. The study protocol was approved by the local ethics committee. Among the hypertensive participants, almost 70% were obese (BMI ≥30), as opposed to 36% of the obese participants in the normotensive group (p< 0.0001). In the total number of subjects the frequency of hypertension was significantly higher in males than in females and males compared to females had an 77% higher chance (OR=1.77; 95 % CI = 1.36 - 2.30) of having hypertension. Smoking at any time increased the risk of hypertension, and former smokers were more likely to be hypertensive than current smokers. Genetic variants of ACE and eNOS gene were not associated with an increased prevalence of hypertension. All studied subjects, both with and without the risk alleles, were at significantly increased risk for hypertension as indicated by obesity, male sex or nicotine smoking, and the influence of these factors on hypertension development is much stronger than the influence of candidate genes. In conclusion, the results of the present study strongly indicate that obesity, nicotine smoking, and male sex, but not genetic variations significantly enhance the risk of hypertension in adults in the Polish population.

Subdivision 3. Clinical Studies
Presentation Preference Mini-oral presentation
Additional information
Different Associations of Serum proBDNF and BDNF with Lipid Profiles in Male and Female Chinese Han Adolescents

Abstract nr. 480
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Lipoproteins, Triglycerides

As one member of the neurotrophic family, brain-derived neurotrophic factor (BDNF) has been suggested by previous studies to play a role in glucose and lipid metabolism. However, this role has not been specified and its mechanism has not been elucidated yet. To explore the specific effect on and mechanism of the role of BDNF in lipid metabolism, 56 health young subjects (22.89 ± 1.80 years, 27 males and 29 females) were enrolled and given a wash-out diet of 30.1% fat and 54.1% carbohydrate for 7 days. Twelve-hour fasting venous blood was collected after the diet. Serum levels of precursor of BDNF (proBDNF) and BDNF were measured by enzyme-linked immunosorbent assays. Serum levels of glucose, triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and apolipoproteins (Apo) A-I and B100 were analyzed by routine methods. The results showed that proBDNF was positively associated with TG ($r = 0.289$, $p = 0.04$) and BDNF with TG ($r = 0.464$, $p = 0.001$) as well as insulin ($r = 0.307$, $p = 0.028$) in the whole study population. When gender was taken into account, proBDNF was observed to be positively associated with TG ($r=0.488$, $p=0.018$), TC ($r = 0.58$, $p = 0.004$), LDL-C ($r = 0.646$, $p = 0.006$) and ApoB100 ($r = 0.567$, $p = 0.005$), and BDNF with TG ($r = 0.663$, $p = 0.001$) and insulin ($r = 0.452$, $p = 0.03$) in the male subjects. Meanwhile, only proBDNF was found to be negatively associated with ApoA-I ($r = -0.492$, $p = 0.008$) in the female subjects. These results suggest that serum proBDNF and BDNF may be associated with lipid profiles differently in male and female adolescents. The association of serum BDNF levels with the concentration of serum TG may be through the action of insulin.

The study was supported by the Open Program from State Key Laboratory of Oral Diseases (Grant No. SKLODOF2014OF03). Dr. Ding Zhi Fang is the recipient of the grant.
PROTEOMIC PROFILE OF DISTINCT MORPHOTYPES CO-EXISTING IN HUMAN MACROPHAGES SPONTANEOUSLY DIFFERENTIATED IN VITRO

Abstract nr. 481
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation

**Background:** Macrophages are heterogeneous in morphology and function and this heterogeneity has been evidenced in homeostatic and pathological conditions. In particular, heterogeneity has been described in atherosclerotic lesions where distinct macrophage subsets may drive plaque progression, stability and even regression. Tissue macrophages are not easily obtained and blood-derived monocytes are usually accepted as a good surrogate of macrophages infiltrating tissues. We previously reported that human macrophages spontaneously differentiated from monocytes (MDMs) show two dominant and distinct morphotypes (spindle and round) co-existing in the same culture.

**Aim:** To delineate the proteomic profile of the distinct MDM morphotypes spontaneously differentiated in vitro.

**Methods:** Mononuclear cells were isolated from venous blood of healthy donors by Ficoll-Paque Plus, plated in DuplexDish50 and cultured in medium 199 supplemented with 10% autologous serum. At the 7th day, MDMs were fixed and singly dissected by means of a laser capture microdissection system (PALM MicroLaser, Zeiss). Proteins were extracted from 6000 cells/morphotype, digested and qualitatively and quantitatively compared using a label-free mass spectrometry-based method.

**Results:** One hundred-thirty two proteins were identified. Among them, 28 were more abundant in round and 28 in spindle MDMs. In detail, spindle MDMs were characterized by the increase of several proteins belonging to the Rab family, small GTPases that regulate the formation, transport, tethering, and fusion of vesicles with membrane. In round morphotypes, we have detected the abundance of proteins involved either in the clearance of apoptotic cells, as transglutaminase 2, or in the lipid transport (fatty acid binding protein 4, FABP4). The increase of FABP4 was also detected in macrophage matured with M-CSF and activated with IL-4, a condition that triggers a polarization toward a non-inflammatory phenotype. In addition, heat shock proteins that are involved in the protection against stress condition were more abundant in round MDMs.

**Conclusions:** The proteome of the two dominant and distinct MDM morphotypes shows distinctive traits. In particular, the profile of round MDMs is reminiscent of an anti-inflammatory and reparative
phenotype. Results may be useful to focus on macrophage heterogeneity in health and disease and to address the effects of environmental challenges and pharmacological treatments.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
TWEAK/Fn14 interaction increases NAPDH Oxidase activity and oxidative stress in macrophages

Abstract nr. 482
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis

**Aim:** Interaction of Tumor necrosis factor-like weak inducer of apoptosis (TWEAK, Tnfsf12) with its sole receptor, fibroblast growth factor-inducible 14 (Fn14), regulates vascular damage through different mechanisms such as inflammation. Nonetheless, no study regarding TWEAK and oxidative stress has been performed so far, even though oxidative stress is a key player involved in atherosclerosis development.

**Methods and Results:** We found that TWEAK, along with its receptor Fn14, are mainly expressed in areas of human advanced atherosclerotic plaques characterized by extensive macrophage infiltration and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits expression (p22phox and Nox2), suggesting a possible link between TWEAK an oxidative stress. A positive correlation between TWEAK mRNA levels and NADPH oxidase activity (r=0.282; p<0.01) was observed in human peripheral blood mononuclear cells from human asymptomatic subjects (N=152). We demonstrated in murine macrophages RAW 264.7 that TWEAK promotes reactive oxygen species (ROS) production and NADPH oxidase activity in a dose-dependent manner. Cellular redox status was further unbalanced after TWEAK stimulation since it promoted intracellular glutathione (GSH) depletion (7.8±0.3 vs 1.5±0.5 nmoL/mg protein; p<0.001). We mechanistically showed the implication of the TWEAK-Fn14 axis in oxidative stress since genetic silencing of Fn14 or the NADPH oxidase catalytic subunit Nox2 abrogates induction of ROS mediated by TWEAK. Furthermore, we described that small G protein Rac1 activation mediates the downstream effect of TWEAK on oxidative stress. We finally moved to an in vivo murine model to demonstrate the major role of TWEAK in oxidative stress. Genetic deletion of TWEAK under an ApoE^-/- background reduced a ~50% the number of DHE positive macrophages.

**Conclusions:** TWEAK regulates vascular damage by stimulating ROS production dependent on Nox2 activity. These new insights into TWEAK/Fn14 axis underline their potential use as therapeutic targets in atherosclerosis.

**Funding support:** This work was supported by Fondo de Investigaciones Sanitarias (Programa I3-SNS to LB-C) and Instituto de Salud Carlos III (PI13/00395; RETICS RD12/0042/0038).
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Elementary Flux Mode analysis: a novel mathematical approach for metabolic pathway analysis to gain insight in gene-metabolite interactions

Abstract nr. 483
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Genetics,Metabolism

Blood metabolite profiles are thought to reflect the interaction of an individual’s genes with his/her environment and thus provide additional insight in the aetiology of metabolic and cardiovascular disease. Genome-Wide Association Studies (GWAS) of metabolite levels have led to the discovery of many novel genes and loci, that have yet to be fully interpreted for their role in complex disease.
Here, we propose Elementary Flux Mode (EFM) analysis as a novel approach to assist in the functional interpretation of GWAS results on metabolomics data. EFM analysis consists of enumerating the complete set of steady state fluxes that are possible in a metabolic network. EFM therefore represent mathematically defined pathways. We validated the potential of EFM analysis using known genetically determined metabotypes (GDMs) concerning amino acid metabolism in humans from the OMIM and GWAS catalogue database. We performed EFM analysis on a condensed version of the genome-scale stoichiometric model (GSMM) of the hepatocyte developed by Gille et al. (2010).
The resulting model yielded 8.5·10^9 EFMs that were involved in the degradation, synthesis or conversion of amino acids. Subsequently, for each reaction and amino acid, we determined the essentiality of that reaction in the metabolism of the amino acid by counting the number of EFMs that both contained the reaction and were involved in the conversion of the amino acid. Comparing these results with the selected GDMs, we found that the known genotype-metabotype relationships could be predicted with a high degree of accuracy (ROC curve AUC=0.93). In contrast, a more traditional prediction based on co-occurrence of the gene and metabolite in KEGG pathway gene sets had a low degree of specificity and was less accurate (AUC=0.79).
In conclusion, we present a novel strategy for analysing metabolomics GWAS using a Systems Biology approach. A workflow has been developed to 1) compute the EFMs in a GSMM, 2) map GWAS results to the EFMs, and 3) visualise enriched EFMs. Our approach integrates GWAS with mathematical models of human metabolism and has the potential to provide new mechanistic
insights into the causes of metabolic and cardiovascular disease.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
PCSK9 predicts coronary artery calcification in asymptomatic familial hypercholesterolemia patients

Abstract nr. 484
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia, Imaging, PCSK9

Rationale: PCSK9 inhibition is a novel therapeutic strategy aimed at reducing LDL-cholesterol. This is particularly promising for patients with familial hypercholesterolemia (FH) who are at very high risk of developing premature coronary heart disease (CHD).

Objective: to evaluate the relationship between circulating PCSK9 levels and coronary calcification score in asymptomatic FH patients.

Methods: 162 molecularly defined FH patients (25-65 years old, 52% males), were randomly selected from 5 centers participating in the SAFEHEART study. All patients had been treated with stable doses of statins for more than a year. Coronary artery calcification (CAC) was measured using the Agatston method and quantified as categorical values. Fasting plasma samples were collected and analyzed for lipids and lipoproteins. PCSK9 was measured by ELISA. Lp(a) and Apo(a) concentrations were assessed by immunoturbidimetry and LC-MS, respectively.

Results: Circulating PCSK9 levels were significantly less in patients without CAC (n=63), compared to those with CAC (n=99): 421 ng/mL (340-494) vs. 500 ng/mL (403-578), respectively, p=0.01. The main predictors for a positive CAC score was age (z=5.92, p<0.0001); PCSK9 levels (z=2.98, p=0.002); total and LDL-cholesterol (z=2.60, p=0.008); BMI (z=2.34, p=0.01); triglyceride (z=2.33, p=0.02); and glycemia (z=2.29, p<0.02). No other significant relationship was found between CAC score and any other biochemical or demographic parameter including gender, tobacco use, family history of CHD, type of LDLR mutation and Lp(a) levels. After correction for age, PCSK9 levels remained predictive of a positive CAC score (z=2.01, p=0.02) and of borderline significance (z=1.82, p=0.061) when corrected for statin treatment intensity (high vs. moderate doses).

Conclusion: In statin treated asymptomatic FH patients, elevated PCSK9 levels are associated with the presence of CAC, an independent predictor of CHD.
Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Bone-marrow activity and CFU-GM capacity of hematopoietic stem and progenitor cells are enhanced in patients with cardiovascular disease

Abstract nr. 485
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Imaging, Immunity, Inflammation

Background
Atherosclerosis is a chronic inflammatory disease that underlies acute cardiovascular events such as myocardial infarction and stroke. These cardiovascular events stimulate the bone marrow to release hematopoietic stem and progenitor cells (HSPCs) to subsequently increase the availability of circulating monocytes, and paradoxically, aggravate pre-existing atherosclerosis. Whereas this paradigm has predominantly been studied in animal models, we here set out to evaluate the effect of cardiovascular events on bone marrow activity and HSPCs in humans.

Methods and Results
First, we performed positron emission tomographic (PET) imaging in 25 cardiovascular patients with a history of myocardial infarction or stroke (aged 55±5) and 25 matched control subjects (aged 53±6) to assess 18fluorodeoxyglucose (FDG) uptake in arterial wall and bone marrow. Cardiovascular patients exhibited increased FDG uptake in the arterial wall compared to control subjects (carotid target to background ratio 2.04±0.32 versus 1.63±0.25; p<0.001). In addition, the standard uptake value (SUV) in bone marrow was increased in cardiovascular patients compared to control subjects (bone marrow SUV 2.18±0.26 in patients versus controls 1.80±0.35; p=0.018). To dissect the nature of enhanced FDG uptake in bone marrow, we assessed G-CSF harvested HSPCs in cohort of cancer patients in whom an autologous stem cell transplantation were performed (n=74). Cancer patients with a positive history of cardiovascular disease (n=44, aged 54±5) exhibited HSPCs with an increased progenitor capacity compared to control cancer patients (n=30, aged 57±8); number of CFU-GM colonies per CD34+ cells in cardiovascular patients 0.31±0.15 versus controls 0.19±0.07 (p<0.001). In support, G-CSF mobilized HSPCs harvested from healthy unrelated donors also displayed an increased CFU-GM capacity following stimulation with atherogenic substrates in vitro.

Conclusion and Clinical Relevance
We here show increased bone marrow activity coinciding with an increased CFU-GM capacity of HSPCs in patients with CVD. These findings indicate long-term changes in the bone marrow after an acute cardiovascular event. Validation of the clinical credence of this cardiovascular-bone
marrow axis may provide novel targets to attenuate the increased disease risk and injury in patients following cardiovascular events.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
The RNA-binding protein Quaking post-transcriptionally promotes differentiation of monocytes into macrophages

Abstract nr. 486
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Genetics, Inflammation

A hallmark of inflammatory diseases is the excessive recruitment and influx of monocytes to sites of tissue damage and their ensuing differentiation into macrophages. Numerous stimuli are known to induce new transcription necessary for macrophage identity, but post-transcriptional control of human macrophage differentiation is less well understood. Here, we detail our discovery that levels of the RNA-binding protein Quaking (QKI) are low in monocytes of early atherosclerotic lesions, but abundant in macrophages of advanced plaques. Specific depletion of QKI protein impaired monocyte adhesion, migration and differentiation into macrophages, and lesion formation. RNA-seq and microarray analysis of human monocyte and macrophage transcriptomes, including those of a unique QKI haploinsufficient patient, reveal developmental changes in RNA levels and alternative splicing of RNA transcripts enriched in QKI-bound sequence elements. The importance of these transcripts and requirement for QKI during differentiation illustrates a central role for QKI in post-transcriptionally guiding macrophage identity and function. These studies implicate QKI as a novel target for therapeutic intervention in inflammatory diseases.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Reference Values for Arterial Wall Inflammation in Humans using 18Fluorodeoxyglucose Positron Emission Tomographic Imaging

Abstract nr. 487
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Imaging, Inflammation

Background
18fluorodeoxyglucose positron emission tomography with computed tomography (18F-FDG PET/CT) is increasingly being applied in atherosclerosis imaging to provide an estimate of arterial wall inflammation. We aimed to determine reference values for carotid and aortic arterial wall 18F-FDG uptake in humans.

Methods and Results
18F-FDG PET/CT imaging of the carotid arteries and aorta was performed in 25 healthy subjects, 23 patients at increased coronary heart disease (CHD) risk, and 35 patients with a documented history of atherosclerotic cardiovascular disease (CVD). Arterial wall inflammation was quantified using the target-to-background ratio (TBR). The difference between healthy subjects and patients at CHD risk was 0.41±0.08 (p<0.001) for the carotid index TBRmax and 0.27±0.09 (p=0.003) for the aortic TBRmax, and the mean difference between patients at CHD risk and patients with CVD was 0.20±0.7 (p<0.003) for the carotid index TBRmax and 0.12±0.09 (p=0.059) for the aortic TBRmax. Using the 95% prediction interval in healthy subjects, the threshold for TBRmax was 1.72 in the carotid index (sensitivity 47%, specificity 95%) and 2.45 in the aorta (sensitivity 40%, specificity 95%). Additional ROC derived thresholds for TBRmax were 1.69 in the carotid index (sensitivity 97%, specificity 67%) and 2.34 in the aorta (sensitivity 93%, specificity 71%). Interscan, intra- and interobserver agreement was excellent in all arterial segments imaged in both patients as healthy subjects.

Conclusion and Clinical Relevance
In view of the emerging role of 18F-FDG PET/CT atherosclerosis imaging in clinical research and practice, the present reference values can serve as guidance for the degree of arterial wall inflammation in humans.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Removal of plasma mature and furin-cleaved PCSK9 by LDL-apheresis in familial hypercholesterolemia: application of a new assay for PCSK9

Abstract nr. 488
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia,LDL,Lipoproteins,PCSK9

Aim: Proprotein convertase subtilisin/kexin 9 (PCSK9) is known to be a good target to decrease LDL cholesterol (LDL-C) and two forms of PCSK9, mature and furin-cleaved PCSK9, circulate in blood. However, it has not been clarified whether and how the levels of each PCSK9 are affected by LDL-apheresis (LDL-A) treatment, a standard therapy in patients with severe forms of familial hypercholesterolemia (FH). Our objective was to investigate the differences in LDL-A-induced reduction of mature and furin-cleaved PCSK9 between homozygous and heterozygous FH, and between dextran sulfate (DS) cellulose adsorption and double membrane (DM) columns and to clarify the mechanism of their removal.

Methods: A sandwich ELISA to measure two forms of PCSK9s using monoclonal antibodies was developed. Using the ELISA, PCSK9 levels were quantified before and after LDL-A with DS columns in 7 homozygous and 11 heterozygous FH patients. A crossover study between the two column types was performed. The profiles of PCSK9s were analyzed after fractionation by gel filtration chromatography. Immunoprecipitation of apolipoprotein B (apoB) in FH plasma was performed.

Results: Both mature and furin-cleaved PCSK9s were significantly decreased by 55-56% in FH homozygotes after a single LDL-A treatment with DS columns, and by 46-48% or 48-56% in FH heterozygotes after treatment with DS or DM columns. The reduction ratios of LDL-C were strongly correlated with that of PCSK9 in both FH homozygotes and heterozygotes. In addition, more than 80% of plasma PCSK9s were in the apoB-deficient fraction and a significant portion of mature PCSK9 was bound to apoB, as shown by immunoprecipitation.

Conclusions: Both mature and furin-cleaved PCSK9s were removed by LDL-A in homozygous and heterozygous FH either by binding to apoB or by other mechanisms. This report is also the first to demonstrate for an ELISA method to measure both forms of plasma PCSK9—mature and furin-
cleaved form—and this technique is expected to be useful for investigating the effects of medications or the physiological or pathological roles of PCSK9 (Hori M et al. 2015 J. Clin. Endocrinol. Metab; 100: E41-9).

Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
Apolipoprotein CII and CIII and cardiovascular mortality

Abstract nr. 489
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Apolipoproteins, Cardiovascular Disease, Lipoproteins, Triglyceride-Rich Proteins

Hypertriglyceridemia caused by an increase of triglyceride-rich lipoproteins is considered to be a major risk factor of cardiovascular disease (CVD) and the plasma triglyceride concentration is an important target of lipid-lowering therapy. Apolipoprotein (apo) CII and CIII are endogenous mediators of the lipoprotein lipase activity and affect the concentration of triglycerides in the blood. The aim of our study was to investigate the association of apoCII and apoCIII with cardiovascular mortality in patients of the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study.

The LURIC study is an ongoing prospective study on environmental, biochemical, and genetic risk factors for coronary artery disease (CAD) in a cohort of Caucasian individuals. All 3266 patients included in this investigation had undergone coronary angiography, 978 deaths occurred during a median follow-up of 9.9 years, 608 patients died of cardiovascular causes.

The concentrations of apoCII and apoCIII were positively associated with the levels of triglycerides and triglyceride-rich lipoproteins. ApoCIII was only slightly correlated with CAD, whereas low concentration of apoCII was significantly associated with the severity of CAD. We used Cox proportional hazard models to analyse the association of apolipoprotein concentrations. We stratified the study cohort into quartiles according to the concentration of apoCII and apoCIII. ApoCIII was significantly associated with cardiovascular mortality with the lowest hazard ratio (HR) in the second quartile (reference quartile). The age and sex-adjusted HR in the highest quartile was 1.62 (95%CI: 1.29-2.04, p<0.001). The association retained statistical significance after adjustment for established cardiovascular risk factors including triglycerides, LDL and HDL cholesterol. The lowest quartile of apoCIII was also associated with increased cardiovascular mortality. The association of apoCII concentration with cardiovascular mortality showed also U-shaped distribution. In all statistical models, we observed the highest HRs in the quartile with the lowest apoCII concentration compared to the reference quartile (third quartile). The results of our investigation challenge the concept of apoCIII inhibition as beneficial therapy to reduce cardiovascular mortality. The association of mediators of lipoprotein lipase activity with cardiovascular events seems to be complex and the role of apoCII should also be considered in the development of new treatment strategies.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Role of proprotein convertase subtilisin/kexin 9 (PCSK9) variants in Japanese heterozygous familial hypercholesterolemia

Abstract nr. 490
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Familial Hypercholesterolemia, Genetics, PCSK9

Aim: Familial hypercholesterolemia (FH) is characterized by hypercholesterolemia, skin and tendon xanthomas and premature coronary artery disease (CAD). Low-density lipoprotein receptor (LDLR), apolipoprotein B (APOB) and proprotein convertase subtilisin/kexin type 9 (PCSK9) were identified as causative genes of FH. Gain-of-function mutations of PCSK9 are known to cause FH by enhancing degradation of LDLRs. PCSK9 E32K or R496W variant has been reported to exacerbate clinical phenotype of FH patients carrying LDLR mutations in Japanese or Caucasian. However, it has not been fully clarified whether other PCSK9 variants modify clinical phenotype of FH. The aim of our study is to investigate the distribution of PCSK9 variants and the combination of LDLR mutations and PCSK9 variants (LDLR mutations/PCSK9 variants) in Japanese FH heterozygotes and the effects of those variants/mutations on the clinical features.

Methods: Direct sequence analysis was performed for all 18 exons of LDLR and 12 exons of PCSK9 in 296 clinically diagnosed heterozygous FH.

Results: The prevalence of PCSK9 variants, LDLR mutations, LDLR mutations/PCSK9 variants, and no mutations/variants in either LDLR or PCSK9 were 10.1%, 44.6%, 8.4%, and 35.8%, respectively. Nine PCSK9 variants containing 2 novel variants such as T264I and G504W were detected. PCSK9 E32K, V4I and L21_22insL variants were the most frequently observed and showed the same prevalence (8.7%). There were no significant differences in clinical phenotype among patients carrying only PCSK9 E32K, V4I and L21_22insL variants. Furthermore, novel 18 LDLR mutations were observed. The prevalence of patients carrying both LDLR mutations and a PCSK9 variant: V4I, L21_22insL, or E32K was 3.0%, 2.0% and 0.3%, respectively. In patients carrying a PCSK9 V4I or L21_22insL and without any variants in addition to LDLR mutations, there were no significant differences in either age or lipid parameters. In patients carrying LDLR mutations, additional PCSK9 V4I variant significantly increased the prevalence of coronary artery disease.

Conclusion: Our present study demonstrates that there are 3 frequent PCSK9 variants: E32K, V4I and L21_22insL in Japanese heterozygous FH. PCSK9 V4I variant may be one of candidates to
modify the clinical phenotypes of FH patients carrying LDLR mutations.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Small dense LDL apoB is catabolized more slowly than large LDL apoB in hyperlipidemic subjects, and rosuvastatin enhances its clearance.

Abstract nr. 491
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, LDL, Metabolism, Therapy

LDL consists of particles varying in size, density, and composition. Small dense LDL (sdLDL) cholesterol (C) has been shown to be a better predictor of coronary heart disease risk than total LDL-C. Intensive statin therapy significantly lowers sdLDL-C as well as large buoyant LDL (lbLDL)-C. We tested the hypotheses that lbLDL and sdLDL apoB100 have different catabolic rates, and that statin therapy enhances the fractional catabolism of apoB100 in both particles. Six subjects (3 men and 3 women; age 63±5 years [mean±SEM]; BMI 25.5±1.5 kg/m²) with combined hyperlipidemia (triglycerides [TG] 191±50 mg/dl; LDL-C 160±16 mg/dl; HDL-C 43±6 mg/dl) received placebo and rosuvastatin 40 mg/day for 8 weeks each in sequential order. At the end of each phase, the kinetics of apoB100 in triglyceride rich lipoproteins ([TRL] d<1.019 g/ml), lbLDL (d=1.019-1.044 g/ml), and sdLDL (d=1.044-1.063 g/ml) were determined using stable isotope methodology, gas chromatography-mass spectrometry, and multicompartmental modeling. Compared to placebo, rosuvastatin markedly decreased the plasma levels of LDL-C (52%; P =0.0002), TG (32%; P=0.06), and total apoB (42%; P<0.0001). ApoB100 pool size in TRL, lbLDL, and sdLDL decreased 32±6%, 39±5%, 41±4%, respectively. These changes were attributable to significant (P<0.05) increases in apoB100 fractional catabolic rates in TRL (45±16%), lbLDL (131±66%), and sdLDL (97±32%). During both the placebo and rosuvastatin phases, sdLDL apoB100 was catabolized more slowly than lbLDL apoB100 (P=0.01 and 0.004, respectively). Rosuvastatin did not alter apoB100 production rate or the percent of TRL apoB100 converted to each LDL subfraction. Proteomic analysis of the LDL particles indicated that rosuvastatin increased apoD abundance in lbLDL and decreased apoAIV abundance in sdLDL. No significant effects on other apolipoproteins were noted. These data indicate that sdLDL apoB100 is catabolized significantly more slowly than lbLDL apoB100 in dyslipidemic subjects on placebo or statin therapy. Maximal dose rosuvastatin decreases plasma apoB concentrations by enhancing apoB100 catabolism in TRL, lbLDL, and
sdLDL and alters the distribution of minor apolipoproteins in LDL subfractions.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
DEPLETION IN LPA-I:A-II PARTICLES ENHANCES HDL-MEDIATED ENDOTHELIAL PROTECTION IN GENETIC LCAT DEFICIENCY

Abstract nr. 492
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Endothelium, HDL

LCAT deficiency is a rare disorder characterized by reduced plasma cholesterol esterification, LCAT activity, HDL cholesterol and apoA-I levels, and enhanced unesterified/total cholesterol ratio. The severe deficiency of atheroprotective HDL in carriers of LCAT deficiency should increase their risk of developing coronary heart disease (CHD); however, subjects with LCAT deficiency do not show remarkably increased preclinical atherosclerosis. The various HDL subpopulations are differently efficient in maintaining endothelial cell homeostasis and genetic HDL disorders represent a unique tool to understand the relationship between HDL quantity and quality and HDL function.

In the present study, we evaluated the vasoprotective effects of HDL isolated from carriers of LCAT deficiency, which are characterized by a selective depletion of large LpA-I:A-II particles and predominance of small, pre-β migrating HDL. For this purpose HDL from LCAT-deficient carriers and controls were isolated by ultracentrifugation and characterized on the basis of their size, shape, surface charge and apolipoprotein composition. Anti-inflammatory and vasoprotective properties of HDL were tested in endothelial cells. In addition, plasma adhesion molecule levels and flow-mediated vasodilation were measured in subjects with LCAT deficiency and controls.

In endothelial cells, HDL from LCAT deficient carriers showed increased anti-inflammatory properties and enhanced capacity to promote eNOS activation and consequently NO production with a gene dose-related effect. In agreement with the in vitro data, carriers of LCAT mutations have flow-mediated vasodilation values comparable to control subjects despite the low plasma HDL levels. The enhanced vasoactive action of carrier HDL could be explained by the selective depletion in LpA-I:A-II particles, which are less effective than LpAI particles in modulating NO production.
The in vitro and in vivo data described in the present work suggest that the specific HDL subpopulations which accumulate in LCAT deficiency are more effective in maintaining endothelial cell homeostasis.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Interferon Regulatory Factors 3 and 7 regulate the inflammatory response in vein graft remodelling

Abstract nr. 493
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Cardiovascular Disease, Inflammation

Introduction
Type I interferons (IFN) are implicated in the development of vascular proliferative diseases. Pathway analysis by gene set enrichment analysis of hypercholesterolemic ApoE3*Leiden murine vein grafts revealed that the type I IFN pathway belonged to the top 15 of significant regulated pathways. The transcriptional regulators of type I IFN and type I IFN responsive genes are the interferon regulatory factors (IRF). IRF 3 and IRF 7 are downstream factors of TLRs, which play a role in vein graft remodeling. The aim of this study was to investigate the role of IRF3 and IRF7 in vein graft remodeling.

Methods and Results
In vein grafts in atherosclerotic ApoE3*Leiden mice both IRF3 and IRF7 are expressed. The importance of IRFs in vein graft remodeling is illustrated by the increase in vein graft thickening in Irf3−/− and Irf7−/− mice 28 days after surgery, compared to control mice (n=9/group, Irf3−/−; 39%, p=0.185, Irf7−/−; 68% p=0.003). Also an increase in outward remodeling (Irf3−/−; 26%, p=0.081, Irf7−/−; 42%, p=0.049) was observed after 28 days. Immunohistochemical analysis revealed that both Irf3−/− and Irf7−/− mice showed a significant higher influx of macrophages in the vessel wall than the control mice whereas the Irf7−/− mice also showed a significant decrease in collagen content. RNA levels of typical type I IFN responsive genes such as Mx1, Ifit1-3 and Oas2 were down regulated in the knockout vein grafts in comparison to control vein grafts. Activation of both Irf3−/− and Irf7−/− bone marrow derived macrophages with LPS and poly:(IC) as ligands for TLR4 and TLR3 respectively, resulted in a significant increase in TNFα production. This suggests a role of IRF 3 and IRF7 in modulating the inflammatory responses during vascular remodelling.

Conclusions
IRFs regulate vein graft remodeling since Irf3−/− and especially Irf7−/− vein grafts show increased vessel wall thickening and outward remodelling 28 days after surgery, due to a pro-inflammatory response as reflected by the increase in macrophages in the vein graft wall.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Ankylosing spondylitis (AS) is a chronic inflammatory disease associated with increased risk of cardiovascular disease (CVD). High-density lipoprotein (HDL) exerts a series of anti-atherogenic properties and protects from CVD. The aim of this work was to evaluate whether HDL’s anti-atherogenic properties are impaired in AS patients. HDL (apoB-depleted serum) was isolated from 35 patients with AS and 35 age- and sex-matched controls. We measured the antioxidant capacity of HDL, the ability of HDL to induce cholesterol efflux from macrophages, the activity of HDL-associated enzymes paraoxonase-1 (PON1) and myeloperoxidase (MPO), as well as the ability of HDL to activate the endothelial Akt kinase by phosphorylation at Ser473. We found that HDL from AS patients had decreased antioxidant capacity and decreased ability to promote cholesterol efflux from macrophages compared to controls. HDL-associated PON1 activity was lower and HDL-associated MPO activity higher in patients with AS compared to controls. In addition, HDL form AS patients had impaired endothelial Akt kinase activating properties that correlated inversely with the MPO/PON1 ratio. Overall, our findings show that HDL from patients with AS display impaired anti-atherogenic properties. Attenuation of HDL properties may constitute a link between AS and CVD.

Funding: The work was supported by a grant from the Ministry of Education of Greece (Thalis Grant MIS 377286).
IL-25 inhibits atherosclerosis development in apoE deficient mice

Abstract nr. 495
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Immunity, Inflammation

Aim: IL-25 has been implicated in the induction of type 2 immunity and several studies have identified innate lymphoid type 2 cells (ILC2s) as the cytokine’s major target cell population. The aim of this study was the investigation of the role of IL-25 in atherosclerosis.

Methods: Atherosclerosis-prone apolipoprotein (apo)E deficient mice were treated for 1 and 4 weeks with 1μg recombinant mouse (rm)IL-25/day both on early and late disease. Oil Red O staining of the aortas determined the outcome of the disease while immunohistochemistry was performed in order to assess the macrophage, collagen and CD3+ T cell content of subvalvular plaques. Additionally, ApoE/Rag-1, IL5 deficient mice were treated with daily injections of 1μg rmIL-25 for 1 week while apoE deficient mice were also treated accordingly with rmIL-5. Cell populations were analyzed with the use of flow cytometry. Splenic ILC2s were immunomagnetically enriched, FACS-sorted, in vitro expanded and transferred to apoE deficient mice. Cytokine levels and plasma immunoglobulins were assessed with the use of Luminex technology and ELISA respectively.

Results: One week treatment of apoE deficient mice with rmIL-25 robustly expanded ILC2s in the spleen which was accompanied by increased levels of the natural antibody-producing B1a cell population in the spleen, anti-phosphorylcholine (PC) natural IgM antibodies in plasma and increased levels of IL-5 in plasma and spleen. Transfer of ILC2s to apoE deficient mice was accompanied with an increase of B1a cells in the spleen. Treatment of apoE/Rag-1 deficient mice with IL-25 was also associated with extensive expansion of splenic ILC2s and increased plasma IL-5, suggesting ILC2s to be the source of IL-5. Administration of IL-25 in IL-5 deficient mice resulted in an expanded ILC2 population but did not stimulate generation of anti-PC IgM, indicating that ILC2-derived IL-5 is required for the downstream production of natural antibodies. Additionally, administration of IL-25 for 4 weeks in apoE deficient mice reduced atherosclerosis in
the aorta both during initiation and progression of the disease. **Conclusions:** IL-25 has a protective role in atherosclerosis mediated by innate responses, including ILC2 expansion, increased IL-5 secretion, B1a expansion and natural anti-PC IgM generation.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Weight loss improves plasma markers of endothelial function and arterial stiffness: A randomized controlled trial with healthy abdominally obese men

Abstract nr. 496
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Endothelium, Lifestyle, Obesity, Prevention

Background: Effects of body weight on vascular function are not clear. Therefore, we examined (i) cross-sectional differences between healthy normal-weight and abdominally obese men and (ii) effects of dietary weight loss on vascular function markers related to cardiovascular disease.

Design: Twenty-five healthy normal-weight and 50 healthy abdominally obese men participated. The obese subjects [waist circumference between 102 and 110 cm] were allocated to dietary weight-loss or no-weight loss control groups. Subjects assigned to the weight-loss program followed a very low-calorie diet for 6 weeks to obtain a waist circumference below 102 cm, followed by a weight-maintenance period for 2 weeks. Subjects assigned to the control treatment maintained their habitual diet and physical activity levels. The normal-weight men were measured once and the obese men at baseline and after the intervention period.

Results: The mean weight reduction was 10.2 kg [95% CI: 9.1 to 11.3 kg; P < 0.001]. Plasma markers of endothelial function were lower [sE-selectin; P < 0.001] or tended to be lower [sICAM-1; P = 0.083] in normal-weight compared with obese men, but improved following dietary weight loss compared with the control treatment by 29 ng/mL [95% CI: 16 to 42 ng/mL; P < 0.001] and 37 ng/mL [95% CI: 25 to 50 ng/mL; P < 0.001], respectively. Cross-sectional differences were observed at baseline for some [carotid-femoral pulse wave velocity (cfPWV)] (P < 0.01), but not all vascular function markers [brachial artery flow-mediated vasodilaton (FMD), reactive hyperemia (RHI) and central augmentation indices (AIx)]. Markers of arterial stiffness [AIx and cfPWV] tended to improve in the weight-loss group compared with the control group by 3.7% [95% CI: -0.6 to 8.0%; P = 0.086] and 0.5 m/s [95% CI: 0.0 to 1.1 m/s; P = 0.065], respectively. Changes in vascular measurements of endothelial function [FMD and RHI] did not reach statistical significance.

Conclusion: Cross-sectional differences were observed in plasma markers of endothelial function and arterial stiffness. In fact, these markers improved in the obese men following dietary weight loss, and became comparable with those of normal-weight subjects. No effects of body weight on
vascular measurements of endothelial function were found.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Cholesterol lowering efficacy of plant stanol ester chewable food supplement

Abstract nr. 497
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Intervention, LDL, Nutraceuticals, Nutrition

Modification of diet remains a recommended approach for prevention of rise in serum cholesterol as well as for management of elevated serum cholesterol concentration either alone or alongside lipid lowering medication. There is a need for new dietary tools that are in addition to proven efficacy, convenient to undertake as part of normal daily activities. Plant stanols, administered in foods including spreads and yoghurt drinks, have been shown to efficiently lower serum cholesterol by 7 to 10% at a daily dose of 2g. Here, we aimed to study serum cholesterol lowering efficacy of plant stanol ester in a new product matrix, an ambient chewable, easy-to-swallow, food supplement.

A randomized, double-blind, placebo-controlled clinical trial was conducted at Helsinki University Hospital, Helsinki, Finland during autumn 2014. Healthy adult men and women with serum LDL-cholesterol exceeding 3 mmol/l, not consuming lipid lowering medication were randomized to the study. The participants consumed four chewable supplements (Raisio Nutrition Ltd, Raisio, Finland) either with (n=50) or without (n=53) plant stanol ester daily for four weeks. The supplements were advised to be consumed in two lots per day with meal, the daily dose of plant stanols being 2g per day.

Total cholesterol was lowered on average by 0.32 mmol/l (4.9%, 95%CI -8.0 to -1.8%) and LDL-cholesterol by 0.31 mmol/l (7.5%, 95%CI -12.0 to -3.1%) by plant stanol ester supplement compared to the controls (p<0.01). No statistically significant change in HDL-cholesterol (mean difference -0.01 mmol/l) or serum triglycerides (mean difference 0.002 mmol/l) was measured. Based on calculating the returned supplements, 98% (74 to 107%) of the supplements were consumed by the participants. The majority considered the taste of the plant stanol ester supplement to be good or very good (68% of the responders) and they also considered the supplement to be easy or very easy to consume daily (78% of the responders) at the advised dose for four weeks.

The results demonstrate that consumption of plant stanol ester chewable supplement lowers serum cholesterol efficiently compared to the control supplement and thus provides a convenient tool for prevention of and for dietary management of elevated blood cholesterol levels.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Mini-oral presentation
Additional information
The Relationship Between Gamma-Glutamyl Transferase Levels and Coronary Plaque Burdens and Plaque Structures in Young Adults With Coronary Atherosclerosis

Abstract nr. 498
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Risk Factor

**Background:** Elevated gamma-glutamyl transferase (GGT) levels have been demonstrated to be associated with poor prognoses in patients with coronary artery disease. Coronary computed tomography angiography (CCTA) is a noninvasive imaging modality that may differentiate the structure of coronary plaques. Elevated plaque burdens and noncalcified plaques, detected by CCTA, are important predictors of atherosclerosis in young adults.

**Hypothesis:** The present study investigated the possible relationship between GGT levels and coronary plaque burdens/structures in young adults with coronary atherosclerosis.

**Methods:** CCTA images of 259 subjects were retrospectively examined, and GGT levels were compared between patients with coronary plaques and individuals with normal coronary arteries. Coronary plaques, detected by CCTA, were categorized as noncalcified, calcified, and mixed, according to their structures. The significant independent predictors of coronary atherosclerosis were also analyzed using multivariate logistic regression analysis.

**Results:** GGT levels were significantly higher in patients with coronary plaque formation than in controls (35.7 ± 14.7 vs 19.6 ± 10.0 U/L; \( P < 0.001 \)). GGT levels were also positively correlated with the number of plaques; presence of noncalcified plaques; and levels of high-sensitivity C-reactive protein (hs-CRP), hemoglobin A1c, uric acid, and triglycerides. Moreover, smoking and levels of GGT, hs-CRP, uric acid, and low-density lipoprotein cholesterol were independent predictors of coronary atherosclerosis.

**Conclusions:** GGT is an inexpensive and readily available marker that provides additional risk stratification beyond that provided by conventional risk factors for predicting coronary plaque burdens and plaque structures in young adults.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Association between Serum Vitamin D Levels and Subclinical Coronary Atherosclerosis and Plaque morphology in Young Adults

Abstract nr. 499
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Cardiovascular Disease, Risk Factor

BACKGROUND:
We investigated the link between serum vitamin D levels and subclinical coronary atherosclerosis in young adults. We measured serum 25OHD levels, high-sensitivity C-reactive protein (hs-CRP) and uric acid levels in asymptomatic patients under 45 years old without known cardiovascular disease.

METHODS:
We analyzed the relation between serum levels of 25OHD and subclinical atherosclerosis and coronary plaque morphology. Subclinical coronary atherosclerosis was defined as the presence of any plaques on a coronary CT angiography in asymptomatic patients.

RESULTS:
Patients with subclinical atherosclerosis had significantly higher serum total cholesterol, triglycerides, hs-CRP, uric acid, hemoglobin A1c (HbA1c) and creatinine levels and lower serum 25OHD levels compared with those without subclinical atherosclerosis. There was no significant correlation with 25OHD and plaque morphology. In multivariate logistic regression analysis subclinical coronary atherosclerosis was associated hs-CRP (adjusted OR: 2.832), vitamin D (adjusted OR: 0.689), and uric acid (adjusted OR: 3.671).

CONCLUSIÓN:
Serum levels of 25OHD are significantly associated with subclinical coronary atherosclerosis.
Proteomic analysis of HDL particles from abdominal aortic aneurysm patients

Abstract nr. 500
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords HDL, Pathogenesis

Introduction and Objectives: High-density lipoprotein (HDL) cholesterol levels have been inversely associated with abdominal aortic aneurysm (AAA). Only recently, however, has protein composition rather than HDL concentration been suggested to be involved in the protective mechanisms of HDL in atherothrombosis. The objective of this work is to detect protein alterations in HDL particles from AAA patients.

Methods: HDLs were isolated from plasma of AAA patients (n=14) and controls (n=7) by ultracentrifugation. To assess quantitative changes in the HDL proteome, proteins were subjected to relative quantification in multiplexed mode based on iTRAQ labelling followed by LC-MS/MS analysis using a QExactive. Quantitative data were analysed by means of a home-made workflow built around the statistical model recently developed in our laboratory that allows integration and comparison of data from biological replicates, detailed analysis of variance at the protein level, and systems biology analysis of protein functionality.

Results and Discussion: We quantified up to 535 proteins in the HDL proteomes, and detected 15 proteins whose abundance consistently changed in patients with AAA relative to controls. Among others, serum paraoxonase/arylesterase 1, peroxiredoxin-6 and HLA class I histocompatibility antigen were found increased, whereas complement C3, alpha-2-macroglobulin and alpha-1-antitrypsin were decreased in HDL from AAA patients. Further analysis by western-blot validated the differences observed in the proteomic studies. Quantitative systems biology analysis revealed that the abundance of proteins involved in antigen processing/presentation process and acute-phase response was consistently increased, whereas those associated with regulation of proteolysis, inflammatory response and complement activation were decreased in HDL from AAA patients as compared to controls.

Conclusions: Proteins involved in redox balance, immuno-inflammation and proteolysis are altered in HDL from AAA patients. This study identifies novel markers potentially underlying the beneficial
effects of HDL in AAA.

Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
Total cholesterol levels are highly correlated with LDL cholesterol and non-HDL cholesterol but less so with sdLDL cholesterol and Lp(a).

Abstract nr. 501
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, Lipids, Risk Factor

Recent guidelines in the United States recommend using only total cholesterol (TC) as a surrogate marker of atherogenic lipoproteins. Other lipid parameters have been reported to serve as better markers of heart disease risk. Our goal was to examine the interrelationships of total cholesterol with other measures of atherogenic lipids and lipoproteins.

We assessed lipid and apolipoprotein parameters in 228,142 subjects (108,494 men; 119,638 women) with a mean age of 57.8 years and a mean body mass index of 29.7 kg/m². Subjects who were indicated to be non-fasting or on lipid-lowering therapy (statins, ezetimibe, niacin, or fibrates), or to have established cardiovascular disease were excluded. Serum TC, triglycerides (TG), direct LDL cholesterol (LDL-C), small dense LDL-C (sdLDL-C), lipoprotein(a) (Lp(a)), and apolipoprotein B (apoB) were determined by direct automated and standardized assays; LDL particle number (LDL-P), by nuclear magnetic resonance. Direct very low density lipoprotein cholesterol (VLDL-C) and non-high density lipoprotein cholesterol (non-HDL-C) levels were calculated by subtracting directly-measured LDL-C and HDL-C from TC. LDL-C and VLDL-C were also calculated using the Friedewald formula.

In all subjects, median values [IQR] of the measured parameters in mg/dL were TC 186 [158-217], TG 111 [80-160], LDL-C 111 [87-139], non-HDL-C 130 [104-161], VLDL-C 18 [12-25], sdLDL-C 25 [19-36], apoB 91 [75-110], and Lp(a) 16 [11-45]. LDL-P median values [IQR] were 1247 [947-1591] nmol/L. All parameters were significantly higher in women than in men, except TG, VLDL-C, and sdLDL-C, for which men had higher levels. Calculated LDL-C values were significantly lower than direct LDL-C values (105 versus 111 mg/dL), while the converse was true for calculated VLDL-C (22 mg/dL) versus direct VLDL-C (18 mg/dL). TC was significantly correlated (Spearman) with direct LDL-C (0.92), non-HDL-C (0.90), calculated LDL-C (0.89), and apoB (0.84), but less so with LDL-P (0.75), sdLDL-C (0.69), VLDL-C (0.26), TG (0.25), and Lp(a) (0.06).

These data indicate that TC correlates well with direct and calculated LDL-C and non-HDL-C, but not as well with sdLDL-C, and certainly not with Lp(a). In our opinion, sdLDL-C and Lp(a) add significant information about cardiovascular disease risk not obtained from the measurement of
TC.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference
Electronic poster presentation

Additional information
BACKGROUND: Homoarginine, an L-arginine homologue, is a cationic amino acid derived from lysine. Its physiological role is unknown, but the structural similarity to L-arginine suggests that it may be an alternative substrate for nitric oxide synthase. Data from the Ludwigshafen Risk and Cardiovascular Health Study have shown that low serum concentrations of homoarginine are an independent risk factor for all-cause and cardiovascular mortality. Recently, genome-wide association studies identified three genomic loci significantly related to serum levels of homoarginine. The strongest association was observed on chromosome 15 at the L-arginine:glycine amidinotransferase locus, the enzyme responsible for the synthesis of homoarginine.

AIM: The purpose of this study was to evaluate the effect of homoarginine on neointimal formation in a rat model of balloon injury.

METHODS: Thirty-six male Sprague-Dawley rats underwent endothelial injury at the level of the left carotid, followed by the insertion of a cannula into the right jugular vein. The cannula was connected to an Alzet pump containing saline, L-arginine (30 mg/kg per day, used as a positive control) or homoarginine (30 mg/kg per day). Fourteen days after balloon injury, blood was collected and left carotids were harvested for histological analyses. Systolic blood pressure was measured before and at the end of drug treatments.

RESULTS: As expected, L-arginine administration significantly reduced the carotid intimal/medial area ratio compared to that of controls (0.69±0.40 vs 1.33±0.67, p<0.05). Homoarginine-treated rats also showed a significant reduction of the vessel intimal/medial area ratio versus controls (0.71±0.43 vs 1.33±0.67, p<0.05). No changes in systolic blood pressure were detected by treatment in all groups. Plasma L-arginine concentration was significantly increased in both L-arginine- and homoarginine-treated rats compared to controls (p<0.05).

CONCLUSIONS: Our study shows that homoarginine is able to inhibit neointima formation in
balloon-injured rat carotid arteries. Moreover, our data indicate that this effect could be due, at least in part, to an increased availability of L-arginine.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
BAT activation enhances the lipid-lowering effect of statin treatment in APOE*3-Leiden.CETP mice

Abstract nr. 504
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Dyslipidemia, Hypolipidemic Drugs, Lipids

Introduction:
Statins are currently the most widely used lipid-lowering drugs, but prevent only 15-30% of all cardiovascular events, urging the need for new treatment strategies. Brown adipose tissue (BAT) produces heat by burning triglyceride-derived fatty acids and contributes largely to triglyceride clearance. As a result BAT activation accelerates the hepatic clearance of cholesterol-enriched lipoprotein remnants via the apoE-LDL receptor (LDLR) pathway, thereby alleviating hypercholesterolemia and atherosclerosis development in hyperlipidemic APOE*3-Leiden.CETP mice. Since statins upregulate the hepatic LDLR expression, the aim of this study was to investigate whether BAT activation and statin treatment cooperate in lowering hyperlipidemia.

Methods and Results:
Female APOE*3-Leiden.CETP mice were fed a Western-type diet (0.15% cholesterol; 3 weeks) and subsequently treated with the selective β3-adrenergic receptor agonist CL316243 to activate BAT (20 µg/day; subcutaneous), atorvastatin (0.0036% w/w, supplemented through the diet) or both for 2 weeks. BAT activation alone and in combination with atorvastatin markedly lowered plasma triglyceride levels (both approx. -60%; P<0.01), whereas atorvastatin treatment did not affect triglyceride levels. Total cholesterol levels were lowered by both BAT activation (-29%; P<0.005) and atorvastatin treatment (-31%; P<0.001), and were more drastically lowered by the combination (-44%; P<0.0001). In order to study triglyceride and cholesterol kinetics we intravenously injected glycerol tri[3H]oleate and [14C]cholesteryl oleate double-labeled 45 nm-sized VLDL-mimicking particles. The selective uptake of triglyceride-derived fatty acids into BAT was markedly enhanced by BAT activation alone (+234%; P<0.0001) and on top of atorvastatin (+220%; P<0.0001), but not by atorvastatin alone. Moreover, the hepatic uptake of cholesterol-enriched remnants only tended to be increased by BAT activation (+18%) or atorvastatin (+22%), and was clearly further increased when BAT activation was combined with atorvastatin treatment (+70%; P<0.005).

Conclusions:
BAT activation enhances the lipid-lowering effect of statin treatment, via accelerating the formation and subsequent hepatic uptake of cholesterol-enriched lipoprotein remnants. We, therefore, postulate that combining statin treatment with BAT activation is a promising new avenue to combat hyperlipidemia and likely also cardiovascular diseases.

**Funding:**
Netherlands Heart Foundation (2009T087) and CardioVascular Research Netherlands (CVON-GENIUS and CVON-ENERGISE).

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Low levels of IgM antibodies against an AGE-modified apolipoprotein B100 peptide predict cardiovascular events in non-diabetics

Abstract nr. 505
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease

Increased glucose levels are associated with generation of advanced glycation endproduct (AGE)-modifications. Interaction between AGE-modified plaque components and immune cells is believed to play an important role in the development of vascular complications in diabetes. Methylglyoxal (MGO) is one type of reactive aldehyde that gives rise to AGE-modification. We have previously shown that high levels of IgM against MGO-modified apolipoprotein B (apo B)100 are associated with less coronary artery calcification in patients with type 2 diabetes. Several studies have inferred a relationship between IL-5 and natural IgM antibodies. The present study analysed if autoantibodies against MGO-modified epitopes of the LDL-protein apo B100 predict cardiovascular events.

A library consisting of 302 peptides comprising the complete apoB100 molecule was screened to identify peptides targeted by MGO-specific antibodies. Peptide (p) 220 (apo B amino acids 3286-3305) was identified as a major target. Baseline IgM and IgG against MGO-p220 were measured in 700 individuals from the Malmö Diet and Cancer Cohort. A total of 139 cardiovascular (CV) events were registered during the 15-year follow-up period. Controlling for major CV risk factors demonstrated that subjects in the lowest tertile of MGO-p220 IgM had an increased risk for CV events (hazard ratio (95% confidence interval): 1.77 (1.09-2.86), $P_{\text{trend}}=0.017$). Interestingly, the association between MGO-p220 IgM and CV events remained and even tended to become stronger when subjects with diabetes were excluded from the analysis (2.08 (1.19-3.63), $P_{\text{trend}}=0.008$). MGO-p220 IgM was inversely associated with plasma glucose, but not with oxidized LDL. Furthermore, MGO-p220 IgM levels were significantly associated with plasma IL-5 levels, indicating that these antibodies could be natural antibodies recognizing epitopes in modified LDL.

To test whether IgM against MGO-p220 are natural antibodies secreted by B1 cells, we purified different B-cell populations from four healthy donors and measured binding of secreted antibodies...
to MGO-p220. B1 cells from three out of the four donors produced high amounts of IgM binding to MGO-p220.
B1 cells produce IgM that recognize MGO-modified peptide sequence in apo B. Subjects with low levels of these antibodies have an increased risk to develop CV events and this association is present also in non-diabetic subjects.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Persistent cellular and arterial wall inflammation in patients with rheumatoid arthritis in remission

Abstract nr. 506
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Inflammation

Background— Patients with Rheumatoid Arthritis (RA) are characterized by a two-fold increased cardiovascular (CV) risk, which has been attributed to the pro-inflammatory state. The introduction of biological agents (mainly anti-TNFα) has markedly reduced the patient-exposure to an active inflammatory state in RA. However, whereas part of RA patients maintain complete remission using only methotrexate (MTX) therapy, others need persistent biological therapy. We hypothesize that RA patients needing biologicals to maintain disease remission are characterized by a persistent cellular inflammatory drive that might affect the inflammation status in the arterial wall. We therefore evaluated the inflammatory state of circulating monocytes and the arterial wall in remissive RA patients on either MTX or MTX and biological maintenance therapy.

Methods and Results— We included RA patients using MTX maintenance (MTX; n=15, Disease activity score (DAS) 1.76[1.49-2.48]) and RA patients using biological maintenance therapy on top of MTX (BIOL; n=9, DAS 1.56[1.46-1.97]). Flowcytometry revealed that isolated monocytes of BIOL patients showed increased expression of pro-adhesive (CCR2) and migratory (CD11b) surface markers, as well as an increased adhesion to human arterial endothelial cells, compared to MTX patients. In addition, monocytes from BIOL patients showed higher production of the cytokines TNFα and IL1b after LPS challenge. The inflammatory phenotype of circulating monocytes coincided with increased arterial wall inflammation assessed with ¹⁸ Fluorodeoxyglucose positron emission tomography in BIOL patients (target-to-background ratio (TBR) in carotid artery in BIOL 1.97±0.25 versus in MTX 1.48±0.14; p=0.017).

Conclusions— Patients with RA needing maintenance biological therapy are characterized by activation of circulating monocytes and an increased inflammatory state of the arterial wall, despite the clinical remission. The persistent, systemic inflammatory state bears clinical consequences for cardiovascular prevention in RA patients, particularly if biological maintenance is required.

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Elevated LDL and the inflammatory response

Abstract nr. 507
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Cardiovascular Disease, Inflammation, LDL

Introduction Atherosclerosis is a lipid-driven, low-grade inflammatory disease. Besides inducing inflammation in the atherosclerotic lesions, low-density-lipoprotein (LDL) was recently shown to also elicit a long-lasting pro-inflammatory phenotype in circulating monocytes via histone modifications. Statins, known to lower LDL-c, have also been suggested to have a direct anti-inflammatory effect in patients. In the present study, we investigate the effect of statin treatment on the phenotype of circulating monocytes.

Methods and results We included patients with familial hypercholesterolemia (FH), characterized by severely elevated LDL-c levels. Patients were divided in no statin (n=20, LDL 6.9±1.7) versus statin therapy (n=9, LDL 2.7±0.54). Using flow cytometry, increased monocyte counts were observed in no-statin patients. These patients also had higher monocyte expression of cell-adhesion markers CCR2 and CD11c. Upon challenge for 24 hours with LPS, monocytes of statin users revealed a significantly reduced cytokine response of TNF and a significantly increased anti-inflammatory response of IL-10. Chromatin immunoprecipitation will be performed investigating underlying histone modifications.

Conclusions Here we show that statin use attenuates the inflammatory phenotype of circulating monocytes in patients with FH. Awaiting the effect of non-statin LDL-c lowering compounds, this study implies that anti-inflammatory effects on circulating monocytes may contribute to an atheroprotective effect of LDL-c lowering strategies.

Subdivision 2. Translational Research

Presentation Preference Mini-oral presentation
Additional information
Pharmacogenetic profile of lipid response to atorvastatin in children and adolescents with familial hypercholesterolemia

Abstract nr. 508
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Familial Hypercholesterolemia, Hypolipidemic Drugs, Lipids, Pharmacology

In children and adolescents with familial hypercholesterolemia (FH) pharmacotherapy with statins is the cornerstone in the current regimen to reduce low density lipoprotein cholesterol (LDLc) and premature coronary heart disease risk. There is, however, a great interindividual variation in response to therapy, partially attributed to genetic factors. It has been suggested that genetic polymorphism of the enzymes POR, CYP3A4, CYP3A5 and solute carrier SLCO1B1 may influence this variation. We analyzed the association of alleles POR*28, CYP3A4*22, CYP3A5*3 and SLCO1B1 521T>C and 388A>G with lipid levels response to atorvastatin. The study included 105 FH children and adolescents treated with atorvastatin (10 to 40mg). Total cholesterol (TChol) and LDLc were measured at baseline and after 6 months of treatment. POR*28 CYP3A4*22, CYP3A5*3 alleles and SLCO1B1 521T>C and 388A>G genotypes were determined with TaqMan or PCR-RFLP methods. POR*28 carriers had significantly lower % mean reduction of TChol (33.1% in *1/*1, 29.8% in *1/*28 and 25.9% in *28/*28 individuals, p=0.045) and of LDLc (43.9% in *1/*1, 40.9% in *1/*28 and 30.8% in *28/*28 individuals, p=0.013). In multivariable linear regression adjusted for confounding factors, POR*28 genotypes, additionally to baseline cholesterol level, account for an estimated 8.3% and 7.3% of overall variability in % TChol and LDLc reduction (β:4.05; 95% CI 1.73-6.37; p=0.001 and β:5.08; 95% CI 1.62-8.54; p=0.004, respectively). CYP3A4*22, CYP3A5*3 and SLCO1B1 521T>C and 388A>G polymorphisms were not associated with lipid reductions and did not modify the effect of POR*28 on atorvastatin response. In children with FH, carriage of POR*28 allele was associated with reduced effect of atorvastatin on TChol and LDLc and therefore identifies FH children that may require higher atorvastatin doses to achieve full therapeutic benefits. Further studies in different populations are needed to replicate the association.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Available data associate high-density lipoprotein (HDL-C) concentrations in men with body mass index (BMI), anabolic steroids, age, and certain cytokines. Data are less clear in women, especially across the full adult lifespan. We postulated that sex steroids, interleukins, body composition, and age control HDL-C in healthy women distinctly. To test this concept, 120 healthy women in Olmsted County, MN, USA, a stable well studied clinical population, provided 10-hr fasting HDL-C, TC, LDL-C and TG (dependent variables) and concomitantly testosterone, estrone, estradiol, 5-alpha DHT and sex-hormone binding globulin (SHBG) all by mass spectrometry; insulin, glucose, and albumin (Mayo Res Lab); abdominal visceral and total fat (AVF and TAF, by CT scan); and a panel of 7 cytokines (by ELISA) (all independent variables). Univariate and multivariate regression analyses were applied at overall protected P<0.001 for multi-R squared. HDL-C, at the univariate level, correlated significantly negatively with (P value): BMI (0.0002), insulin (0.0001), AVF (0.0005), and IL-6 (0.0060). Multivariately, age (0.0001, positively), AVF (0.0001, negatively), and IL-6 (0.0063, negatively) together explained 28.1% of HDL variance (P<0.00001). TC correlated positively with single-variable age (P=0.0070) and AVF (P=0.033), and multivariately with age only (P=0.00069, 9.3% of TC variance). TG’s correlated to univariate BMI (P<0.0001), glucose (P=0.013), insulin (P<0.0001), AVF (P<0.0001), and IL-6 (P=0.0028), and multivariate SHBG (P=0.115), AVF (P<0.0001), and IL-6 (P=0.0016) all positively (P<0.00001, 38.9% of TG variance). NonHDL-C correlated positively with age (P=0.0123), BMI (P=0.0003) and AVF (P=0.0002) individually, and with TAF and IL-8 jointly (P<0.00001, 16.5% of variance). LDL-C (calculated) correlated univariately to age (0.023), AVF (0.013), and TAF (P=0.0060) all positively, and multivariately to TAF (P=0.0033) and IL-8 (P=0.045) at overall R^2=0.094, P=0.0031. These data delineate strong conjoint effects of age (positive), AVF (negative) and IL-6 (negative) on HDL-C (Figure 1), and of TAF and IL-8 (both positively, Figure 2) on nonHDL-C, in women ages 21-79 yr. Longitudinal studies will ultimately be required, using similar criterion methods, to confirm or refute these cross-sectional inferences. **Abstract word count**: 323
Positive Relation of nonHDL to TAF and IL-8

$P = 2.7 \times 10^{-6}$
$R^2 = 0.165$
$N = 120$ women

Positive Relation of nonHDL to TAF and IL-8
**HDL-C in Women Joint Effects of Age (+) and AVF (-)**

![Graph showing HDL-C levels](image)

**Funding:** supported in part via R01 DK073148 and AG029362 and P30 DK050456 (Metabolic Studies Core of the Minnesota Obesity Center) from the National Institutes of Health (Bethesda, MD). The project described was supported by UL1 TR000135 from the National Center for Advancing Translational Sciences (NCTs) and 60NANB10D005Z from the National Institute of Standards and Technology (NIST).

**Disclosures:** The authors have nothing to disclose.

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**HDL-C in Women Joint Effects of Age (+) and AVF (-)**

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Blood formed elements under cholesterol load

Abstract nr. 510
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Dyslipidemia, Immunity, Lipids

The aim of the work is to investigate and to make a comparative analysis of the changes in the ultrastructure of blood cells under cholesterol load conditions and to estimate their pathogenetic role.

Material and methods: 119 male patients with atherosclerosis were examined. The control group consisted of 51 persons. With the aim of food charge, we used so-called “cholesterol breakfast” with a high content of cholesterol and saturated fatty acids, the total amount of cholesterol being 1720 mg. Blood samples were examined 12 hours after the last food intake and 3 hours after cholesterol charge (the peak of triglyceridemia and Cholesterolemia). Total cholesterol, Triglycerids, cholesterol of high density lipoproteins were determined. Ultra structure was examined on electron microscope Tesla BS-500.

Results and conclusion: In healthy persons “cholesterol stress” causes activation of leucocytes and platelets an increase in heterogeneity of immunocompetent cells. The activation is expressed in an irregular outline of cells, ameboid movement (chemotaxis index), displacement of specific granules to plasmolemma and degranulation of some cells; fatty drops in cytoplasma are seen. Monocyts display different populations. An episodic increase in atherogenic lipids in healthy persons’ blood can cause an atherogenic stimulus mediated by the presence of the subpopulation of large active monocytes, by contents of fatty drops,. It can be assumed that heterogeneity of white blood cells and disbalance between their subpopulations followed by cooperation and secretion of biologically active agents play an important role in atherogenesis. An increase in chemotaxis of neutrophilic granulocytes, irregularity of the membrane surface, an increase in specific and azurophilic granules, predominance of lipid drops indicate an increased ability for phagocytosis. Apoptosis of lymphocytes in peripheric blood in the control group after a food charge indicates a preservation of regulatory abilities of the organism. We believe that activation of granulocytes and lymphocytes, even in a moderate increase in atherogenic lipoproteins (LP), has an adaptive character directed at lipid withdrawal and manifestation of cellular immune reaction.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Differences on carotid and femoral plaques detected by ultrasonography as markers of cardiovascular disease in patients with genetic hypercholesterolemias

Abstract nr. 511
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease,Familial Hypercholesterolemia,Imaging,Risk stratification

Background- Genetic hypercholesterolemia (GH) includes primary dyslipidemias associated with high serum values of total cholesterol (TC) and early cardiovascular disease (CVD). Nonetheless the association of traditional CV risk factors with CVD, a cost-effective procedure for detecting subjects at higher risk of CV events is still an important issue in this population.

Objective- To describe the prevalence of plaques in peripheral arteries detected by ultrasonography and its association with the prevalence of CVD in GH patients.

Material and Methods-We enrolled all the patients with GH referred to our lipid clinic from 2010 to 2014. We studied lipid profile, anthropometric variables, traditional CVD risk factors and prevalence of CVD (coronary, cerebrovascular or peripheral artery disease). Carotid and femoral arteries scan by ultrasonography was performed in all of them in the first visit to our clinic.

Results-We included 481 patients, 79.4 % men, in our analysis, 241 with Familial Hypercholesterolemia (FH) and 239 with Familial Combined Hyperlipidemia (FCHL) (age, 49 y/o (15-77); TC, 299±65 mg/dL. 311 patients (64.5%) had atherosclerotic plaques in any location with a higher prevalence in femoral than carotid arteries (276 vs 182). 137 subjects (30.6%) had plaques in both territories. There were 61 subjects included (12.7%) with established CVD (mean age 55, range 37-77). In all groups, femoral plaques were more prevalent than carotid plaques except for the oldest group. CVD prevalence was higher in those with femoral plaques compared to those subjects with carotid plaques (58 vs 41; sensitivity 91.8 vs 67.2%, p<0.001). Only 2 patients with CVD had no plaques in any location. Within those ever smokers, femoral plaques were more prevalent than carotid plaques (213; 67.8% vs 139, 44.3%, p<0.001).

Conclusions- In this GH population, CVD prevalence was associated to femoral atherosclerotic plaques with a higher sensitivity than carotid plaques. This association was especially important in subjects with any history of tobacco consumption.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Mini-oral presentation

Additional information
CLOPIDOGREL BINDS TO LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 (LP-PLA2) AND INHIBITS THE ENZYME ACTIVITY. A NEW PLEIOTROPIC EFFECT OF THIS ANTIPLATELET DRUG?

Abstract nr. 512
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Co-author(s) - Tselepis, Alexandros
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Lipoproteins

Introduction: Lp-PLA$_2$ circulates in plasma in active form bound to lipoproteins, primarily to LDL and in a smaller proportion to HDL. Lp-PLA$_2$ catalyzes the hydrolysis of platelet activating factor (PAF) and oxidized phospholipids. These phospholipids are formed under oxidative stress conditions in the arterial intima and may play important role in the pathophysiology of atherosclerosis. Many clinical studies have demonstrated that elevated Lp-PLA$_2$ levels in plasma are correlated with increased cardiovascular risk. Clopidogrel is one of the antiplatelet drugs of choice for cardiovascular patients. Apart from potent antiplatelet activity, clopidogrel exhibits various pleiotropic effects.

Aim of the study: We investigated the possible effect of clopidogrel on the activity of Lp-PLA$_2$, in vitro.

Materials and Methods: The effect of clopidogrel and its intermediate metabolite 2-oxo clopidogrel on recombinant Lp-PLA$_2$ (rLp-PLA$_2$) activity as well as on the enzyme activity in total plasma, and on LDL was determined by the trichloroacetic acid precipitation method using $[^3H]$ PAF as a substrate. We also studied the possible interaction of clopidogrel with rLp-PLA$_2$ using fluorescence spectroscopy. The possible degradation of clopidogrel by rLp-PLA$_2$ was evaluated with LC-MS/MS (LC/MSD Trap SL) before and after incubation of clopidogrel (C=100mM) with rLp-PLA$_2$ (2μg/ml).

Results: Clopidogrel in various concentrations from 1000 to 10000 μM inhibits rLp-PLA$_2$ enzymatic activity as well enzyme activity in plasma and on LDL, in a dose-dependent manner. The same phenomenon but to a lesser extent was observed with the 2-oxo clopidogrel. Titration of clopidogrel concentrations (0.5-20 mM) to the solution of rLp-PLA$_2$ (2 mM) revealed a gradual decrease in fluorescence, thus providing strong evidence that clopidogrel binds to Lp-PLA$_2$. However, according to the results obtained by LC/MS-MS, clopidogrel is not degraded by Lp-PLA$_2$, since the spectrum of clopidogrel MRM (m/z 322) was the same either in the presence or absence of Lp-PLA$_2$. 
Conclusions: Clopidogrel binds to Lp-PLA₂ and inhibits the enzyme activity, suggesting a new pleiotropic (antiatherogenic) effect of this antiplatelet drug. However, the possible consequences of clopidogrel binding to Lp-PLA₂ on the drug antiplatelet and clinical efficacy in patients treated with clopidogrel, needs further investigation.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
Apolipoprotein AI Deficiency Stabilizes Plasma High Density Lipoprotein against Serum Opacity Factor Activity

Abstract nr. 513
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, HDL, Lipids, Lipoproteins

Human plasma high-density lipoprotein-cholesterol (HDL-C) concentrations are a negative risk factor for cardiovascular disease, and their elevation via pharmacological measures sometimes reduces incident CVD. However, HDL-C concentrations may not be as reliable a predictor of CVD as once thought: one study showed that controlling for apolipoprotein (apo) AI inverted the negative relationship of HDL-C with pre-clinical atherosclerosis, so apo AI may be the dominant determinant of reduced CVD in subjects with elevated HDL-C concentrations. The reaction of Streptococcal serum opacity factor (SOF) against HDL produces a large cholesteryl ester-rich microemulsion (CERM), a smaller neo HDL that is apolipoprotein (apo) AI-poor, and lipid-free apo AI. SOF injection into mice reduces plasma cholesterol ~43% in 3 hours while forming the same products observed in vitro, but at different ratios. Previous studies supported the hypothesis that labile apo AI is required for the SOF reaction vs. HDL. Here we further tested that hypothesis by studies of SOF against HDL from apo AI-null mice. SOF injections into apo AI-null mice reduced plasma cholesterol ~35% in three hours. The reaction of SOF vs. apo AI-null HDL in vitro produced a CERM and neo HDL, but no lipid-free apos. Moreover, according to the rate of CERM formation the SOF reaction is slower in apo AI-null mouse HDL vs. wild-type (WT) mouse HDL. Chaotropic perturbation studies using guanidine hydrochloride showed that apo AI-null HDL was more stable than WT HDL. Human apo AI added to apo AI-null HDL was quantitatively incorporated, giving a reconstituted HDL. Both SOF and guanidine hydrochloride displaced apo AI from the reconstituted HDL. These results support the hypothesis that apo AI-null HDL is more stable than WT HDL because it lacks apo AI, a labile protein that is readily displaced by physico-chemical and biochemical perturbations. Thus, apo AI-null HDL is less SOF-reactive than WT HDL. It remains to be determined what other HDL-modifying activities depend on related HDL stability and altered by apo AI deletion.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Afamin as novel marker for metabolic syndrome and related diseases

Abstract nr. 514
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis,Epidemiology,Metabolism,Risk Factor

Afamin, a human vitamin E binding glycoprotein, is expressed in the liver and secreted into the blood stream. Afamin plasma concentrations were previously found strongly associated with the prevalence and incidence of the metabolic syndrome and all of its components in three large human epidemiologic studies totalling >5000 participants from northern Italy, Austria and southern Germany (Bruneck, SAPHIR and KORA F4). In line with these results, transgenic mice overexpressing the human afamin gene exhibited a hyperlipemic, hyperglycemic, obese metabolic-syndrome-like phenotype suggesting a causal role of afamin for developing metabolic syndrome.

Furthermore, regression analysis in participants of the prospective Young Finn Study revealed afamin as an independent predictor of hepatic steatosis (fatty liver) in a 10-year follow-up independent of elevated liver enzymes and metabolic syndrome parameters (odds ratio of 1.26 per 10 mg/L increase; 95%CI 1.13-1.41; p<0.0001). Hepatic steatosis is associated with obesity, systemic hypertension, dyslipidemia and insulin resistance.

In order to investigate the association between afamin and pathophysiological clinical endpoints related to metabolic syndrome, we determined afamin plasma concentrations in the LURIC study, a large prospective cohort study designed to evaluate risk factors for cardiovascular events. In total, we determined the prognostic value of afamin in 1345 participants with stable coronary artery disease (CAD) according to coronary angiographic data, clinical data and biomarkers. Afamin was significantly lower in participants who died during follow up compared to survivors (81 (69-95) vs. 77 (64-93); p=0.021). Univariate regression analysis identified afamin as a significant predictor of all-cause and cardiovascular death (risk ratio of 0.99 per 1SD increase in log-transformed values; 95%CI 0.98-1.00; p<0.001 for all-cause mortality; risk ratio of 0.99 per 1SD increase in log-transformed values; 95%CI 0.98-1.00; p=0.001 for cardiovascular mortality). In multivariate Cox proportional-hazards regression analysis, increases in afamin plasma concentration yielded no significant change in all-cause or cardiovascular mortality after controlling for established confounders such as NT-proBNP and hscTNT.

We therefore conclude that afamin is not an independent predictor of mortality in stable CAD and that afamin’s association with metabolic syndrome derives from its correlation with hepatic steatosis which is a strong predictor for components of the metabolic syndrome.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Salsalate activates brown adipose tissue

Abstract nr. 515
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Lipids, Metabolism, Obesity

Background and aim: The anti-inflammatory drug salsalate diminishes plasma triglycerides and glucose in type 2 diabetes patients, but the underlying mechanisms are unknown. Brown adipose tissue (BAT) has recently emerged as a prominent player in energy expenditure and lipid metabolism by combustion of fatty acids towards heat through uncoupling by UCP-1. Therefore, the aim of the current study was to investigate whether salsalate exhibits its beneficial metabolic effects via activation of BAT.

Methods and results: Male APOE*3-Leiden.CETP mice, a well-established mouse model for human-like lipoprotein metabolism, were fed a high fat diet for 12 weeks to induce obesity and were then randomized to receive salsalate (0.5%, w/w) or vehicle for 4 weeks. Salsalate effectively reduced body weight (-21%; P<0.001) and fat mass (-50%; P<0.001) and lowered plasma triglyceride levels (-46%; P<0.05). Salsalate increased the uptake of fatty acids (i.e. [3H]oleate) from intravenously injected glycerol tri[3H]oleate-labeled lipoprotein-like particles specifically by interscapular BAT (+156%; P<0.01) and dorsal cervical BAT (+102%; P<0.05), decreased intracellular lipid content in BAT (-45%; P<0.001) and increased rectal temperature (+0.5°C; P<0.05), all pointing to more active BAT. Strikingly, treatment of T37i brown adipocytes with salsalate increased uncoupled respiration, upregulated Ucp1 (3.3-fold; P<0.001) and enhanced glycerol release (+92%; P<0.01), both of which were abolished by addition of the protein kinase A (PKA) inhibitor H89.

Conclusions: In conclusion, salsalate profoundly activates BAT, presumably by directly activating brown adipocytes via the PKA pathway. This suggests a novel mechanism that may explain the beneficial metabolic effects of salsalate and warrants its clinical development as a treatment option.

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Cardiovascular Science (RICS) to P.C.N. Rensen, E. Lutgens and M.P.J. de Winther.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Preventing atherosclerosis with a targeted gene therapy to inhibit VCAM-1

Abstract nr. 517

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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention

Keywords Atherosclerosis, Prevention, Therapy

The recruitment of leukocytes is an important driver of atherosclerosis development, particularly in the early stages. This recruitment relies heavily on Vascular Cell Adhesion Molecule (VCAM-1), which is expressed on the inflamed endothelial walls. As shown by knockout models and pharmacological interventions, blocking VCAM-1 is highly effective in preventing plaque development. However, safe, specific and long term therapies to block VCAM-1 are still lacking. A fusion construct, CD7/VCAM, has previously been developed which, when expressed in activated endothelial cells in vitro prevents leukocyte transmigration by functionally disrupting VCAM-1 adhesion clusters. For efficient and long term in vivo delivery we are developing a gene therapy delivery system. We produced clinically relevant and highly potent adeno associated virus 6 (AAV6-CD7/VCAM) gene therapy vectors. In order to further increase efficacy and safety a single-chain antibody (scFv) against VCAM-1 is employed to specifically deliver this novel therapeutic construct to early stage plaques. Pure scFv has been produced with yields of 5-10mg/L culture, using an insect cell production system. Via flow cytometry we could demonstrate specific binding to the VCAM expressed on SVEC4-10 cells. A variety of site-specific conjugation techniques have been explored to attach the scFv to the AAV6. This approach is based on the combination of chemical glycation, Cu free click chemistry and enzymatic sortase reactions and has been validated using the covalent attachment of biotin to the AAV6. In future we are planning extensive in vitro and in vivo testing of this targeted gene delivery system, including biodistribution with radiotracers and reporter genes, as well as therapeutic effectiveness. Given that currently vascular gene delivery is notoriously difficult, successful development of this system would be advantageous. It also represents a one shot, non-invasive and preventative treatment for atherosclerosis. It additionally provides a flexible platform technology for similar strategies in other inflammatory diseases and generally for targeted therapeutic gene delivery.

Funding: Co-funded Australian National Heart Foundation, National Health Medical Research Council and Baker IDI Bright Sparks Top Up Scholarships. National Health Medical Research Council Project Grant.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Effect of polymorphisms in CYP3A5, SLCO1B1, and ABCG2 on the plasma concentrations of simvastatin and the lipid response in Chinese

Aims: This study examined whether polymorphisms in genes potentially related to the pharmacokinetics of simvastatin influenced the plasma concentration of simvastatin and the lipid response in Chinese patients with hypercholesterolaemia.

Methods: Morning fasting blood samples were collected from 259 patients who had received a simvastatin 40 mg night time dose for 6 weeks. Plasma concentrations of simvastatin and simvastatin acid were quantified using liquid chromatography tandem mass spectrometry. The CYP3A5 *3, SLCO1B1 388A>G and 521T>C and ABCG2 421C>A were genotyped.

Results: Eleven subjects with plasma simvastatin levels less than the limit of detection were excluded. Compared to subjects with the SLCO1B1 521TT genotype, those with one or two copies of the 521C variant allele (n=60) had higher plasma concentrations of simvastatin acid after adjustment for body weight (mean±SE: 3.70±0.34 vs. 2.59±0.19 μg/L, P=0.005) with the 5 subjects homozygous for the 521C variant having the highest plasma concentrations (8.36±1.14 μg/L). The ABCG2 421C>A and the SLCO1B1 521T>C polymorphisms were associated with the plasma concentrations of simvastatin lactone after adjustment for body weight. Neither the plasma concentrations of simvastatin nor simvastatin acid were associated with the percentage reduction in LDL-C with simvastatin. There was no significant association between the genetic polymorphisms examined and the LDL-C response to simvastatin although the 5 subjects with the 521CC genotype tended to have smaller LDL-C response (41.9±1.8 % vs. 47.3±11.9 %, P>0.05).

Conclusion: These findings confirm that the SLCO1B1 521T>C and the ABCG2 421C>A polymorphisms influence the systemic exposure to simvastatin acid or lactone but have little effect on the lipid-lowering efficacy in Chinese patients.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Effect of probucol on reducing tendon xanthoma and xanthelasma in Chinese patients with heterozygous familial hypercholesterolaemia

Abstract nr. 519
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Familial Hypercholesterolemia, Imaging

Introduction: Probucol is a di-phenolic anti-oxidant drug with anti-atherosclerotic effects. It is known to reduce HDL-C but may increase reverse cholesterol transport. A recent long-term follow-up study in patients with heterozygous familial hypercholesterolaemia (FH) in Japan showed that probucol therapy significantly reduced cardiovascular events. The present study examined the effect of probucol on reducing tendon xanthoma and xanthelasma in Chinese patients with FH.

Methods: In this single arm, open label study, Chinese patients with FH were treated with probucol 500 mg twice daily with the morning and evening meals for 24 weeks. Fasting lipids were measured at baseline and after 12 and 24 weeks. The volumes of the Achilles tendons were measured by magnetic resonance imaging at baseline and after treatment with probucol for 12 and 24 weeks. The size of xanthelasma was assessed by the investigators.

Results: A total of 24 patients participated in the study and 6 patients withdrew due to side effects or personal reasons. In the 18 patients (13 females, 58.1±8.9 years: baseline mean(±SD) LDL-C level: 2.96±0.75 mmol/L who completed the study, there was a significant reduction in plasma HDL-C (from 1.72±0.40 mmol/L to 0.93±0.32 mmol/L), but no significant change in LDL-C or triglycerides. The mean(±SE) volume of the Achilles tendon on both sides were significantly reduced (Left: 13.85±1.73 cm³ to 12.96±1.60 cm³, P=0.002; Right: 13.86±1.73 cm³ to 12.98±1.60 cm³, P=0.002) after 24 weeks treatment. The lipid content of Achilles tendon on both sides was also significantly reduced at the end of the study (Left: 18.7±0.8 % to 17.4±0.5 %, P<0.05; Right: 18.5±0.6 % to 17.6±0.7 %, P<0.05). There was no correlation between the changes in Achilles tendon volume and the changes in HDL-C levels or other lipids with probucol. The size of xanthelasma was reduced in 4 out of the 5 patients with these deposits.

Conclusion: This small study showed that treatment with probucol for 24 weeks resulted in regression of tendon xanthoma which was independent of its lipid-modifying effects.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Effect of probucol on cholesterol efflux and plasma lipids and lipoproteins in Chinese patients with heterozygous familial hypercholesterolaemia

Abstract nr. 520
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Familial Hypercholesterolemia, Lipids, Reverse Cholesterol Transport

Introduction: Probucol is a di-phenolic anti-oxidant drug with lipid-modifying effects. It may reduce plasma LDL-C and HDL-C levels, but the mechanism of these effects are not fully understood. Probucol inactivates hepatic ABCA1 in mice which leads to an increased reverse cholesterol transport through increasing the fecal excretion of HDL-derived cholesterol. The present study examined the effect of probucol on plasma lipid and lipoproteins in Chinese patients with familial hypercholesterolaemia (FH).

Methods: In this single arm, open label study, Chinese patients with FH were treated with probucol 500 mg twice daily with the morning and evening meals for 24 weeks. Fasting lipids were measured at baseline and after 12 and 24 weeks. The expression of ABCA1 in peripheral blood mononuclear cells (PBMCs) isolated from heparinized blood and the cholesterol efflux from human macrophages differentiated from the PBMCs were determined at baseline and after 12-week treatment.

Results: From 24 patients entering the study, 6 patients withdrew due to side effects or personal reasons. In the 18 patients (13 females, 58.1±8.9 years: baseline LDL-C level: 2.96±0.75 mmol/L) who completed the study, there were significant (P<0.001) reductions in plasma HDL-C (from 1.72±0.40 mmol/L to 0.93±0.32 mmol/L) and apoAI (from 160.1±23.4 mg/dL to 98.8±24.3 mg/dL), but no significant change in LDL-C, triglycerides or apoB levels. The Lp(a) level was increased from 31.3±30.6 mg/dL to 34.8±31.9 mg/dL (P<0.05). The expression of ABCA1 and cholesterol efflux capability were significantly reduced with probucol and the changes in ABCA1 expression were closely correlated to the changes in cholesterol efflux (r=0.850, P<0.001). There was no significant association between changes in cholesterol efflux and the changes in plasma HDL-C levels.

Conclusion: This small study showed that treatment with probucol significantly reduced the plasma HDL-C and apoAI levels but had no effect on LDL-C levels in patients with FH. In addition, probucol inhibited ABCA1-mediated cholesterol efflux from macrophages differentiated from the patients’ PBMCs.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Australian intakes of LC n-3 PUFA and the cardiovascular disease risk factor: the omega-3 index

Abstract nr. 521
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Cardiovascular Disease, Lipids, Prevention

**Background:** Approximately 1.1 million people (5%) in Australia have heart disease (1). Long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) are predominantly comprised of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The Omega-3 Index (2) is the sum of EPA and DHA and is expressed as a percentage of total fatty acids in erythrocyte membranes. It has been postulated as a new risk factor for cardiovascular disease. However, there are limited data on the Australian population.

**Aims:** 1) To report on the Australian intakes (n=12,153 aged 2 years and over) LC n-3 PUFA. 2) To determine the Omega-3 Index in a smaller cohort of Australian adults and the proportion of Australian adults who fall into the cardioprotection range (greater than 8%) and the at risk range (less than 4%).

**Research Methods & Procedures:** 1) Data on LC n-3 PUFA intake was obtained from the Australian Bureau of Statistics. 2) Erythrocyte membrane EPA and DHA was determined in 137 adults (n= 62 male; n= 75 female) using standard fatty acid extraction and transesterification protocols. The fatty acids were separated using gas chromatography and the unknown fatty acids were identified against known standards.

**Main findings:** The mean LC n-3 PUFA intake for Australians was 267 mg per day (with a standard error estimate of 6.1%). The median omega-3 index was 5.2% (n=137) with a range from as low as 1.6% to as high as 9.9%. The people with extremely low values were strict vegans; as they do not consume EPA and DHA directly in their diets as they do no consume foods such as fish, meats and eggs. Sixteen percent had omega-3 index less than 4% whilst only 3% fell into the cardioprotection range. Correlation analysis showed that LC n-3 PUFA intake plus age were positively associated with Omega-3 Index, whilst being vegan was negatively associated with Omega-3 Index. Multiple regression shows that LC n-3 PUFA intake, age and vegan status explains 38% of the variance of the Omega-3 Index (p<0.001).

**Conclusion:** The majority of Australians are not consuming enough LC n-3 PUFA for optimal cardiovascular health.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Impaired Macrophage Cholesterol Efflux in Poorly Controlled Type 2 Diabetes Mellitus

Abstract nr. 522
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes, Reverse Cholesterol Transport

Macrophage foam cell formation plays an important role in the development of atherosclerosis. Accumulation of cholesterol in macrophages is partly influenced by the cells’ capability to efflux cholesterol to extracellular cholesterol acceptors. We have evaluated whether there were changes in macrophage cholesterol efflux in patients with poorly controlled type 2 diabetes.

Forty type 2 diabetic patients and fifty non-diabetic controls were recruited. Peripheral blood monocytes were isolated and differentiated into macrophages using autologous serum. Cholesterol efflux assay was performed by measuring the percentage of [3H]cholesterol transferred from subject's monocyte-derived macrophages to fixed concentrations of exogenous apolipoprotein (apo) AI, HDL, HDL₂ and HDL₃ as cholesterol acceptors.

Diabetic patients had elevated triglyceride and lower HDL than controls. As expected, HbA1c was much higher in diabetic patients than control (9.3% ± 0.9 vs 5.4% ± 0.5, p<0.01). In type 2 diabetic patients, macrophage cholesterol efflux to apo AI (18.9% ± 9.5 vs 26.8% ± 10.5, p<0.01) and to HDL (34.2% ± 7.8 vs 43.8% ± 8.6, p<0.01) was significantly impaired compared to controls.

Similar reduction in cholesterol efflux to HDL₂ and HDL₃ was also observed. Macrophage cholesterol efflux to apo AI (r = -0.39, p<0.01) and HDL (r = -0.53, p<0.01) correlated inversely with HbA1c.

In conclusion, macrophage cholesterol efflux was significantly impaired in poorly controlled patients with type 2 diabetes mellitus and might contribute to their increased risk of developing cardiovascular diseases. Whether improvement in glycaemic control will restore macrophage cholesterol efflux warrants further investigations.
Plasma lipidomic profiling: Improved prediction of cardiovascular events in type 2 diabetes

Abstract nr. 524
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Diabetes, Lipids, Risk stratification

Type 2 diabetes (T2D) is a major risk factor for cardiovascular disease (CVD). However, risk stratification is challenging. Traditional lipid measurements (elevated cholesterol, triglycerides and/or lowered HDL-C) do not show the full complexity of the altered lipid metabolism associated with T2D or CVD.

We applied a lipidomic strategy to identify plasma lipids associated with future cardiovascular events (CVE; myocardial infarct (MI), stroke and CVD death) in patients with T2D. Plasma lipids (310 species) were measured using electrospray-ionisation tandem mass spectrometry on 3779 individuals selected from the ADVANCE study in a case/cohort design. The cohort consisted of T2D patients who had a CVE during the 5-year follow-up (n=698) and T2D patients who did not have a CVE (n=3081). Weighted Cox regression was used to identify lipid species associated with future CVE. Multivariate models combining traditional risk factors alone or with lipid species were developed using the Akaike information criteria (AIC). C-statistics (AUC) and net reclassification improvement (NRI), calculated within a bootstrapping framework, were used to evaluate the ability of plasma lipids to improve upon traditional risk factors to discriminate and reclassify five-year risk.

Sphingolipids, phospholipids (including lyso- and ether-linked species), cholesteryl esters and glycerolipids were associated with future CVE (p<0.05, corrected for multiple comparisons using the Benjamini-Hochberg method). Compared to the base model containing 14 traditional risk factors, the combined lipid and risk factor model (42 features) resulted in an increase in AUC of 0.053 to 0.746 (95% CI, 0.719-0.771), a NRI of 15.6% (95% CI, 7.5%-23.9%) based on a categorical model of <10, 10-15, and >15% risk and a continuous NRI of 52.6% (95% CI, 39.2%-66.1%). Models specific for future MI (20 features, AUC 0.765 (0.725-0.805), categorical NRI 18.7% (5.5%-32.7%)) and stroke (20 features, AUC 0.799 (0.756-0.840), categorical NRI 27.3% (13.0%-42.3%)) also showed improved performance.
The strong associations between plasma lipids and future CVE provide insight into the role of lipids in the pathogenesis of CVD and identify potential therapeutic targets. The improvement in the prediction of CVE, above traditional risk factors, demonstrates the potential of plasma lipids as biomarkers for CVD risk stratification in T2D.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Current control status of dyslipidemia and the prevalence of residual risk in patients with ischemic stroke

Abstract nr. 525
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Prevention, Risk Factor, Triglycerides

Objectives: We investigated the control status of dyslipidemia and the prevalence of residual risk in patients with ischemic stroke.
Methods: Patients who were hospitalized due to acute ischemic stroke within 7 days in two referral hospitals were enrolled retrospectively. Patients without follow-up lipid battery between 1 month and 1 year after discharge were excluded. Individual target LDL level was determined using fasting lipid battery and risk factors during admission according to 2011 AHA/ASA guideline for secondary prevention. Residual risk was determined as 1) high TG (>200 mg/dL) or 2) low HDL (<40 mg/dL) or 3) high Non-HDL (≥130 mg/dL) in follow-up lipid battery between 1 month and 1 year after discharge.
Results: Among the 1919 patients, a total of 951 (49.6%) patients had follow-up lipid battery. Target LDL goal was achieved in 664 (69.8%) patients. Diabetes mellitus, hyperlipidemia and atherothrombotic subtype were more frequent in not achieved 287 patients, while atrial fibrillation and discharge statin prescription were less prevalent. Residual risk was observed in 507 (53.3%) patients. Male gender, diabetes mellitus, hyperlipidemia, smoking, fasting blood glucose and initial NIHSS score were more frequent or higher in patients with residual risk, while discharge statin prescription was less prevalent. Among target LDL goal achieved population, 319 (48.0%) patients showed residual risk.
Conclusions: Target LDL goal achievement rates in ischemic stroke patients needs to be improved. Residual risk was observed almost half of the ischemic stroke patients irrespective of LDL goal achievement.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Background: We showed that microRNAs (miRNAs), including miR-223, associate with high-density lipoproteins (HDL). The origin and regulation of HDL-miRNAs export and delivery is to be established. miR-223 is highly abundant in polymorphonuclear neutrophils (PMNs) and macrophages and important for the activation of these inflammatory cells. Here we aim to determine if HDL-miR-223 and other HDL-miRNAs (miR-451, miR-16, miR-17, miR-24, miR-106a and miR-146a) originate from PMNs and macrophages. We also aim to study the regulation of their export to HDL and the delivery to recipient cells (adipocytes and human skeletal muscle cells).

Results: In order of assess if PMNs export miRNAs to native HDL, isolated neutrophils were incubated in the presence or absence of human HDL (1 mg total protein/ml) for 4 h. HDL were then isolated from culture media using apoA-I immunoprecipitation columns. HDL-miRNA levels were measured by TaqMan assays. miRNA export assays showed a significant increase in miR-223 and other miRNAs on HDL post-PMN incubation compared to pre-incubation. To examine if other inflammatory cells also export miRNAs to HDL, human monocyte-derived macrophages (HMDM) were cultured. Similar to PMNs, HMDM were also found to export miR-223 to HDL after 16 h. As a consequence of miRNAs export, we predicted that PMN cellular levels of mature miR-223 would decrease. Interestingly, we found no significant differences in cellular mature miRNA levels in PMNs or HMDM treated with HDL. To determine if cellular miR-223 levels are supported by increased miR-223 transcription in response to HDL induction and miRNA export, we quantified primary (pri)-miR-223 levels using real-time PCR and TaqMan assays. We found that HDL induce a significant 3.75-fold increase in PMN pri-miR-223 levels after 4 h. Likewise; we found a significant 2.24-fold increase in HMDM pri-miR-223 levels after 16 h of HDL treatment. The transfer of miR-223 from HDL to recipient cells was also assessed and confirmed by quantifying HDL-miR-223 levels before and after incubations.

Conclusion: HDL regulate the transcription of miRNAs in origin cells required for delivery to recipient cells. This study brings new insights in the field of HDL-miRNA cell-to-cell communication.
and HDL functional properties.
Funded by Australian National Heart Foundation

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Elevated Lipoprotein(a), Hypertension and Renal Insufficiency as Predictors of Coronary Artery Disease in Patients with Genetically Confirmed Heterozygous Familial Hypercholesterolemia

Abstract nr. 527
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Genetics, Lp(a), Risk Factor

Background: Familial hypercholesterolemia (FH) is classically caused by LDLR mutations that result in marked hypercholesterolemia and premature coronary artery disease (CAD). Lipoprotein(a) [Lp(a)] is a co-existing genetic trait that increases CAD in FH, although the independence of this association relative to other CAD risk factors remains unclear.

Objective: To examine the association between Lp(a) and other cardiovascular risk factors and prevalent CAD in patients with FH.

Methods: A cross-sectional study of 390 patients with genetically confirmed FH in the Familial Hypercholesterolemia Western Australia Program. Clinical and biochemical parameters of FH patients with and without CAD were compared. Plasma Lp(a) concentrations were measured by immunoturbidimetry. Renal function was determined by estimated glomerular filtration rate (eGFR).

Results: FH patients with CAD were older, more often male and had a higher prevalence of hypertension, smoking, type 2 diabetes, obesity, reduced eGFR, elevated Lp(a) and low HDL-cholesterol than patients without CAD (P<0.05 for all). Age, male sex, smoking, hypertension, reduced eGFR, type 2 diabetes, obesity, plasma creatinine, HDL-cholesterol and Lp(a) were significant predictors of CAD in the FH patients with univariate analyses, (P<0.05 for all). Elevated Lp(a), hypertension and reduced eGFR remained significant independent predictors of CAD (P <0.05 for all) in FH after adjusting for other modifiable risk factors.

Conclusion: Elevated Lp(a), hypertension and renal insufficiency are significant, independent risk factors of CAD in patients with FH. These findings underscore the need for identifying and treating these abnormalities to reduce excess CAD risk in patients with FH.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
MCP-1 AND M-CSF SIGNIFICANTLY IMPROVE RISK PREDICTION OF FIRST-TIME CORONARY EVENTS

Abstract nr. 528
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords ACS, Inflammation, Risk Factor, Risk stratification

Background:
Myeloid cells play a central role in atherosclerosis. Here we hypothesized that mediators involved in myeloid cell homeostasis might offer additional prognostic information to traditional risk factor in the prediction of future coronary events (CE).

Methods:
We measured plasma levels of monocyte chemotactic protein-1 (MCP-1), macrophage colony-stimulating factor (M-CSF), CCL3, CCL4, CCL20, CXCL1, CXCL16, and CX3CL1 in 385 individuals who suffered a coronary event during follow-up (median 15.2 years), and 401 age and sex-matched controls that remained event free. Study subjects were recruited from the larger cardiovascular cohort of the Malmö Diet and Cancer Study. Biomarker measurement was performed in parallel in the same samples by using a Proximity Ligation Assay, expressing the results as relative units.

Results:
In a Cox proportional hazards regression model with stepwise variable selection from myeloid mediators, classic Framingham risk factors, CRP and presence of diabetes, MCP-1 and M-CSF were retained as having the strongest correlations with incident coronary events. Importantly, MCP-1 was positively correlated (Hazard ratio, HR 2.07, 95% CI 1.77-2.43, p<0.001), whereas M-CSF had a strong negative correlation with the considered end-point (HR 0.49, 95% CI 0.41-0.58, p<0.001). Addition of MCP-1 and M-CSF to a binary logistic regression model significantly improved the predictive ability compared of a model using traditional risk factors alone (c-statistic 0.82 [95% CI 0.78-0.85] with MCP-1 and M-CSF vs. c-statistic 0.66 [95% CI 0.62-0.70] with traditional risk factors alone, P<0.05). Addition of MCP-1 and M-CSF to the risk model correctly up or down classified 194 of 276 individuals (70%) at an intermediate (10-20%) risk compared to 14% incorrect re-classification. For individuals at high (>20%) risk, we recorded a 26% correct and 16% incorrect down-classification, whereas 9% of individuals at low (<10%) risk were correctly and 9% were incorrectly up-classified after addition of MCP-1 and M-CSF to the model (net reclassification improvement, NRI 0.51 (95% CI 0.42-0.60) and integrated discrimination improvement, IDI 0.19
(95% CI 0.16-0.21).
Conclusion:
Addition of MCP-1 and M-CSF to traditional risk factors significantly improve prediction of coronary events in healthy individuals. The apparently protective effect of M-CSF against CE in this population warrants further investigation.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Circulating desmosine relates to cardiovascular comorbidity, coronary artery calcification score, systemic inflammation and mortality in patients with COPD

Abstract nr. 529
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Cardiovascular Disease,Inflammation,Risk stratification

The problem being addressed and purpose of the paper
Chronic obstructive pulmonary disease (COPD) is a risk factor for the development of cardiovascular disease. Elastin degradation may represent a shared mechanism for the pulmonary (emphysema) and vascular (arterial stiffness). Desmosine, the crosslinking moiety only present in mature elastin, is a specific biomarker for elastin breakdown. The aim of the study was to explore the relationship of circulating desmosine (pDES) with emphysema, emphysema progression, cardiovascular comorbidities, coronary artery calcification score as a surrogate of coronary atherosclerosis and mortality in a cohort of patients with COPD.

Procedure and Methods
pDES was measured in 955 patients with COPD (609 male, age 63.1±7.2 years, FEV₁ 50.6±15.1% predicted) by an isotope dilution LC-MS/MS method. Coronary artery calcification (Agatston) score (CACS) was assessed in 440 standard CT scan images and classified as low (<100 Agatston units (AU)), intermediate (101–400 AU), high (401–1000 AU) or very high (>1000 AU).

Main Findings
pDES was significantly elevated in patients with compared to those without cardiovascular comorbidities (p<0.01) and correlated with lung function (FEV₁) (r=0.39, p<0.0001), symptoms (MMRC) (r=0.16, p<0.0001), 6MWD (r=0.16, p<0.0001), BODE index (r=0.10, p<0.005), systemic inflammation (fibrinogen, IL6, IL8, CCL18, and SPD) but not with emphysema. All these variables showed statistically significant higher values in the patients with highest pDES values (higher quartile). pDES was elevated in patients with very high CACS in comparison with patients with lower CACS (Figure 1) and in patients that died during a 3 year follow-up (p<0.0001).

Principle Conclusion
We conclude that pDES relates to lung function, systemic inflammation, cardiovascular comorbidities, and CACS in patients with COPD. pDES is a predictor of all cause overall mortality.

Figure 1. Differences in pDES between patients with very high CACS and lower CACS levels (* \( p<0.01 \)).

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Mast cells are significantly increased in unstable but also stable coronary lesions in patients with myocardial infarction.

Abstract nr. 530
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Inflammation, Vulnerable Plaque

Objectives: Mast cells (MCs) may play an important role in plaque destabilization and atherosclerotic coronary complication. Here we have studied the presence of MCs in the intima, media and adventitia of unstable and stable coronary lesions at different time points after myocardial infarction (MI).

Methods: Sections of the infarct-related left anterior descendent coronary artery (LAD) were obtained at autopsy from patients with acute MI (up to 5 days old; n=22) and with chronic MI (5 - 14 days old; n=17) as well as sections of the LAD from controls without cardiac disease (n=11). Herein, tryptase-positive MCs were quantified in the intima, media and adventitia of both unstable and stable lesions.

Results: In all lesions, both in control and MI patients, the numbers of MCs in the intima and adventitia were significantly higher than in the media, independent of plaque stability. Moreover, in acute and chronic MI patients the presence of MCs was increased significantly in all three layers of unstable- but also stable lesions, wherein the numbers of MCs were significantly higher in unstable lesions than in stable lesions. Lastly, chronic MI patients had relatively more unstable plaques than acute MI patients and controls.

Conclusion: The increase of MCs in both stable and unstable atherosclerotic coronary lesions after MI we show here on the one hand suggests that MCs are associated with the onset of MI and on the other hand that MI, in turn, triggers intra-plaque infiltration of MCs also in stable plaques, thereby increasing the risk of re-infarction.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Genetic Analysis of Familial Hypercholesterolemia in India

Abstract nr. 531
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia, Genetics

Background: Familial Hypercholesterolemia (FH), an autosomal codominant disorder, is characterized by very high total and LDL cholesterol since birth, and is strongly associated with premature Coronary Artery Disease (CAD). It is a highly underappreciated entity in India. Puri et al documented a frequency of FH as 1 in 350, among 2500 school children in Delhi. We report molecular study of LDLR, ApoB100 and PCSK9 genes in cases of FH.

Methods: We enrolled 100 unrelated cases, who satisfied modified Dutch Lipid Network Criteria (DLNC). They were diagnosed by LDL cholesterol above 95th centile (Indian standards), and family history of hypercholesterolemia/tendon xanthomas/ premature CAD. Sanger sequencing was done for mutations in LDLR gene, Exons 26 and 29 of ApoB 100 and common mutation (p.D374Y) in PCSK9 gene. Large deletions/duplications in LDLR gene were analyzed by MLPA technique. Cascade screening was carried out in 224 family members.

Results: Distribution of cases according to DLNC was 37 Definite, 25 Probable and 38 possible. We detected 36 different disease causing mutations in 45 families in the LDLR gene, of which 9 were novel (annotated kindly by Dr. Humphries Group, UK), while 27 mutations were previously reported. No mutations were detected in ApoB and PCSK9 genes. Mutations were identified in 79% of Definite, 35% of Probable and 19% of Possible FH cases. Ten cases were homozygous and one case was compound heterozygous for the LDLR gene mutations. Splice site mutation in intron 10 (c.1587-1G>A) of LDLR gene was observed in 7 unrelated North Indian families, which is likely to be a founder mutation. It was observed that mutation carriers had higher LDL cholesterol and early presentation of CAD than the non- mutation carriers. The family specific mutation was observed in 95 (41%) of 224 subjects analyzed.

Conclusion: The current study has added to the mutation spectrum of LDLR gene in India. One founder mutation was identified. DLNC criteria proved useful for selecting cases for genetic studies. Cascade screening was well-accepted by family members.

Subdivision 2. Translational Research
Presentation Preference Oral presentation
Additional information
Increased serum malondialdehyde levels in high cardiovascular risk patients with anxiety disorder

Abstract nr. 533
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Pathogenesis, Risk Factor

Metabolic syndrome (MetS), smoking, depression and anxiety were found to be independent risk factors of coronary heart disease (CHD) and cardiac mortality in initially healthy individuals. The mechanisms through which anxiety might affect the development and onset of CHD need further research. Oxidative stress is one possible mechanism that may be related to inflammation and progression of atherosclerosis. The aim of our study was to evaluate the link between anxiety and oxidative stress measured by malondialdehyde (MDA) concentration in serum.

Methods: The study included 249 male smokers (age 40-54 y.) with MetS. Detailed cardiovascular risk factors assessment and psychiatric screening with Mini International Neuropsychiatric Interview for anxiety disorders were performed. MDA concentration in blood serum was measured using UHPLC method. The statistical software IBM SPSS (v.21) was used for the statistical analysis.

Results: 58 men were diagnosed with at least one specific anxiety disorder (panic disorder, obsessive - compulsive disorder, generalized anxiety disorder, social phobia, posttraumatic stress disorder) and 191 men were without anxiety. Patients with anxiety disorders had significantly higher MDA levels compared with patients without anxiety (respectively 259 ± 345 ng/ml vs. 179 ± 184 ng/ml; p= 0.02). Other cardiovascular risk factors and biochemical parameters were not significantly different between the patients with and without anxiety disorders: age (47.4 ± 4.2 y. vs. 47.7 ± 4.1 y.), body mass index (30.1 ± 3.98 kg/m² vs. 31.2 ± 4.14 kg/m²), waist circumference (107 ± 9 cm vs. 110 ± 10 cm), C-reactive protein (3.4 ± 5.9 mg/l vs. 3.3 ± 3.9 mg/l), fibrinogen (3.8 ± 0.0 g/l vs. 3.7 ± 0.7 g/l), total cholesterol (6.8 ± 1.6 mmol/l vs. 6.5 ± 1.3 mmol/l), LDL cholesterol (4.3 ± 1.5 mmol/l vs. 4.2 ± 1.1 mmol/l), HDL cholesterol (1.1 ± 0.3 mmol/l vs. 1.1 ± 0.2 mmol/l), triglyceride (3.4 ± 3.0 mmol/l vs. 2.7 ± 2.3 mmol/l), fasting glucose (5.7 ± 0.5 mmol/l vs. 5.7 ± 0.5 mmol/l).

Conclusions: Our results suggest that patients with anxiety disorders experienced higher levels of oxidative stress which can promote atherosclerosis despite no significant impact of anxiety on other cardiovascular risk markers.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Docosahexaenoic acid inhibits age-induced accumulation of N(ε)-(carboxymethyl)lysine in non-atherosclerotic intramyocardial blood vessels in ApoE -/- mice.

Abstract nr. 534
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Elderly, Nutrition, Pathogenesis

**Background** Formation of the advanced glycation endproduct N(ε)-(carboxymethyl)lysine (CML) in the vasculature has been associated with vascular dysfunction. CML was found to deposit in atherosclerotic blood vessels but also in the non-atherosclerotic microvasculature. Although aging was shown to facilitate CML accumulation in atherosclerotic blood vessels, its effect on the (non-atherosclerotic) microvasculature is unknown. This we have analyzed in the present study in the heart and brain of mice. Additionally, as CML formation can be induced by inflammation and oxidative stress, we studied whether diet supplementation of the fish oil constituent docosahexaenoic acid (DHA), an omega-3 fatty acid with anti-inflammatory and anti-oxidant properties, could counteract CML accumulation.

**Methods:** ApoE -/- mice (n=50) were fed a western diet and were sacrificed after 40, 70 and 90 weeks. Part of these mice (n=20) were fed a western diet enriched with DHA from 40 weeks on. CML in cardiac and cerebral microvessels was quantified using immunohistochemistry.

**Results:** Cardiac CML depositions significantly increased with an immunohistochemical score of 11.85 [5.92-14.60] at 40 weeks, to 33.17 [17.60-47.15] at 70 weeks (p=0.005). At the same time points, cerebral CML increased from 6.45; [4.78-7.30] to 12.99; [9.85-20.122] (p=0.003). CML did not further increase at 90 weeks, neither in the heart nor in the brain. DHA significantly decreased CML in the intramyocardial vasculature at 70 weeks only (33.17;[17.60-47.15] vs. 14.73;[4.44-28.16] p=0.037). No such effects were found in the brain.

**Conclusions:** Aging coincides with CML accumulation in non-atherosclerotic small blood vessels of the heart and the brain in ApoE -/- mice. Diet supplemented DHA prevented age-associated microvascular CML accumulation in the heart but not in the brain. This could point to different mechanism(s) of age-induced CML accumulation in small blood vessels of the heart and the brain.
Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
Effect of APOE genotype on LDL cholesterol levels in FH and FDB patients: Is there sex-specifically protective genotype?

Abstract nr. 535
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Genetics, LDL, Lipoproteins

Objective: Familial hypercholesterolemia (FH) and familial defective apoB 100 (FDB) are autosomal dominant diseases of lipid metabolism, characterized by increased plasma low-density lipoprotein cholesterol (LDL-C) levels, and caused mostly by the mutations within the LDL receptor (FH patients) or the R3500Q mutation in apoB gene (FDB patients). Apolipoprotein E (APOE) isoforms influence the risk of cardiovascular diseases in general population, with the ancestral APO*E4 allele bringing the higher risk to its carriers.

Methods: Total number of 131 FH/FDB patients was studied. The DNA was isolated using the standard salting out method. APOE *E2, E3 and E4 alleles and R3500Q mutation in APOB gene were determined using PCR – RFLP method. Defects of the LDLR gene were detected using sequencing and/or MLPA analysis. STATISTICA software has been used for ANOVA procedure.

Results: In a representative cohorts of 40 unrelated FH and 91 unrelated FDB patients, genotyped for APOE, we detected statistically significant difference in LDL-C levels between men and women in FDB (m: 4.23 ±0.98mmol/l, w: 5.29 ±1.61mmol/l; p=0.001), but not in FH group (m: 6.33 ±1.76mmol/l, w: 6.55 ±1.64mmol/l). Moreover, LDL-C levels of homozygous APO*E3 men (3.47 ±0.43 mmol/l) were significantly lower than in women of the same genotype (5.96 ±1,92 mmol/l) in FDB group of patients (p=0.002), this effect was not detected in FH group.

Conclusion: We tested interactions of APOE genotype, sex, and genetically approved diagnosis (FH vs. FDB) in the patients with hypercholesterolemia. The analysis revealed sex differences in LDL-C levels in a cohort of FDB, but not FH patients. Genotyping for APOE isoforms showed significant reduction in LDL-C levels in FDB men with homozygous APO*E3 genotype in comparison to women of the same genotype and/or carriers of other genotypes of any sex. Thus, significant portion of the FDB patients carrying the most frequent APOE genotype may escape adequate preventive medical care, which must be confirmed by further investigation of larger patients’ cohorts.

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Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Integrated Management of Coronary Diseases at Risk Groups Via Family Medicine in Alexandria

Abstract nr. 536
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Lifestyle, Prevention, Risk Factor

Introduction: Cardiovascular disease is a leading cause of global morbidity and mortality and is responsible for approximately one-third of all global deaths annually. Screening rates in Primary Health Care (PHC) for single behavioral risk factors are widely documented; however, such risk factors occur in cluster in individuals and populations. Since many patients present with multiple health risk behaviors, integrated approach to health assessment and intervention may be more patient centered, holistic, and timely, thereby generating greater patient satisfaction. Aim: This research aimed to study the integrated management of coronary heart disease at risk groups via family medicine units in Alexandria. Methods: A cross-sectional study followed by an intervention program. All family physicians working in the study were subjected to interview questionnaire concerning CHD risk factors management in at risk patients, as well as observed for their operational performance. A sample of 380 patients aged above 45 years was selected randomly from the family clinics and were screened for CHD risks as well as assessed for the management of their conditions by their family physicians. A short training program was done to the family physicians with assessment of its impact on their operational performance and the achievement of integrated care. Results: Shortcomings were found in the registration of risk factors, and the lifestyle advice provided by family doctors. Sixty-three percent of the sample were hypertensive, 38.2% were diabetic, 13.9% were hyperlipidemic, and 12.4% had IHD. Obesity was found in 87.7%, current tobacco use in 23.4%, and passive smoking in 26.1% of the screened cases. Multiple risk factors were present in the majority of cases. A training program for doctors was developed and implemented in two family medicine centers. The results showed a significant improvement in knowledge, attitude and practice of family doctors in integrated management of coronary heart disease at risk. Recommendations: It can be recommend that family health services must cover all the population; with integrated management of multiple risk factors CHD; and guidelines for the integrated management should be available at all facilities.

Subdivision 5. Not applicable. Abstract matches with track d
Presentation Preference: Electronic poster presentation
Additional information
Atherosclerotic plaque area increases after orthopedic surgery in Apo E -/- mice

Abstract nr. 537
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Pathogenesis, Vulnerable Plaque

Background: Observational studies show a peak incidence of cardiovascular events after major surgery. For example, the risk for acute myocardial infarction is increased 25-fold early after total hip replacement. The acuteness of this increased risk suggests plaque complications, which are closely correlated with intra-plaque inflammation. We hypothesized that systemic inflammation caused by major orthopedic surgery leads to plaque complications.

Methods: ApoE -/- mice (n=48) were fed a western diet for 10 weeks after which the operation group underwent midshaft femur osteotomy followed by realignment with a intramedullary K-wire, to mimic major orthopedic surgery. Mice in both operation and control group were sacrificed 5 or 15 days post-surgery resp. post-saline injection. Paraffin embedded slides of the aortic root were stained with Von Kossa, EVG, Toluidine blue (mast cells), MAC-3 (macrophages), Ly-6g (granulocytes) and CD45 (lymphocytes).

Results: Plaque area in operated animals versus controls had increased by 5,5 % compared after 5 days (n.s.), and by 34% after 15 days (p=0.02). This plaque enhancement was especially related to an increase (180 %) in the necrotic core, which was 99.0 x 10³ μm² [71.2-155] in controls, versus 257 x 10³ μm² [151-315] post-surgery (p=0.013). No significant differences were found in the density of inflammatory cells.

Conclusions: Atherosclerotic plaque area is enhanced after major orthopedic surgery in Apo E -/- mice, mainly due to enlargement of the necrotic core, pointing towards increased plaque vulnerability.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
A hemodynamic property of the 3D computational DM aortic model

Abstract nr. 538  
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins  
Keywords Cardiovascular Disease, Diabetes

3D computational aorta models have been studied to reproduce aortic diseases such as aneurysm and aortic dissection; however there is no study about the 3D aorta model applied Diabetes Mellitus (DM). To predict biomechanical properties of human aorta with DM, we developed a 3D computational aorta model that embodies pressure and von Mises stress under DM pathological boundary conditions. Our model was applied to pulsatile blood pressure mimicking biomechanical environment using COMSOL Multiphysics v4.4. The hemodynamics was hereby compared between normal and DM model. Results comparing biomechanical properties show that the mean values of flow velocity, aortic pressure, and von Mises stress were lower in DM aorta model than normal aorta model, and that the variation of aorta movement was reduced in DM model compared to normal model. From these results, DM aortic model appear more susceptible to rupture in aortic wall. Also when compared the values among discrete regions of aorta, the values of them were higher in ascending aorta where stress concentration was found in both normal and DM model, which is corresponding to the aortic lesion. Combining the result of the correlation between hemodynamics and the development of vascular lesion, our study may provide a better understanding of the relationship between biomechanical properties of aorta and developing pathobiology of aortic diseases. This work was supported by the National Research Foundation of Korea (NRF), and funding was granted by the Ministry of Science, ICT & Future Planning of Korea (2011-0028925) and by the Ministry of Education of Korea (2010-0020224).

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
The Impact of the Metabolic Syndrome on Erythrocyte Aggregation Characteristics in Ischemic Stroke Patients

Abstract nr. 539
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Diabetes, Metabolism, Obesity

Objective: the study of erythrocyte aggregation properties in patients with ischemic stroke (IS) against the metabolic syndrome (MS) background.

Materials and methods: 105 patients suffering from ischemic stroke were examined; the MS was verified in 53 patients. The erythrocyte aggregation and deformability were measured on a laser-assisted optical rotational cell analyzer LORRCA on the 1-st and 21-st days after the IS occurrence. The investigated parameters included the aggregation amplitude (AA), the time of single-stranded erythrocyte aggregate formation by the "rouleaux» type (Tf), the three-dimensional erythrocyte aggregate formation time (Ts), the maximum deformability index (DI).

Results: The AA was significantly higher in the MS patients for whom it was 13.4 ± 1.7 a.u. (arbitrary units) versus 9.7 ± 2.3 a.u. in patients without MS (normal value up to 10 a.u.). This trend continued throughout the whole observation period.

Initially, the Tf value for the MS patients was 1.57±0.22, compared with 1.73±0.16 for the non-MS patients (normal value is from 2.25). By the 21-st day, this indicator did not change for the MS patients and increased by 22% for the non-MS patients.

The Ts value on the MS background at the beginning of the observation was 13.11±3.56 s versus 13.79±4.22 s in the MS absence. By the 21-st day this indicator grew by 15% on the MS background and by 25% for the non-MS patients.

The DI on the MS background was initially 0.50±0.03, compared with 0.51±0.04 without the MS. Later, the ID value increased but at a considerably less extent for the MS patients. By the 21-st day from the II occurrence, this indicator grew to 0.60±0.03 for the MS patients and to 0.68±0.05 for the non-MS patients.

Conclusion: The presence of the MS in patients with ischemic stroke decreased the functional properties of erythrocytes – both aggregation and deformability, which enhances blood clot development and inhibits microcirculation.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Annexin A1 Counteracts Chemokine-Induced Arterial Myeloid Cell Recruitment.

Abstract nr. 540
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model,Atherosclerosis,Inflammation

Rationale: Chemokine-controlled arterial leukocyte recruitment is a crucial process in all stages of atherosclerosis. Formyl-peptide receptor 2 (FPR2) is a chemoattractant receptor which recognizes pro-inflammatory and pro-resolving ligands. One of these pro-resolving ligands is Annexin A1, a cytoplasmic protein which is mobilized to the cell surface and secreted upon activation. The contribution of FPR2 and its ligand Annexin A1 to atherosclerotic lesion formation is largely undefined.

Objective: Due to the ambivalence of FPR2 ligands, we here investigate the role of FPR2 and its resolving ligand Annexin A1 in atherogenesis.

Methods and Results: Deletion of FPR2 or its ligand Annexin A1 exhibits increased lesion sizes with a higher count of recruited macrophages and neutrophils in the plaque. In addition, intravital microscopy of the carotid artery of those knock-out mice shows enhanced arterial myeloid cell adhesion. A potential therapeutic effect was shown by administration of Ac2-26, an Annexin A1 fragment. The fragment largely diminishes arterial recruitment of myeloid cells in a FPR2-dependent fashion. This effect is also observed in presence of selective antagonists to CCR5, CCR2, or CXCR2, while Ac2-26 was without effect when all three chemokine receptors were antagonized simultaneously. Furthermore, repeated treatment with Ac2-26 reduces atherosclerotic lesion sizes and lesional macrophage accumulation. Mechanistically we identify Annexin A1 as an endogenous inhibitor of integrin activation evoked by the chemokines CCL5, CCL2, and CXCL1. Specifically, the Annexin A1 fragment Ac2-26 counteracts conformational activation and clustering of integrins on human and mouse myeloid cells evoked by CCL5, CCL2, and CXCL1 through inhibiting activation of the small GTPase Rap1.

Conclusions: Instructing the Annexin A1-FPR2 axis harbors a novel approach to target arterial leukocyte recruitment. With the ability of Ac2-26 to counteract integrin activation exerted by various chemokines, delivery of Ac2-26 may be superior in inhibition of arterial leukocyte recruitment as compared to blocking individual chemokine receptors.

Keywords: Annexin A1, atherosclerosis, chemokine, integrin, leukocyte

Funding: The study was supported by the DFG (SO876/3-1, SO876/6-1, FOR809, SFB914 TP B08, SFB1123 TP A06, ZA428/6-1, SFB1009 TP A05), the Else Kröner Fresenius Stiftung, the NWO (VIDI project 91712303), the LMUexcellence and the FöFoLe program of the LMU Munich.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Effect of Mipomersen on LDL-Cholesterol levels in Patients with Severe LDL-Hypercholesterolemia and Atherosclerosis Treated by Regular Lipoprotein-Apheresis

Abstract nr. 541
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia

Objectives: Mipomersen, an antisense oligonucleotide targeting apolipoprotein B synthesis, significantly reduces plasma concentrations of LDL-cholesterol by 25-47% and of lipoprotein(a) by 20-30% in hetero- and homozygous familial hypercholesterolemia when added to ongoing maximally tolerated lipid-lowering drug therapy. In this study we evaluated the effect of mipomersen in patients with severe LDL-hypercholesterolemia and atherosclerosis treated by lipid lowering drugs and regular LDL-apheresis.

Methods: This prospective, randomized, controlled phase II monocenter trial enrolled 15 patients (9 males, 6 females; 56±11 ys., BMI 28±5 kg/m²) fulfilling German criteria for regular LDL-apheresis (established atherosclerosis and LDL-cholesterol ≥130 mg/dl despite maximal possible drug therapy). All patients were on stable lipid lowering drug therapy and regular apheresis for >3 months. 8 patients were treated with dextran-sulfate adsorption (Liposorber®, Kaneka), 3 with polyacylate-adsorption (DALI®, Fresenius), and 4 with cascade filtration (Octo Nova®, Diamed). Patients randomized to treatment (n=11) received mipomersen 200 mg sc per week at day 4 after weekly apheresis for 26 weeks. Patients randomized to control (n=4) did not receive any injection.

Results: Four patients discontinued mipomersen early because of side effects, 3 for severe injection site reactions and 1 for elevated liver enzymes. In an on treatment analysis mipomersen reduced pre-apheresis LDL-cholesterol significantly by 19.2±15%, from a baseline of 189±47 mg/dl to 151±35 mg/dl, while there was no significant change in the control group (+4.7±6%), with the difference between the groups being significant (p=0.009). Mipomersen also decreased pre-apheresis lipoprotein(a) concentration from a baseline of 76.2±67 mg/dl by 12.1±16% (p=0.16).

Conclusion: Mipomersen reduces LDL-cholesterol (significantly) and Lp(a) (non-significantly) in patients on maximal drug therapy and regular apheresis but often is associated with significant side effects.

Research Funding Support: Funding for this investigator-sponsored study was provided by Genzyme, A Sanofi Company, through a non-restricted grant.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Effectiveness of acenocoumarol genetic and clinical dosing algorithms in predicting stable dose in the Greek cohort of the EU-PACT trial

Abstract nr. 542
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Anticoagulants, Genetics, Pharmacology, Risk stratification

The use of genotype-guided dosing algorithm that may increase the effectiveness and safety of the coumarinic anticoagulant acenocoumarol has been recently assessed in a single-blind, randomized trial (EU-PACT, ClinicalTrials.gov number, NCT01119261) comparing a genotype-guided dosing algorithm that included clinical variables and genotyping for CYP2C9 and VKORC1 with a dosing algorithm that included only clinical variables, for the initiation of acenocoumarol treatment in patients with atrial fibrillation or venous thromboembolism (Verhoef et al. NEJM2013;369(24):2294-303). Primary outcome of the trial was the percentage of time in the target range for the international normalized ratio (INR; target range, 2.0 to 3.0) in the 12-week period after the initiation of therapy. Several secondary outcomes, such as time to and number of patients with INR≥4, percentage time spent with an INR≥4 or with an INR<2, number of minor and major adverse events and incidence of coumarin sensitivity and resistance, were also assessed. In the present study we performed an ethnicity analysis for the Greek population recruited in EU-PACT trial. A total of 207 patients (104 in the pharmacogenomic arm, 103 in control arm) starting acenocoumarol therapy were recruited in Greece.
Percent time in range (PTIR) was 61.1% for patients receiving genotype-guided dosing and 62.7% for those receiving clinically guided dosing (P = 0.68). No differences were observed in PTIR for weeks 1-4, 4-8 and 9-12 of the trial and for the other secondary outcomes assessed. We have further tested in the pooled sample the effect of CYP450 enzymes and VKORC1 gene polymorphisms on acenocoumarol stable dose and time to reach stable dose. Aacenocoumarol stable dose was significantly associated with CYP2C9 and VKORC1 genotype (p<0.001 in each case).
In conclusion genotype-guided dosing of acenocoumarol did not improve the PTIR during the 12 weeks after therapy initiation in comparison with the clinical algorithm. However, genetic variants
of CYP2C9 and VKORC1 are significant determinants of individual dose of acenocoumarol needed to maintain a therapeutic INR in the Greek population.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Objective: The most effective mechanisms of developing the diabetic dyslipidemia includes quantitative and qualitative changes in the properties of lipoproteins, degradation of lipoprotein metabolism, genetic predispositions and environmental factors. Today to elucidate the basic molecular mechanism of disease development which cause lipid and lipoprotein metabolism disorders becomes more important. We investigated the effects of LDLR (rs1799898), LPL (rs320), LCAT (rs2292318), ADIPOQ (rs1501299), RETN (rs3745367), PON1 (rs662), CETP (rs708272), LIPC (rs2070895), SCARB1 (rs5888), MNSOD (rs48809) gene polymorphisms on development of diabetic dyslipidemia.

Methods: The study group includes 217 diabetic dyslipidemia patients and 212 healthy controls. Patient group and the healthy control groups matched by age and gender. Genomic DNA was performed from the blood samples and genotype analysis were carried out on the LightCycler® 480 Instrument.

Results: Significant associations were observed between LPL (rs320), LCAT (rs2292318), ADIPOQ (rs1501299), RETN (rs3745367), CETP (rs708272), LIPC (rs2070895), SCARB1 (rs5888), MNSOD (rs48809) gene polymorphisms and development risk of diabetic dyslipidemia. However no associations were determined between the LDLR (rs1799898) and PON1 (rs662) gene polymorphisms and diabetic dyslipidemia.

Conclusion: LPL (rs320), LCAT (rs2292318), AdipoQ (rs1501299) RETN (rs3745367), CETP (rs708272), LIPC (rs2070895), SCARB1 (rs5888), MnSOD (rs48809) gene polymorphisms play important role in the basic molecular metabolism of diabetic dyslipidemia, therefore these polymorphisms may be used as a marker to detect the disease development early.
The Cardiovascular Comorbidity in Children with Chronic Kidney Disease (4C) study: baseline data of a multicentre prospective observational study

Abstract nr. 545
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Chronic Kidney Disease, Epidemiology, Risk Factor

Background and objectives: Children and adolescents with chronic kidney disease (CKD) are at high risk for cardiovascular morbidity and mortality. A systemic arteriopathy and cardiomyopathy has been characterized in pediatric dialysis patients by the presence of morphological and functional abnormalities. While cardiovascular morbidity in adults is related to older age and additional risk factor load (e.g. diabetes), the role of CKD-specific factors in the initiation and progression of cardiac and vascular disease (CVD) are likely to be characterized with greater sensitivity in the pediatric age group.

Design, setting, participants, measurements: The Cardiovascular Comorbidity in Children with CKD (4C) Study is a multicentre, prospective, observational study in children with CKD aged 6 to 17 years, with an initial glomerular filtration rate of 10-45 ml/min/1.73m². The prevalence, degree and progression of cardiovascular comorbidity as well as its association with CKD progression is explored through longitudinal follow-up. The morphology and function of the heart and large arteries is monitored by sensitive non-invasive methods and compared with aged-matched healthy controls. All patients are seen at 6-months intervals by 8 regional coordinators visiting the centres for follow-up including collection of blood and urine samples and annually for assessment of the cardiovascular status.

Results: Since its start in 2009, the 4C Study has recruited a total of 705 patients in 55 participating centers from 12 European countries. At baseline examination, 30% of children were hypertensive (ambulatory blood pressure monitoring), 40% had left ventricular hypertrophy (echocardiography), 40% showed an increased initima-media thickness of the carotid artery (cIMT; ultrasound), and 23% an increase in aortic pulse-wave velocity (PWV; oscillometry). By multivariate analysis, systolic blood pressure and serum levels of 25-hydroxyvitamin D, parathyroid hormone, and serum calcium- and phosphorus levels showed significant associations
with age-corrected IMT and PWV, respectively.

**Conclusions:** Children aged 6-17 years with CKD stage 3-5 have significant subclinical CVD. At initial examination, surrogate endpoints of CVD were associated with systolic blood pressure and disturbances of mineral metabolism. Follow-up data (including analysis of clinical, pharmacological, biochemical and genetic risk factors) are expected to provide innovative insight into cardiovascular and renal disease progression in CKD

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Pattern of Statin Potency Usages and Lipid Outcomes in Thai Diabetic Patients: A Single Center Study.

Abstract nr. 546
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Diabetes, Dyslipidemia, Hypolipidemic Drugs, LDL

ACC/AHA 2013 guideline has recommended using moderate or high potency statins in diabetic patients; however statins have been reported to result in greater LDL-C reductions in Asian than in Caucasian.

We examined the pattern of statin potency usages and their outcomes on lipid profiles of diabetic patients who attended diabetic clinic at Siriraj Hospital during January 1st 2013 - June 30th 2014. Statin potencies were graded according to the ACC/AHA guidelines. Mean age of our study subjects (n=1,427) was 56.8±17 years (60% female). The prevalence of ASCVD was 12%. Mean HbA1C was 7.5±1.5%. Mean fasting plasma total cholesterol, HDL-C and LDL-C levels were 160±33, 53±16, and 84±31 mg/dl respectively. Median plasma triglyceride level was 115 (30-1,575)mg/dl. Among the 1,427 subjects studied, 78.5% was on statins, 2.7% were on non-statin drugs, while 18.8% did not take any lipid lowering drugs. Among subjects who received statins (n=1,121), 33.3% were on low potency statins, 55.8% were on moderate potency statins, and 10.9% were on high potency statins. Plasma LDL-C < 70 mg/dl was achieved in 407 (36.3%) of the statin users. Among subjects who achieved LDL-C <70 mg/dl, only 8.6% were on high potency statin while 42.7% were on low potency statin, 48.6% were on moderate potency statin. Among subjects who had plasma LDL-C ≥ 70 mg/dl, 12.2% were on high potency statin, while 27.9% were on low potency statin, and 59.8% were on moderate potency. There was no significant difference in statin potency usages among subjects who achieved and not achieved LDL-C goal (p=0.065). Interestingly, 46.5% of the subjects who received low potency statins achieved LDL-C < 70 mg/dl.

Conclusion: Low potency statins were prescribed in about one-third of the statin users, and attained an LDL-C goal in nearly half of them. High potency statins were less frequently prescribed in Thai diabetic patients. Ninety-one percent of the subjects who had plasma LDL-C < 70 mg/dl can be achieved by using low or moderate potency statins, suggesting that Thai diabetic patients responded well with statins. Therefore, stepping up statin potency method may be an alternative way in clinical practice.

Subdivision 3. Clinical Studies
Presentation Preference: Electronic poster presentation
Additional information
Perfusion at arterial blood pressure induces transient presence of the endogenous complement inhibitor C4b-binding protein in human saphenous vein grafts.

Abstract nr. 547
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Cardiovascular Disease, Inflammation

**Background.** Complement activation appears to play an important role in vein graft failure as exogenous inhibitors of complement were shown to protect veins against arterial blood pressure-induced remodeling. However, we noted previously that the cell protective complement inhibitor C1 esterase inhibitor is also activated in human saphenous veins perfused at arterial blood pressure. Here we have analyzed whether C4b-binding protein (C4bp), another endogenous complement inhibitor, is present in the vein wall as well.

**Materials and methods.** Human saphenous vein segments obtained from patients undergoing coronary artery bypass grafting (n=55) were perfused in vitro at arterial blood pressure with either autologous blood for 1, 2, 4 or 6 hours or with autologous blood supplemented with reactive oxygen species scavenger N-acetylcysteine. The segments were subsequently analyzed quantitatively for the presence of C4bp and complement activation product C3d using immunohistochemistry.

**Results.** Perfusion induced deposition of C3d and C4bp within the media of the vessel wall, which increased reproducibly and significantly over a period of 4 hours up to 3.8 % for C3d and 81 % for C4bp of the total vessel area. Remarkably after 6 hours of perfusion, a sharp and significant decrease was found in C3d-positive area to 1.3 % as well as the C4bp-positive area to 19% of the total area of the vein. The areas positive for both C3d and C4bp were increased in the presence of N-acetylcysteine.

**Conclusion.** Vein graft perfusion induces a transient but limited increase in the presence of C3d and a much more extensive increase in the presence of C4bp. Both decline dramatically after 6 hours of perfusion, which in part can be prevented by inhibition of reactive oxygen species.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Association of complement C1q, C4d/C4-ratio and C1-INH with cardiovascular disease over a 7-year follow-up period: The CODAM study

Abstract nr. 548
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Cardiovascular Disease, Immunity, Inflammation

Objectives
Complement C3 was in several studies, including the CODAM cohort, associated with cardiovascular disease (CVD). C3 can be activated via the classical, the lectin and the alternative complement pathway. Factors of the classical pathway are present in human atherosclerotic plaques. Furthermore, animal and in-vitro studies showed that the classical pathway is instrumental in clearance of pathogenic material in atherosclerotic lesions. In the current study, we measured the target-binding and initiating factor C1q; furthermore an estimate of classical pathway activation - C4d/C4-ratio; and the inhibitor of classical pathway activation C1-INH. We determined their associations with carotid intima-media thickness (cIMT), CVD, cardiovascular events (CVE) and also with low-grade inflammation (LGI) and endothelial dysfunction (ED).

Methods
Complement C1q, C4d, C4 and C1-INH were measured at baseline in a prospective cohort – the CODAM study (baseline N=547, 61% men, age 60±7 years). At baseline and after a 7-year follow-up period (N=459), we determined the presence of CVD and CVE, and measured cIMT, LGI (score of CRP, serum amyloid A, soluble intercellular adhesion molecule-1 (sICAM-1), TNFα, interleukin-8, interleukin-6) and ED (score of sICAM-1, soluble vascular cell adhesion molecule-1, soluble endothelial selectin, von Willebrand factor). Associations of baseline C1q, the C4d/C4-ratio and C1INH with cIMT, CVD, CVE, LGI and ED over the 7-year follow-up period were analysed with generalized estimating equations, adjusted for potential confounders.

Results
C1q, the C4d/C4-ratio and C1-INH were all not associated with cIMT, CVD or CVE (all Pvalues>0.1). C4d/C4-ratio and C1-INH were also not associated with LGI or ED. In contrast, C1q was positively associated with ED (standardized β in the fully adjusted model was 0.10 [95% CI 0.03; 0.10]). C1q was also positively associated with LGI in a model adjusted only for age, sex, impaired glucose metabolism and type 2 diabetes (standardized β=0.09 [0.01; 0.16]), but this association was attenuated and became non-significant in the fully adjusted model (P=0.12).
Conclusions
C1q may play a role in ED, but systemic C1q, C4d/C4-ratio or C1-INH do not reflect participation of the classical complement pathway in atherosclerosis or in the development of CVD or CVE. (Funded by the Dutch Heart Foundation - NHS2010B194).

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Stress-induced inflammation is associated with altered cortisol reactivity and telomere shortening in patients with coronary artery disease

Abstract nr. 551
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Cardiovascular Disease, Inflammation

Introduction: Stress is known to elicit an inflammatory response which is normally dampened by glucocorticoids. There is evidence for a dysregulated cortisol response in patients with coronary artery disease (CAD). Neutrophils in CAD patients have also been shown to be more prone to release matrix metalloproteinase (MMP)-9 ex vivo. The aim was to investigate whether CAD patients exposed to acute mental stress exhibited a release of MMP-9 and other neutrophil granule products into the circulation and if this was related to cortisol responsiveness, leukocyte telomere length (TL) and carotid atherosclerotic burden.

Methods: Sixty-four CAD patients underwent a standardized psychological stress test between 6-12 months after an index event (acute coronary syndrome and/or coronary intervention). MMP-9, MMP-8, myeloperoxidase (MPO) and salivary cortisol were measured before and 30min after the stress test. Leukocyte TL, basal cortisol levels after awakening and at bedtime, and background psychological factors was assessed. Carotid arteries were examined using duplex ultrasound.

Results: The variation in stress-induced release of neutrophil markers was substantial. Patients were therefore divided into lower and upper tertiles depending on changes in serum MMP-9, T1: -12 %, T3: +27 %, with corresponding changes in MMP-8 and MPO. Clinical or psychological characteristics did not differ between groups, neither did basal levels of neutrophil markers or cortisol. Changes in heart rate and blood pressure were similar in T1 and T3, while cortisol levels declined significantly after stress in T3 (-30%) but not in T1. MMP-9 % change was inversely associated with cortisol % change in the whole cohort, p = 0.025. Leukocyte TL was shorter in T3 than in T1, 0.78 vs 0.88, p = 0.006. Moreover, presence of plaques in right carotid artery differed between T1 and T3, 66 % vs 100 %, p = 0.004.

Conclusion: The association between stress-induced neutrophil activation and altered cortisol reactivity may illustrate a failure to counteract inflammation. Moreover, this was associated with leukocyte telomere shortening which may reflect a long-lasting impairment in the individual’s capacity to handle stressful stimuli. Data suggest that mental stress testing can identify high-risk patients who are in need of novel prevention and treatment strategies.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Reversal of hypoxia in murine atherosclerosis prevents necrotic core expansion by enhancing efferocytosis

Abstract nr. 552
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Pathogenesis, Vulnerable Plaque

Background Advanced murine and human plaques are hypoxic, but it remains unclear whether plaque hypoxia is causally related to atherogenesis. We hypothesized that reversal of hypoxia in atherosclerotic plaques by breathing hyperoxic carbogen gas will prevent atherosclerosis.

Methods and Results: LDLR^{-/-} mice were fed a western-type diet, exposed to carbogen (95% O_2, 5% CO_2) or air and the effect on plaque hypoxia, size and phenotype was studied. First, the hypoxic marker pimonidazole was detected in murine LDLR^{-/-} plaque macrophages from plaque initiation onwards. Second, the efficacy of breathing carbogen (90min, 5L/min, normobaric, single exposure) was studied. Carbogen increased arterial blood pO_2 5-fold in LDLR^{-/-} mice (n=5 male/group, 12-wk diet, p<0.0001), and reduced plaque hypoxia in advanced plaques of the aortic root (-32%, p=0.028) and arch (-84%, p=0.029). Finally, the effect of repeated, daily carbogen exposure on progression of atherosclerosis was studied in LDLR^{-/-} mice (n=15/group) fed a 0.25% cholesterol diet for an initial 4 weeks, followed by 4 weeks of diet and carbogen or air. Carbogen reduced aortic plaque hypoxia (-40%), necrotic core size (-37%, p=0.0003), and TUNEL+ apoptotic cell content (-50%, p=0.03) and increased efferocytosis by MAC3+ macrophages (+36%, p=0.03). These plaque-stabilizing effects were independent of plaque size, plasma cholesterol, hematopoiesis and systemic inflammation. Mechanistically, hypoxia hampered in vitro efferocytosis by bone marrow-derived macrophages, which was dependent on the receptor MerTK.

Conclusion: Carbogen restored murine plaque oxygenation and prevented necrotic core expansion by enhancing efferocytosis, likely via MerTK. Thus, plaque hypoxia is causally related to necrotic core expansion.
Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Lipoprotein(a) and small dense LDL subfraction as independent risk determinant of CHD and myocardial infarction.

Abstract nr. 553
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Co-author(s) - Ezhov, Marat
Co-author(s) - Irina, Adamova
Co-author(s) - Sergei, Pokrovsky
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, LDL, Lp(a), Risk Factor

Purpose. To determine the relationship between lipoprotein(a) [Lp(a)] concentration and lipoprotein subfractions with coronary heart disease (CHD).

Methods. The study population consisted of 228 patients (mean age 57.6±9.6 years, 73% men) who underwent coronary angiography. All patients received statins. Patients with 50% stenosis of at least one coronary artery consisted 190 cases (CHD group), 38 (17%) patients had no atherosclerotic lesions (nonCHD). Myocardial infarction was registered in 117 (51%) patients. Blood tests included Lp(a) (ELISA), lipids («Biocon», Germany) and quantitative content of lipoprotein subfractions («Lipoprint® Quantimetrix», USA). According to the Lp(a) concentration below 30 mg/dl [normoLp(a)] or greater than or equal to 30 mg/dl [hyperLp(a)], and with [sdLDL(+)] and without [sdLDL(-)] atherogenic small dense LDL (sdLDL) patients were divided into 4 subgroups (Table).

Results. Lp(a) and sdLDL levels were significantly higher in CHD patients compared to nonCHD: median Lp(a) - 55.0 (95% CI 40.3-60.2 mg/dl) and 13.9 (40.3-60.2 mg/dl); median sdLDL - 2.4 (2.0-3.0 mg/dl) and 1.0 (0.0-2.0 mg/dl), respectively. Classic risk factors and lipids level were comparable in the both groups. Concentrations of Lp(a) (r=0.313, p<0.001), sdLDL (r=0.168, p<0.05) and mean LDL particle size (r= - 0.165, p<0.05) were associated with CHD according to rank correlation analysis. In multiple regression model after adjustment for age, sex and other lipids both Lp(a) and sdLDL were associated with the coronary atherosclerotic lesions (p<0.05). In groups with hyperLp(a) regardless of sdLDL levels odds ratio (95% confidence interval) for the CHD was significantly higher than in group with normoLp(a) and sdLDL(-) (Table). Odds ratio for myocardial infarction was higher for patients with the presence of sdLDL(+) and/or hyperLp(a).

Conclusion. Lp(a) concentration above 30 mg/dl and presence of sdLDL subfractions are associated with higher risk of coronary heart disease and myocardial infarction.

Funding. Supported by Moscow State Government Grant № 8/3-280н-10. Transparency declarations. All authors: no conflicts of interest to declare.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information

<table>
<thead>
<tr>
<th>Groups</th>
<th>1 – normo-Lp(a) / sdLDL(-)</th>
<th>II – normo-Lp(a) / sdLDL(+)</th>
<th>III – hyperLp(a) / sdLDL(-)</th>
<th>IV – hyperLp(a) / sdLDL(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD</td>
<td>n=32</td>
<td>n=58</td>
<td>n=34</td>
<td>n=104</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.2 (0.9-5.4)</td>
<td>7.1 (1.8-28.1)*</td>
<td>8.2 (3.0-22.5)*</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>1</td>
<td>2.7 (1.1-6.7)*</td>
<td>2.8 (1.0-7.6)*</td>
<td>2.6 (1.1-6.0)*</td>
</tr>
</tbody>
</table>

*p<0.05
Carnosine attenuates the development of diabetic nephropathy in BTBR ob/ob mice

Abstract nr. 554
Author Schilperoort, Maaike, Leiden University Medical Centre, Leiden, Netherlands
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Animal model, Diabetes, Intervention

Over one-third of patients with diabetes develop diabetic nephropathy (DN): a progressive kidney disease that can lead to kidney failure. Characteristic pathological changes associated with DN are loss of glomerular podocytes, mesangial matrix expansion and renal hypertrophy. Patients with a genotype resulting in lower levels of the enzyme that breaks down carnosine are less susceptible for DN (Jansen et al. 2005; Riedl et al. 2007), which indicates a protective role of carnosine in diabetic renal injury. These data suggest that treatment with carnosine is a promising therapy for DN. Therefore, we hypothesize that carnosine supplementation protects diabetic BTBR ob/ob mice from developing DN.

BTBR ob/ob mice were supplemented for 18 weeks with L-carnosine (4 mM) in drinking water and fed regular chow ad libitum. BTBR mice are naturally hyperinsulinemic and when this genetic background is combined with the ob/ob mutation, mice are insulin resistant, hyperglycemic, and rapidly develop a phenotype that closely resembles advanced human DN (Hudkins et al. 2010). At week 18, blood samples were collected to determine fasting plasma glucose (FPG) and glycated hemoglobin (HbA1c) concentrations. Urinary samples were collected to measure albumin and creatinine levels. At the end of the study, mice were sacrificed and tissues were examined by histological and immunohistochemical analyses.

Carnosine reduced FPG (-27%; P=0.005) and HbA1c (-12%; P=0.019) levels. In addition, the albumin-creatinine ratio was markedly reduced in carnosine-treated mice as compared to controls (-55%; P<0.001). Carnosine did not affect the amount of mesangial matrix within the glomeruli, which was mainly composed of fibronectin. The amount of fibronectin tended to be lower in the carnosine-treated group as compared to untreated controls (-14%; P=0.09). Carnosine did not prevent the diabetes-induced reduction of glomerular podocytes. However, carnosine-treated mice showed less hypertrophy of the renal corpuscles (-9%; P=0.037).

These results show that treatment with carnosine improves glucose metabolism and kidney function in BTBR ob/ob mice. In addition, carnosine supplementation ameliorates the diabetic kidney morphology by reducing renal hypertrophy. These results suggest that carnosine could be a novel therapeutic strategy to either treat patients with DN or to be used as prevention in patients
with diabetes.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Endothelial heterogeneity: rat aortic endothelial cells show a greater oxidative stress response to TNF-α compared to cardiac microvascular endothelial cells.

Abstract nr. 555
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Endothelium, Inflammation

Endothelial cells (ECs) from different vascular beds exhibit heterogeneity in terms of structure, function and response to injury. Despite this, current knowledge and assumptions are largely based on data obtained from a small pool of readily available cell lines such as HUVECs, hence failing to account for differences between venous and arterial ECs, or macrovascular and microvascular ECs.

We aimed to compare the responses to a pro-inflammatory stimulus of two distinct EC types from vascular locations known to be primary targets of atherogenesis and ischaemic heart disease. Cultured primary rat aortic endothelial cells (AECs) and cardiac microvascular endothelial cells (CMECs) were treated with TNF-α (20ng/ml; 24h), and their responses compared in terms of cell viability, nitric oxide (NO) biosynthesis, oxidative stress, NF-KB activation and large-scale protein regulation (proteomics).

Both cell types showed evidence of dysfunction: (i) 3.3-fold and 2.3-fold increase in necrosis in AECs and CMECs respectively; and (ii) ~30% and ~20% reduction in NO production respectively. Proteomics analysis further revealed greater up-regulation of the endothelial dysfunction marker, endothelin converting enzyme-1 in AECs vs. CMECs (5.2-fold; p=0.003). NO-production was restored and necrosis abolished in both cell types by pre-treatment with the endothelioprotective agent, oleanolic acid (40ng/ml). AECs and CMECs showed significantly heterogeneous responses in terms of reactive oxygen species (ROS) production (DCF fluorescence): AECs: 2.8-fold increase vs. CMECs: 1.4-fold. This was accompanied by 2.8-fold and 2.2-fold increase in superoxide dismutase (SOD) expression in AECs and CMECs respectively. Proteomics analysis furthermore showed 5.3-fold enrichment of the Gene Ontology (GO) terms: “Response to ROS” (p=0.01) and “Response to oxidative stress” (p=0.01) in AECs vs. CMECs. In contrast, CMEC proteomics were characterized by a marked enrichment of GO terms related to a pro-inflammatory or immune response, supported by significant activation of the NF-KB pathway in CMECs (40% reduction in IKB-alpha expression; p<0.05).

In conclusion, TNF-α treatment induced a reversible state of endothelial dysfunction in AECs and CMECs; however, AECs showed a markedly greater oxidative stress response vs. CMECs, while the CMEC response was characterized by up-regulation of pro-inflammatory pathways. These
findings support the importance of endothelial heterogeneity in the field of vascular biology.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Mutations in ABCA8 underlie reduced plasma high density cholesterol levels in humans

Abstract nr. 556
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Genetics,HDL,Reverse Cholesterol Transport

The relationship between cardiovascular disease risk and high density lipoprotein cholesterol (HDLc) levels is unclear, raising the need for further studies. We identified and characterized the impact of a novel HDLc gene, ATP binding cassette transporter A8 (ABCA8). We sequenced ABCA8 in 80 low (HDLc %ile<10\textsuperscript{th}) and 120 high HDLc (HDLc %ile≥90\textsuperscript{th}) individuals, and identified three ABCA8 variants exclusively in low-HDLc subjects: Proline609Arginine (in the ATP-binding domain), E17-2 A>G (disruption of essential splice site) and Threonine741Stop. Genotyping of expanded families identified additional mutation carriers and first-degree relative controls. Compared with controls, heterozygous mutation carriers showed a significant 26.5% decrease in plasma HDLc levels and 55.5% decrease HDLc percentiles (age and sex adjusted). Overexpression of human ABCA8 in mouse livers via adenoviral injection led to a 23.1% increase in HDLc levels. Wild-type ABCA8 localized at the plasma membrane and the ER. However, P609R- and T741X-ABCA8 are only present at the ER. A significant 181% increase in cholesterol efflux to lipid free APOA-I was observed with wild type ABCA8, but not with the mutants P609R or T741X. It has been described that ABCA8 regulates levels of sphingomyelin, an essential lipid in the formation and maturation of HDL. Compared to controls, HDL sphingomyelin content of ABCA8 mutation carriers was decreased, and HDL sphingomyelin levels of mice overexpressing ABCA8 in the liver was significantly increased. We show here that ABCA8 is a cholesterol transporter that modulates HDLc and sphingomyelin levels in humans and mice.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Triglycerides, non-HDL-C, LDL-C and apoB and cardiovascular risk in patients with clinical manifest arterial disease.

Abstract nr. 559
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins,Cardiovascular Disease,LDL,Lipids

**Background** Since low-density lipoprotein cholesterol (LDL-C) only partly represents the atherogenic lipid burden, a growing body of evidence suggests that non-high-density lipoprotein cholesterol (non-HDL-C), triglycerides (TG) and/or apolipoprotein B (apoB) may provide a more accurate way to estimate atherosclerotic cardiovascular (CV) disease risk due to lipids. Patients with a history of manifest arterial disease compose a growing population at increased risk of a recurrent CV event.

**Objective** To compare the relation between LDL-C, non-HDL-C, TG and apoB and the occurrence of vascular events and mortality in patients with clinical manifest arterial disease.

**Methods** Prospective cohort study of 6904 patients with manifest arterial disease. Imputation methods were used to reduce missing covariate data. Cox proportional hazard models were used to evaluate the relation between LDL-C, non-HDL-C, TG, apoB and vascular events (stroke, myocardial infarction, vascular mortality) and all-cause mortality.

**Results** Mean age at baseline was 60±10.4 years and 74% were males. New vascular events occurred in 1075 subjects and 1189 patients died during a median follow-up of 6.0 years (IQR 3.1-9.3). LDL-C, non-HDL-C and apoB were statistically associated with increased risk of stroke, vascular events and (vascular) mortality. Hazard ratios (HR) per 1 SD for a vascular event were for LDL-C 1.11 (95% Confidence interval (CI) 1.04-1.18), for non-HDL-C 1.13 (95%CI 1.06-1.20), for TG 1.04 (95%CI 1.00-1.08) and for apoB 1.11 (95%CI 1.04-1.18), after adjustment for age, sex, body mass index, smoking, alcohol and diabetes mellitus. For all-cause mortality, HR per 1 SD were for LDL-C 1.04 (95%CI 0.98-1.10.98), for non-HDL-C 1.06 (95%CI 1.00-1.13), for TG 1.04 (95%CI 0.99-1.09) and for apoB 1.11 (95%CI 1.05-1.18).

**Conclusions** In patients with clinical manifest arterial disease, higher levels of LDL-C, non-HDL-C and apoB are related with a higher risk of future cardiovascular events. Although differences between the separate lipids are confined, non-HDL-C has the strongest association with cardiovascular risk.
Comparing the relation between LDL-C, non-HDL-C, TG and apoB per 1 SD with vascular events and mortality.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Silencing Antithrombin and Protein C in ApoE Knock Out Mice Causes Severe Coagulopathy But Not Atherothrombosis

Abstract nr. 560
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Dyslipidemia, Thrombosis

Atherothrombosis is a major cause of cardiovascular events. Unfortunately, models rarely develop atherothrombosis due to their strong anticoagulation activity and relatively stable atherosclerotic plaques. In current mouse models for atherothrombosis often the latter obstacle is overcome by physically inducing plaque rupture. However, it has not been studied whether reducing anticoagulation leads to atherothrombosis in mice. Recently, a novel mouse model was developed in which spontaneous venous thrombosis was induced by siRNA targeting of the anticoagulation factors Serpinc1 (antithrombin) and Proc (protein C). We have used this model to study the role of anticoagulation in arterial and venous thrombosis in dyslipidemic and atherosclerotic ApoE knockout (ApoE−/−) mice.

ApoE−/− mice were fed Western Type Diet for 8 weeks, followed by injection with siRNA targeting Serpinc1, Proc, or both. Combined knockdown of Serpinc1 (-98.7±0.4%) and Proc (-95.4±0.4%) led to severe coagulopathy, including a massive decrease in circulating platelets (-94.3±0.8%), severe weight loss (-16.6±3.2%), microscopically visible venous thrombi and subsequent hepatic ischemia within 48 hours post injection. Single gene targeting (Serpinc1: -96.0±1.2%, Proc: -90.8±0.6%) caused no macroscopically visible signs of coagulopathy, but circulating platelets were decreased (Serpinc1: -36±11%, Proc: -53±21%), albeit to a lesser extent than when both genes were targeted. Atherothrombosis was not observed in either single or double gene targeting. In conclusion, combined silencing of Serpinc1 and Proc causes severe coagulopathy in ApoE−/− mice. Furthermore, this study shows that atherothrombosis cannot be induced in ApoE−/− by combined or separate silencing of Serpinc1 and Proc alone. Nonetheless, this model will remain valuable for future research on the interplay between hyperlipidemia and thrombosis.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Blood lipids influence DNA methylation in circulating cells

Abstract nr. 561
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Epidemiology, Genetics, Lipids

Interactions between lipid metabolism and the immune system dictate the development of atherosclerosis. We studied whether blood lipids induce epigenetic priming of circulating immune cells, which may promote their pro-atherogenic properties.

We performed a fixed-effect meta-analysis on 3296 subjects from six biobanks in the BIOS consortium to evaluate the association between whole blood genome-wide DNA methylation obtained with the Illumina 450k array and serum lipid levels. For LDL cholesterol, HDL cholesterol and triglyceride levels, we observed 2, 33 and 33 differentially methylated CpGs, respectively, in a linear model correcting for age, gender, cell composition and batch effects (P_{BH} < 0.05; inflation factor λ = 1). Next, a bidirectional Mendelian Randomization approach was performed to infer causal relationships with methylation QTLs as proxies for DNA methylation and polygenic scores constructed from GWAS-identified SNPs as proxies for lipid levels. We found evidence for an effect of LDL cholesterol levels on DNA methylation mapping to the DHCR24 gene (P_{BH} < 0.05), of HDL cholesterol levels to ABCG1, and of triglycerides levels to ABCG1 (2 CpGs), CPT1A (2 CpGs), SREBF1 and SCD. Interestingly, all these genes are known to be involved in lipid metabolism. Moreover, expression of ABCG1, CPT1A, DHCR24 and SREBF1 (measured using RNA-seq in the same samples) was associated with DNA methylation of the lipid-associated CpGs (P_{BH} < 0.05).

Our analysis shows that variation in blood lipid levels affect DNA methylation in circulating cells. Future studies are required to evaluate whether these changes lead to increased atherogenic properties of circulating cells and in part mediate the effect of lipid levels on cardiovascular risk.

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Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Transforming growth factor-beta signaling in T cells promotes stabilization of atherosclerotic plaques through an interleukin-17 dependent pathway

Abstract nr. 562
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Pathogenesis, Vulnerable Plaque

Adaptive immunity has a major impact on atherosclerosis, with pro- and anti-atherosclerotic effects exerted by different subpopulations of T cells. Transforming growth factor-beta (TGF-beta) may promote development either of regulatory T cells or Th17 cells, depending on factors in the local milieu. In the present study, we have addressed the effect on atherosclerosis of enhanced TGF-beta signaling in T cells. Bone marrow from mice with a T-cell specific deletion of Smad7, a potent inhibitor of TGF-beta signaling, was transplanted into hypercholesterolemic Ldlr-/- mice. Smad7-deficient mice had significantly larger lesions that contained a large collagen-rich smooth muscle cap, consistent with a more stable phenotype. The inflammatory cytokine interleukin-6 was expressed in the atherosclerotic aorta and increased mRNA for IL-17A and the Th17 specific transcription factor ROR-gamma-t were detected in draining lymph nodes. Treating Smad7-deficient chimeras with neutralizing IL-17A antibodies reversed the stable cap formation. IL-17A stimulated collagen production by human vascular smooth muscle cells and ROR-gamma-t mRNA correlated positively with collagen type-1 and alpha-smooth muscle actin mRNA in a biobank of human atherosclerotic plaques. These data demonstrate that TGF-beta modulates atherosclerosis in a context dependent manner and links IL-17A to induction of a stable plaque phenotype.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Glucose addition affects postprandial response of glucagon-like peptide 1 (GLP-1) to a test meal

Abstract nr. 563
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Lipoproteins, Metabolism, Triglyceride-Rich Proteins

The increased and prolonged postprandial lipemia is associated with higher risk of cardiovascular disease (CVD). Recent data point out that non-fasting TG concentration is associated with CVD closely than fasting TG concentration. However, there is not so much information how particular dietary components of experimental meal affect the response of important signalling molecules during postprandial lipemia. Therefore, we studied the effect of saccharide added to a fat load on the response of insulin, GLP-1 and triglyceride-rich lipoproteins (TRL) to the meal.

Two examinations were carried out in 30 healthy male volunteers (age 35 ± 8; BMI 26.1 ± 3.2). Men consumed experimental meal containing 75 g of fat (cream) + 25 g glucose (F+G meal) or 75 g of fat (F meal) in control experiment. The blood was taken before meal and 30, 60, 90, 120, 240, 360 and 480 minutes after meal consumption. Both total and active GLP-1 concentrations were measured using ELISA (Millipore).

After F+G meal, glucose concentration rose from 5.4 ± 0.4 to 6.5 ± 1.0 mmol/l in 30 minutes (p<0.001) and that went along with an increase in insulinemia from 7.3 ± 3.4 to 32 ± 21.5 mIU/l (p<0.001), and with a supression of noneesterified fatty acid (NEFA) concentration during first 90 minutes. After F meal, insulin increment was smaller (from 6.6 ± 2.6 to 11.3 ± 4.3 mIU/l, p<0.001) and glycemia was not affected. Interestingly, 2 hour AUC of both total and active GLP-1 were 21.5 % and 18 % lower (p<0.001) (Fig.), respectively, when glucose was given with the fat load. No differences were observed in the response of triglyceride (TG) and cholesterol concentration in TRL isolated by ultracentrifugation (d<1.006 g/l) to F+G and F meals.

The addition of glucose to a test meal induces physiological response of insulin and lower response of GLP-1 to a test meal during early postprandial phase but has no effect on the changes of TRL-cholesterol and TRL-TG within 8 hours after meal.

Supported by grant No. NT 14027-3/2013 from IGA MH CR.
GLP1 - Total

- Fat + Glucose
- Fat

`pM`

Time [min]

0 60 120 180 240 300 360 420 480

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
CYP27A1 gene dosage accounts for the anti-atherosclerotic effect of Rifampicin in mice

Abstract nr. 564
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Atherosclerosis, Dyslipidemia, HDL

Sterol 27-hydroxylase (CYP27A1) regulates bile acid synthesis and cholesterol metabolism. To explore the impact of CYP27A1 deficiency on atherosclerosis, cyp27A1 knockout (KO) mice were crossed with apoE KO mice and the resulting apoE KO, cyp27A1 heterozygote/apoE KO (HET) and cyp27A1 KO/apoEKO (DKO) were challenged with a Western diet (WD). The apoE KO phenotype was reversed in DKO, possibly because of enhanced cholesterol detoxification via up-regulation of hepatic CYP3A11 mRNA. HET in contrary developed much more severe lesions than apoE KO mice because of reduced 27-hydroxycholesterol (27-OHC) plasma concentration. CYP3A1 can be induced by Rifampicin (RIF).

The aim of this study was to induce CYP3A1 expression by RIF in apoE KO and HET and to analyze the effect of a reduced CYP27A1 expression on atherosclerosis development. ApoE KO and HET mice were fed a WD and divided at the age of 6 weeks in 2 groups (n=6), one receiving RIF (10 mg/kg ip) daily for a month, the other vehicle only. At the end of the experiment, blood and organs were collected. Plasma was used for total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), and as acceptor in cholesterol efflux (CE) using RAW264.7 cells. Atherosclerosis was quantified in the aortic valve. Histology and real time PCR were performed in liver.

RIF induced hepatic mRNA CYP3A11 levels 4-fold in apoE KO and HET mice (p<0.006) but had no effect on the other cytochromes CYP27A1, CYP7A1 and CYP8B1 and on LDL-receptor expression. RIF hepatotoxicity was ruled out by histologic sections. Atherosclerosis was significantly reduced by RIF in apoE KO mice and the latter had ~3-fold less atherosclerosis than their HET littermates with similar treatment (p<0.0234). In apoE KO, RIF decreased TC ~2-fold, increased the HDL/LDL ratio ~2-fold, whereas in HET, RIF had no effect on plasma lipids composition. CE did not change significantly between the groups.
RIF induced hepatic CYP3A11 expression and had an anti-atherogenic effect dependent on CYP27A1 expression. This reveals the importance of targeting CYP27A1 expression for novel therapeutic interventions in the field of atherosclerosis.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
A molecular link between energy expenditure, lipid metabolism, and cardiovascular health

We previously showed that USF1 transcription factor was associated with familial combined hyperlipidemia in Finns. In the current study, we have conducted a detailed metabolic characterization of Usf1 in mice. We generated a congenic strain of Usf1 knockout mice, fed with high-fat diet for 5 months. The knockouts displayed both elevated cholesterol and phospholipids in their plasma HDL-fractions and lower total and VLDL-triglycerides. This was due to both enhanced TRL clearance linked to elevated LPL activity of the knockouts, and reduced hepatic VLDL secretion. The elevated HDL-C levels were associated with increased efflux capacity of HDL particles derived from Usf1-/- mice. The detailed analysis of Usf1-/- HDL composition revealed an enrichment of phospholipids, known to enhance the efflux capacity of HDL particles. The Usf1-/- mice were protected against HFD induced obesity despite being physically less active and eating more than Usf1+/+ mice. Furthermore, they were protected against insulin resistance, vascular inflammation, atherosclerosis, and fatty liver. While absorption of lipids was similar between Usf1-/- and Usf1+/+ mice, the Usf1-/- mice displayed elevated VO2 and VCO2, even in thermoneutral conditions, suggesting an increased metabolic rate. Analysis of post-injection organ distribution of [3H]triolein revealed a selective uptake of TRLs by brown adipose tissue in Usf1-/- mice, mediated by an LPL-dependent mechanism, as inhibiting LPL by tetrahydrolipstatin completely eliminated lipoprotein uptake by BAT. There was a dramatic 8-fold reduction in lipid droplet size as well as lipid content of BAT with abundance of mitochondrial complex II in BAT of Usf1-/- mice. Furthermore, PET/CT measurements demonstrated an increased glucose uptake by BAT of Usf1-/- mice. Together, these findings demonstrate overall avidity of BAT to process energy substrates.

Remarkably, the protection against cardiometabolic complications in mice is paralleled in a Finnish 1000 Genomes imputed eQTL dataset, in whom SNP alleles reducing USF1 mRNA expression
are associated with increased HDL-C levels, reduced triglycerides, insulin resistance and atherosclerosis.
Our data establish the critical role of Usf1 as a metabolic master-regulator, and demonstrate that Usf1 deficiency leads to remarkably beneficial metabolic profile in mice and men. Our discoveries make USF1 appear a particularly attractive therapeutic target.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Human carotid plaques with high levels of interleukin 16 are associated with reduced risk for cardiovascular events.

Abstract nr. 566
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Co-author(s) - Nilsson, Jan
Co-author(s) - Gonçalves, Isabel
Co-author(s) - Björkbacka, Harry
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Cardiovascular Disease, Immunity, Inflammation, Vulnerable Plaque

Background and purpose – The aim of this study was to determine whether interleukin 16 (IL-16) measured in human carotid plaques was associated with symptoms (such as stroke, transient ischemic attack or amaurosis fugax), markers of plaque stability and future cardiovascular events. Methods – Plaques obtained from patients that had suffered symptoms within one month before removal by endarterctomy (n=107) were compared to plaques from patients without symptoms but more than 80% stenosis of the carotid artery (n=95). Neutral lipids, collagen, elastin, smooth muscle cell and macrophage contents were evaluated histologically and caspase-3 activity was measured biochemically. IL-16, MMPs and TIMPs were measured in plaque homogenates using a multiplex immunoassay. IL-16, CD3, CD4 and FOXP3 mRNA expression was analyzed with quantitative real-time PCR.

Results – Carotid plaques from asymptomatic patients had higher levels of IL-16 mRNA. High IL-16 protein levels (above median) were associated with reduced incidence of new cardiovascular events compared to low IL-16 levels during a mean follow up of 2 years (HR=0.47, C.I. 0.22-0.99, p=0.047). The IL-16 levels correlated with the plaque stabilizing components, elastin, collagen, MMP2, TIMP1, TIMP2 and FoxP3 mRNA, but also with the destabilizing factors CD68 and neutral lipids.

Conclusion – This study shows that high levels of IL-16 are associated with fewer pre-operative symptoms and decreased risk of suffering a new cardiovascular event within two years, suggesting that IL-16 might have a protective role in human atherosclerotic disease.
CHARACTERISATION OF TLR7 IN ATHEROSCLEROTIC TISSUE.

Abstract nr. 567
Author Paulsson-Berne, Gabrielle, Karolinska Intitutet, Stockholm, Sweden
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Vulnerable Plaque

Background:
Inflammatory pathways in atherosclerosis are involved in the disease at different stages, from early onset to final ischemic events. Atherosclerotic lesions are infiltrated by immune cells, predominantly macrophages and T cells. Specific markers connecting to local inflammatory responses may lead to the life threatening condition of plaque rupture in patients with severe atherosclerosis. Toll like receptors are key components in many inflammatory pathways. Here we have investigated the presence of TLR7 within atherosclerotic lesions.

Methods:
Carotid artery samples from patients undergoing endarterectomy were obtained and used for sectioning we investigated the presence of TLR7 protein by immunohistochemistry.

Results:
TLR 7 was found in all plaques analyzed. The staining pattern was indicating that TLR7 was mainly present in infiltrating leukocytes, not in vascular cells. Consecutive sections where stained with T cell and macrophages markers and in both cases the TLR7 signal co-localized to the same area. To identify the specific cell types where TLR7 is present a panel of surface markers was used for the most typical cells in the plaque to perform double or triple staining with immunoflourescence. With this analysis we have identified macrophage (M1 and M2) and T cell (CD4 and CD8) populations that co-express TLR 7.

Conclusion:
It has become evident that there are subtypes of T cells as well as macrophages that might differ in their function within the plaque. Specific markers may either enforce or diminish local inflammatory responses in the plaque. Further investigations of how TLR7 effect the phenotype of these cells are needed.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Quantitative serum apolipoprotein profiling should complement non-selective serum HDL-c and LDL-c tests in dyslipidemic patients

Abstract nr. 568
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Co-author(s) - Atsma, Douwe
Co-author(s) - Roosendaal, Frits
Co-author(s) - Cobbaert, Christa

Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Cardiovascular Disease, Dyslipidemia, Risk stratification

Introduction: Serum low- and high density lipoprotein cholesterol (LDL-c and HDL-c) are hallmark medical tests for diagnosis and treatment monitoring of dyslipidemia. Currently, there is an ongoing debate whether the lipoprotein cholesterol content, being LDL-c or HDL-c, or the actual number (and type) of lipoprotein particles (LDL-P or HDL-P) is the clinically relevant and optimal measure to assess cardiovascular disease risk. Furthermore, since contemporary direct LDL-c and HDL-c tests lack selectivity in hypertriglyceridemic specimens (Langlois et al. 2014), these assays may lead to misdiagnosis and misclassification.

Method: We developed a mass spectrometric method for multiplexed quantification of six serum apolipoproteins (apoA-I, apoB100, apoC-I, apoC-II, apoC-III, and apoE). In contrast to classical HDL-c and LDL-c tests and lipoprotein particle counting methods, the quantitative proteomics test allows standardization of unequivocally characterized apolipoproteins with an analytical performance meeting test requirements derived from biological variation (van den Broek et al. 2015). In this study, the selectivity of the multiplexed proteomics-based method was evaluated by duplicate analysis of normotriglyceridemic (triglycerides < 2.3 mmol/L; n = 54) and hypertriglyceridemic sera (triglycerides between 2.7-18.1 mmol/L; n = 46), as compared with clinical immunoassays.

Results: The mass spectrometric quantification of apoA-I, apoB, apoC-II, apoC-III, and apoE correlated well with CE-marked, uniplex immunoturbidimetric assays ($R^2 \geq 0.97$ for n=100). Moreover, test results were interchangeable with an average %bias (mass spectrometric method relative to immunoturbidimetric assay) of -5.3 and +4.6% in normotriglyceridemic sera and between -5.0 and +6.9 % in hypertriglyceridemic sera, whereas the imprecision (coefficient of variation, CV) varied between 4.4 and 9.2%. Furthermore, the targeted mass spectrometric detection of specific signature peptides allowed the discrimination between apolipoprotein variants.
or phenotypes (such as apoB<sub>48</sub> vs. apoB<sub>100</sub>, or total apoE vs. apoE2) that went unnoticed with immunoassays.

**Conclusion:** The proteomics-based method for multiplex quantification of serum apolipoproteins is metrologically sound, fulfils clinical needs, and is not affected by hypertriglyceridemia. Clinical validation studies are underway to assess its performance in predicting patient outcome, particularly for dyslipidemic patients.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
High-resolution optical imaging of atherothrombosis

Abstract nr. 569
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Imaging, Thrombosis, Vulnerable Plaque

The disruption of unstable plaques at the latest stages of atherosclerosis determines the exposure of thrombogenic material, mainly collagen, to circulating platelets, leading to atherothrombosis with potential clinical consequences (Fuster V. et al., 2005). Due to the size of both collagen fibers and platelets, investigation of these structures can benefit from modern optical imaging modalities that combine high speed multidimensional and multichannel acquisition with microscopic or nanoscopic resolution. Hence, the mechanism of collagen-driven platelet activation may be further unraveled and subsequently stimulate the development of novel anti-thrombotic therapies. We employed different optical microscopic and nanoscopic modalities to address structural and functional determinants involved in atherothrombosis. Two-photon laser-scanning microscopy (TPLSM) was used to exploit near-infrared excitation and visualize both exogenous (i.e. fluorescent labels) and endogenous signals, such as atherosclerotic plaque autofluorescence and second harmonic generation (SHG) of fibered collagen (Lilledahl MB. et al., 2007). TPLSM revealed that commercially available Horm collagen (Takeda, Austria), which is extensively used as a substrate for platelet adhesion and aggregation in functional studies mimicking atherothrombosis, is a heterogeneous preparation of mainly type I fibers, interspersed with type III (both SHG-positive) and at least one other, unspecified type of collagen fibrils which are SHG-positive but type I and type III negative. The latter may be important for studies aimed at blocking type-specific collagen interactions with platelets. Furthermore, endogenous fluorescence generated by the broad two-photon excitation spectrum was used to label-free visualize plaque material in dynamic 4D in vitro studies where we assessed the behavior of native fluorescently labeled platelets in response to human plaque homogenate under various shear conditions and in the presence of different inhibitors. 3D stimulated emission-depletion (STED) microscopy and 3D structured illumination microscopy (SIM) are two fluorescence-based imaging modalities that overcome the diffraction barrier, thus offering a lateral resolution of <200 nm (Hell SW., 2009). Preliminary work based on these techniques showed details of collagen fiber organization and platelet-collagen interactions with nanoscopic resolution in three dimensions. Future imaging studies aim to further unveil the dynamics of receptor-mediated adhesion and activation of platelets on the different types of collagen fibers and atherosclerotic plaque material.
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Intervention with anti-inflammatory RvE1 attenuates atherosclerosis without reducing plasma cholesterol and adds to the anti-atherogenic effect of Atorvastatin

Abstract nr. 570
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Co-author(s) - Gjorstrup, Per
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Co-author(s) - Kooistra, Teake

Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Inflammation, Intervention, Therapy

Aims It is now well recognized that, besides LDL-cholesterol, local vascular inflammation plays a key role in atherogenesis. A failure to effectively resolve this inflammation in the vascular wall is associated with persistent atherosclerotic lesion progression. The discovery of specialized pro-resolving regulators of both acute and chronic inflammatory responses may represent a therapeutic alternative. The aim of the present study is to find evidence that a locally active pro-resolving mediator with anti-inflammatory characteristics, ω-3 fatty acid eicosapentaenoic acid-derived resolvin E1 (RvE1), can reduce atherosclerosis, independent of and complimentary to cholesterol-lowering and anti-inflammatory atorvastatin.

Methods and results ApoE*3Leiden transgenic mice, a humanized mouse model for atherosclerosis, were fed a hypercholesterolemic diet for 9 weeks and subsequently treated with atorvastatin (1.5mg/kg/day), RvE1-low (1mg/kg/day), RvE1-high (5mg/kg/day), and the combination of atorvastatin and RvE1-low for the following 16 weeks. RvE1-low and RvE1-high reduced atherosclerotic lesion size to the same extent (36%; p<0.05), without affecting plasma cholesterol levels. Plasma cholesterol-lowering (24%; p<0.01) atorvastatin reduced the lesion size with 27% (p<0.05). Notably, refined lesion analysis revealed a marked increase in mild lesions with RvE1 versus atorvastatin treatment. Outstandingly, in combination with atorvastatin, RvE1 tended to add to the anti-atherogenic effect of atorvastatin alone, resulting in an additional decrease in lesion size (31%) and an increase in mild lesions (+243%; p<0.01). RvE1 had no effect on plasma levels of the systemic inflammatory marker, serum amyloid A (SAA), but did down-regulate the local aortic gene expression of genes reportedly involved in atherogenesis, including CTSS, CD74, CD44, HPSE, IFR5, Ccl2, CSP1, IL-20R beta, Ccr5 and Adam17 and significantly inactivated processes triggered by IFN- (p<0.001) and TNF-α (p<0.001).

Conclusions The resolvin RvE1 attenuates atherogenesis at the level of lesion initiation and lesion size, both alone and on top of a statin. The local anti-inflammatory activity of RvE1 is demonstrated by the modulated aortic expression of genes involved in inflammatory and immune
responses, without altering plasma cholesterol or the systemic inflammation marker, SAA.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Lipid-modifying efficacy and tolerability of anacetrapib added to ongoing statin therapy in patients with hypercholesterolemia or low high-density lipoprotein cholesterol

Abstract nr. 571
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Co-author(s) - Gill, Geraldine G.
Co-author(s) - Cressman, Michael D.
Co-author(s) - Ashraf, Tanya B.
Co-author(s) - Johnson-Levonas, Amy O.
Co-author(s) - Dr. Mitchel, Yale
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Atherosclerosis, Cardiovascular Disease, Familial Hypercholesterolemia

Purpose: Anacetrapib, a cholesteryl ester transfer protein inhibitor, reduces low-density lipoprotein cholesterol (LDL-C), apolipoprotein (Apo) B and lipoprotein a (Lp(a)), and raises high-density lipoprotein cholesterol (HDL-C) and ApoAI. This 24-week, Phase 3, worldwide, multicenter, randomized, double-blind, placebo-controlled study assessed the lipid-modifying efficacy and safety profile of anacetrapib added to ongoing therapy with statin ± other lipid-modifying therapies in patients with hypercholesterolemia who were not at their LDL-C goal (as per NCEP ATP III guidelines) and in patients with low HDL-C.

Methods: Patients on a stable dose of moderate/high-intensity statin ± other lipid-modifying therapies and having an LDL-C ≥70 mg/dL, ≥100 mg/dL, ≥130 mg/dL or ≥160 mg/dL for very high, high, moderate and low CHD risk, respectively, or at LDL-C goal with HDL-C ≤40 mg/dL were randomized in a ratio of 1:1:1, stratified by background therapy use, to anacetrapib 100 mg (n=153), anacetrapib 25 mg (n=152), or placebo (n=154) for 24 weeks, followed by a 12-week off-drug reversal phase. The primary end points were the percent change from baseline in LDL-C (beta-quantification method) and HDL-C, as well as the safety profile of anacetrapib. This trial is registered in ClinicalTrials.gov, #NCT01717300.

Results: Both doses of anacetrapib reduced LDL-C, non-HDL-C, ApoB and Lp (a) and increased HDL-C and Apo AI vs placebo (p<0.001 for all; see table below). There were no differences between the anacetrapib 100 mg or 25 mg groups and placebo in the proportions of patients who discontinued drug due to an adverse event or in abnormalities in liver enzymes, creatinine kinase, blood pressure, electrolytes or adjudicated cardiovascular events.

Conclusions: In patients with hypercholesterolemia or low HDL-C, treatment with anacetrapib resulted in substantial reductions in LDL-C and increases in HDL-C and was well tolerated. The 30,000 patient REVEAL study will provide information on the impact of the lipid changes of
anacetrapib 100 mg on cardiovascular outcomes.

<table>
<thead>
<tr>
<th>Lipid Endpoints</th>
<th>Baseline Mean (SD)*</th>
<th>Week 24 Mean (SD)*</th>
<th>Percent Change from Baseline Least Squares Mean (SE)*</th>
<th>Difference from Placebo in Least Squares Means (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C mg/dL (beta-quantification method)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n=133)</td>
<td>92.9 (25.5)</td>
<td>96.6 (33.2)</td>
<td>0.9 (3.0)</td>
<td></td>
</tr>
<tr>
<td>Anacetrapib 25 mg (n=126)</td>
<td>95.3 (31.7)</td>
<td>79.7 (39.3)</td>
<td>-21.9 (1.0)</td>
<td>-22.8 [-30.4, -15.1]*</td>
</tr>
<tr>
<td>Anacetrapib 100 mg (n=133)</td>
<td>95.7 (33.6)</td>
<td>68.6 (36.8)</td>
<td>-28.0 (2.9)</td>
<td>-28.9 [-36.4, -21.3]*</td>
</tr>
<tr>
<td>HDL-C mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n=140)</td>
<td>47.7 (14.2)</td>
<td>48.5 (14.0)</td>
<td>3.9 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Anacetrapib 25 mg (n=136)</td>
<td>45.2 (12.4)</td>
<td>75.8 (24.9)</td>
<td>69.4 (3.5)</td>
<td>65.5 [56.0, 75.0]*</td>
</tr>
<tr>
<td>Anacetrapib 100 mg (n=138)</td>
<td>46.2 (12.6)</td>
<td>91.7 (32.2)</td>
<td>98.6 (3.5)</td>
<td>94.7 [85.2, 104.1]*</td>
</tr>
<tr>
<td>Lp(a) nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n=144)</td>
<td>32.1 (81.6)</td>
<td>29.1 (79.7)</td>
<td>-3.0 (21.9)</td>
<td></td>
</tr>
<tr>
<td>Anacetrapib 25 mg (n=137)</td>
<td>32.4 (83.1)</td>
<td>16.6 (74.3)</td>
<td>-16.8 (36.4)</td>
<td>-22.8 [-39.4, -16.8]*</td>
</tr>
<tr>
<td>Anacetrapib 100 mg (n=147)</td>
<td>22.8 (59.3)</td>
<td>12.3 (43.8)</td>
<td>-29.5 (39.9)</td>
<td>-32.6 [-59.2, -26.2]*</td>
</tr>
</tbody>
</table>

*p<0.001; t: median for Lp(a)

**Table**

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Virgin pumpkin oil provides benefits beyond those of refined pumpkin oil on cardiometabolic risk factors and disease development.

Abstract nr. 572
Author Morrison, Martine, TNO, Leiden, Netherlands
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Dyslipidemia, Lifestyle, Nutrition

Background: Pumpkin oil, rich in linoleic and oleic unsaturated fatty acids, is reported to have beneficial effects on cardiometabolic risk factors, but effects on end-point cardiometabolic disease (non-alcoholic fatty liver disease/NAFLD and atherosclerosis) have not been studied to date. Although health-promoting effects are often ascribed to the type of fatty acids present in pumpkin oil, additional beneficial effects of other minor components (e.g. bioactive phytochemicals like phenols and sterols) remain unclear. Therefore, we investigated the effects of a refined and a virgin (unrefined) pumpkin oil, comparable in fatty acid profile but with different phytochemical content, on development of NAFLD and atherosclerosis. Methods: ApoE*3-Leiden mice were fed an atherogenic diet (CON; 15% cocoa butter, 1% cholesterol) or the same atherogenic diet with 9% of cocoa butter replaced by 9% refined pumpkin oil (REF) or 9% virgin pumpkin oil (VIR) for 20 weeks to investigate effects on cardiometabolic risk factors dyslipidemia and inflammation as well as development of NAFLD and atherosclerosis. Results: Both pumpkin oils improved dyslipidemia, with decreased plasma cholesterol and triglyceride levels in comparison with CON. VIR had additional cholesterol-lowering effects compared with REF. While systemic inflammation marker SAA was not affected by REF, plasma SAA levels were significantly reduced in VIR after 12 and 20 weeks of dietary intervention. Vascular inflammation marker sVCAM-1 was not affected by either treatment. Development of NAFLD and atherosclerosis was less pronounced in REF compared with CON, and VIR significantly affected disease end-points; decreasing hepatic steatosis and hepatic inflammation as well as atherosclerotic lesion area and lesion severity. Conclusion: Both virgin and refined pumpkin oil beneficially affect cardiometabolic risk factors and disease development, with additional beneficial effects of virgin oil compared with refined oil. These results indicate that fatty acid composition and other minor components found in virgin oil contribute to observed health-promoting effects.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Increased lipid peroxidation has been identified as one of the key mechanisms for the
development of atherosclerosis and coronary artery disease (CAD). The 8-isoprostanes are
prostaglandin analogues derived from peroxidation of arachidonic acid and seem to be a reliable
marker of oxygen modification of lipids. Paraoxonase 1 (PON-1) is a hydrolytic enzyme associated
with high density lipoprotein which shows capability to protect against lipid oxidation.
The aim of the presented study was to investigate the possible relation between serum PON-1
activity, plasma 8-isoprostane concentration and the extent of angiographically proven CAD.
Blood specimens were collected from 116 patients with angiographically documented CAD of
several degrees (CAD group) and from 45 patients with no angiographically proven coronary
artery lesions (control group). The severity of arterial lesion were evaluated by the Gensini scoring
system. PON-1 activity towards paraoxon was measured in serum by spectrophotometric method
and plasma 8-isoprostane (8-iso-PGF2α) concentration was determined by ELISA.
CAD patients had increased 8-iso-PGF2α concentration and decreased PON-1 activity compared
to the control group (Table 1). There were significant correlations between the severity of CAD
assessed by Gensini score and both, PON-1 activity (R=-0.22; P<0.05) and 8-iso-PGF2α
concentration (R=0.29; P<0.05). However there were no significant correlations between PON-1
activity and 8-iso-PGF2α level neither in CAD nor in the control groups.
Analyzing the impact of atherogenic risk factors on the PON-1 activity and 8-iso-PGF2α
concentration we have observed the significant difference in 8-iso-PGF2α concentrations in CAD
group between patients with metabolic syndrome relative to those without metabolic syndrome
and between patients with and without hypertension. PON-1 activity was lower in CAD patients
with diabetes compared to normoglycemic patients and in smoking patients compared to
nonsmoking patients (Table 2).
The results of our study suggest that the impaired PON-1 activity and elevated 8-iso-PGF2α
concentration are associated with the presence and the extent of coronary stenosis and may be
considered as additional markers of CAD development and potential pharmacological strategy for reducing and preventing the progression of atherosclerosis.

Concentration of 8-iso-PGF2α and PON-1 activity in patients with coronary artery disease (CAD) and in control group.

<table>
<thead>
<tr>
<th></th>
<th>8-iso-PGF2α [pg/mL] mean ± SD</th>
<th>Unpaired t-test</th>
<th>PON-1 [U/L] median (25th-75th)</th>
<th>Mann-Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD group</td>
<td>122±72</td>
<td>P&lt;0.05</td>
<td>123 (91-197)</td>
<td>209 (111-281)</td>
</tr>
<tr>
<td>Control group</td>
<td>108±42</td>
<td></td>
<td></td>
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</tbody>
</table>

Concentration of 8-iso-PGF2α and PON-1 activity in CAD patients with or without risk factors for atherosclerosis.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Cystatin C and cardiovascular disease: a Mendelian randomization study

Abstract nr. 574
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Co-author(s) - Pasterkamp, Gerard
Co-author(s) - Ärnlöv, Johan
Co-author(s) - Holmes, Michael V.
Co-author(s) - Asselbergs, Folkert W.
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Genetics, Risk Factor, Risk stratification

Observational studies show a strong dose-response association between circulating cystatin C (encoded by CST3) and incident coronary heart disease (CHD), independent of traditional risk factors and renal function. This supports the hypothesis that circulating cystatin C could represent a causal factor for CHD. However, residual confounding and reverse causality could be alternative explanations that are difficult to tease from observational studies. We sought to investigate the causal role of cystatin C in CHD development by conducting a Mendelian randomization (MR) analysis using a common variant in the CST3 locus.

We incorporated data from population-based prospective studies (17 cohorts with 77,501 individuals) with 37,808 measures of cystatin C and added genetic association data from 28 additional datasets (n=123,766) from the CARDIoGRAM, METASTROKE, and CHARGE consortia to yield a total combined sample size of 201,267 individuals including 41,229 CHD events. We used a common variant (rs911119) in CST3 as a genetic instrument for cystatin C to investigate its causal role in CHD.

Cystatin C associated with risk of CHD in an observational analysis adjusted for age and sex (odds ratio [OR] 2.20; 95% confidence interval [CI]: 1.90, 2.57 per doubling of cystatin C concentration; \( p = 8.87 \times 10^{-31} \)); additional adjustment for confounders (smoking, HDL-cholesterol, BMI, CKD-EPI, and systolic blood pressure) diminished the association (OR 1.60; 95%CI 1.34, 1.96 per doubling of cystatin C concentration; \( p = 9.09 \times 10^{-7} \)). Rs911119 was associated with a decrease on circulating cystatin C levels (5.94% per minor allele; 95%CI 5.51, 6.36; \( p = 4.49 \times 10^{-149} \)), explaining 2.8% of the phenotypic variation. However, the variant did not show significant association with risk of CHD (OR 1.01 (95%CI 0.99, 1.03; \( p = 0.41 \)). Using Mendelian randomization, no causal effect of cystatin C on CHD risk was identified (OR 1.10 per doubling cystatin-C; 95%CI 0.86, 1.41, \( p = 0.459 \)), even though we had >90% power to detect. A causal effect for cystatin C was not identified for any other cardiovascular outcome.

We identified no causal relationship between cystatin C and CVD outcomes. Therapeutics aimed at lowering cystatin-C should not be prioritized for randomized clinical trials of CHD prevention.
Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Enhancement of anti-inflammatory activities of reconstituted HDL by phosphatidylserine in vivo and in vitro

Abstract nr. 575
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords HDL, Inflammation, Lipoproteins, Therapy

Aims: Atherosclerosis is a chronic inflammatory disease of the arterial intima. Plasma high-density lipoprotein (HDL) exerts multiple atheroprotective activities, which include anti-inflammatory actions. Both protein and lipid moieties of HDL contribute to its capacity to inhibit inflammatory response; negatively-charged phospholipids, primarily phosphatidylserine (PS), possess potent anti-inflammatory properties. We evaluated the capacity of PS-enhanced reconstituted HDL (rHDL) to inhibit inflammatory response in vivo and in vitro.

Methods: Anti-inflammatory activities of rHDL containing human apolipoprotein (apo) A-I and synthetic phosphatidylcholine 16:0/18:1 (PC), or apoA-I, PC and synthetic PS 16:0/18:1, were evaluated in LDL-receptor knockout mice that were fed a high-fat diet and received three weekly retro-orbital injections of rHDL (40 mg apoA-I/kg), or vehicle, and in human THP-1 macrophages incubated with rHDL and activated with endotoxin. Concentrations of inflammatory biomarkers were determined in plasma and cell medium by Multiplex technology.

Results: Treatment with PS-enhanced rHDL significantly reduced plasma levels of interleukin (IL)-6, a key biomarker of systemic inflammation in mice (-32% relative to PC-apoA-I rHDL); similar effects were observed for plasma levels of IL-1β, IL-12 p40 and tumor necrosis factor-α. Enhancement of PC-apoA-I-rHDL particles by PS also improved their anti-inflammatory properties in vitro. Notably, PS-PC-apoA-I rHDL inhibited endotoxin-induced inflammatory response in THP-1 macrophages significantly stronger as compared to PC-apoA-I rHDL.

Conclusions: PS enhances the anti-inflammatory activities of rHDL both in vivo and in vitro. PS-enhanced rHDL bears a potential to reduce inflammation in rupture-prone, lipid-rich atherosclerotic plaques in man.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Proteomic profile of HDL in relation to environmental pollutants

Abstract nr. 576
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins,HDL,Lipoproteins

Persistent organic pollutants (including PCBs, organophosphate pesticides and brominated flame retardants) are lipophilic environmental toxins that are widespread in the environment. We have previously shown increased levels of POPs in high-density lipoproteins (HDL) from subjects with cardiovascular disease (CVD) and that POP levels in the circulation are negatively correlated to the activity of HDL-associated paraoxonase-1, an enzyme important for HDL antioxidant/-inflammatory properties. To further study the impact of POPs on HDL, plasma from these individuals were used for HDL proteome analyses.

HDL was isolated from plasma, by ultracentrifugation, from 17 individuals (7 healthy and 10 with CVD). Proteins in HDL were trypsin digested and tryptic peptides were isolated in a nanoflow liquid chromatography system and analyzed in LTQ Velos Orbitrap Pro mass spectrometer. Samples were run in triplicate, and spectra were analyzed with Maxquant software. Protein levels were quantified by using the software’s label free quantitation (LFQ) peptide intensity. The protein analysis identified 118 proteins in HDL. Multivariate linear regression, adjusted for age, showed that increased POP-levels in HDL were associated with increased levels of apolipoproteins E (apoE), cholesteryl ester transfer protein (CETP), Integrin Beta-1 (ITGB1) and phospholipid transfer protein (PLTP). In addition, an association between increased POP-level in HDL and decreased plasma triglycerides was also found while there were no significant differences in proteomic profile or plasma lipid values in the individuals with medicated CVD compared to healthy controls.
ApoE, CETP and PLTP are important proteins in HDL metabolism, while the role of the extracellular matrix receptor ITGB1 in HDL is unknown. Alterations found in the present study indicate that POP-load in HDL affect the proteomic profile and may have impact on HDL functionality.

Subdivision 2. Translational Research

Presentation Preference Mini-oral presentation
Additional information
Abstract nr. 577
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Co-author(s) - Pokrovsky, Sergei
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, HDL, LDL, Lipoproteins

Aim. To investigate the relationship between the content of HDL and LDL subfractions and the diseases of main coronary arteries.

Methods. The study included 120 men, 102 patients (study group) was verified coronary atherosclerosis, the control group consisted of 18 patients with intact coronary arteries. Lipid profiles and lipoprotein subfractions were determined with «Biocon» kits (Germany) and “Lipoprint system ® Quantimetrix», (USA). Oxidized LDL concentration was determined with «Cayman» kits (USA).

Results. Study group patients were older (59±9 and 5±10 years, respectively, p=0.01) and had higher levels of triglycerides (TG) than patients in the control group (1.13±0.48 and 1.54±0.86 years, respectively, p=0.007). Other classic risk factors and lipids level were comparable in the both groups. In the control group, the average concentration of small dense LDL (sdLDL) was lower than that in patients with coronary atherosclerosis (1.16±2.17 and 2.53±5.80 mg/dl respectively, p=0.08). According to the regression analysis, the presence of coronary lesions was significantly positively associated with age (0.18, p=0.05), TG (0.22, p <0.05) and inversely associated with the concentration of the intermediate subfractions of high density lipoproteins (HDL4-HDL7, -0.31, p <0.05). With the amount of coronary lesions were positively associated concentrations of oxidized LDL (0.21, p <0.05), small, dense LDL-3 (sdLDL) (0.19, p = 0.05) and inversely correlated concentrations of intermediate HDL subfractions (HDL6 -0.23, p <0.05, HDL7 -0.27, p <0.05) and intermediate-density lipoproteins class A (-0.31, p <0.05).

Conclusions. Concentrations of intermediate HDL subfractions (HDL4-HDL7) inversely associated with both the presence and with the amount of diseased coronary arteries in adult males. Level sdLDL associated only with the presence of atherosclerosis.

Funding. Supported by Moscow State Government Grant № 8/3-280н-10. Transparency declarations. All authors: no conflicts of interest to declare.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Carboxylated and undercarboxylated osteocalcin as the marker of metabolic complications of human obesity.

Abstract nr. 578
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Metabolism, Obesity

Carboxylated osteocalcin (Gla-OC) participates in bone remodeling, whereas the undercarboxylated form (Glu-OC) takes part in energy metabolism. This study was undertaken to compare the blood level of Glu-OC and Gla-OC in non-obese, healthy obese as well as prediabetic volunteers and correlate it with the insulin resistance and inflammation markers. Non-obese (BMI < 30kg/m², n=34) and obese subjects (>30 BMI<40 kg/m², n=98), aged 25-65 yrs were included in the study. The non-obese control, healthy obese and obese with biochemical markers of prediabetes patients, as well as the subgroups with obesity and low or high Gla-OC or Glu-OC were considered for analysis. Venous blood was sampled for determination of Gla-OC, Glu-OC, lipid profile, selected parameters of inflammation (hsCRP, IL-6, sE-selectin, sPECAM-1, MCP-1) and adipokines: leptin, adiponectin, visfatin and resistin. Blood was collected during oral glucose tolerance test (2h OGTT) and oral lipid tolerance test (8h OLTT) for measurement of glucose, insulin, FFA, TG and glucose-dependent insulinotropic polypeptide (GIP). Insulin resistance was estimated by HOMA-IR index and using calculated an oral glucose insulin sensitivity index. In the whole group of obese patients the level of Gla-OC was lower comparing to non-obese subjects. The serum level of Gla-OC inversely correlated with hsCRP, visfatin, BMI. In turn Glu-OC inversely correlated with fasting insulin level and HOMA IR index. In comparison to healthy obese volunteers, prediabetic subjects presented reduced Glu-OC level. Blood glucose, insulin and GIP level as well as HOMA-IR index were higher in this group pointing to decreased insulin sensitivity.

Our results argue for the suggestion, that in obesity, decreased blood concentration of Glu-OC may be early marker of insulin resistance, whereas the lower Gla-OC level could be associated with the appearance of early markers of inflammation.

Supported by: EU FP7 BIOCLAIMS, Grant agreement no. 244995 grant K/ZDS/004498 and NCN grant no. K/PBN/000001.
Presentation Preference: Electronic poster presentation
Additional information
Dietary Ellagitannin Metabolites Exert Antiatherosclerotic Effects in Vitro

Abstract nr. 579
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Nutrition

Reduction of the prevalence of atherosclerotic cardiovascular disease through the consumption of polyphenol-enriched diets has been established in several epidemiological studies. Ellagitannins are polyphenols mainly found in berries, walnuts and pomegranate. These compounds are poorly absorbed after oral ingestion and are metabolized by the colonic microbiota to form a series of catabolites called urolithins. We hypothesized that these circulating metabolites may be, at least in part, the molecules responsible for the beneficial effects against atherosclerosis attributed to ellagitannin-containing foods. In the present study we investigated the activity of urolithin A,B,C,D and B glucoronide in vitro on processes involved in atherogenesis.

Cell cholesterol uptake was quantified by a fluorimetric assay in THP-1 macrophages, incubated with urolithins 1-10µM in presence of human serum or acetylated LDL as cholesterol source. Cholesterol efflux was evaluated by a radioisotope-based assay in THP-1 macrophages exposed to urolithins 10µM and human HDL as cholesterol acceptors. Monocyte adhesion to endothelial cells was investigated in human umbilical vein endothelial cells (HUVECs) treated with urolithins 1-10µM for different times, stimulated with TNF-α, and exposed to THP-1 monocytes. Monocyte adhesion to extracellular matrix was performed in THP-1 monocytes treated with urolithins 10µM and successively transferred to fibronectin-coated wells. In these assays, adherent cells were quantified by a fluorimetric or spectrophotometric count respectively.

Urolithins may retard the formation of atherosclerotic plaque by inhibiting monocyte adhesion to extracellular matrix and to HUVECs and by contrasting macrophage foam cell formation. The present study identifies the key players and describes the mode of action through which the
ellagitannin-containing foods elicit their protecting effects against cardiovascular diseases.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Mini-oral presentation
Additional information
Proteoglycan 4 deficiency alters macrophage function and increases leukocyte counts leading to increased atherosclerosis

Abstract nr. 580
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Pathogenesis

A genetic variant in proteoglycan 4 (PRG4) was recently identified in a pedigree with premature atherosclerosis. We have previously shown that PRG4 is expressed both in early, macrophage-rich and more advanced atherosclerotic lesions. However, the function of PRG4 in atherosclerosis is unknown. The current study aims to elucidate the effect of PRG4 deficiency on macrophage function and atherosclerotic lesion development.

The effect of PRG4 deficiency on macrophage function was determined ex vivo. The overall cytokine gene expression profile of LPS-stimulated peritoneal macrophages suggests a less pro-inflammatory phenotype of PRG4 knockout (KO) macrophages as compared to wild-type (WT). In support of this hypothesis, the production of the pro-inflammatory cytokines TNFα (-31%, p<0.01) and MCP1 (-37%, p<0.01) was reduced in PRG4 KO peritoneal macrophages compared to WT macrophages. Moreover, cholesterol efflux to ApoAI (-9%, p<0.05) and HDL (-15%, p<0.001) was reduced in PRG4 KO macrophages. Combined, these findings suggest an overall change in macrophage function in PRG4 KO mice.

To investigate the effect of specific deletion of PRG4 in bone marrow-derived cells, a bone marrow transplantation (BMT) was performed in hyperlipidemic LDL receptor KO mice. The reduction in macrophage cytokine production was preserved in PRG4 KO bone marrow transplanted mice compared to the WT transplanted control group. White blood cell counts tended to be higher (+31%, p=0.11). This could be explained by a 1.5-fold increase in lymphocyte counts (p<0.05) and 2-fold higher monocyte counts (p<0.01) in PRG4 KO transplanted mice compared to WT control transplanted animals. Importantly, the atherosclerotic lesion area was 1.4-fold (p<0.05) increased in PRG4 KO bone marrow transplanted mice as compared to WT transplanted controls after five weeks of Western-type diet feeding. In line with a more advanced atherosclerotic lesion type in PRG4 KO transplanted animals, the percentage of collagen in the lesions was significantly increased (2.7%±0.4 vs 4.4%±0.7, p<0.05).

In conclusion, PRG4 deficiency alters macrophage function and increases leukocyte counts, leading to an increased susceptibility to atherosclerosis in LDL receptor KO mice. Our data suggest that PRG4 might be a potentially interesting novel target for the treatment of
cardiovascular disease.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Dyslipidaemia progresses in parallel with increasing glucose intolerance in Caucasians and South Asians

Abstract nr. 581
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Co-author(s) - Mulder, Monique
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes, Dyslipidemia, HDL, LDL

Aim: We questioned whether the progression of dyslipidaemia in South Asians with increasing glucose intolerance is similar to that in Caucasians.

Introduction: The development of dyslipidaemia in parallel with increasing glucose intolerance and prior to the onset of type 2 diabetes (T2D), have been reported previously. Several studies have shown that South Asians have dyslipidemia characteristics similar to that in T2D patients, even when they are healthy and normoglycaemic. Therefore we assessed the lipoprotein profiles in subjects from both ethnicities with different degrees of glucose intolerance, in association with insulin sensitivity and β-cell function.

Materials and Methods: In a cross-over study design T2D patients and healthy family members were included, in total 42 Caucasians and 34 South Asians. The study participants underwent a 75-gram oral glucose tolerance test (OGTT) and were divided into 3 groups: normal glucose tolerance (NGT), impaired fasting glucose/impaired glucose tolerance (IFG/IGT), and T2D. Lipoprotein subclass levels were assessed using density-gradient ultracentrifugation. Insulin sensitivity was calculated with Matsuda Insulin Sensitivity Index (ISI) and β-cell function with Disposition Index (DI).

Results: The HDL₃-C/HDL₂-C ratio was proportionate to the degree of glucose intolerance in Caucasians (p=0.047) and in South Asians (p=0.043). Although the dense LDL-C/buoyant LDL-C ratio was proportionate to the degree of glucose intolerance in Caucasians, this was not significant (p=0.062). Dense LDL-C levels were higher in NGT South Asians than in NGT Caucasians (p=0.002). The most striking difference between Caucasians and South Asians was the VLDL-TG levels, as they were higher in South Asians than in Caucasians (p=0.027). In South Asians, VLDL-TG correlated negatively with DI (p=0.04), but in Caucasians there was a trend towards a negative correlation with ISI (p=0.07). All results were adjusted for age, sex and family ties.

Conclusions: South Asians already showed a less favourable lipoprotein profile when
normoglycaemic. The progression of their dyslipidaemia was proportionate to the degree of glucose intolerance, as observed in Caucasians; most likely by different pathways.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Cerebrovascular reactivity and the risk of dementia in apolipoprotein E e4 carriers: a population-based study

Abstract nr. 582
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Apolipoproteins, Cardiovascular Disease, Elderly, Epidemiology

**Background:** Cerebrovascular reactivity (CVR) is a key factor in maintaining continuous cerebral blood flow. Impaired CVR is associated with an increased risk of stroke and mortality, and a few small clinical studies have reported lower CVR in patients with dementia compared to healthy controls. In addition, CVR may be impaired at an early age in carriers of the apolipoprotein E ε4 (APOE4) allele, predisposing for dementia. Yet, the relation between CVR and risk of dementia, particularly on a population level, is uncertain.

**Methods:** We assessed CVR in all non-demented participants undergoing transcranial Doppler investigation with 5%CO₂ induced hypercapnia between 1997 and 1999, as part of the ongoing population-based Rotterdam Study. Follow-up was complete till 1st January 2011. We determined the risk of developing dementia in relation to CVR, calculating hazard ratios with 95% confidence intervals (HR, 95%CI), adjusted for age, sex, cardiovascular risk factors and carotid intima-media thickness, and stratified for APOE4 carrier status. We further assessed associations independent of stroke, and for Alzheimer’s dementia only. Finally, we assessed decline in scores (G-factor) on a cognitive assessment battery in relation to CVR, using linear mixed models.

**Results:** Among 1723 participants (mean age 70.7±6.3 years, 54.4% male) with a mean follow-up of 9.5 years, 162 were diagnosed with dementia, of whom 118 were classified Alzheimer’s disease. Higher CVR at baseline was associated with lower risk of dementia in carriers of the APOE4 allele (adjusted HR [95%CI] per SD increase: 0.76 [0.59-0.99]), but not in non-carriers (aHR 1.02 [0.82-1.25]). The association in APOE4 carriers became slightly stronger after excluding prevalent stroke and censoring for incident stroke (aHR 0.74 [0.56-0.97]), and was similar for Alzheimer’s dementia only. During three repeated cognitive assessments over an 11.0 year follow-up in non-demented participants, APOE4 carriers with higher baseline CVR showed less decline in test scores (p=0.050), whereas no such association was present for non-carriers (p=0.78).

**Conclusion:** Cerebrovascular reactivity is associated with cognitive decline and the risk of dementia in carriers of the APOE4 allele in the general population. The role of microvascular impairment in the etiology of dementia warrants further investigation.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information

Figure 1. Smoothed Kaplan-Meier curves for dementia-free survival in relation to baseline cerebrovascular reactivity, for APOE4 carriers (left) and non-carriers (right).
The dynamics of lipoprotein and glucose concentration after coleveselam treatment

Abstract nr. 583
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Hypolipidemic Drugs, Lipoproteins

Bile acid sequestrants were the first drugs shown to lower cholesterol and cardiovascular mortality and morbidity. However, they are not frequently used in modern hypolipidemic treatment due to their lower tolerability and higher statin efficiency. Interestingly, some evidence has been accumulated that sequestrants may have a positive effect on insulin resistance. Therefore, we analyzed changes of lipid and glucose concentration during the treatment with colesevelam.

Sixteen healthy male volunteers (age 42 ± 11 years, BMI 24.7 ± 2.5 kg/m², cholesterol 5.3 ± 1.2 mmol/l, LDL-C 3.3 ± 1.0 mmol/l, HDL-C 1.4 ± 0.3 mmol/l, TG 1.2 ± 0.5 mmol/l, glucose 5.4 ± 0.4 mmol/l) were treated with colesevelam (3.75 g/day) for 4 weeks. The blood for determination of cholesterol, triglyceride (TG), VLDL, IDL, LDL, and HDL, nonesterified fatty acids (NEFA), glucose, insulin, GLP-1 and other parameters was taken before treatment and on days 1, 3, 7, 14 and 28 on treatment. The changes were evaluated using ANOVA for repeated measures.

The total cholesterol dropped 8% after 7 days of treatment and did not change further (p < 0.0001). Such a decrease is due to 17% decrease in LDL-C concentration (p < 0.0001). The treatment had no statistically significant effect on HDL-C, NEFA and triglyceridemia although sporadic jumps in TG concentration to more than double of baseline were observed in 5 subjects on 6 occasions. Importantly, glycemia dropped 5% on day 3 and slowly returned to the baseline (p < 0.01).

Our data confirm positive effect of colesevelam treatment on lipid metabolism as well as glucoregulation.

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COMMENT THAT SHOULD NOT BE PUBLISHED.
The analysis of insulin and GLP-1 will be finished within a month and the data will be presented at the congress.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Flow Modulates the Endothelial Expression of Neuronal Guidance Cues

Abstract nr. 584
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Endothelium,Immunity

Disturbed hemodynamic conditions lead to impaired homeostasis of the vascular endothelium and, subsequently, influx and retention of monocytes in the arterial wall. Emerging data suggest that, aside from a role in the development and maintenance of the nervous system, neuronal guidance cues (NGCs) are also required for physiological and pathological immune responses. We recently reported that NGCs are differentially expressed in atherosclerosis-prone and -resistant aortic sites in LDLR⁻/⁻ mice during early atherogenesis. Here, we detail our investigation of the role of shear stress on the expression levels of NGCs (ephrins, slits, netrins and semaphorins) in human umbilical vein endothelial cells (ECs) under atherosclerosis-prone (static) and -protective (laminar flow) conditions.

To gain insight into the consequences of laminar flow on the mRNA expression levels of NGCs in ECs, the cells were exposed to 0, 1 or 7 days of laminar flow (shear stress 10 dynes/cm², IBIDI-flow system). The mRNA levels of the leukocyte attractant NGCs EphrinA1, B1 and B2 were all reduced by laminar flow, in particular for EphrinB2 (52%− , p=0.02). In contrast, the potentially anti-inflammatory Slit2 displayed a striking early response to flow, with a marked increase in mRNA expression after 1 day (2.2-fold , p=0.02), but was not sustained at Day 7. Interestingly, the expression levels of netrin-4, semaphorin 3F and semaphorin 4B were upregulated in ECs exposed to laminar flow for 1 and 7 days (2.1-fold, p=0.05; 3.0-fold, p=0.04; 2.1-fold, p=0.05; respectively), suggesting these NGCs as potentially athero-protective anti-inflammatory leukocyte repellent. Moreover, overexpression of the shear-induced transcription factor Krüppel-like factor 2 (KLF2) in static cultured ECs induced the expression of both semaphorin3F mRNA and protein (3.8 fold and 3.9 fold, respectively), while netrin-4 mRNA and protein upregulation appeared to be independent of KLF2.

Taken together, our study provides novel insight into regulation of the endothelial NGC expression profile by flow, which could implicate NGCs as mediators of the EC function by facilitating repulsion or attraction of monocytes under varying hemodynamic conditions.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
PERIPHERAL ARTERIAL DISEASE IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

Abstract nr. 585
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease,Familial Hypercholesteroleemia

Background: Familial hypercholesterolemia (FH) is characterized by elevated plasma levels of total and low-density lipoprotein cholesterol and is associated with early coronary arterial disease (CAD) onset. However, few studies investigated the association between FH and peripheral arterial disease (PAD).

Methods: We consecutively enrolled 202 FH patients (91% with positive genetic test) between 2009 and 2013 that were compared with 524 normolipidemic controls from a historical cohort. PAD was diagnosed by ankle-brachial index (ABI) values ≤0.90. The association between FH diagnosis and PAD was tested by adjusted logistic regression analysis.

Results: Compared with normolipidemic controls, FH patients were older (51±14 vs 44±13 years, p=<0.001), more often female (64.9 vs 50.6%, p=0.001), with higher rates of hypertension (49.5 vs 30.5%, p=<0.001), diabetes (17.3 vs 5%, p=<0.001), CAD (28.2 vs 6.3%, p=<0.001) and higher total cholesterol levels (336±87 vs 181.7±26, p=<0.001). Smoking was more common among normolipemic controls (23.5 vs 14.9%, p=0.002). The prevalence of PAD was 17.3 and 2.3 % respectively in FH and controls (p < 0.001). Regression analyses demonstrated that age (OR = 1.03 CI 95% 1.00-1.05, p=0.033), CAD (OR = 3.12 CI 95% 1.56-6.25, p=0.001) and FH diagnosis (OR = 5.55 CI 95% 2.69-11.44, p=<0.001) were associated with PAD.

Conclusions: FH was associated with almost six times greater chance of PAD in comparison with normolipidemics. Screening for PAD should be a routine clinical evaluation for these individuals.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Effect of Very Low Calorie diet on Lipoprotein(a) levels in patients with Type 2 diabetes

Abstract nr. 586
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes, Lifestyle, Lp(a), Obesity

**Aim:** The aim of this study was to investigate the effect of diet-induced weight reduction on plasma Lipoprotein(a) (Lp(a)) levels in patients with type 2 diabetes (T2D).

**Introduction:** T2D is a worldwide growing healthcare problem. Patients with T2D have an increased lifetime risk for developing cardiovascular disease (CVD). This increased risk is not only due to the diabetes itself, but also due to other risk factors. Such as obesity, high blood pressure and an atherogenic lipoprotein profile. Another independent CVD risk factor is high Lp(a) plasma levels. However, the prevalence of T2D seems to be higher in patients with low Lp(a) levels. We examined the effect of diet-induced weight loss on plasma Lp(a) levels in patients with T2D.

**Methods:** Between 2010 and 2013, 207 patients with T2D and a BMI>27 kg/m² started with a very low calorie diet (VLCD) (750 kcal/day for 8 weeks) followed by a low calorie diet (LCD) (900-1300 kcal/day for 12 weeks). Fasting blood of 132 patients was collected before and after the intervention (20 weeks) and plasma was stored at -80°C. All measurements were performed in one run, and a kringle size independent assay was used to measure the Lp(a) plasma levels and currently Lp(a) kringle size is being determined.

**Results:** After the diet patients lost weight (mean -10%, SD 5%), and Lp(a) increased significantly in the total group. We divided the patients into 3 groups according to their delta Lp(a): 37% increased, 5% decreased and 58% unchanged. The Lp(a) change in the total group was not correlated with weight change. However, in the group with increased Lp(a), Lp(a) was negatively correlated with weight change (r²=0.13, p=0.017). No other metabolic parameters correlated with Lp(a) change.

**Conclusion:** These data show that weight loss is associated with an increase in plasma Lp(a) levels in a subset of T2D patients. The differences in Lp(a) change between the patients could not be explained by changes in metabolic parameters. However, we hypothesize that the Lp(a) kringle size might be associated with the effect of diet on Lp(a).
Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Atherosclerosis is considered a chronic inflammatory disease. Activation of the vagus nerve can inhibit inflammatory responses via α7 nicotinic acetylcholine receptors (nAChR), a process known as the Cholinergic Anti-inflammatory Pathway. We hypothesize that electrical stimulation of the vagal nerve will provide a systemic suppression of inflammation and therefore will counteract atherosclerotic development.

To study effects of intermittent, long term vagus nerve stimulation in experimental atherosclerosis models the availability of a small implantable device is essential.

First, we electrically stimulated the cervical vagal nerve of a rat (30sec, 1V, 10Hz, 0.5ms). Ten minutes after stimulation, blood was drawn. Immune responsiveness was studied by assessing cytokine release from whole blood upon LPS stimulation. Next, we defined the features for an implantable device. The device must be small enough for use in mice and cause minimal inconvenience. Tissue response to the implanted device should be minimal and the device must have a working capacity of at least 5 months. Dummy stimulators were implanted in mice to study the acceptance by the body and the level of inconvenience in the animal.

Results: Tumor necrosis factor alpha release in LPS stimulated blood, was dramatically decreased upon electrical vagal stimulation. A similar decrease was observed for the pro inflammatory cytokines IL-1β, IL-6 and IL-8. This anti-inflammatory effect upon a single stimulation persisted up to 72 hours. The first implanted dummy stimulators did not result in any obvious adverse effects and nicely stayed in position for 5 months without any visible inconvenience.

In our laboratory we developed a small vagus stimulator for mice (fig 1), which is currently at the final stage of development. Experiments to investigate durable functioning are being executed. A fully functional device will be available at the end of 2015.

We expect the implantable Vagus Nerve Stimulator for mice to be of great use in future inflammation related studies and specifically in atherosclerosis research.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
APOA-I DELETION IN APOE-KO MICE PROMOTES MASSIVE CHOLESTEROL ACCUMULATION AND INFLAMMATION IN SKIN AND SKIN-DRAINING LYMPH NODES

Abstract nr. 588
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Dyslipidemia, HDL, Inflammation

Background. Previous experimental findings demonstrate that diet-induced hypercholesterolemia, in atherosclerosis-prone mice lacking apoA-I, leads to a massive accumulation of cholesterol in skin and skin-draining lymph nodes.

Aim. To determine how apoA-I could affect skin and skin-draining lymph nodes morphology and composition of athero-prone mice in normocholesterolemic conditions.

Methods. C57Bl/6 (WT), apoE-KO (EKO), apoA-I-deficient/apoE-KO (dKO) or dKO overexpressing human apoA-I (hA-I) mice were fed a chow diet for 30 weeks. Plasma cholesterol concentration and distribution among lipoproteins were evaluated. Skin biopsies were processed for light microscopy and transmission electron microscopy. Histology and lipid deposition in axillary/inguinal skin-draining lymph nodes were also characterized.

Results. Plasma total cholesterol concentration in dKO mice was comparable with that of WT mice and 3-fold lower than the concentration observed in EKO and hA-I mice. Cholesterol in WT mice was almost exclusively confined to the HDL fraction whereas in EKO mice an elevated cholesterol accumulation in VLDL/LDL and low HDL levels were observed. In dKO mice, HDL cholesterol was absent and cholesterol accumulation in the VLDL/LDL fractions was lower than that found in EKO mice. hA-I mice were characterized by a less prominent presence of VLDL/LDL, but a larger HDL cholesterol peak than that found in EKO mice.

Skin morphology of EKO and hA-I mice was comparable with that of WT mice. Conversely, dKO mouse skin was characterized by increased dermal thickness, accumulation of foam cells and lymphocytes. Additionally, electron microscopy highlighted the presence of cholesterol crystals both in the extracellular milieu and within foamy macrophages.

dKO mice also had enlarged axillary and inguinal skin-draining lymph nodes, characterized by the
accumulation of foamy macrophages, increased cholesterol and lipid deposition, together with the presence of cholesterol crystals surrounded by granulomatous reactions, and dilated sinuses. Conclusions. Our study demonstrates that HDL/apoA-I deficiency itself, in the absence of hyperlipidemia, is sufficient to induce an aberrant accumulation of cholesterol with a concomitant infiltration of foamy macrophages and lymphocytes in the skin and in axillary/inguinal skin-draining lymph nodes.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Dietary sphingomyelin supplementation reduces aortic lesion development in chow-fed apoE-/− mice

Abstract nr. 589
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Animal model, Atherosclerosis, Metabolism, Nutrition

Background: Sphingomyelin (SM) is a ubiquitous component of the diet and has been shown to attenuate diet-induced hepatic steatosis in mice. However, accumulating evidence suggested that circulating SM may be atherogenic in humans, with an elevated plasma SM level being an independent risk factor for cardiovascular diseases. It is unclear if dietary SM increases circulating SM levels and cardiovascular risk. A recent report has indicated that the choline headgroup of dietary phosphatidylcholine is metabolized by gut flora to trimethylamine N-oxide (TMAO), which is pro-atherogenic in mice and increases cardiovascular disease risk in humans. As SM also contains a choline headgroup, this study asks if dietary SM can increase circulating TMAO levels and atherosclerotic lesion development in mice.

Methods: Study 1: Four-week old male C57BL/6 mice were maintained on a high-fat (HF) diet without or with 1.2% (wt/wt) SM for 4 weeks. Serum TMAO and SM levels were quantified. Study 2: Five-week old female apoE−/− mice were maintained on a HF diet without or with 1.2% (wt/wt) SM for 16 weeks prior to assessing aortic lesion area. Study 3: Five-week old female apoE−/− mice were maintained on a chow diet without or with 1.2% (wt/wt) SM for 19 weeks. Serum TMAO and SM levels and aortic lesion area were quantified.

Results: Serum TMAO levels in Studies 1 and 3 were too low to impact on atherosclerotic lesion development. Dietary SM did not increase aortic lesion area in the SM-supplemented apoE−/− mice (Control vs SM: 9.01±1.00% vs 7.75±1.1%) after 16 weeks of intervention. Dietary SM did not significantly elevate serum SM levels in either Study 2 (Control vs SM: 149.9±13.2mg/dL vs 151.5±4.8mg/dL) or Study 3 (Control vs SM: 46.1±2.7mg/dL vs 53.8±2.6mg/dL). However, there was a significant reduction in atherosclerotic lesion area after 19 weeks of SM supplementation in chow-fed apoE−/− mice (Control vs SM: 3.51±0.43% vs 1.67±0.21%, p<0.05). TMAO levels were not measured in study 2.

Conclusion: Sixteen and nineteen weeks of SM supplementation did not significantly elevate
serum SM levels in HF or chow-fed apoE⁻/⁻ mice. Nineteen weeks of dietary SM supplementation significantly reduced atherosclerotic lesion development in chow-fed apoE⁻/⁻ mice.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
MicroRNA biogenesis in macrophages regulates mitochondrial energy metabolism and increases atherosclerosis

Abstract nr. 591
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Inflammation, Metabolism

Macrophages are the main cell type in atherosclerotic lesions and can polarize into different functional phenotypes with pro- and anti-inflammatory roles. In contrast to inflammatory macrophages, the anti-inflammatory phenotype is mediated by increased mitochondrial respiration. We aim to determine the role of microRNA biogenesis in macrophage polarization and the effect on atherosclerosis.

In this study, the expression of the inflammatory genes Il1b and Nos2 was increased, and the markers of anti-inflammatory macrophages, Mrc1 and Fizz1, were down-regulated in bone marrow-derived macrophages (BMDMs) from myeloid cell-specific Dicer knockout mice (LysMCre-Dicer−/−). The expression of 137 miRNAs was reduced in LysMCre-Dicer−/− BMDMs determined by microRNA qPCR array. Argonaute2-RIP-Chip analysis revealed that Dicer deletion resulted in reduced enrichment of 97 up-regulated genes in LysMCre-Dicer−/− BMDMs with Argonaute2 protein that is required for RNA-induced silencing complexes. Among these genes, the highest number of predicted highly conserved binding sites of the down-regulated miRNAs was found in the 3'UTR of ligand-dependent co-repressor (Lcor). Lcor can bind to RXRs and may thus impair mitochondrial respiratory. Accordingly, the mitochondrial oxygen-consumption rate determined by extracellular flux analysis and the expression of genes involved in mitochondrial respiration and fatty acid oxidation were reduced in LysMCre-Dicer−/− BMDMs. Silencing Lcor in macrophages up-regulated genes involved in mitochondrial respiration. In vivo, atherosclerosis was enhanced in LysMCre-Dicer−/− Apoe−/− mice compared to LysMCre-Dicer+/+ Apoe−/− mice fed a high cholesterol diet for 12 weeks (n = 13-14, P < 0.05).

In conclusion, microRNA biogenesis promotes mitochondrial respiration and inhibits pro-inflammatory activation in macrophages and may thus limit the development of atherosclerosis.

Subdivision 1. Basic Science
Presentation Preference Oral presentation
Additional information
Dysregulation of Hepatic Cholesterol Biosynthesis in Diet-Induced Non-Alcoholic Fatty Liver Disease

Abstract nr. 592
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Metabolism, Nutrition, Obesity

**Background:** Chronic consumption of high-fat diets is associated with obesity and non-alcoholic fatty liver disease (NAFLD). Patients with NAFLD are at a high risk for cardiovascular disease and diabetes since NAFLD is closely associated with hyperlipidemia and hyperglycemia. Regulation of the activity of HMG-CoA reductase in the liver is crucial in maintaining cholesterol homeostasis in the liver as well as in the circulation. Despite considerable advances in our understanding of cholesterol metabolism, the regulation of liver cholesterol biosynthesis in response to high-fat diet feeding is not well understood. The aim of the present study was to investigate the mechanisms by which chronic high-fat diet consumption affected hepatic cholesterol biosynthesis and its impact on fatty liver and hyperlipidemia.

**Methods and Results:** Male C57BL/6 mice were fed either a control diet (10% kcal fat) or a high-fat diet (60% kcal fat) for 5 weeks. High-fat diet feeding resulted in an elevation of lipid levels (total cholesterol and triglyceride) in the serum. Mice fed a high-fat diet had an increased body weight and displayed NAFLD features including hepatic lipid accumulation, oxidative stress, inflammatory chemokine expression and hyperlipidemia. Hepatic cholesterol biosynthesis was increased due to activation of HMG-CoA reductase, a key enzyme for de novo cholesterol synthesis. During high-fat feeding, increased Sp1 mediated SREBP-2 activation up-regulated HMG-CoA reductase gene expression which, in turn, contributed to hepatic lipid accumulation and hypercholesterolemia.

**Conclusion:** The present study has identified a potential mechanism by which chronic consumption of a high-fat diet causes HMG-CoA reductase activation, hepatic lipid accumulation and hypercholesterolemia. Identification of molecular mechanisms that are responsible for the increased de novo cholesterol synthesis may lead to a better therapeutic approach to regulate lipid metabolism and to curtail NAFLD associated cardiovascular risk.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Abstract nr. 593
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords LDL

Aim: Familial hypercholesterolaemia (FH) is an inherited autosomal-dominant disorder resulting from defects in the low-density lipoprotein receptor (LDLR) gene, in the apolipoprotein B (ApoB-100) gene or in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene. Few mutations in ApoB-100 have been proved to cause disease, among them, p.Arg3527Gln was the first mutation being identified and characterized. Very recently two novel ApoB-100 mutations have been described: p.Arg1164Thr and p.Gln4494del which show impaired binding capacity to LDLR, and diminished LDL uptake. In this work we analyzed secondary structure and size of these ApoB-100 mutants within LDL particle with the aim of explaining their impaired binding abilities.

Methods: The secondary structure of the human LDL ApoB-100 has been investigated by infrared spectroscopy (IR). Changes in secondary structure of wt ApoB-100, p.Arg3527Gln, p.Arg1164Thr or p.Gln4494del have been analyzed and compared. Particle size of the LDL has been determined by dynamic light scattering (DLS) and electron microscopy.

Results: We have found changes both in particle size and in secondary structure composition of these LDL variants. We have determined that the content of the strands in p.Gln4494del mutant is 48 % lower than the observed in the wt. However, the secondary structure of p.Arg1164Thr is very similar than the observed for the wt. The mean LDL diameters of p.Arg1164Thr and p.Gln4494del -LDL are smaller compared to WT ApoB100-LDL.

Conclusions: The secondary structure of ApoB-100 and the LDL particle size are critical to maintain or stabilize the proper conformation of the ApoB-100 within the LDL particle for its correct binding to the LDLR. The alterations found both in particle size and in secondary structure in p.Arg1164Thr and p.Gln4494del variants may underlie the defective binding and uptake of these LDL.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Calcification associates with aortic distensibility in Marfan Syndrome mice.

Abstract nr. 594
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Cardiovascular Disease

Aim: De-differentiation of and calcification by smooth muscle cells in the aortic root of mice with aortic dilatation is often observed and related to enhanced transforming growth factor beta (TGFβ) signaling. Marfan patients are known to develop aortic root aneurysms and have enhanced TGFβ activation. We aim to study calcification in mouse and human Marfan aortic tissues and its potential participation in aortic dilatation.

Methods and Results: Calcification is observed in the aortic sinus and aorta ascendens of Marfan patients, as determined by alizarin red staining. Alcian blue staining revealed increased accumulation of glucosaminoglycans (GAGs) in Marfan patients compared to control. In human Marfan aorta, alcian blue staining is also used as marker for medial necrosis, since these GAGs tend to accumulate at sites of smooth muscle cell loss. Mostly GAGs are known to be present in bone, where they are visualized by alcian blue. Whether these GAGs contribute to aortic calcification/stiffness is still unclear. In a Fibrillin-1 mutation mouse model of Marfan (FBN1 C1039G/+), alcian blue staining revealed osteoblast-like cells surrounded by GAGs in the medial layer of the aortic sinus. In these mice, the percentage of mice with osteoblast-like cells increased with age, unlike in wild type mice. Ex vivo imaging of the Marfan aorta revealed increased (in vivo) incorporation of the fluorescent OsteoSense probe, specifically in the aortic sinus and ascending aorta. In the ascending aorta no osteoblast-like cells were observed, however, there is a strong positive correlation between Osteosense signal and aorta ascendens diameter. In addition, Osteosense signal negatively correlated with aorta ascendens distensibility.

Conclusions: Calcification is present in the aortic sinus and aorta ascendens of Marfan patients and Marfan mice. The degree of calcification negatively correlates with the aorta ascendens distensibility, suggesting that not only elastic lamina degradation (common in Marfan) may be responsible for increased aortic stiffness in Marfan, but also increased calcification processes.
funding: Interuniversity Cardiology Institute of the Netherlands

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
LncRNA Hand2-AS1, Hand2, and miR-138-5p form a regulation loop to participate in VSMC phenotypic switch

Abstract nr. 595
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease

Vascular smooth muscle cell (VSMC) phenotypic switch is a common pathological feature of atherosclerosis. Long non-coding RNAs (lncRNAs) have many important regulatory functions, but the functions in VSMC phenotypic switch are largely unkown. Here, we identified that Hand2 (heart and neural crest derivatives expressed 2) gene and lncRNA Hand2 antisense RNA 1 (Hand2-AS1) are co-expressed, and their expression levels are significant decreased in dedifferentiated VSMC by RNA-seq and qRT-PCR analysis. By using both gain-of-function and loss-of-function approaches, we found Hand2 promote VSMC phenotypic switch by regulating SM22a, a differentiated VSMC maker gene. Furthermore, we demonstrated that lncRNA Hand2-AS1 binds to the Hand2 gene promoter, and increases Hand2 expression at transcriptional level. MiR-138-5p inhibits Hand2 expression by targeting its 3'-untranslated region. LncRNA Hand2-AS1 is a competitive endogenous RNA, blocks miR-138-5p to targeting Hand2, and increases Hand2 expression at post-transcriptional level. In summary, these findings provide a novel mechanism that one lncRNA can regulate one target gene from both transcriptional and post-transcriptional level, our results indicate that lncRNA Hand2-AS1, Hand2, and miR-138-5p can form a regulation loop to participate in VSMC phenotypic switch.

Presentation Preference Mini-oral presentation
Additional information
Epidemiological profile of high blood pressure about 718 subjects in rural Cameroon.

Abstract nr. 596
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, Hypertension, Risk Factor

Acknowledgments: High blood pressure or hypertension occupies a prominent place with a worldwide prevalence estimated at 26% by the year 2008 and almost 25% in Cameroun.

Objective: To determine the epidemiology of hypertension and cardiovascular risk factors.

Methodology: A descriptive cross-sectional study conducted over two days as part of activities of the “Fondation Cœur et Vie” at Bangou (a western Cameroon village). Were included adults, regardless of gender and aged over than 18 years. Pregnant women were not included.

Results: A total of 718 subjects were recruited, sex ratio M / F was 0.33. The average age was 50.2 ± 17.1 years old. We obtained an overall prevalence of hypertension of 24.51% (176 cases). The average age among hypertensive subjects was 61.3 ± 11.3 years old. Among hypertensive, 56.80% were newly diagnosed. Only 25% of hypertensive patients were controlled. Depending on the level of severity, only 42.60% of hypertensive subjects had a hypertension of grade 2 and 17% had hypertension of grade 3. Abdominal obesity (74.4%) is the most common risk factor followed by physical inactivity (45.4 %). We found 6.8% of diabetes, 11.3 % of smoking and 15.9% of alcoholism in hypertensive patients. According to risk factor’s associations, at least 43.4% of hypertensive patients had more than 3 factors and 13% had 2 factors. Among patients with hypertension, a significant association was observed between family history of hypertension (p = 0.032), diabetes (P<0.001), age over 55 years (p = 0021) in women, male abdominal obesity (p = 0.029) and the occurrence of hypertension.

Conclusion: The high prevalence of hypertension (24.5%) in rural areas requires more preventive measures.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Supplementation of the low-calorie diet with n-3 PUFA significantly improves plasma lipid profile depending on the GIP level.

Abstract nr. 597
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Keywords Lipids, Nutrition, Obesity

Higher plasma GIP level may reflect compensatory mechanism to overcome the diminished islet response in obese patients. The study was aimed to analyze GIP and lipids response to 3-month intervention in terms of n-3 PUFA supplementation along with caloric restriction.

A total of 62 obese patients without any metabolic disorders were included into the study. Patients were assigned to low calorie diet (1200-1500 kcal/day) and were supplemented either with n-3 PUFA (DHA:EPA; 5:1) 3x600mg daily, or placebo (corn-oil), for 3 months. Effects of the intervention on plasma fatty acids profile was assessed by LC-MS/MS.

The detail analysis of plasma fatty acids was performed in relation to GIP status. Supplementation with n-3 PUFA significantly decreased total content of plasma PC FA only in patients with higher GIP level. Caloric restriction alone decreased the saturated/unsaturated FA ratio in Low GIP in contrast to High GIP group. In the High GIP group n-3 PUFA supplementation resulted in decrease of saturated fatty acids: palmitic (16:0), stearic (18:0), miristic (14:0) as well as mono-unsaturated FAs: palmitoleinic (16:1), oleic (18:1) but not PUFA. The n-3 PUFA supplementation resulted in reduction of plasma n-6 PUFA: linoic (18:2) and arachidonic (20:4), which was also pronounced within High GIP group.

We conclude that supplementation of the low-calorie diet with n-3 PUFA significantly improves plasma lipid profile in obese patients characterized by high GIP plasma level. The GIP plasma level may indicate subjects who would potentially benefit from n-3 PUFA supplementation.

Study supported by NCN grant no. K/PBN/000001, EU FP7 BIOCLAMS Grant agreement no. 244995, and grant K/ZDS/004497.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
TARGETING MICROPARTICLES AND MMP9 COLLAGENASE WITH A NEW DRUG - PTCTS - TO TREAT ATHEROSCLEROSIS

Abstract nr. 598

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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium

Keywords Animal model, Atherosclerosis, Pathogenesis, Therapy

Background: A previous study of the group showed that the vulnerability of the atherosclerotic plaque was related with microparticles (MPs) - vesicles larger than 100nm, which release MMP9 collagenase. In another study, intramuscular injection of a new drug (PTCTS) composed by antioxidant nanoparticles from plants and transialidase from Trypanosoma cruzi normalized lipid levels and reduced rabbit atherosclerosis. The mechanism of action includes increased activity of Siglecs (sialic-acid-binding immunoglobulin-like lecitins) and inactivation of inflammasomes. In this work we studied if oral administration of PTCTS is also effective to treat atherosclerosis and if its actions include removal of MPs and MMP9.

Methods: We compared two groups of rabbits. GI (n=6) – 1% cholesterol enhanced diet for 12 weeks; GII (n=8) – 1% cholesterol enhanced diet for 12 weeks with administration of PTCTS (400ul/ day) during the last 6 weeks of diet. Presence and size of MPs were analyzed in photos x30.000 magnification, taken through electron microscopy technique (EM), and immunoelectron microscopy (IME) detecting MMP9. The highest point of atherosclerotic plaque was evaluated in 0,5cm extension of histological ascendant aorta cross section, using anti-MMP9 antibody (clone 56-24A) in immunohistochemistry technique (IHC).

Results: The EM analyses revealed elliptic and rounded MPs. There was a significant difference between GI versus GII in the mean numbers/ photo of elliptic MPs (P=0.01), while the rounded ones showed no difference (P=0.72). Related to the size of MPs, neither the elliptic nor the rounded showed significant difference between GI vs GII (P=0.48 and P=0.09). IHC showed no significant differences of plaque area and plaque/media transition between GI and GII, however it revealed a statistically significant reduction of the percentage of positive area marked with MMP9 antigens (view table). IME revealed MMP9 intra MPs (view image).

Conclusion: Oral administration of PTCTS could become a potential form to treat atherosclerosis,
once we observed reduction in numbers of elliptical MPs as well as in the expression of MMP9 collagenase from rabbit atherosclerotic plaques, suggesting that this drug could be useful in reducing plaque vulnerability.

**Microparticle presenting immuno gold stained MMP9 antigen.**

<table>
<thead>
<tr>
<th></th>
<th>Plaque Area</th>
<th>Plaque/Media Transition</th>
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<tr>
<td></td>
<td>Mean mm² (SD)</td>
<td>% Positivity</td>
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<tr>
<td>GI (n=6)</td>
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<tr>
<td>GII (n=8)</td>
<td>0.14 (0.05)</td>
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<tr>
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**Immunohistochemistry analyses for MMP9 antigens.**

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
COMPARATIVE LIPIDOMIC PROFILING OF PLASMA AND AORTA FROM LDLR-KO, PCSK9-KO AND C57Bl/6 MICE

Abstract nr. 599
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Dyslipidemia, LDL, PCSK9

Background. Lipidomics is a powerful tool to highlight the involvement of lipid species in pathological conditions, including atherosclerosis.

Aim. Lipidomic changes induced by genotype and/or diet were evaluated in plasma and aorta of hyperlipidemic/athero-prone Ldlr-KO, hypolipidemic/athero-resistant Pcsk9-KO and C57Bl/6 (WT) mice.

Methods. Plasma and aorta from mice fed chow or Western diet (WD) for 16 weeks were extracted for lipids and analyzed by mass spectrometry: 200 lipid species belonging to 21 lipid classes were quantified.

Results. On chow diet, plasma lipidome was lower in Pcsk9-KO (2039 μM) vs WT mice (3770 μM) and was much higher in Ldlr-KO (9392 μM) vs WT and Pcsk9-KO mice. WD increased with different degree all lipid classes in each group and the lipidome increased by 2-3 fold in Pcsk9-KO and WT mice and by 5 fold in Ldlr-KO mice. The lipids quantitatively more relevant in each group and dietary condition were cholesteryl esters (CE), phosphatidylcholines, free cholesterol and triglycerides. Among minor lipid species, interestingly, Ceramides(d18:0) levels on chow diet were comparable in Pcsk9-KO and WT mice and were moderately increased by WD, whereas they were 10 fold higher in Ldlr-KO mice on chow diet vs the other groups and increased by another 10 fold on WD. Aorta lipidome of Pcsk9-KO mice was unaffected by diet (chow: 20.7 pmol/μg; WD: 20.2 pmol/μg) whereas it was increased by WD in WT (chow: 18.2 pmol/μg; WD: 38.7 pmol/μg) and Ldlr-KO mice (chow: 19.7 pmol/μg; WD: 49.9 pmol/μg). Among major lipids, WD drastically affected CE concentration in Ldlr-KO mice (chow: 0.1 pmol/μg, WD: 5.3 pmol/μg), whereas it only doubled CE levels in Pcsk9-KO and WT mice. Triglycerides were unaffected by diet in Pcsk9-KO mice, but increased by 2.5 fold with WD in both WT and Ldlr-KO mice. WD did not substantially
modify minor lipids in Pcsk9-KO and WT mice, whereas it drastically changed phosphatidylglycerol, glucosyl/galactosylceramides and Lactosylceramides levels in Ldlr-KO aorta. Conclusions. This lipidomic profiling indicates that diet and/or a genetic background can drastically affect not only major lipid classes, but also minor lipid species, suggesting a possible implication of these molecules in hyperlipidemia and atherosclerosis development.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Abstract nr. 600
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Cardiovascular Disease, Endothelium, Lipoproteins

The deepening of atherosclerosis’ mechanisms has led to an increased interest in the study of biomarkers allowing the quantification of disease severity and establishing a therapeutic conduct appropriate to each patient.

Objectives: Starting from the pathophysiological premise that associated A2 LDL phospholipase (Lp-PLA2) is a marker with high specificity for vascular inflammation, we analyzed the possible correlation of Lp-PLA2 with the diagnostic aspects of severity and evolution of patients with stable coronary artery injury (SCAD).

Methods: A prospective study has been made on a group of patients with evidence of ischemia (137 patients with SCAD, 54 patients with acute coronary syndrome (ACS) and 20 control patients). The following tests has been made: the usual blood tests, an EKG, an echocardiography, a cardiac catheterism (coronarography) and specific tests like: the enzymatic activity of Lp-PLA2 in the plasma using the Azwell Auto Kit, Japan.

Results: The values of Lp-PLA2 in the control group were between 197-206 U/L (mean 201.65 ± 4.21 U/L). A Lp-PLA2 > 206.8 U/L was significantly correlated with stable coronary injury (Sig <0.0001), a Lp-PLA2 > 437.36 U/l at the first measurement was significantly correlated with disease destabilization (SCA) (Sig <0.0001) and a Lp-PLA2 > 321 U/L, calculated during the stable period of the disease was correlated with acute cardiovascular events in evolution or with an evolution to five years severely aggravated, this way proving the negative predictive value.

Conclusions: Lp-PLA2 has proven the capability to descry between evolutionary stages of myocardial ischemia (SCAD/ACS); this way the algorithm of SCA diagnosis is being improved by promptly identifying the destabilization of the condition. Lp-PLA2 as a marker of vascular injury is positively correlated with the evolution of coronary artery disease as well as with the risk of acute cardiovascular events in evolution to a five-year period (Sig <0.001, the measure of association 36.9%).
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
Acute effect of Tetranectin-ApoA-I infusion on atherosclerosis progression/regression in hypercholesterolemic rabbits

Abstract nr. 601
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Animal model, Atherosclerosis, HDL, Reverse Cholesterol Transport

Introduction. Several studies have shown that intravenous administration of synthetic HDL (sHDL) containing human apoA-I is effective in inducing atherosclerosis regression. A possible limitation of this therapeutic approach may be a rapid apoA-I turnover. Tetranectin-apoA-I, a trimeric human apoA-I, was designed to reduce renal clearance and thus prolong half-life and, possibly, efficacy. Aim of this study was to evaluate the effect of Tetranectin-apoA-I infusion on atherosclerosis in a rabbit model widely used to test the efficacy of sHDL.

Methods. 18 rabbits underwent a perivascular injury at both carotids, followed by 1.5% cholesterol diet. At 90 days after surgery, rabbits were randomly divided into 2 groups and i.v. treated, for one time, with 200 mg/kg of sHDL containing Tetranectin-apoA-I (TN-sHDL) or with placebo. All rabbits were fasted over-night and blood samples were collected before and at different time points after the infusion. Plaque changes were analyzed in vivo by IVUS, performed before and at the end of the treatment. Animals were sacrificed three days after treatment and carotids were harvested for histology. Total serum cholesterol efflux capacity (CEC) was evaluated using J774 macrophages incubated with cAMP analogue.

Results. Total atheroma volume in the placebo group increased between the first and the second IVUS evaluation (+7.09 ± 2.33% from baseline). A slight regression was instead observed vs baseline in TN-sHDL treated group (-0.35 ± 1.97%, p< 0.0001 vs placebo). At the maximum plaque burden, TN-sHDL treated rabbits displayed a significant lower macrophage content compared to that found in the placebo group (69.5 ± 13.4% vs 84.3 ± 9.3%, p< 0.05). Four hours after the end of the infusion total CEC of serum TN-sHDL was significantly increased compared to baseline (4.49 ± 1.06% vs 8.69 ± 0.72%, p<0.0001). No changes were observed in the placebo group (5.03 ± 0.96% vs 4.48 ± 0.94%, p>0.05).
**Conclusions.** Our results demonstrate that a single infusion of TN-sHDL is effective in reducing carotid plaques progression in hypercholesterolemic rabbits. This effect is associated with a reduction in the plaque macrophage content, and with an improvement in serum CEC, both features leading to a potential stabilization of atherosclerotic plaques.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
Human validation of genes associated with a murine atherosclerotic phenotype.

Abstract nr. 602
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model,Epidemiology,Genetics,Pathogenesis

Background. The genetically modified mouse is the most widely applied animal model for studying the pathogenesis of atherosclerosis. However, its validity for human disease is unknown. We assessed the association of genes identified in atherosclerotic murine models with human atherosclerotic traits.

Methods. We identified 11,219 publications involving atherosclerotic mice. 2,076 papers reported 659 genes that influence atherosclerosis. We mapped these to their human orthologues. Using VEGAS we performed gene-base tests in CARDIoGRAM, METASTROKE and the Athero-Express Biobank study. We used Ingenuity Pathway Analysis to identify canonical pathways. Their relevance was validated using the results from CARDIoGRAM, METASTROKE and the Athero-Express. We queried expression quantitative trait loci (eQTL) resources to identify common variants modulating gene expression in humans.

Results. 11 and 0 genes associated with coronary artery disease (CAD) and large artery stroke (LAS), respectively, after Bonferroni correction. Nominally (p<0.05), 84 and 41 genes associated with CAD and LAS respectively. 194 genes nominally associated with a plaque phenotype in the Athero-Express, only two were significant after multiple testing correction. We found overlap between pathways in mice and men, but also discordances. The LXR/RXR-activation pathway studied in atherosclerotic mice, is enriched for genes associated with CAD and LAS. We found little evidence of human CAD and LAS for the T-cell differentiation pathway. These differences were found despite all genes having a nominally significant eQTL (411 after multiple testing correction).

Conclusions. We show that human orthologues of genes that are associated with atherosclerosis development in mice are poorly associated with CAD, LAS or advanced human plaque characteristics. Data obtained from atherosclerotic murine models may not always be translatable to human disease and merit careful consideration before being considered for diagnostic of
therapeutic purposes.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
HDL-associated serum amyloid A and surfactant protein B in heart failure with preserved ejection fraction (HFPEF)

Abstract nr. 603
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Chronic Kidney Disease, HDL, Inflammation

Aim: Heart failure with preserved ejection fraction (HFPEF) is a pathophysiologically complex disease with intertwined contributing factors including systemic inflammation and metabolic abnormalities. Advanced disease stages are characterized by post-capillary pulmonary hypertension and often chronic kidney disease (CKD) further propagating the inflammatory state and disease progression. In conditions with increased cardiovascular risk and chronic inflammation, the protein composition of high-density lipoproteins (HDL) is often severely altered. Here we assessed if two proteins highly enriched in CKD-HDL, serum amyloid A (SAA), associated with inflammation and surfactant protein B (SP-B), associated with pulmonary hypertension, are implicated with distinct clinical and diagnostic parameters of HFPEF.

Methods: We developed a novel ELISA assay to quantify HDL-bound SAA and SP-B directly from serum samples. Baseline SAA(HDL) and SP-B(HDL) levels were assessed in 105 HFPEF patients, diagnosed according to current ESC guidelines (1. signs and symptoms of heart failure, 2. left ventricular ejection fraction > 50% and 3. evidence of abnormal left ventricular relaxation, filling or diastolic stiffness) and confirmed diagnosis by right heart catheter. SAA and SP-B levels were then correlated with functional and clinical parameters in the cohort, grouped by occurrence of cardiac events (defined by hospitalization due to heart failure and/or death) during the follow-up period of 3 years.

Results: HFPEF patients had extremely increased levels of HDL-bound SAA and SP-B compared to controls. SAA(HDL) was found to correlate with inflammation (C-reactive protein: r=0.490, p=0.003) and kidney function (glomerular filtration rate: r=-0.525, p=0.001). SP-B(HDL) was inversely associated with distinct markers of pulmonary functions including DLCO (r=-0.478, p=0.018) and FEV1 (r=-0.378, p=0.033). Importantly, these correlations were independent of HDL-cholesterol levels and only found in patients who experienced cardiac events after inclusion.

Conclusion: High levels of HDL-bound SAA and SP-B demonstrate both systemic inflammation and pulmonary congestion as cardinal features of HFPEF, reflecting critical disease-specific pathophysiological alterations. Both are excellent candidates as biomarkers with potential diagnostic value. Interestingly, SAA(HDL) emerges as a novel predictor of CKD progression in this patient group advancing our understanding of causal processes in HFPEF.
Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
The PCSK9 protein: A Key Modulator of Cardiovascular Outcome

Nabil G Seidah & Annik Prat
IRCM, Canada

Autosomal dominant hypercholesterolemia (ADH), a genetic disorder that affects 1:200 subjects worldwide, is caused by mutations in the LDLR & ApoB genes. In 2003, the gene coding for the proprotein convertase subtilisin/kexin 9 (PCSK9) was identified as the 3rd ADH locus & PCSK9 GOF mutations resulted in increased LDLc. The underlying mechanism involves binding of PCSK9 to LDLR and the sorting of the PCSK9=LDLR complex to endosomes/lysosomes for degradation, by an unknown mechanism. Mice & human lacking PCSK9 have higher levels of LDLR in the liver with concomitant very low levels of circulating LDLc. They also exhibit a lower incidence of atherosclerosis & aortic calcification partly due to reduced inflammation.

The PCSK9=LDLR complex is sorted to endosomes/lysosomes for degradation by both an intracellular & extracellular pathway, the latter predominating in the liver. We identified a novel LOF LDLR R410S mutant in ADH patients. This mutant reaches the cell surface and binds extracellular PCSK9 (WT or D374Y) & LDL to a similar extent, but different from WT, the R401S levels are not affected by PCSK9. In contrast, the intracellular activity of PCSK9 (WT or D374Y) on co-expressed LDLR (WT or R410S) is not affected. In that context, studies with Sortilin & APLP2 revealed that neither protein is critical for the extracellular pathway, but overexpression of both enhances the intracellular pathway of PCSK9=LDLR degradation.

In PCSK9 KO mice & not WT, the cell surface levels of the LDLR is dramatically higher in the liver & pancreatic islets of males, but not females. In contrast, the LDLR preferentially accumulates at the cell surface of KO female enterocytes. Ovariectomy of KO females resulted in a typical KO male pattern, while estrogen E2 treatment restored the female pattern. Thus, although the absence of PCSK9 leads to a similar degree of hypocholesterolemia in male & female mice, it results in a sex- & tissue-specific subcellular distribution of the LDLR & VLDLR, which is determined by E2 levels.

Grant support: CIHR # 216684 & Leducq Foundation # 13 CVD 03

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Effects of Administration Sequence of Atorvastatin and Ezetimibe on Lipid Profiles and Serum CoQ10 Levels in Patients with Anterior STEMI

Abstract nr. 605
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords ACS,Hypolipidemic Drugs

Background Aggressive lipid lowering strategy is essential after STEMI. Statins are usually used for such cases but there is concern that those drugs may deteriorate left-ventricular function due to inhibition of geranylgeranyl pyrophosphate production leading to CoQ10 deficiency in the myocardium. Ezetimibe, a cholesterol transport inhibitor is occasionally administered in combination with statins in the drug-resistant cases because cholesterol absorption increases in a complementary fashion. However, there are few data about combination use of those 2 drugs, particularly those regarding myocardial energy metabolism by the drug administration sequence.

Methods and Results Consecutive 40 patients with anterior STEMI were included in the study. After successful primary PCI, they were randomly assigned to one of the following 2 groups, administration of atorvastatin 10mg for 2 weeks first then additional ezetimibe 10mg for 2 weeks (AE group), and ezetimibe 10mg first then additional atorvastatin 10mg (EA group). General lipid profiles as well as campesterol (a marker of cholesterol absorption) and lathosterol (a marker of cholesterol synthesis) were measured on days 0 (pretreatment), 15 (2 weeks after single lipid-lowering therapy with atorvastatin or ezetimibe) and 29 (2 weeks after dual lipid-lowering therapy with atorvastatin and ezetimibe). Measurement of serum levels of CoQ10 was also done at the same time. Changes in the ratios of lathosterol to total cholesterol were decreased by first atorvastatin use (-52%, p=0.0006) and increased by first ezetimibe use (+74%, p=0.0002). Meanwhile, those of campesterol were increased +13.3% (p=0.0188) and decreased -34% (p=0.0002), respectively. Those changes were reversed by additional use of ezetimibe or atorvastatin, respectively. Impacts of atorvastatin on LDL-C reduction rate (%) were significantly larger in the EA (additional use) than in AE groups (initial use) (-43.3±11.1 vs. -30.6±15.2, p=0.0105) although final LDL-C levels were comparable in both groups. Serum CoQ10 was decreased in the AE group (p=0.0004), while unchanged in the EA group (p=0.0680).

Conclusions Additional use of atorvastatin to initial administration of ezetimibe could save CoQ10 from its consumption after STEMI onset. This combination use strategy of ezetimibe and atorvastatin in the sequence may be useful to keep left-ventricular function better and achieve enough LDL-C lowering.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
The Impact of Family History on Cardiovascular Risk is Higher among Hypertensive Individuals of African Origin than Other Ethnic Groups

Abstract nr. 609
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Co-author(s) - Pinto , Sara-Joan
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease,Epidemiology,Hypertension,Risk stratification

Hypertension is considered an important risk factor for cardiovascular diseases (CVD). Nevertheless, the absolute risk for CVD among hypertensive patients is rather small. Moreover, the prevalence of hypertension related CVD varies greatly across ethnicities, with individuals of African origin having more often hypertensive CVD. Whether this is due to a certain genetic background is still unknown. A positive family history (FHx) for CVD suggests a genetic predisposition for CVD.

We hypothesized that a positive FHx for CVD among hypertensive individuals identifies those at risk for CVD differently among different ethnic groups. Therefore, we cross-sectionally investigated the impact of FHx on CVD in a multi-ethnic group of hypertensive individuals.

We investigated all hypertensive individuals (n=3447) of the multi-ethnic cohort study HELIUS (HEalthy LIfe in Urban Settings) in Amsterdam, the Netherlands. It included participants of Dutch (n= 447), South-Asian Surinamese (700), African Surinamese (n=772), Ghanaian (744), Turkish (459) and Moroccan (n=325) origin. We defined hypertension as either a blood pressure > 140/90 mmHg on physical examination or the use of antihypertensive medication with past diagnosis. We defined CVD as a clinical diagnosis of a myocardial infarction or stroke, or angina pectoris according to the Rose criteria, before age 65 years, since early-onset CVD suggests a genetic predisposition. We analysed our data using logistic regression and corrected for age, gender, smoking status, blood pressure level, BMI and diabetes.

FHx for CVD was most prevalent among the South-Asian Surinamese (54.9%) and least prevalent among the Ghanaians (6.9%). CVD was most prevalent among the Turks (32.9%) and least prevalent among the Dutch (11.6%). A positive FHx for CVD yielded the highest risk for CVD among African Surinamese and Ghanaian hypertensive individuals (Odds Ratio and 95% Confidence Intervals (CI): 2.45 (1.66-3.62) and 2.41 (1.26-4.47), respectively, p<0.02). Dutch individuals had the lowest risk (1.20 (0.64-2.20)).

Our data suggest that family history for CVD is a strong discriminant for CVD especially among hypertensive individuals of African origin. Besides, its impact differs sharply across ethnicities. It can therefore be used in clinical practice to discriminate hypertensive patients of African origin at high risk for CVD.
Results of logistic regression on outcome CVD before age 65, per ethnic group

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation

Additional information

<table>
<thead>
<tr>
<th>Outcome: CVD &lt;65 years</th>
<th>Dutch N=447 (52 events = 11.6%)</th>
<th>South-Asian Surinamese N=700 (223 events = 31.8%)</th>
<th>African Surinamese N=772 (153 events = 19.8%)</th>
<th>Ghanaians N=744 (134 events = 18.1%)</th>
<th>Turks N=459 (151 events = 32.9%)</th>
<th>Moroccans N=325 (78 events = 24.0%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>OR CI</td>
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<td>OR CI</td>
<td>OR CI</td>
</tr>
<tr>
<td>Family history of CVD</td>
<td>1.30 [0.64, 2.62]</td>
<td>2.00 [1.42, 2.82]†</td>
<td>2.45 [1.06, 5.62]†</td>
<td>2.41 [1.26, 4.47]†</td>
<td>1.58 [1.06, 2.40]†</td>
<td>1.39 [0.71, 2.41]†</td>
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<tr>
<td>Male</td>
<td>1.61 [0.63, 3.97]</td>
<td>1.20 [0.63, 1.77]</td>
<td>0.64 [0.41, 0.96]‡</td>
<td>0.56 [0.36, 0.96]‡</td>
<td>0.77 [0.47, 1.29]</td>
<td>1.39 [0.74, 2.62]</td>
</tr>
<tr>
<td>Age</td>
<td>1.01 [0.88, 1.16]</td>
<td>1.01 [0.89, 1.03]</td>
<td>1.01 [0.88, 1.03]</td>
<td>1.03 [1.00, 1.06]</td>
<td>1.02 [1.00, 1.05]</td>
<td>1.00 [0.97, 1.03]</td>
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<td>Smoker</td>
<td>2.56 [1.38, 4.74]</td>
<td>1.44 [0.89, 2.21]</td>
<td>2.31 [1.56, 3.43]‡</td>
<td>2.02 [0.98, 3.99]‡</td>
<td>0.97 [0.52, 1.51]</td>
<td>0.72 [0.29, 1.67]</td>
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<tr>
<td>Systolic BP</td>
<td>1.00 [0.97, 1.03]</td>
<td>0.99 [0.86, 1.00]</td>
<td>0.99 [0.86, 1.01]</td>
<td>1.00 [0.98, 1.01]</td>
<td>0.96 [0.87, 1.00]</td>
<td>0.98 [0.86, 1.00]</td>
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<tr>
<td>Diastolic BP</td>
<td>0.96 [0.82, 1.08]</td>
<td>0.99 [0.87, 1.01]</td>
<td>0.99 [0.87, 1.01]</td>
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<td>BMI</td>
<td>1.05 [0.98, 1.12]</td>
<td>1.02 [0.99, 1.06]</td>
<td>1.01 [0.98, 1.06]</td>
<td>1.00 [0.95, 1.04]</td>
<td>1.02 [0.98, 1.06]</td>
<td>1.06 [1.02, 1.14]†</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.01 [0.94, 2.05]</td>
<td>2.08 [1.43, 3.00]†</td>
<td>1.37 [0.85, 2.18]</td>
<td>1.07 [0.69, 1.66]</td>
<td>1.56 [0.93, 2.63]</td>
<td>1.23 [0.66, 2.25]</td>
</tr>
</tbody>
</table>

*Significant for p<0.05.
In patients with severe aortic stenosis increased plant sterol deposition in vascular tissue characterizes patients with concomitant coronary artery disease

Abstract nr. 610
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Lipids,Metabolism,Risk Factor

**Purpose:** The aim of the study was to evaluate the relationship of phytosterols, oxyphytosterols and other markers of cholesterol metabolism in relation to concomitant coronary artery disease in patients with severe aortic stenosis scheduled for elective aortic valve replacement.

**Methods:** Besides markers for cholesterol metabolism (plant sterols and cholestanol as markers for cholesterol absorption and lathosterol as an indicator of cholesterol synthesis) oxyphytosterols were determined in plasma and aortic valve cusps of 104 consecutive patients with severe aortic stenosis (statin treatment: 68 patients; no statin treatment: 36 patients). Coronary angiography prior to aortic valve replacement determined the extent of coronary artery disease.

**Results:** Patients treated with statins were characterized by lower cholesterol, lower cholestanol and lower lathosterol plasma levels. Statin treatment did not affect sterol concentrations in cardiovascular tissue. Absolute values for the cholesterol absorption markers (sitosterol and campesterol) were significantly higher in vascular tissue of patients with documented coronary artery disease compared with those without concomitant CAD. Campesterol oxides were significantly higher in tissue of aortic valve cusps and oxidized sitosterol-to-cholesterol ratios were significantly higher in plasma of patients with coronary artery disease. Interestingly, neither the cholesterol absorption marker cholestanol nor the ratio of cholestanol-to-cholesterol was associated with CAD.

**Conclusions:** Patients with concomitant coronary artery disease are characterized by increased concentrations of plant sterols and their respective oxides in vascular tissue. The fact that cholestanol was not associated with CAD add to the hypothesis that plant sterols are atherogenic.

Subdivision 3. Clinical Studies
Presentation Preference Mini-oral presentation
Additional information
Postprandial response in lipid metabolism in human subjects with ANGPTL3 deficiency

Abstract nr. 611
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Lipids, Lipoproteins and Apolipoproteins, Therapy

Introduction: Homozygosity of loss-of-function mutation (LOF) in human ANGPTL3 gene (S17X) is a direct cause of familial combined hypolipidemia (FHBL2, OMIM #605019) featuring low plasma VLDL, LDL and HDL levels. Angptl3 deficiency is linked with improved TG clearance via activation of lipoprotein lipase and with improved insulin sensitivity. It was therefore of interest to characterize post-prandial response of a high fat meal in S17X LOF-carriers.

Methods: We obtained plasma samples from 7 homozygous carriers of S17X LOF mutation, 33 heterozygote carriers (50% circulating Angptl3) and 37 noncarriers during fasting and 2, 4 and 6 hrs after a high fat meal (oral fat tolerance test). Samples were measured for plasma TG, cholesterol, phospholipids, NEFA, Angptl3 and 4, leptin, adiponectin, FABP4, chylomicron apoB48, apoB100 and VLDL/LDL, apoA-I and HDL, apoE, glucose, and insulin.

Results: Angptl3 deficient subjects show significantly lower plasma TG, cholesterol and phospholipid levels in VLDL, LDL and HDL fractions during fasting as well as in 2, 4 and 6 hr postprandial samples. Postprandial TG elevation was 33 % in homozygotes, 37 % in heterozygotes and 48 % in noncarriers after 2 hours. Postprandial TG levels started to decrease already after 2 hours in homozygotes whereas in heterozygous and control subjects TG elevated up to 4 hours (45 % and 77 %, respectively) after which they started to decrease. Plasma leptin, adiponectin, Angptl4 and FABP4 levels did not correlate with Angptl3 deficiency. High fat meal decreased Angptl3 secretion in heterozygotes and control group.

Conclusions: The present data show that plasma postprandial lipid levels remain low after high fat diet and show a faster turnover rate in Angptl3 deficient subjects compared to heterozygous and control subjects. The results suggest that subjects with Angptl3 deficiency are less prone to develop postprandial hyperlipidemia and the postprandial phenotype therefore would be anti-atherogenic.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
FISH CONSUMPTION, OMEGA-3 FATTY ACIDS (N-3), AND NMR LIPOPROTEIN SUBFRACIONS IN 26034 APPARENTLY HEALTHY WOMEN

Abstract nr. 612
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Epidemiology, Lipoproteins, Metabolism, Nutrition

Background: The role of fish and n-3 fatty acids on lipoprotein metabolism is not well-characterized. Reported standard lipid effects include a decrease in triglycerides and a modest increase in HDL-cholesterol and LDL-cholesterol.
Objectives: We examined the association between intake of fish, total n-3, and the main n-3 subtypes (EPA, ALA, DHA) in relation to standard lipids and lipoprotein size and subfractions.
Design: This cross-sectional study comprised 26034 women who participated in the Women's Health Study. Standard lipids where directly measured and lipoprotein subfraction concentrations and size were measured by nuclear magnetic resonance (NMR) spectroscopy. Information on n-3 and fish intake was obtained using a validated food-frequency questionnaire. Multivariable linear regression models were used to calculate means and β coefficients for various lipids and lipoproteins across quintiles of fish and n-3 intake after adjusting for clinical and dietary factors.
Results: Table 1 summarizes significant differences (increase [+] or decrease [-]) in lipids and lipoprotein subfractions across increasing quintiles of dietary intake of fish, n-3, EPA, DHA, and ALA.
Conclusions: Among 26034 healthy women, different patterns of association were noted for fish, n-3, and specific n-3 fatty acids on lipid and lipoprotein subfractions.
Table 1

<table>
<thead>
<tr>
<th>Food</th>
<th>Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>↑ Total and LDL Cholesterol, Apo B concentration, LDL size and large LDL particle concentration.</td>
</tr>
<tr>
<td></td>
<td>↓ Large and medium VLDL particle concentration</td>
</tr>
<tr>
<td>Omega 3</td>
<td>↑ LDL-size and LDL particle concentration and HDL-size and large HDL particle concentration.</td>
</tr>
<tr>
<td></td>
<td>↓ Triglycerides, VLDL-size, large and medium VLDL and medium HDL particle concentration.</td>
</tr>
<tr>
<td>ALA</td>
<td>↑ HDL and LDL size.</td>
</tr>
<tr>
<td></td>
<td>↓ VLDL-size, large VLDL particle concentration, LDL cholesterol and small HDL particle concentration.</td>
</tr>
<tr>
<td>DHA</td>
<td>↑ LDL and HDL cholesterol, LDL size and large LDL particle concentration, Apo A, large HDL particle concentration and HDL size.</td>
</tr>
<tr>
<td>EPA</td>
<td>↓ VLDL particle concentration and Apo A and medium HDL particle concentration.</td>
</tr>
</tbody>
</table>

Presentation Preference Oral presentation
Additional information
Statins in the treatment of patients after coronary revascularization: only secondary prevention?

Abstract nr. 613
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Co-author(s) - Totolian, Arek
Co-author(s) - Mitrofanova, Lubov
Co-author(s) - Shlyakhto, Evgeny
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Endothelium, Pathogenesis

Background
The increasing number of coronary artery bypass grafting (CABG) surgeries demands to develop new options and methods of treating this category of patients. The remote results after CABG surgery within 5 years are the minimum rate of mortality and extremely rare relapse of myocardial infarction, however recurrence angina pectoris is noted in 50% of patients. There is significant problem of using vena saphena magna as the shunt. It is exposed that 50% of these shunts be occluded in 10 years. Its reason is hyperplasia wich lead to arterialization of venous shunts. Then atherosclerosis develops in this vein. Last years high-dose rosuvastatin treatment has been reported in associated with improved endothelial function, decreasing of oxidative-stress and reduction of chemokines and its receptors expression.

Objectives
The objective of this study was to determine whether high-dose rosuvastatin therapy would prevent these expansive remodelling changes in venous shunts.

Methods
The study was an investigator-initiated, prospective, single-center, double-blind, placebo-controlled, randomized study which enrolled 189 patients suffering CAD with planned CABG surgery. Patients were randomly divided for 2 groups: control group with standart administration simvastatin 20 mg/daily and treatment group, where they were treated using high-dose rosuvastatin therapy 40 mg/daily during 14 days before surgery.

Results
The therapy of high-dose rosuvastatin 40 mg/daily during 14 days before was associated with reduction of intimal hyperplasia of venous conduits. In addition, the number of smooth muscle cells and proliferation index were also significantly reduced according to performed immunochemistry investigation.

Conclusion
The high-dose therapy rosuvastatin 40 mg /daily during 14 days before CABG surgery reduces desquamation of the endothelium, intimal hyperplasia and reduce the number of layers of smooth
muscle cells in the medial parts of the great saphenous veins used for aorta-coronary
anastomosis. It also reduces the proliferation index of the value of the Ki-67 expression in
endothelial and smooth muscle cells of the great saphenous vein.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Apolipoprotein A-II dissociates and rapidly returns to High Density Lipoprotein (HDL) during interaction with phosphatidylcholine-rich particles

Abstract nr. 614
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins,HDL, Metabolism

The dominant subpopulation of HDL are HDL(AI/AII). Metabolism of HDL(AI/AII) has not been fully elucidated. It is believed, that an important role in conversion played apolipoprotein A-II (apoA-II), by impact on particles'stability and by protecting them from remodeling by plasma factors. ApoA-II belongs to group of exchangeable apolipoproteins. However, the dynamics of apoA-II circulation and its functions are not known.

The aim of our study was to evaluate the dynamics of apoA-II circulation between HDL subpopulations in the presence of phosphatidylcholine-rich particles.

After precipitation of apo-B-containing lipoprotein, serum containing HDL as a sole fraction was incubated at 37°C with phosphatidylcholine liposomes at different time intervals. The incubation was stopped by precipitation of liposomes and newly generated pre-β-HDL. In non-precipitated α-HDL and dissolved sediments the concentrations of phospholipids, apoA-I and apoA-II were measured. Two-dimensional (2D) electrophoresis was performed to evaluate the mobility and size of particles.

In the presence of PC-L, in the first ~1 second of the reaction, on average of 18.7±1.4% of apoA-II dissociated from HDL. 2D-electrophoresis has shown the presence of apoA-II containing pre-β-mobility lipoprotein fraction. Longer time of incubations have shown that the concentration of freed apoA-II gradually decreased and followed at the same time increasing the content of apoA-II in α-HDL. On average 55% and 84% of released apoA-II returned to α-HDL after five and 120 minutes of incubation. During the incubation the phospholipids and apoA-I content in α-HDL also have been changing. While the phospholipids content gradually increased at ~1 second, 5 and 120 minutes of incubation (respectively by 48.2±3.4%, 71.4±2.8% and 132.3±3.1%), the apoA-I level decreased (respectively by 13.8±1.3%, 14.2±1.2% and 18.0±4.4%).

We have proved for the first time that apoA-II may dissociate from HDL and cycle between pre-β-HDL and α-HDL. The return of released apoA-II to α-HDL indicates the higher affinity of apoA-II to α-HDL than to lipid-load liposomes and also the higher preferences of apoA-II for spherical structures of α-HDL than pre-β-HDL disc forms. We assume that the release of apoA-II from HDL(AI/AII) and incorporation of phospholipids may facilitate the remodeling of HDL and
dissociation of apoA-I.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
A comparison of the prevalence of cardiometabolic risk factors in different altitude populations in Kyrgyz Republic

Abstract nr. 615
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Co-author(s) - Lunegova, Olga
Co-author(s) - Zalesskaya, Juliya
Co-author(s) - Bektasheva, Erkaim
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Dyslipidemia, Hypertension, Metabolism, Obesity

**Background:** Prevalence of cardiovascular disorders has been described in different ethnic populations. The data on cardiometabolic risk factors are scarce.

**The aim** of this study was to determine the prevalence of the cardiometabolic risk factors and compare it on populations in three altitudes of Kyrgyz Republic: low (LA; Bishkek, 750m), middle (MA; At Bashy, 2200m) and high (HA; Ak Say, 3600-3800m).

**Materials and methods:** 453 ethnic Kyrgyzes (287 men, 166 women) 30 to 75 years of age of above mentioned regions were studied. Clinical investigation, anthropometrical evaluation (weight, height, waist (WC) and hip circumference (HC)), body mass index (BMI), measurement of systolic (SBP) and diastolic (DBP) blood pressure were measured in all patients. Laboratory analysis included: fasting plasma glucose, insulin, lipids. Metabolic syndrome (MS) was defined according to modified ATP III criteria.

**Results:** HA male natives have lesser BMI, WC, WC/HC (p<0,001), DBP (p<0,01), glucose (p<0,05), triglycerides (p<0,0001) compare with MA and LA; they also have rare prevalence of obesity (p<0,001), abdominal obesity (p<0,05) and hypertriglyceridemia (p<0,001). Compare with MA and LA female natives in HA have lesser SBP (p<0,01), glucose (p<0,0001); they have rare prevalence of hyperglycemia (p<0,01), hypertension (p<0,05). Natives of LA, MA and HA have lesser prevalence of MS: 54,4%, 39%, 28,3% (p<0,01) in males and 49,5%, 44,7%, 25,9% (not significant) in females respectively. The number of metabolic risk factors decreased from LA to HA in male (p<0,001) and females (p<0,01) respectively.

**Conclusions:** BMI, WC, WC/HC were decreased in men at HA; lower prevalence of high SBP and of high fasting glucose was found in the female HA natives; we found less prevalence of men with MS due to lower prevalence of obesity, abdominal obesity and hypertriglyceridemia in the HA natives; the number of cardiometabolic risk factors were decreased in both sexes in the HA areas; the prevalence of MS and cardiometabolic risk factors apparently varies among men and women and this indicates the need for more epidemiologic studies

Subdivision 3. Clinical Studies
Presentation Preference Oral presentation
Additional information
ApoE modulates the kinetics of HDL apoA-I in humans

Abstract nr. 616
Author Morton, Allyson, Harvard School of Public Health, Boston, United States of America
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Co-author(s) - Furtado, Jeremy D.
Co-author(s) - Sacks, Frank
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, HDL, Lipoproteins

Human HDL kinetic studies typically measure total apoA-I turnover, rather than partitioning by HDL size or apolipoprotein content. ApoE has potential roles in HDL metabolism by promoting enlargement and clearance. About 5-10% of plasma HDL apoA-I is associated with apoE. To determine whether apoE modulates the kinetics of apoA-I HDL, we compared the metabolism of apoA-I in HDL that has apoE with apoA-I in HDL that does not have apoE. We recruited 5 participants with low HDL-C (average 39 mg/dl, range 24-48 mg/dl) and BMI between 25-35 kg/m². We gave the participants an intravenous bolus of d3-leucine, and collected blood at 14 time points to 70 hours. HDL was isolated from plasma by anti-apoA-I immunoaffinity chromatography, separated by anti-apoE chromatography into HDL that contain or do not contain apoE, and separated using ND-PAGE into 4 size fractions: alpha-1, alpha-2, alpha-3, and prebeta-1. ApoA-I was purified from the 8 HDL subtypes with SDS-PAGE, hydrolyzed into amino acids, and d3-leucine enrichment was measured by GC-MS. Pool size of apoA-I was determined from the protein bands, adjusted to plasma total apoA-I. We used SAAM-II modeling software to compute apoA-I fractional catabolic rates (FCR) and fluxes for each HDL subtype. The FCRs of apoA-I of HDL size fractions that contain apoE were about 2.5 to 5 times faster than that of apoA-I of the corresponding HDL size that does not contain apoE (table). Driven by the higher FCRs, a substantial amount of apoA-I flux in plasma occurs in apoE-containing HDL, varying from 20-40% among the 4 sizes. Tracer enrichment curves for a representative participant and size fraction are also shown (figure), demonstrating the much faster turnover for apoA-I HDL that has apoE. In summary, apoA-I HDL with apoE is an important, metabolically unique subspecies of HDL that could account for a substantial minority of total HDL flux in plasma. This research is funded by NIH 5R01HL095964.

<table>
<thead>
<tr>
<th>HDL Size</th>
<th>apoA-I FCR (pools/d)</th>
<th>apoA-I Pool Size (mg)</th>
<th>apoA-I Flux (mg/kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-1</td>
<td>2.2 (apoE+); 0.30 (E-)</td>
<td>33 (E+); 508 (E-)</td>
<td>0.74 (E+); 1.5 (E-)</td>
</tr>
<tr>
<td>Alpha-2</td>
<td>1.8 (apoE+); 0.40 (E-)</td>
<td>44 (E+); 1068 (E-)</td>
<td>0.90 (E+); 4.5 (E-)</td>
</tr>
<tr>
<td>Alpha-3</td>
<td>1.2 (apoE+); 0.43 (E-)</td>
<td>104 (E+); 1658 (E-)</td>
<td>1.2 (E+); 6.4 (E-)</td>
</tr>
<tr>
<td>Prebeta-1</td>
<td>1.0 (apoE+); 0.45 (E-)</td>
<td>45 (E+); 302 (E-)</td>
<td>0.50 (E+); 1.3 (E-)</td>
</tr>
</tbody>
</table>

ApoA-I FCRs, pool sizes, and fluxes, average of n=5
Alpha-3 tracer enrichment curves for a representative participant
Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Identifying and quantifying six apoA-I HDL subspecies by a novel modified sandwich ELISA

Abstract nr. 617
Author Yamamoto , Rain, Harvard School of Public Health, Boston Ma, United States of America
Co-author(s) - Furtado , Jeremy D.
Co-author(s) - Sacks , Frank
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins,Cardiovascular Disease,HDL,Lipoproteins

HDL-cholesterol is a well-established independent risk factor used in CVD risk stratification. However, the cardioprotective effect of HDL-cholesterol is challenged by genetic studies and pharmaceutical trials, which do not support the causal relationship between increased HDL-cholesterol levels and CVD. Previously, our lab found that HDL can be classified into subspecies based on the presence of apoC-III, where for example 7% of the total plasma apoA-I in HDL has apoC-III. We also showed that the apoA-I concentration of HDL that has apoC-III predicts increased risk of CHD, whereas that of HDL that does not have apoC-III predicts decreased risk of CHD. We hypothesized that some of the many other proteins that are located in HDL are present in apoA-I HDL subspecies.

We developed a novel sandwich ELISA to measure apoA-I in HDL that is associated with a specific protein of interest, and applied it to apoA-II, apoC-III, and apoE, which define known subspecies of apoA-I HDL. We also applied the ELISA to apoC-I, apoC-II, and plasminogen, to determine whether their association with HDL constitutes a subspecies of apoA-I HDL, and in what concentrations they exist in HDL. We confirmed the existence of all the apoA-I lipoprotein subtypes using pooled plasma samples collected from healthy men and women (age: 40±14 (mean±SD), triglyceride: 116±63 mg/dl, LDL-cholesterol: 118±28 mg/dl, HDL-cholesterol: 45±10 mg/dl). The concentration (percentage) of total plasma apoA-I in HDL associated with apoA-II was 103±11 mg/dl (71±12%), with apoC-I was 23±4 mg/dl (16±2%), with apoC-II was 4±1 mg/dl (2±1%), with apoC-III was 9±3 mg/dl (6±1%), with apoE was 8±5 mg/dl (5±3%) and with plasminogen was 4±1 mg/dl (3±1%). The results for apoA-II, apoC-III, and apoE are consistent with previously reported values. In conclusion, we established a convenient sandwich ELISA, which demonstrated the existence of new apoA-I HDL subspecies that are defined by presence of apoC-I, apoC-II, and plasminogen, as well as known subspecies of apoA-I HDL; and we quantified the concentration of such subspecies. Future studies will investigate these abundant HDL subtypes in relation to CVD risk. This study is funded by NIH 1R01HL123917-01.
The novel sandwich ELISA method summary, using apoA-I associated with apoC-III as an example

**ApoA-I concentration associated with HDL proteins (mg/dl)**

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Intimal smooth muscle cell cholesterol content and implications for plaque regression

Abstract nr. 618
Author Francis, Gordon, University of British Columbia, Vancouver, Canada
Co-author(s) - Chehroudi, Cyrus
Co-author(s) - McManus, Bruce
Co-author(s) - Abraham, Thomas
Co-author(s) - Allahverdian, Sima
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, HDL, Pathogenesis, Reverse Cholesterol Transport

Cholesterol accumulation in atherosclerotic plaque has previously been thought to occur primarily in monocyte-derived macrophages. Using coronary artery sections from hearts explanted at time of heart transplantation we have determined the relative contribution of smooth muscle cells (SMCs) and macrophages to foam cell formation. Following staining of formalin-fixed tissues to clearly identify intracellular lipid along with the SMC-specific marker SMα-actin or myeloid lineage-specific marker CD45, we determined that at minimum, SMCs comprise 50±7% (Avg±SEM, n=14 subjects) of foam cells in human coronary atherosclerosis. Further estimation of plaque foam cell content using fluorescence-activated cell sorting suggests the contribution of SMCs to foam cells in human atherosclerosis may be much higher. As a possible explanation for this finding, SMCs in advanced lesion intima showed a specific reduction in ABCA1 expression not seen in early or advanced lesion myeloid lineage cells or in early lesion SMCs. These results suggest an inability of SMCs to release cholesterol via the ABCA1-apoAI-HDL axis contributes to the large contribution of SMCs to the foam cell population, and possibly also to their conversion to a macrophage phenotype. Previous studies have suggested cultured SMCs can express macrophage markers upon lipid loading. We found that 40±6% (n=15) of cells expressing the macrophage marker CD68 also expressed the SMC marker SMα-actin. In addition, 34±8% (n=11) of CD68-positive cells lacked expression of the myeloid lineage marker CD45. These results indicate that up to one-third or more of cells considered to be myeloid lineage macrophages in human atherosclerosis are in fact SMCs exhibiting a macrophage phenotype. Further studies are examining the relationship of lipid loading to expression of macrophage markers by SMCs, and the contribution of SMCs to cells capable of undergoing either regression or necrosis in the atherosclerotic plaque.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Proteome of human plasma Very Low-Density Lipoprotein and Low-Density Lipoprotein exhibits a link with coagulation and lipid metabolism

Abstract nr. 619
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Co-author(s) - Peppelenbosch, Maikel
Co-author(s) - Rezaee, Farhad
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords LDL, Lipoproteins, Thrombosis

Background
Apart from transporting lipids through the body, the human plasma lipoproteins VLDL and LDL are also thought to serve as a modality for intra-organismal protein transfer, shipping proteins with important roles in inflammation and thrombosis from the site of synthesis to effector locations.

Methods
To better understand the role of VLDL and LDL in the transport of proteins, we applied a combination of LTQ ORBITRAP-XL (nLC-MS/MS) with both in-SDS-PAGE gel and in-solution tryptic digestion of pure and defined VLDL and LDL fractions.

Results
We identified the presence of 95 VLDL and 51 LDL associated proteins including all known apolipoproteins and lipid transport proteins, and intriguingly a set of coagulation proteins, complement system and anti-microbial proteins. Prothrombin, protein S, fibrinogen γ, PLTP, CETP, CD14 and LBP were present on VLDL but not on LDL. Prenylcysteine oxidase 1, Dermcidin, Cathelicidin antimicrobial peptide, TFPI-1 and Fibrinogen α chain were associated with both VLDL and LDL. Apo A-V is present on VLDL only and not on LDL.

Conclusions
Collectively, this study provides a wealth of knowledge on the protein constituents of the human plasma lipoprotein system and provides strong support for the notion that protein shuttling through this system is involved in the regulation of biological processes. Human diseases related to proteins carried by VLDL and LDL can be divided in three major categories: 1-dyslipidemia 2-atherosclerosis and vascular disease and 3-coagulation disorders.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
The composition of specific apolipoproteins may determine HDL functions. We present novel mass spectrometry (MS)-based methods that capture the absolute quantities and kinetics of 7 apolipoproteins in 5 HDL size fractions. We recruited three participants, a white female, black female and white male, ages 25, 33 and 49 years old, who were overweight or obese with a body-mass index of 25.6, 30.7 and 30.5 kg/m$^2$, and had low HDL cholesterol levels of 48, 37 and 24 mg/dL. These participants were infused with a bolus of D3-Leu tracer, and blood samples were collected for 70 hours. ApoA-I-containing HDL was prepared by immunoaffinity purification, separated into 5 size fractions, prebeta, alpha3, alpha2, alpha1, and alpha0 by non-denaturing-PAGE, and in-gel trypsinized for MS analysis. We monitored 7 proteins that likely affect HDL metabolism – apoA-I, apoA-II, apoA-IV, apoC-III, apoD, apoE and apoM. Each protein pool size had a distinct distribution across the HDL sizes. ApoE and apoM were enriched in larger HDL, whereas apoC-III and apoA-IV were enriched in smaller HDL sizes. We evaluated the tracer enrichment curves of these 7 proteins in the 5 size fractions using high resolution parallel reaction monitoring performed on a quadrupole Orbitrap (Thermo). The enrichment curves for each protein varied from each other by slope and time of peak enrichment (Fig. 1a). In contrast, the enrichment curves across HDL sizes for a single protein showed smaller, but likely meaningful, differences in either slope or time of peak enrichment. Irrespective of the HDL size on which it resides, apoE had the fastest FCR, followed by apoA-IV, and apoC-III. ApoA-I, apoA-II, apoM, and apoD had slower but similar FCRs (Fig. 1b). Overall, his study showed distinct distribution and kinetic behaviors of 7 HDL proteins across 5 HDL size fractions that were conserved in the three diverse participants. These findings may help elucidate the functional role of these proteins and the HDL particles that contain them. This study was supported by research grants from Kowa Company, Ltd., (Nagoya, Japan) to M.A. and from NIH 5R01HL095964 to F.S.
Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
The effect of omega-3 carboxylic acids on apolipoprotein CIII-containing lipoproteins in moderate to severe hypertriglyceridemia

Abstract nr. 621
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Co-author(s) - Amerine, William
Co-author(s) - Davidson, Michael
Co-author(s) - Sacks, Frank
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Apolipoproteins, Dyslipidemia, Intervention, Lipoproteins

Lipoprotein subspecies containing apoCIII adversely affect CVD risk. It has been shown that LDL with apoCIII is a stronger predictor of CVD than LDL without apoCIII, and that HDL with apoCIII is associated with increased CVD. Small studies suggest that omega-3 fatty acids may reduce plasma total apoCIII in addition to their TG-lowering effects, but there is a lack of detailed analysis in regards to which lipoprotein subtypes are affected by treatment.

We analyzed plasma samples from the EVOLVE trial (EpanoVa for Lowering Very high triglycerides), a 12-week double-blind study of 399 subjects with fasting TG between 500 and 2000 mg/dl who were randomized to Epanova (omega-3 carboxylic acids) 2, 3 or 4 g/d or olive oil (placebo). Our analysis included 273 subjects who were randomized to placebo, Epanova 2 or 4 g/d and had baseline and end of treatment samples. Epanova contains EPA (50-60%) and DHA (15-25%) in free fatty acid form.

We studied the effect of omega-3 carboxylic acids on apoCIII concentrations in HDL, LDL, and VLDL; and on the concentrations of subspecies of HDL, LDL, and VLDL that contain or do not contain apoCIII. Epanova significantly reduced plasma apoCIII relative to placebo (2g: -4.2 mg/dl, p=0.002; 4g: -4.0 mg/dl, p<0.0001), as well as apoCIII in HDL (2g: -0.6 mg/dl, p=0.12; 4g: -1.0 mg/dl, p=0.01), and apoCIII in LDL (2g: -2.9 mg/dl, p<0.0001; 4g: -3.3 mg/dl, p<0.0001). Epanova increased selectively the concentration of LDL apoB that does not contain apoCIII, a subtype with a weak relation to CHD, by 5.1 mg/dl (2g, p=0.047) and 7.1 mg/dl (4g, p=0.006). Treatment did not significantly increase the concentration of LDL with apoCIII (2g: 0.15 mg/dl, p=0.7; 4g: 0.2 mg/dl, p=0.6).

Omega-3 carboxylic acids at dosages of 2 and 4 g/d are effective for lowering total plasma apoCIII and apoCIII in HDL and LDL. The effect of omega-3 carboxylic acids to raise LDL concentration is limited to the less harmful subspecies of LDL without apoCIII. Reduction in apoCIII may be a mechanism for the TG lowering effects of omega-3 carboxylic acids.

Funding for the EVOLVE trial was provided by Omthera Pharmaceuticals, Inc.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
CHOLESTEROL TRAFFICKING-RELATED SERUM LIPOPROTEIN FUNCTIONS IN CHILDREN WITH CHOLESTERYL ESTER STORAGE DISEASE

Abstract nr. 622
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Co-author(s) - Cagliero, Paola
Co-author(s) - Ronda, Nicoletta
Co-author(s) - Gomaraschi, Monica
Co-author(s) - Calabresi, Laura
Co-author(s) - Bernini, Franco
Co-author(s) - Guardamagna, Ornella

Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Dyslipidemia, Functionality, HDL

Serum lipoproteins influence cell cholesterol content by delivering and removing cholesterol to/from cells, functions mainly exerted by low density lipoprotein (LDL) and high density lipoprotein (HDL), respectively. Especially in the case of HDL, lipoprotein structure and composition are crucial for function, beyond serum levels. Cholesteryl ester storage disease (CESD) is caused by lysosomal acid lipase gene (LIPA) mutations and reduced activity of lysosomal acid lipase (LAL), the enzyme responsible for hydrolysis of cholesteryl esters and triglycerides (TG). CESD patients typically present dyslipidemia, liver damage and premature atherosclerosis. The purpose of this work was to evaluate the serum HDL cholesterol efflux capacity (CEC) via the multiple pathways and serum cholesterol loading capacity (CLC) in CESD pediatric patients and to study lipoprotein qualitative modifications. CEC and CLC studies were performed using radioisotopic and fluorimetric assays, respectively. The study involved 3 children aged 3.5-5 years presenting combined dyslipidemia, hypertransaminasemia and anemia. Patients underwent routine and specific laboratory set analysis, medulla and liver biopsy and abdominal Rx scan and ultrasound; LAL activity was checked by DBS tests and LIPA gene sequencing was performed. Patients showed positive DBS tests and exon 8 c.894 G>A and c.883 C>T mutations were detected by LIPA gene sequencing. Abdominal ultrasound showed mild hepatomegaly and liver steatosis in all CESD subjects. CESD patients displayed high total cholesterol, LDL cholesterol, TG, phospholipids and low HDL cholesterol compared to age-matched control subjects (n=9). CESD serum CEC was impaired as a whole but also through the aqueous diffusion (AD) process and with respect to specific membrane cholesterol transporters. A marked reduction in the pre-β HDL concentration (-69%; p=0.009) and an increase in smaller α-migrating particles were detected. Finally, CESD pediatric patients showed an increased serum capacity to load normal macrophages with cholesterol (CLC). In conclusion, CESD pediatric patients displayed an
impaired HDL serum CEC and an increased serum CLC that could explain the accelerated atherosclerosis observed in these patients. These new data demonstrate that the pro-atherogenic modifications of LDL and HDL include disturbances in lipoprotein functions involved in cell cholesterol homeostasis from very early age in CESD patients.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Chemerin level, high sensitivity-CRP, insulin resistance and severity of coronary heart disease in type 2 diabetes.

Abstract nr. 623
Author Amin, noha, Faculty of Medicine, Alexandria University, alexandria, Egypt
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Diabetes, Pathogenesis

Background: Chemerin has been recently described as a novel adipokine having a role in inflammation and atherosclerotic cardiovascular disease. It has also been suggested to have a role in glucose homeostasis and insulin action.

Objective: To assess the relationship between serum Chemerin levels, hs-CRP (high sensitivity C-reactive protein), insulin resistance and the severity of coronary artery disease (CAD) in type 2 diabetic patients.

Methods: The study included 160 subjects; divided equally into 4 groups; patients having CAD and type 2 diabetes (A), CAD without type 2 diabetes (B), patients having type 2 diabetes without CAD (C) and a control group (D). Serum Chemerin levels, hs-CRP, HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) and lipid profile were assessed. SYNTAX score was calculated for the CAD groups (A and B).

Results: Our study showed a statistically significant increase in both serum Chemerin and hs-CRP levels in subjects with CAD compared to those without CAD (p < 0.001 & 0.009 resp.). There was no statistically significant difference in both serum Chemerin level and hs-CRP among diabetic compared to non-diabetic subjects (p = 0.16 & 0.395 resp.). When patients with CAD (groups A and B) were divided according to their SYNTAX score into three groups, there was a statistically positive correlation between the severity of CAD and both Chemerin level and hs-CRP level (p<0.001 & 0.04 resp.). There were no statistically significant correlations between serum Chemerin and fasting plasma glucose, HbA1c, fasting serum Insulin and HOMA2-IR. There was a significant positive correlation of serum chemerin with waist circumference (p=0.024) and waist/hip ratio (p =0.044). There was a significant positive correlation of hs-CRP with waist/hip ratio (p<0.031) and statistically significant negative correlation with HDL-C (p< 0.001).

Conclusion: There is a significant positive statistical correlation between serum Chemerin level, hs-CRP and the severity of atherosclerotic CAD. However, further research is recommended to determine the exact role of Chemerin and hs-CRP in glucose homeostasis and their relation to diabetes.

Abbreviations: HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, hs-CRP: High sensitivity C-reactive protein, CAD: Coronary artery disease, DM: Diabetes mellitus.
Additional information
The effects of Lipoprotein Apheresis on Cardiovascular Events incidence: A Single-Center Experience

Abstract nr. 624
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Co-author(s) - Emilio, Pasanisi Maria
Co-author(s) - Christina, Petersen
Co-author(s) - Michele, Coceani
Co-author(s) - Roberta, Luciani
Co-author(s) - Mascia, Pianelli

Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease,Familial Hypercholesterolemia,Lp(a),Therapy

Aim. Lipoprotein apheresis (LA) is the elective therapy for homozygous and other forms of Familial Hypercholesterolemia (FH), resistant/intolerant to lipid lowering drugs, and hyperlipoproteinemia(a) for which drugs are not available.
To assess the effect of LA on the incidence of adverse cardiac or vascular events (ACVE) at the time period of pre-initiation of apheresis and during the LA treatment.

Methods. We collected data of 30 patients (mean age 62±8 years, male 73%), with FH and cardiovascular disease on maximally tolerated lipid lowering therapy and LA treatment (median 5 years, interquartile range 3-8 years). Associated hyperlipoproteinemia(a) was present in 16/30 subjects. The LA treatment was performed biweekly as clinically indicated by dextran-sulfate or heparin-induced LDL precipitation apheresis.
The ACVE incidence, before and after treatment, was evaluated by statistical analyses.

Results. The ACVE incidence occurred before and after the LA treatment inception, were 86 and 15 events respectively. Notably, 6/15 of ACVE were secondary to stent restenosis and 7/15 follow-up events occurred during the first 5 years. The AVCE rates/year were 0.58 and 0.13 respectively (p<0.001).

Conclusions. Our data confirm long-term efficacy and positive impact of LA on morbidity in patients with FH and atherosclerotic disease at maximally tolerated lipid lowering therapy.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
**Moderate increase of Indoxyl Sulfate promotes monocyte differentiation into profibrotic macrophages. A neglected risk factor in Abdominal Aortic Aneurysms**

Abstract nr. 625
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Co-author(s) - Ghigliotti, Giorgio

**Topic** The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium

**Keywords** Cardiovascular Disease, Immunity, Inflammation, Renal function

**Background:** Patients with abdominal aortic aneurysms (AAAs) have a high prevalence of moderate chronic kidney disease (CKD) and augmented CD14+CD16+ monocytes, a subset found enhanced also in CKD patients (Ghigliotti Dis Markers). Increased serum levels of Uremic toxins (UT) represent one of the CKD-associated triggers of CV damage. The UT Indoxyl-3-sulphate (IS), ligand of Aryl hydrocarbon Receptor (AhR), accumulates early during CKD progression because of tubular damage and is associated to overall mortality and CV diseases.

We investigated the effect of 1, 10, 20 microM IS, concentrations representative of mild-to-moderate CKD, on monocyte-macrophage differentiation.

**Methods and Results:** in THP-1 monocytes, IS (20 microM) increased the migration rate 2 folds and promoted in a concentration-dependent manner CD163 expression up to 3 folds (p<0.05). These effects were mediated by the AhR-Nrf2/HO-1 cross-talk pathways, and were effectively counteracted by the AhR antagonist CH-223191. Moreover, IS down-regulated the CCR2 (2 folds; p<0.05) and upregulated the MCP-1 gene expression (5 folds; p<0.05), phenomena associated to monocyte-macrophage transition and features of CD14+CD16+ monocytes.

IS-primed THP1 monocytes differentiated into macrophages overexpressing IL-6 mRNA (>10 folds with IS 10 and 20 microM) and components of the AhR/AhRR and Nrf2/HO1 axes; 10 and 20 microM IS evoked macrophages with features of both classical (MCP-1, COX2) and alternative immunity (MMP-9 downregulation; PPARg, TIMP-1 and TGF-β overexpression). Thus, moderate IS increases skewed monocyte differentiation towards macrophages with low-inflammatory, profibrotic potential.
As proof of concept, we measured IS serum levels in AAA patients and in age-matched controls; we found that AAA patients had increased IS (p=0.0017) that correlated with CD14+CD16+ monocytes (p=0.0028; r=0.3454). Next, we treated THP1 monocytes with serum from AAA patients and with serum of controls and compared the phenotype of the differentiated macrophages. AAA serum induced monocyte polarization into low-inflammatory, profibrotic macrophages, with higher IL-6 gene expression (>10 fold, p<0.001) and protein expression of TGF-β (3 folds, p<0.05), PPARγ and TIMP-1 (2 folds, p<0.001).

**Conclusion:** A moderate IS increase primes monocyte differentiation into low-grade-inflammatory, profibrotic macrophages; this process takes place at systemic level and may participate to maladaptive arterial remodeling in AAA patients.

**Subdivision 1. Basic Science**

**Presentation Preference** Electronic poster presentation

**Additional information**
HDL-associated proteins and cardiovascular event prediction in a secondary prevention study

Abstract nr. 626
Author O'Brien , Kevin, University of Washington, Seattle Wa, United States of America
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Co-author(s) - Isquith , Daniel
Co-author(s) - Neradilek , Moni
Co-author(s) - Zhao , Xue-Qiao
Co-author(s) - Probstfield , Jeffrey
Co-author(s) - Vaisar , Tomas
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Apolipoproteins,Cardiovascular Disease,HDL,Risk stratification

**Problem:** Cardiovascular disease (CVD) remains the leading cause of death in Western populations. However, current multivariate models have limited ability to predict CVD risk, spurring interest in identifying novel CV risk markers. Recent studies have raised the possibility that HDL-associated proteins may add to CVD risk prediction.

**Purpose:** To determine whether specific, HDL-associated proteins may add to CVD event risk prediction in individuals with known CVD.

**Procedures/Methods:** We performed a prospective case-cohort study of 155 participants (45 cases) nested in the AIM-HIGH HDL Proteomics Substudy. This study included participants for who at least two carotid magnetic resonance imaging scans had been obtained along with all remaining Substudy participants with subsequent cardiovascular (CVD) events (death, myocardial infarction, stroke, revascularization, or hospitalization for unstable angina). High density lipoprotein (HDL) was isolated by sequential density ultracentrifugation from baseline plasma samples, and 30 HDL-associated proteins were quantified by targeted mass spectrometry. Analyses were performed using a weighted Cox regression model based on Barlow’s case-cohort method to account for the oversampling of participants with events. To account for multiple comparisons, p-values were adjusted to control the false discovery rate (FDR) at 5%. P<0.05 was considered statistically significant after this adjustment.

**Main Findings:** A total of 45 participants suffered a new CVD event after the study baseline. In the primary analysis, 7 of 30 proteins were predictive of CVD events with hazard ratios (HRs) of 1.6-2.7 in models adjusting for age and gender (Table). These findings were supported by a sensitivity analysis which further adjusted the models for HDL isolation batch. After further adjustment for a Framingham risk score (model for secondary CVD risk), the HRs for a CVD event remained statistically significant for 4 proteins [lecithin-cholesterol acyl transferase (LCAT), vitronectin, apolipoprotein A-V (apoA5) and phospholipid transfer protein (PLTP)].

**Conclusions:** Our results suggest that quantification of specific HDL-associated proteins in people with known CVD may significantly improve risk prediction above and beyond a standard risk prediction model. Validation of these HDL-associated proteins as CVD risk predictors in larger and
more diverse cohorts is therefore warranted.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Model 1: Primary Analysis (adj. for age + gender)</th>
<th>Model 2: Model 1 + FRS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadj. HR (95% CI)</td>
<td>Unadj. HR P-value†</td>
</tr>
<tr>
<td>Lecithin-cholesterol acyltransferase (LCAT)</td>
<td>2.67 (1.43, 4.98)</td>
<td>0.033</td>
</tr>
<tr>
<td>Vitronectin (VTN)</td>
<td>2.18 (1.27, 3.74)</td>
<td>0.033</td>
</tr>
<tr>
<td>ApoA5</td>
<td>1.58 (1.15, 2.18)</td>
<td>0.033</td>
</tr>
<tr>
<td>Phospholipid transfer protein (PLTP)</td>
<td>1.62 (1.12, 2.35)</td>
<td>0.046</td>
</tr>
<tr>
<td>Haptoglobin (HP)</td>
<td>1.82 (1.17, 2.63)</td>
<td>0.046</td>
</tr>
<tr>
<td>Complement C4a</td>
<td>1.75 (1.13, 2.71)</td>
<td>0.046</td>
</tr>
<tr>
<td>Serum amyloid A4 (SAA4)</td>
<td>1.65 (1.09, 2.50)</td>
<td>0.047</td>
</tr>
</tbody>
</table>

AUC = area under the curve (c-statistic); FRS = Framingham Risk Score for recurrent CHD events over 2 years (age, gender, total-C:HDL-C ratio, systolic BP, diabetes and current smoking).

*Hazard ratio (HR) per 1 SD in log(protein) after adjusting for age and gender (model 1) and log(FRS) (model 2);†Test of HR = 1 (no association);‡Change in AUC between model 2 (with protein) and the model without protein (AUC = 0.69); models were re-fit using logistic regression for AUC calculations.

Table. HDL-associated proteins and recurrent cardiovascular events in AIM-HIGH Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Consideration of Hypertensive Retinopathy as an Important End-Organ Damage in Patients with Hypertension: Results from a Cross-Sectional Study

Abstract nr. 627
Author Sijl, Alper van, Diakonessenhuis, Utrecht, Netherlands
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Co-author(s) - Ree, Marcel van der
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Blood pressure, Cardiovascular Disease, Hypertension

Background – Longstanding and therapy resistant hypertension may cause cerebral, renal, cardiac and retinal organ damage (EOD). Retinal hypertensive abnormalities are correlated with a higher risk of cardiovascular (CV) disease but are not included in CV risk assessment tools. Research into prevalence and determinants of retinal organ damage, or hypertensive retinopathy (HR), in patients with hypertension is scarce. We evaluated the prevalence of HR and the association with other signs of end organ damage in patients with therapy resistant hypertension.

Methods – A retrospective observational investigation was performed in all hypertensive patients referred by a GP to the hypertensive clinic at the Diakonessenhuis, Utrecht, the Netherlands between 2011 and 2013. A general screening of risk factors, albuminuria, left-ventricular hypertrophy and retinal fundoscopy was performed in all patients.

Results – 43.2% (121/280) of patients referred to the clinic were diagnosed with HR, while 7.7% and 12.8% of patients were diagnosed with microalbuminuria and left-ventricular hypertrophy (LVH), respectively. Patients with isolated HR consisted of 62% of all patients with end-organ damage. When HR was added as EOD (with EOD being considered a treatment indication as is a high outcome in the SCORE risk table) the percentage of patients with a treatment indication increased from 14.8% to 20.7%. Prevalence of HR was higher in those who did not obtain treatment goals (systolic blood pressure less than 135/90) than those who did obtain treatment goals, 53.6% vs. 33.8%, respectively.

Discussion – HR is prevalent in nearly a third of therapy resistant hypertensive patients, while the number of patients with isolated HR accounts for the majority of (end-) organ damages. Fundoscopy in the evaluation of hypertension improves the indication for therapy. Furthermore, diagnosing HR could be helpful in selecting more aggressive treatment in patients with therapy resistant hypertension.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
HDL has several putative anti-atherogenic effects, including preserving endothelial function. The cholesterol transporters ATP Binding Cassette A1 and G1 (ABCA1 and ABCG1 (ABCA1/G1)) are highly expressed in endothelial cells (ECs) and mediate cholesterol efflux to apolipoprotein A-1 and HDL. We have shown previously that whole body Abca1/g1 deficiency decreases endothelium-dependent vasorelaxation due to decreased endothelial nitric oxide synthase (eNOS) activity in mice fed cholesterol-rich diets. These observations suggested that endothelial ABCA1/G1 are anti-atherogenic. Studies in zebrafish have suggested that endothelial ABCA1/G1 suppress angiogenesis, which could be anti-atherogenic in advanced lesions. However the role of endothelial ABCA1/G1 in atherosclerosis and angiogenesis in mammals has not been directly investigated.

We generated LDL receptor knockout (Ldlr-/-) mice with endothelial Abca1/g1 or Abcg1 deficiency. After 22 weeks of a cholesterol-rich Western type diet, both endothelial Abca1/g1 and Abcg1 deficiency accelerated atherosclerosis in the aortic root and whole aorta, with a more pronounced effect of endothelial Abca1/g1 than Abcg1 deficiency (2-fold; EC-Abca1/g1 knockouts compared to controls; $P<0.001$). Plasma cholesterol levels (~1000 mg/dL) and blood leukocyte levels were similar. In aortic ECs, Abca1/g1 deficiency suppressed eNOS activity (~50%; $P<0.05$), and increased inflammatory mRNA expression (vascular and intracellular adhesion molecule-1, E-selectin, monocyte chemoattractant protein-1, tumor necrosis factor α, interleukin 6 (IL-6)) and inflammasome priming (NLRP3, IL-1β) following a lipopolysaccharide (LPS) stimulus (2-fold; $P<0.01$). These findings were recapitulated in human aortic ECs. In aortic rings stimulated with vascular endothelial growth factor, endothelial Abcg1 deficiency and Abca1/g1 deficiency increased the formation of new sprouts, suggesting increased angiogenesis (4-fold; EC-Abca1/g1 knockouts compared to controls; $P<0.001$). However, very few new blood vessels were observed in advanced atherosclerotic lesions of the aortic root with no difference between the groups. These observations suggest that endothelial Abca1/g1 and to a lesser extent endothelial Abcg1 deficiency accelerates atherosclerosis due to decreased eNOS activity and increased endothelial inflammation. These are the first studies to show directly that cholesterol efflux pathways in ECs...
are athero-protective.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Elevated plasma levels of matrix metalloproteinase (MMP)-2, MMP-9, and tissue inhibitor of metalloproteinase (TIMP)-1 in patients with pseudoxanthoma elasticum (PXE).

Abstract nr. 629
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Co-author(s) - Kawashiri, Masa-aki
Co-author(s) - Nohara, Atsushi
Co-author(s) - Kobayashi, Junji
Co-author(s) - Mabuchi, Hiroshi

Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Pathogenesis

Backgrounds: Pseudoxanthoma elasticum (PXE) is an inborn disorder of the connective tissue with specific skin, ocular, and cardiovascular system including coronary and/or peripheral artery. Matrix metalloproteinases (MMPs) degrade collagens, elastins; the core proteins of proteoglycans, and extracellular matrix (ECM) glycoprotein such as fibronectin and laminin. Recent pathological studies have shown that MMPs and their inhibitors (TIMPs) are involved in the development of atherosclerotic lesions.

Methods: Plasma levels of MMP-2, MMP-3, MMP-9, TIMP-1 and TIMP-2 were determined in peripheral blood obtained from five PXE patients (male/female = 2/3, age = 51±20 (mean±SD): group P) and 15 age-matched control subjects (groupC) by a sandwich ELISA method, and the results were compared between the groups.

Results: Plasma levels of MMP-2 (ng/ml) (group P vs group C = 1081±328 vs 743±62, p<0.005), MMP-9 (ng/ml) (33±6 vs 24±16, p<0.05) and TIMP-1 (ng/ml) (188±24 vs 119±35, p<0.005) in PXE were significantly higher than those in control subjects, whereas there were no significant differences in plasma MMP-3 and TIMP-2 levels between PXE patients and control subjects.

Conclusions: Our data suggest that elevated plasma levels of MMP-2, MMP-9 and TIMP-1 in PXE patients may be due to atherogenesis followed by ECM remodeling.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
EXPLOITING THE GEOMETRIES OF ATHEROSCLEROTIC PLAQUES TO SITE SPECIFICALLY INHIBIT THROMBOSIS USING FLOW SENSITIVE ANTI-THROMBOTIC NANOPARTICLES

Abstract nr. 630
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Co-author(s) - Yao, Joy
Co-author(s) - Hoefer, Thomas
Co-author(s) - Peter, Karlheinz
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Imaging, Therapy, Thrombosis

Anti-platelet therapy, used to treat thrombosis after atherosclerotic plaque rupture, suffers from substantial bleeding complications because it is not tailored to act exclusively at sites of pathological thrombus formation where blood shear stresses are typically very high. We developed a nanoparticle based drug delivery system that utilizes high shear stress to site specifically inhibit cellular processes at atherosclerotic plaques, without causing systemic platelet inhibition.

Anti-platelet drugs were used as proof of principle to demonstrate the feasibility of localized drug delivery induced by high blood shear stress. Cangrelor (20 µM) and Integrillin (350 µM), antagonists of platelet P2Y12 and GPIIbIIIa receptors respectively, were encapsulated in phosphatidylcholine based liposomes. Inhibition of thrombus formation was tested with in-house developed microfluidic blood perfusion channels (50µm(h) x 1mm (w)) containing a surface of collagen type-I and lumen restrictions to mimic atherosclerotic plaques. Drug encapsulated liposomes (1x10¹²) were infused into wildtype mice and thrombus formation was monitored in the carotid artery and mesenteric arterioles. Systemic concentrations of anti-platelet drugs were monitored with platelet aggregometry using platelet rich plasma obtained from mice infused with drug loaded nanoparticles.

Nanoparticle delivery of anti-platelet drugs inhibited in vitro thrombus formation at sites of stenotic plaque geometries, i.e. at blood shear rates >1000 s⁻¹. Infusion of nanoparticles in wildtype mice prevented full thrombotic occlusion. Microscopic analysis revealed normal initial phases of thrombus build-up but an inhibition of platelet aggregation upon reaching full vessel occlusion. Platelets from these mice demonstrated normal aggregation in response to 10 uM ADP indicating an absence of systemic platelet inhibition. In addition, mice infused with anti-platelet nanoparticles did not display increased tail bleeding times.

Targeted delivery of anti-platelet drugs by flow sensitive liposomes offers a potent and site specific therapy to prevent atherothrombotic events. The drug is only released in areas of pathologic shear stress caused by the formation of platelet thrombi and atherosclerotic plaques but leaves other
areas of the vasculature unaffected. This study is a step towards safer and more potent therapy inhibiting thrombosis in the setting of atherosclerosis and could be extended towards local delivery of anti-inflammatory drugs or lipid lowering drugs.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
Development and Assessment of an Angiographic Scoring System for Peripheral Artery Disease

Abstract nr. 631
Author Morris, Dylan, James Cook University, Douglas, Australia
Co-author(s) - Singh, Tejas
Co-author(s) - Moxon, Joseph
Co-author(s) - Golledge, Jonathan
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Imaging, Risk stratification

Background
Patients with peripheral artery disease (PAD) have athero-thrombosis affecting their lower limb arteries. Many PAD patients now receive CT angiogram (CTA) as part of their clinical assessment. The aim of this study was to develop and assess a scoring system for angiographic assessment of lower limb athero-thrombosis from CTAs.

Methodology
An angiographic scoring system (ANGIO Score) was developed by a team of clinicians and scientists. Scoring comprised assessment of the 10 major arteries in the lower limb. Lower limb arteries were scored 0, 1 or 2 according to the degree of stenosis or occlusion. Values were summated to produce an overall score for the affected limb. Reproducibility of the ANGIO Score was assessed in 30 lower limbs by two observers, and assessed by weighted-Kappa analysis. The associations of the ANGIO Score with ankle-brachial pressure index (ABI) and major cardiovascular events (CV; myocardial infarction, stroke, cardiovascular death) were assessed in 75 and 251 patients, respectively. Kaplan-Meier survival curves and Cox proportional hazard analysis were used to assess the association of ANGIO Score and major CV events.

Results
The agreement between readers was good (weighted-κ = 0.78). The ANGIO Score was negatively correlated with ABI in 75 PAD patients with different clinical presentations (Spearman correlation coefficient = -0.60, p<0.001). A higher ANGIO Score was associated with increased risk of major CV events independent of traditional cardiovascular risk factors (Score ≥10: hazard ratio=2.93 95%CI 1.49-5.77, p=0.013).

Conclusion
The ANGIO Score is a novel method for reporting severity of lower limb athero-thrombosis assessed on CTA that is associated with ABI and CV risk.
Freedom from major cardiovascular events in PAD patients according to their degree of lower limb atherothrombosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio, 95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANGIO Score of 0-4</td>
<td>1.00 (Ref)</td>
<td>-</td>
</tr>
<tr>
<td>ANGIO Score of 5-9</td>
<td>2.086 95%CI 1.070-4.067</td>
<td>0.031</td>
</tr>
<tr>
<td>ANGIO Score ≥10</td>
<td>2.934 95%CI 1.492-5.767</td>
<td>0.002</td>
</tr>
<tr>
<td>Age</td>
<td>1.015 95%CI 0.989-1.042</td>
<td>0.257</td>
</tr>
<tr>
<td>Sex</td>
<td>0.763 95%CI 0.444-1.311</td>
<td>0.327</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.130 95%CI 0.694-1.840</td>
<td>0.622</td>
</tr>
<tr>
<td>Smoker†</td>
<td>0.989 95%CI 0.513-1.906</td>
<td>0.974</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.725 95%CI 0.382-1.377</td>
<td>0.326</td>
</tr>
<tr>
<td>Dyslipidaemia‡</td>
<td>0.635 95%CI 0.376-1.071</td>
<td>0.088</td>
</tr>
<tr>
<td>IHD</td>
<td>1.939 95%CI 1.172-3.206</td>
<td>0.010</td>
</tr>
</tbody>
</table>

CI, confidence interval.
* Represents risk of major cardiovascular events of patients in highest and middle ANGIO Score tertiles compared to the lowest (reference) tertile.
† Patients who have ever smoked.
‡ Based on treatment for dyslipidaemia with statins.

Results of adjusted Cox proportional hazard analyses assessing the association of lower limb atherothrombosis with major cardiovascular events in PAD

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation

Additional information
Risk Factors of early atherosclerosis in children with obesity

Abstract nr. 634
Author Ustiuzhanina, Margarita, Ural State Medical University, Yekaterinburg, Russian Federation
Co-author(s) - Kovtun, Olga
Co-author(s) - Samarina, Olga
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Dyslipidemia, Lipids, Risk Factor

At present, the epidemic of obesity among children and adults around the world is becoming one of the most serious public health problem. According to leading scientists obesity and early atherosclerosis share the same risk factors, which include disorders of lipid metabolism in childhood and adolescence.

The aim of our study was to evaluate the prevalence of dyslipidemia-associated SNP and their role in disorders of lipid metabolism in obese children.

The study included 50 obese children and adolescent (36 boys, 14 girls; mean age 14.8±1.8 years) with BMI 31 kg/m² (95% CI: 29.8, 32.3) and 20 healthy children and adolescent with normal body weight (12 boys, 6 girls; mean age 13.9±2.0 years) with BMI 19.52 kg/m² (95% CI: 18.56, 20.48). Plasma concentrations of glucose, total cholesterol, triglycerides, HDL and LDL cholesterol were defined automatically by homogeneous enzymatic colorimetric method; concentration of ApoA and ApoB were measured by immunoturbidimetric method. Dyslipidemia-associated SNP: ApoB R3500Q (rs5742904), PPARG Pro12Ala (rs1801282), ApoA1 G75A and APOE Cys112Arg (rs429358) and Arg158Cys (rs7412) were determined by real-time PCR.

These levels of total cholesterol, triglycerides, LDL, HDL and ApoB were significantly different in children from the study and control groups, and the levels of ApoA and glucose were distributed equally. The difference between frequencies of dyslipidemia-associated SNP did not differ significantly. As well as significant association of carriage of these genes with obesity or hypertension.

Our findings reflect a lack of significant differences in the frequency of dyslipidemia-associated SNP in obese and healthy children, thus we can suppose secondary character of dyslipidemia in obese children. Perhaps, epigenetic mechanisms play major role in the development of lipid disorders in children with obesity. So they also need to be further investigated and evaluated.
<table>
<thead>
<tr>
<th></th>
<th>Obsere</th>
<th>Reference</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.00 (95% CI: 3.8, 4.3)</td>
<td>3.30 (95% CI: 2.8, 3.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.27 (95% CI: 1.0, 1.5)</td>
<td>0.54 (95% CI: 0.4, 0.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>1.79 (95% CI: 1.3, 2.0)</td>
<td>2.27 (95% CI: 1.9, 2.6)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>0.93 (95% CI: 0.6, 0.9)</td>
<td>1.29 (95% CI: 1.0, 1.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoB, g/l</td>
<td>0.86 (95% CI: 0.3, 0.9)</td>
<td>0.68 (95% CI: 0.6, 0.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoA, g/l</td>
<td>1.57 (95% CI: 1.5, 1.6)</td>
<td>1.66 (95% CI: 1.5, 1.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>4.60 (95% CI: 4.5, 4.8)</td>
<td>4.83 (95% CI: 4.0, 4.7)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Lipoprotein particle number and core lipid composition changes with pitavastatin therapy in hypercholesterolemic patients with or without type 2 diabetes

Abstract nr. 635
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes, Dyslipidemia, Lipoproteins, Metabolism

Background Many studies have shown that the reduction of cardiovascular events was not fully explained by the decreased LDL-cholesterol levels with statin therapy. Therefore, lipoprotein profiles other than LDL-cholesterol should be taken into account for the assessment of drug therapy efficacy. Methods We studied hypercholesterolemic subjects with type 2 diabetes (n=16, DM group) and without diabetes (n=16, N group) before and after 8 weeks of treatment with pitavastatin 2 mg/day. We measured cholesterol and triglyceride levels in 20 lipoprotein subfractions using a high-performance gel permeation chromatography (HPLC). Then, lipoprotein particle numbers (PN) were calculated from cholesterol and triglyceride levels using a newly-developed LipoSEARCH program based on the assumption that the volume ratio of surface layer to lipid core is equivalent for lipoprotein with the same particle size. Lipoprotein core lipid composition was expressed by %cholesteryl ester (%CE), which shows the amount of CE in proportion to the sum of CE and triglyceride. Results and discussion Cholesterol, triglyceride and PN in LDL were decreased significantly after pitavastatin treatment in both the N and the DM groups (p<0.01). The extent of cholesterol and PN reductions in the N group was significantly greater than that in the DM group (p<0.01), while the extent of triglyceride reduction was not significantly different between the two groups (p=0.090). Significant decreases in PN were observed in all LDL subclasses in both groups (p<0.0001). In the N group, the extent of PN reduction was greater in medium LDL as compared with large or very small LDL, and that in small LDL was greater than that in very small LDL. In the DM group, differences in the extent of PN reduction among subclasses were smaller. As for core lipid composition, %CE decreased in all LDL subclasses, suggesting preferential clearance of CE-rich lipoproteins by the treatment. Among LDL subclasses, the extent of reduction in %CE was the greatest in large LDL, raising the possibility that particle size also plays a role in LDL clearance. Conclusions: The simultaneous
analyses of lipoprotein PN and core %CE by HPLC have the potential to enable researchers to elucidate the dynamic mechanism of lipoprotein metabolism.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Cardioprotective properties of Aronia Melanocarpa

Abstract nr. 636
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Animal model,Lipids,Nutrition,Prevention

One of the potential strategies for counteraction to the age-dependant oxydation damages is the delivery of antioxidants with food. Aronia melanocarpa (A.m.) has been rated first in antioxidative potential among anthocyanin fruits. Certain polyphenolic ingredients in A.m. have revealed cardioprotective effects according to the literature data. Aim: To ascertain the cardioprotective properties of juices from A.m. by studying somatometric and serum parameters as well as correlations between them in rats supplemented with such juices. Material and methods: An experimental study has been carried out on 24 rats of Wistar species supplemented with juices of A.m. during 3 months.
Results and discussion: The Lee’s obesity index has remained within the norm in all groups of animals at P>0.05. Statistically significant differences have been found in weights of the heart but not in the heart weight index. There exists a strong and positive correlation between the animal weight and the weights of the heart r_{xy} = 0.77 and a mean of magnitude negative correlation between the rat’s weight and the heart weight index at r_{xy} = -0.6.
An optimization of the lipid profile has been found in cardioprotective direction with both groups of supplemented animals where as the combination of both constituents (A.m. and pectin) has resulted in increase of the effect.
The clues have been confirmed by the atherogenetic and cardioprotective indices. The results of the study have indicated that the juice of A.m. and especially it’s combination with pectin possess organ-preserving and cardioprotective properties and can be recommended as prophylactic means for conversion of the unsuccessful into successful vascular aging.
Atherogenic Small Dense Low-Density Lipoproteins Reduced after 2-Month Liraglutide therapy in Patients with Type-2 Diabetes: a Prospective Pilot Study

Abstract nr. 637
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes,Lipoproteins,Therapy

Liraglutide is a novel anti-diabetic agent with several actions beyond glucose metabolism, including some beneficial effects on plasma lipids, such as a slight reduction in low-density lipoproteins-cholesterol (LDL-C) concentrations. Yet, its effect on distinct lipoproteins, particularly on atherogenic small dense (sd) LDL, is unknown.

Forty four patients (24 men and 20 women; mean age: 61±11 years), with type 2 diabetes (T2DM) naïve to incretin-based therapies and treated with metformin only, were included. Liraglutide (1.2 mg/day) was given on top of metformin (1500 mg/day). At baseline and after 2 months, fasting plasma samples were collected for laboratory analyses, including all the 7 distinct LDL subclasses by gel electrophoresis (Lipoprint, Quantimetrix Corporation, USA). With this methodology, sdLDL is classified as LDL3-C to LDL7-C. Statistical analysis was performed using the paired t-test and Spearman correlation.

Significant reductions were found in fasting glycemia and glycated hemoglobin (HbA1c) (from 8.5±2.9 to 7.0±1.3 mmol/L and from 8.7±0.8 to 8.0±0.9%, respectively; p<0.0001 for both), body mass index (from 28.5±6.2 to 27.7±5.7, p=0.0035) and triglycerides (from 1.9±0.8 to 1.6±0.7 mmol/L, p=0.0015). Also, LDL-C reduced slightly after liraglutide therapy (from 1.7±0.5 to 1.5±0.5 mmol/L, p=0.0146), while no significant changes were found in high density lipoprotein-cholesterol as well as waist circumference. Cholesterol (C) content (in mmol/L) in each LDL subclass was calculated and we found an increase in LDL1-C (from 0.63±0.23 to 0.72±0.23, p=0.0158), accompanied by reduction in LDL3-C and LDL4-C (from 0.32±0.23 to 0.16±0.19, p<0.0001 and from 0.07±0.13 to 0.02±0.05, p=0.0043, respectively). C content in any of the other LDL subclasses did not change significantly. Of interest, changes in fasting glycemia or HbA1c were not correlated with changes in any of the 7 distinct LDL subclasses.
This is the first study that investigated the effect of liraglutide on the full spectrum of LDL subclasses in patients with T2DM. Liraglutide significantly reduced atherogenic sdLDL, beyond glycemic control. Whether these findings influence cardiovascular outcome remains unknown.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Circulating lutein and zeaxanthin levels are potential markers of inflammatory resolution in patients with coronary artery disease

Abstract nr. 639
Author Chung, Wing Shan Rosanna, Linköping University, Linköping, Sweden
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Co-author(s) - Lundberg, Anna K.
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords ACS,Atherosclerosis,Inflammation,Nutrition

Introduction: Low levels of plasma carotenoids as well as low dietary intake of carotenoids are well-known cardiovascular risk factors. Inverse correlations between plasma carotenoids and inflammatory markers have been reported in population-based studies. A few studies also indicate a positive correlation between carotenoid levels and T cell and NK cell numbers. Statin therapy, which has potential anti-inflammatory effects, has been shown to increase the carotenoid levels. Here, we assessed different carotenoids and their relationship with interleukin-6 (IL-6) and immune cells in patients with acute coronary syndrome (ACS) and stable coronary artery disease (CAD).

Methods: The cohort included 48 ACS patients and 109 stable CAD patients. Circulating levels of 5 major carotenoid types, namely lutein+zeaxanthin (measured together), β-cryptoxanthin, lycopene, α- and β-carotene, were determined by liquid chromatography. Plasma IL-6 levels, numbers of neutrophils, monocytes, T cell and NK cell subpopulations were also assessed. In a subgroup of 33 patients, these parameters were measured before coronary revascularization and after 3 and 12 months.

Results: Cholesterol-adjusted levels of lutein+zeaxanthin and lycopene were inversely correlated to IL-6 in the whole cohort, p<0.001 and p=0.014, respectively. In the stable CAD patients of whom 95 % were on statin treatment, only lutein+zeaxanthin levels correlated to IL-6 (p<0.001). In the 12-month follow-up, the percentage decrease in IL-6 levels was inversely correlated to the percentage decrease in lutein+zeaxanthin levels at 3 months (p=0.008) and 12 months (p=0.018), and also inversely correlated to the percentage decrease in lycopene levels at 12 months (p=0.043). Other carotenoids did not correlate to IL-6, neither did they change over 12 months. There were no correlations between carotenoid levels and immune cells in cross-sectional or longitudinal analyses.

Conclusion: In CAD patients treated with statin, circulating levels of lutein+zeaxanthin and lycopene were inversely correlated with plasma IL-6. The strong inverse correlations between changes in lutein+zeaxanthin and IL-6 over the 12-month follow-up suggest that lutein+zeaxanthin is a potential marker of inflammatory resolution. Data further indicate that lutein+zeaxanthin may have a more prominent anti-inflammatory role than other carotenoids.
Presentation Preference Oral presentation
Additional information
Familial Hypercholesterolemia in Mexico

Abstract nr. 641
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia, LDL

Introduction. Familial hypercholesterolemia (FH) is an autosomal dominant disorder. It is estimated that there are more than 200,000 Mexicans affected, most are undiagnosed, and so who have high risk of developing cardiovascular disease and premature death. Until now there have been only isolated initiatives for the detection of these patients in Mexico.

Objective. Presenting the data accumulated in the detection of patients with FH in Mexico.

Methods. They have been studied 118 index cases. The clinical and biochemical diagnosis of FH was defined according to International Guidelines. The LDLR gene, a portion of exon 26 of the APOB gene, and the PCSK9 gene were sequenced.

Results. In 64 index cases (54%) the genetic cause was identified; most mutations in the LDLR gene (95%). 37 different mutations (35 for the LDLR gene and 2 for APOB gene). Five mutations found in 4 or more index cases (1055G> A, 1090T> C, 682G> A, 2271delT, 338insG) and reported only in Mexican population (Robles-Osorio et al, 2006; Martínez L et al, 2011; Vaca et al, 2011; Magaña-Torres et al, 2014). The family study (cascade screening) allowed the detection of 251 new cases. Most patients unknown their disease, very few were receiving treatment, and usually when they received was practically at ages in which cardiovascular risk reduction is already very limited or even after a heart attack.

Conclusion. Although we have identified 369 individuals with FH, these numbers are still well below the more than 200,000 Mexicans are estimated to be affected. We need to join efforts and sensitize the medical community and the health authorities that an early diagnosis of FH can save lives.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
LRP1 EXPRESSION IS DOWNREGULATED IN HUMAN AND MOUSE MONOCYTE SUBPOPULATIONS DURING ATHEROSCLEROTIC PROCESSES

Abstract nr. 643
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Co-author(s) - Collino, Cesar
Co-author(s) - Tinti, Maria
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Co-author(s) - Castro, Claudia
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Immunity, Inflammation

Atherosclerosis is an inflammatory process in which circulating monocytes are involved. Three monocyte subpopulations (MoSP) are characterized in human: classical, intermediate and nonclassical, whereas murine MoSP are basically LyC6^hi and LyC6^lo. The low-density lipoprotein receptor-related protein 1 (LRP1) is expressed in several tissues, included monocytes. LRP1 is involved in vascular remodeling and foam cell formation. However, LRP1 expression in MoSP in atherosclerosis is unclear. Herein we determine LRP1 levels in MoSP in patients with atherosclerotic lesions and in a murine model of atherosclerosis (apolipoprotein E deficient mice:KO-ApoE^{-/-}) by flow cytometry (FC). Peripheral blood samples were obtained from: i) healthy individuals (HI group) (cholesterol<200mg/dl; HDL-cholesterol>40mg/dl (male); >50mg/dl (female), triglycerides<150mg/dl, creatinin<1,2 mg/dl, arterial pressure<140/90, without antecedents of arterial hypertension, nonsmoking, and without familiar antecedents of CVD; ii) patients (AP group) with evident atherosclerotic plaques or thickening of the vascular intima in carotid (>1.0 mm) (carotid Doppler ultrasound study) and/or coronary artery calcium (CAC) score >1 (computed tomography scanning); and iii) patients (nonAP group) excluded of HI group without carotid and coronary atherosclerotic lesions. Characteristics of patient groups are shown in Table 1. Peripheral blood samples from KO-ApoE^{-/-} mice (12 weeks of age) maintained with low-fat diet (LFD) or high-fat diet (HFD) for 4 weeks were analyzed. Under these food conditions only HFD mice developed atherosclerotic plaques. Human MoSP were selected by FC strategy using specific fluorochrome-conjugated antibodies (F-MoAb) against CD45/LRP1/CD14/CD16^1. Murine Ly-C6 MoSP were selected using F-MoAb against monocytic markers (CD11b/Ly-6G/Ly-6C/LRP1. The inclusion of LRP1 as fourth marker identified three murine MoSP: Ly-6C^hi/LRP1^hi, Ly-6C^hi/LRP1^lo, and Ly-6C^lo/LRP1^lo (Table 2). The mean fluorescence intensity (MFI) for LRP1 in each MoSP was determined from CD14/CD16 (human)^1 and Ly-6C/LRP1 plots (mouse). MFI-LRP1 values calculated are shown in Table 2. Human LRP1 was significantly decreased in classical
monocytes in AP group in comparison to HI and nonAP groups. Mouse LRP1 was significantly decreased in Ly-6C<sup>hi</sup>LRP1<sup>hi</sup> MoSP in KO-ApoE<sup>−/−</sup> HFD with respect to LFD mice. Thus, LRP1 expression in MoSP constitutes an attractive diagnostic tool for subjects with high risk of cardiovascular disease.

1 Ferrer D. et al., Cytometry Part A. 2014, 85, 601-610.
Funding supports: PICTO-GSK-2012-0084; PICT-2012-2607.

<table>
<thead>
<tr>
<th>LRP1 Expression in Human Monocyte Subpopulations</th>
<th>1 HI Group (n=27)</th>
<th>2 AP Group (n=52)</th>
<th>3 NonAP Group (n=90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical</td>
<td>CD14&lt;sup&gt;+&lt;/sup&gt;CD16&lt;sup&gt;+&lt;/sup&gt; (MFI±SD)</td>
<td>68.4±10.5</td>
<td>52.2±9.9**</td>
</tr>
<tr>
<td>Intermediate</td>
<td>CD14&lt;sup&gt;+&lt;/sup&gt;CD16&lt;sup&gt;+&lt;/sup&gt; (MFI±SD)</td>
<td>77.6±17.4</td>
<td>75.2±16.3</td>
</tr>
<tr>
<td>Nonclassical</td>
<td>CD14&lt;sup&gt;+&lt;/sup&gt;CD16&lt;sup&gt;+&lt;/sup&gt; (MFI±SD)</td>
<td>40.0±10.9</td>
<td>39.1±9.6</td>
</tr>
</tbody>
</table>

** Denote significant differences (p<0.05) between 1 vs. 2 (ANOVA test).
HI, Healthy individual; NonAP, patients without cardiac and coronary atherosclerotic lesions; AP, patients with cardiac and coronary atherosclerotic lesions.
MFI±SD, mean fluorescence intensity ± standard deviation.

<table>
<thead>
<tr>
<th>LRP1 Expression in Mouse Monocyte Subpopulations</th>
<th>1 KO-ApoE&lt;sup&gt;−/−&lt;/sup&gt; LFD (n=4 mice)</th>
<th>2 KO-ApoE&lt;sup&gt;−/−&lt;/sup&gt; HFD (n=4 mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ly6C&lt;sup&gt;hi&lt;/sup&gt;LRP1&lt;sup&gt;hi&lt;/sup&gt; (MFI±SD)</td>
<td>134.5±10</td>
<td>102.0±9.6**</td>
</tr>
<tr>
<td>Ly6C&lt;sup&gt;hi&lt;/sup&gt;LRP1&lt;sup&gt;hi&lt;/sup&gt; (MFI±SD)</td>
<td>35.3±4.5</td>
<td>32.0±3.9</td>
</tr>
<tr>
<td>Ly6C&lt;sup&gt;hi&lt;/sup&gt;LRP1&lt;sup&gt;hi&lt;/sup&gt; (MFI±SD)</td>
<td>31.0±3.3</td>
<td>29.7±4.3</td>
</tr>
</tbody>
</table>

** Denote significant differences (p<0.05) between 1 vs. 2 (Mann-Whitney test).
KO-ApoE<sup>−/−</sup>, apolipoprotein E deficient mice, LFD, low fat diet; HFD, high fat diet.
MFI±SD, mean fluorescence intensity ± standard deviation.
# Table 1: Clinical and biochemical parameters of patients.

<table>
<thead>
<tr>
<th>Parameters (Mean±SD)</th>
<th>1 HI Group</th>
<th>2 AP Group</th>
<th>Non AP Group</th>
<th>One-way ANOVA test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.0±9.4 (a)(b)</td>
<td>49.2±7.4 (b,e)</td>
<td>38.3±11.1 (a)(c)</td>
<td>(a) n.s. (b,c) p&lt;0.001</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>22.7±2.9 (a)(b)</td>
<td>26.6±4.0 (b)(e)</td>
<td>26.1±5.3 (a)(e)</td>
<td>(a, e) n.s. (b) p&lt;0.05</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>93.0±8.8 (a)(b)</td>
<td>93.5±8.7 (b,e)</td>
<td>87.9±15.6 (a)(c)</td>
<td>(a, b) p&lt;0.01 (d) p&lt;0.05</td>
</tr>
<tr>
<td>Blood pressure (s) mmHg</td>
<td>110.1±10.9 (b)(d)</td>
<td>118.7±11.3 (b)(e)</td>
<td>117.5±11.7 (d)(e)</td>
<td>(e) n.s. (b) p&lt;0.01 (d) p&lt;0.05</td>
</tr>
<tr>
<td>Leukocytes/µL (10³)</td>
<td>6.2±1.3 (a)(f)</td>
<td>6.5±1.7 (c)(f)</td>
<td>6.6±1.6 (c)(e)</td>
<td>(a, e, f, n.s.</td>
</tr>
<tr>
<td>Total Monocytes (%)</td>
<td>7.8±2.0 (a)(f)</td>
<td>7.8±2.3 (c)(f)</td>
<td>7.7±1.9 (a)(e)</td>
<td>(a, d) n.s.</td>
</tr>
<tr>
<td>Serum creatinin (mg/dl)</td>
<td>0.9±0.17 (a)(f)</td>
<td>0.97±0.18 (c)(f)</td>
<td>0.95±0.18 (a)(e)</td>
<td>(a, e, f, n.s.</td>
</tr>
<tr>
<td>Glycemia (mg/dl)</td>
<td>94.1±6.9 (a)(b)</td>
<td>101.8±5.7 (b)(c)</td>
<td>99.4±8.7 (a)(c)</td>
<td>(a) n.s. (b) p&lt;0.01 (c,d) p&lt;0.05</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>160.3±24.1 (b)(d)</td>
<td>202.8±35.9 (b)(e)</td>
<td>183.5±37.1 (c)(d)</td>
<td>(b, d) p&lt;0.001 (c,d) p&lt;0.01</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>0.9±11.0 (a)(b)</td>
<td>50.2±13.8 (b)(e)</td>
<td>58.0±20.9 (a)(e)</td>
<td>(a, e) n.s. (b) p&lt;0.01</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>82.7±18.1 (b)(d)</td>
<td>133.1±30.4 (b)(e)</td>
<td>159.3±32.5 (c)(d)</td>
<td>(b,c) p&lt;0.001 (d) p&lt;0.01</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>63.1±10.5 (b)(d)</td>
<td>125.6±60.9 (b)(e)</td>
<td>116.3±119.8 (d)(e)</td>
<td>(a, e, n.s. (b,d) p&lt;0.05</td>
</tr>
<tr>
<td>F50 CV L</td>
<td>8.9±4.5 (b)(d)</td>
<td>32.0±17.1 (b)(e)</td>
<td>16.2±14.8 (e)(d)</td>
<td>(b,c) p&lt;0.001 (d) p&lt;0.03</td>
</tr>
<tr>
<td>F50 CV BMI</td>
<td>11.7±7.0 (b)(e)</td>
<td>33.6±15.2 (b)(e)</td>
<td>18.2±14.6 (e)(c)</td>
<td>(a, e) n.s. (b,c) p&lt;0.001</td>
</tr>
<tr>
<td>F50 Hard</td>
<td>0.9±0.2 (b)(e)</td>
<td>4.2±5.1 (b)(c)</td>
<td>1.8±2.5 (c)(e)</td>
<td>(a, e) n.s. (b,c) p&lt;0.001</td>
</tr>
</tbody>
</table>

(a), non significant (n.s); (b) significant difference between column 1 and 2; (c) significant difference between column 1 and 3; (d) significant difference between column 1 and 3; (e), non significant (n.s) difference between column 2 and 3; (f), non significant (n.s) difference between column 1 and 2.

BMI: body mass index; F50 CV L: Framingham Score cardiovascular events to 30 years based in lipid profile; F50 CV BMI: Framingham Score cardiovascular events to 30 years based in BMI; F50 Hard: Framingham Score for hard events to 30 years.

### Table 1

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Effects of a PCSK9 inhibitor, alirocumab, on lipid and lipoprotein metabolism in normal subjects

Abstract nr. 644
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Co-author(s) - Cowan, Lisa
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords LDL, Lipoproteins, Metabolism, PCSK9

Background: Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors are promising new drugs for the treatment of hypercholesterolemia. They inhibit the binding of PCSK9 to the low density lipoprotein (LDL) receptor that, in turn, decreases lysosomal degradation of LDL receptors and increases their numbers on the cell surface. In Phase 2/3 studies, alirocumab significantly lowered plasma levels of LDL-cholesterol (C) and apolipoprotein B (apoB). The kinetic mechanism underlying the LDL-C lowering effects of PCSK9 inhibition has not been reported.

Method: We enrolled 10 healthy volunteers (4 male, 6 female), into a Phase 1, placebo-controlled, single-blind, single-sequence study (NCT01959971) to examine the effects of alirocumab, 150 mg administered subcutaneously every two weeks, on lipid and lipoproteins levels and the metabolism of apoB in very low density (VLDL), intermediate density (IDL), and LDL. Subjects received 2 doses of placebo followed by 5 doses of alirocumab. At the end of each treatment period, we measured fasting lipids and lipoprotein levels, and performed stable isotope studies of the apoB turnover in VLDL, IDL, and LDL.

Results: Alirocumab significantly reduced plasma levels of total-C by 37%, from 178.4±34 to 112.7±29 mg/dL, LDL-C by 59%, from 110.2±25 to 45.5±26 mg/dL, and apoB by 51%, from 93.6±25 to 45.5±13 mg/dL compared to placebo. Plasma triglycerides (TG) and HDL-C did not change. Levels of C, TG, and apoB fell in IDL and LDL (Table). The reductions in LDL apoB were explained by a dramatic increase in the fractional clearance rate (FCR) of LDL apoB from 0.50±0.18 on placebo to 1.02±0.35 pools/day on alirocumab (p<0.001), and a trend toward lower LDL apoB production rate on alirocumab (15.1±4.6 vs 12.9±3.3 mg/kg/day; p=0.10). There was also a trend toward an increase in IDL apoB FCR on alirocumab (9.2 vs 10.8 pools/day; p=0.06). No serious adverse event or treatment discontinuation occurred. Mild injection-site reactions were
reported. Additional kinetic parameters will be presented at the meeting.

**Summary:** Alirocumab treatment caused significant decreases in the levels of IDL and LDL that were due to increases in the FCRs of these lipoproteins, particularly LDL. Study was funded by Sanofi and Regeneron Pharmaceuticals, Inc.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (mg/dL)</th>
<th>Alirocumab (mg/dL)</th>
<th>% change</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL-C</td>
<td>13.1±9</td>
<td>9.4±6</td>
<td>-7.9±62</td>
<td>0.70</td>
</tr>
<tr>
<td>IDL-C</td>
<td>5.9±3</td>
<td>3.1±1</td>
<td>-36.4±36</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL-C</td>
<td>95.3±19</td>
<td>42.0±14</td>
<td>-55.8±10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VLDL-TG</td>
<td>58.5±44</td>
<td>53.8±42</td>
<td>+6.6±55</td>
<td>0.76</td>
</tr>
<tr>
<td>IDL-TG</td>
<td>7.6±4</td>
<td>5.5±2</td>
<td>-20.3±25</td>
<td>0.03</td>
</tr>
<tr>
<td>LDL-TG</td>
<td>14.6±4</td>
<td>9.3±2</td>
<td>-33.9±13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VLDL-apoB</td>
<td>7.8±8</td>
<td>5.6±4</td>
<td>-3.4±53</td>
<td>0.84</td>
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<tr>
<td>IDL-apoB</td>
<td>3.8±2</td>
<td>2.5±1</td>
<td>-26.3±29</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL-apoB</td>
<td>71.2±24</td>
<td>30.3±11</td>
<td>-56.0±11</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
ASSESSMENT OF sdLDL-C, Lp(a), AND OTHER RISK FACTORS OF HEART DISEASE IN THE AZORES

Abstract nr. 645
Author Ferin Borges, Rita, University of the Azores, Ponta Delgada, Portugal
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Co-author(s) - Baptista, José
Co-author(s) - Pavão, Maria Leonor
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Apolipoproteins, Atherosclerosis, Lp(a), Risk Factor

In the Azores age adjusted death rates from coronary heart disease (CHD) are two-fold higher than in mainland Portugal. We determined the prevalence of standard and emerging CHD risk factors in 206 female and 146 male Azorean subjects (mean age 41 years). With regard to body mass index (BMI) in this population 51% of the men and 36% of the women were overweight (BMI > 25 kg/m2), while 23% of the men and 33% of the women were obese (BMI > 30 kg/m2). Also 36% of the men and 13% of the women were smokers, while 51% of the men and 42% of the women were hypertensive. Only 15% were taking anti-hypertensive medication, and 11% were on lipid medications. Low density lipoprotein (LDL) cholesterol levels > 160 mg/dL were seen in 31% in men and 23% in women, while high density lipoprotein (HDL) cholesterol < 40 mg/dL was found in 21% of the men and 2% of the women. Serum apoB levels > 120 mg/dL were observed in 35% of the men and 27% of the women, while 64% of the men and 52% of the women had small dense LDL cholesterol levels > 40 mg/dL (seen in 25% of participants in the Framingham Offspring Study, mean age 58 years). Lp(a) > 30 mg/dL was noted in 36% of the men and 32% of the women. Levels of C reactive protein > 2.0 mg/L were registered in 36% of the men and 58% of the women. In this middle aged asymptomatic population of Azorean subjects there was a high prevalence of being overweight, obese, hypertensive, and having elevated levels of LDL-C and apoB. Moreover more than half the subjects had elevated levels of small dense LDL cholesterol, an emerging risk factor for premature CHD.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Mini-oral presentation
Additional information
LOMITAPIDE TREATMENT HIGHLY AFFECTS SERUM FUNCTIONS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

Abstract nr. 646
Author Favari, Elda, University of Parma, Parma, Italy
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Co-author(s) - Yahya, Reyhana
Co-author(s) - Averna, Maurizio
Co-author(s) - Gomaraschi, Monica
Co-author(s) - Calabresi, Laura
Co-author(s) - Roeters van Lennep, Jeanine
Co-author(s) - Mulder, Monique
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Functionality, HDL, Pharmacology

Lomitapide is an approved cholesterol lowering agent. Cholesterol Efflux Capacity (CEC), as index of HDL functionality, was impaired in FH patients. The aim of this work was to evaluate Lomitapide effect on serum lipoproteins and HDL functionality in four European severe FH patients. Serum CEC and cholesterol loading capacity (CLC) were measured on cells-based assays to either serum depleted of apoB-containing lipoproteins and whole serum, respectively. Lomitapide treatment reduced VLDL-C and LDL-C up to 80% and 60%, respectively. LDL shifted to larger buoyant particles and HDL-C levels initially decreased but seemed to return to baseline when treatment was maintained. All patients showed decrease of the HDL3 subpopulation. Total CEC from human macrophages to serum from P1 was reduced after treatment with 10mg of Lomitapide compared to baseline (p<0.01) but it was either slight increased or not changed through the increasing dosage of drug for all the other FH patients. SR-BI-CEC decreased (average -30%; p<0.05) in parallel to HDL-C levels and in P2 and P3 it increased back to baseline when drug was titrated up to 20-30mg/day, respectively. ABCG1-CEC increased after treatment in P2, P3 and P4 (average +40%; p<0.05) and was not modified in P1, independently of HDL-C levels. ABCA1-CEC from serum of P3 increased back to baseline at the highest dosage of the drug, consistently with pre-beta particle levels. Except that for P1, sera from P2, P3 and P4 displayed a significantly reduced capacity to load cells with cholesterol after drug treatment compare to baseline. In severe FH patients Lomitapide treatment, beyond its high cholesterol-lowering efficacy, induces significant HDL structural changes that impact on HDL functionality, independently of total HDL-C plasma levels. Whether Lomitapide is overall beneficial or detrimental with respect to serum cellular cholesterol handling on the basis of this small series of cases remains to be determined, but it
appears that drug treatment affects the serum pro-atherogenic properties of FH subjects.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Vascular effects of intraperitoneal applied sterols, phytostereols and oxyphytosterols in apoE-/mice

Abstract nr. 647
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Co-author(s) - Böhm , Michael
Co-author(s) - Plat , Jogchum
Co-author(s) - Laufs , Ulrich
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Endothelium,Lipids,Metabolism

Purpose: Plant sterol esters (PSE) are used as food supplements to reduce serum cholesterol levels. Both plant sterols and cholesterol are prone to oxidation and convert to oxysterols and oxyphytosterols. The effects of oxysterols and oxyphytosterols on reactive oxygen species (ROS), endothelial function and atherogenesis in apoE-/-mice are not known.

Methods: maleapoE-/mice were subjected to intraperitoneal application of cholesterol, sitosterol, 7-beta-hydroxycholesterol, 7-beta-hydroxysitosterol or cyclodextrin solution as control over a time period of 4 weeks. All animals had access to ad libidum water and cholesterol free normal chow. After 4 weeks we determined sterol, oxysterol, phytosterol and oxyphytosterol levels in serum, ROS activity assessed by spin trap in aortic tissue, endothelial function of aortic rings and atherosclerosis in the aortic sinus (n=10 per group).

Results: Compared to control i.p. application of cholesterol showed no difference in regard to plasma cholesterol levels (379±111 mg/dl vs. 381±41 mg/dl), but resulted in a significant decrease in lanosterol levels (156±54 vs. 118±6 µg/dl). Likewise, the i.p. application of 7-beta-hydroxy-cholesterol (0,013±0,094 mg/dl vs. 0,196±0,094 mg/dl) and 7-beta-hydroxysitosterol (346±167 vs. 4899±1111 ng/ml) increased respective plasma levels compared to controls. However, this effect was not seen for the phytosterol sitosterol (40±27 vs.16±6 ng/ml). In regard to oxidative stress in aortic tissue we found a significant increase in 7-beta-hydroxy-sitosterol treated mice compared to control (increase compared to control by 157,7±48,9), but no effect by cholesterol (91,9±67,5%), sitosterol (106,4±12,5%) and 7-beta-hydroxy-cholesterol (109,0±52,1%). Compared to controls, neither cholesterol nor sitosterol, or 7-beta- hydroxyl-cholesterol and 7-beta-hydroxy-sitosterol affected endothelial function. Atherosclerotic lesions were evaluated by oil-red-O-staining in the aortic sinus. Compared to controls there was no difference in cholesterol treated mice (17,2±8,5 vs. 14,5±9,8 %), sitosterol treated mice (17,0±9,2 %), 7-beta-hydroxy-cholesterol (7,9±4,5 %) and 7-beta-hydroxy-sitosterol treated mice (10,1±6,4 %).

Conclusions: Increased oxyphytosterol plasma levels increase ROS activity in aortic tissue, but and do not affect endothelial function and atherosclerosis in apoE-/-mice.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information

Abstract nr. 648
Author Kempen, Herman, The Medicines Company, Zürich, Switzerland
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Co-author(s) - Asztalos, Bela
Co-author(s) - Otvos, James
Co-author(s) - Eralp, Bellibas
Co-author(s) - Wijngaard, Peter
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords HDL, Lipoproteins, Pharmacology, Therapy

Introduction: MDCO-216, a complex of dimeric recombinant apolipoprotein A-1 Milano (ApoA-1 M) and a phospholipid (POPC), is currently under development to improve cardiovascular outcomes by reducing plaque burden in patients with atherosclerotic disease. The purpose of this study was to assess how basal and ABCA1-mediated cholesterol efflux capacities from J-774 macrophages correlated with the levels of various HDL subfractions after a single administration of MDCO-216 in healthy volunteers and coronary artery disease patients.

Methods: 16 healthy volunteers and 16 CAD patients received a single 2 hour IV infusion of MDCO-216 (5, 10, 20, 30 or 40 mg/kg). Ex-vivo basal and ABCA1-mediated cholesterol efflux capacities and levels of HDL-subfractions (prebeta-1, alpha-1, alpha-2, alpha-3, alpha-4 by 2-D electrophoresis), prebeta-1 HDL (by ELISA) and small, medium or large HDL-particles (by 1H-NMR) were used as pharmacodynamic biomarkers. Areas-under-the-effect-curve (AUEC) for these biomarkers were calculated for the 0-24 h period for all 32 participants. AUEC for basal and ABCA1-mediated efflux were then correlated with the AUEC of the various HDL biomarkers.

Results: AUECs for basal and ABCA1-mediated efflux correlated positively with AUECs for prebeta-1 ELISA and alpha-1 HDL, and inversely with that for small HDL-P (Table 1). In multiple regression analysis AUECs for prebeta-1-ELISA and alpha-1 were each independently associated with AUECs for both efflux capacities.

Conclusions: The results support the hypothesis that MDCO-216 rapidly fuses with small HDL and creates new alpha-1 particles (shown earlier to contain both apoA-IM and apoA-I wild type), and new prebeta-1 HDL (shown earlier to contain only apoA-I wild-type) which can both contribute to increased basal and ABCA1 efflux capacity.
Table I. Pearson correlation coefficients for AUEC of basal and ABCA1-mediated efflux capacities with AUEC of HDL subfractions (n=32)
(correlations in parentheses are not significant)

<table>
<thead>
<tr>
<th></th>
<th>AUEC_{0.24} (baseline)</th>
<th>AUEC_{0.24} (ABCA1 mediated efflux)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUEC_{0.24}(Prebeta-1 ELISA)</td>
<td>0.72</td>
<td>0.74</td>
</tr>
<tr>
<td>AUEC_{0.24}(Prebeta-2D)</td>
<td>(0.32)</td>
<td>0.47</td>
</tr>
<tr>
<td>AUEC_{0.24}(alpha-1 2D)</td>
<td>0.68</td>
<td>0.76</td>
</tr>
<tr>
<td>AUEC_{0.24}(alpha-2 2D)</td>
<td>(0.28)</td>
<td>(0.24)</td>
</tr>
<tr>
<td>AUEC_{0.24}(alpha-3 2D)</td>
<td>-0.41</td>
<td>(-0.28)</td>
</tr>
<tr>
<td>AUEC_{0.24}(alpha-4 2D)</td>
<td>(0.06)</td>
<td>(0.34)</td>
</tr>
<tr>
<td>AUEC_{0.24}(large HDL-P)</td>
<td>0.55</td>
<td>(0.20)</td>
</tr>
<tr>
<td>AUEC_{0.24}(medium HDL-P)</td>
<td>(0.25)</td>
<td>(0.23)</td>
</tr>
<tr>
<td>AUEC_{0.24}(small HDL-P)</td>
<td>-0.55</td>
<td>-0.53</td>
</tr>
</tbody>
</table>

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Interferon-beta promotes macrophage foam cell formation

Abstract nr. 649

Author Boshuizen, Marieke, Academic Medical Center Amsterdam, Amsterdam, Netherlands
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Co-author(s) - Velden, Saskia van der
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Co-author(s) - Winther, Menno de

Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Atherosclerosis, Lipids

Foam cell formation is a crucial event in atherogenesis. Previously we demonstrated that interferon-beta (IFN-β) of mice promotes atherogenesis. However, studies on the effect of IFN-β on foam cell formation are minimal and conflicting. In a previous study we did not observe any effect of IFN-β on oxLDL uptake in murine bone marrow derived macrophages (BMDMs). But as it is of great interest to understand the way in which IFN-β may influence foam cell formation, we further extended these studies and focused on the role of IFN-β in different models of foam cell formation. We loaded BMDMs with acLDL overnight, and added IFN-β over the last 6h, resulting in increased lipid loading as measured by Oil red O staining. This increased lipid loading was accompanied by increased expression of scavenger receptor-A (SR-A), while CD36 was unaffected. We further analyzed the uptake pathways of lipid loaded BMDMs that had been pretreated with IFN-β and observed increased endocytosis of DiI-acLDL as compared to controls. These effects were mediated via SR-A, as inhibition of SR-A with Poly:I blocked the IFN-β-induced increase in Oil red O staining and DiI-acLDL endocytosis. To validate our findings in vivo, LDLR-/mice were put on normal chow (NC) or a high cholesterol diet (HCD) for 10 weeks. Peritoneal macrophages (PEMs) were collected 4 days after intraperitoneal thioglycollate administration, combined with an IFN-β (5000 U/ml) or PBS injection 24 and 8 hours before sacrifice. In accordance with our in vitro data, lipid loading increased following IFN-β treatment, accompanied by increased scavenger receptor-A (SR-A) gene expression. In addition, ex vivo culturing of PEMs from IFN-β-treated versus PBS-treated animals showed increased phagocytosis of fluorescently labeled latex beads. These results confirm our previous findings that IFN-β is a pro-atherosclerotic cytokine, as we now show that it promotes lipid accumulation in different models of foam cell formation, likely involving increased lipid influx via SR-A.

This work was supported by the Dutch Heart Foundation, grant #2010B022.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation

Additional information
Association of polymorphisms of endothelial NO-synthetase gene with the type of dyslipidemia in patients with arterial hypertension and metabolic syndrome

Abstract nr. 650
Author Bondar, Vadym, Odessa National Medical University, Odessa, Ukraine
Co-author(s) - Oliynyk, Dmitriy
Co-author(s) - Vasylets, Viktoria
Co-author(s) - Chernyshova, Kateryna
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Dyslipidemia, Genetics, Hypertension

Dyslipidemia and its features in patients with arterial hypertension (AH) and metabolic syndrome (MS) may be associated with endothelial dysfunction and determined by polymorphisms of endothelial NO-synthetase (eNOS) gene. The purpose of the research: analysis of the distribution of polymorphisms of eNOS gene and assessment of the features of dislipidemia depending on the distribution of the isolated and combined polymorphisms of eNOS gene in patients with AH and MS in Ukrainian population. Materials and methods: 128 patients with AH and MS were examined (ISH 2011, IDF 2007). In the surveyed group polymorphisms T(-786)C and G894T of eNOS gene were determined by PCR. Subsequently, the patients were divided into subgroups with isolated polymorphism T(-786)C (subgroup 1), with isolated polymorphism G894T (subgroup 2), with combination of two polymorphisms (subgroup 3) and with "normal genotypes" of eNOS gene (subgroup 4). In each subgroup we additionally studied the type of dyslipidemia (Fredrickson’s classification). In the surveyed group, the frequency of isolated polymorphism T(-786)C was (17,9%/23), isolated polymorphism G894T - (29,6%/38), combination of polymorphisms T(-786)C and G894T - (35,9%/46), "normal genotypes" of eNOS gene - (16,4%/21). There is a high frequency of occurrence of isolated polymorphism G894T and combination of polymorphisms compared to isolated polymorphism T(-786)C and “normal genotypes” of eNOS gene. Type IIa dyslipidemia (with elevation of LDL and total cholesterol levels) was predominant in subgroup (1) (58%, r = 0,64, p<0,01); in subgroup (2) - type III dyslipidemia (64%, r = 0,72, p<0,001) with elevation of IDL, total cholesterol and triglycerides levels; in the subgroup (3) - type IIb dyslipidemia (58%, r = 0,66, p<0,01) with elevation of LDL and VLDL, total cholesterol and triglycerides levels; in the subgroup (4) - type III dyslipidemia (42%, r = 0,56, p<0,01). Thus, the type of dyslipidemia in patients with AH and MS is associated with polymorphism of eNOS gene. This association indicates the relationship of endothelial dysfunction with impaired lipid metabolism. The most unfavorable polymorphism, which is associated with dyslipidemia of high CVR, is a combination of polymorphisms T(-786)C and G894T of eNOS gene in Ukrainian population.
Presentation Preference: Electronic poster presentation
Additional information
Application of ACC/AHA guidelines significantly increases eligibility for statin therapy in management of atherosclerotic cardiovascular risk in Chilean adult population

Abstract nr. 651
Author Attilio, Rigotti, Pontificia Universidad Católica de Chile, Santiago, Chile
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Co-author(s) - Dussallaint, Catalina
Co-author(s) - Villarroel, Luis
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology, Guidelines, LDL

Introduction. Guidelines of the Adult Treatment Panel III (ATPIII) from the National Cholesterol Education Program (NCEP) were one of the major tools influencing clinical practice for cardiovascular disease (CVD) risk assessment and high cholesterol management. In 2013, the American College of Cardiology (ACC) and the American Heart Association (AHA) jointly released new guidelines that substantially modified and expanded previous ATPIII NCEP recommendations. The relative impact of these guidelines on potential statin use has been compared in US and European, but not Latin American, populations.

Aim. To estimate eligibility for statin therapy based on ACC/AHA -compared with ATPIII NCEP-guidelines in Chilean adult population using data from the National Health Survey performed in 2009-2010 (NHS 2009-2010).

Methods. We analyzed data of 2,529 adults over 20 years of age included in NHS 2009-2010 who had valid information regarding CVD risk assessment and LDL cholesterol levels (47% of the complete original sample studied in NHS 2009-2010).

Results. Based on ACC/AHA guidelines, 33.6% of the Chilean adult population would be eligible for statin treatment, in contrast to 21.6% estimated under ATPIII NCEP criteria. ACC/AHA guidelines increase the population eligible for statin treatment regardless of gender, education and age, even though its effect is more pronounced among women (27.2% under ACC/AHA versus 10.5% under ATPIII NCEP) and with advanced age (among elderly (≥ 65 years old): 70.2% under ACC/AHA versus 35.4% under ATPIII NCEP). In addition, new ACC/AHA guidelines lead to a much larger statin eligibility among low and moderate ATPIII NCEP CVD risk categories. Furthermore, concordance analysis between guidelines shows 19.4% discordant recommendation for statin treatment: 3.8% of subjects eligible for therapy under ATPIII NCEP would no longer be eligible under ACC/AHA, whereas 15.6% of individuals eligible under ACC/AHA should not use statins based on ATPIII NCEP.

Conclusion. Compared to ATPIII NCEP, new ACC/AHA guidelines would increase the number of Chilean adults eligible for statin therapy by 56%, with a more pronounced impact among women, older adults and low risk subjects without cardiovascular disease.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Tetrahydrobiopterin improves diabetic vascular dysfunction by regulating ROCK pathway

Abstract nr. 652
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Co-author(s) - Ko, Kyung Soo
Co-author(s) - Rhee, Byoung Doo
Co-author(s) - Han, Jin
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Cardiovascular Disease, Diabetes

Abstract
Vascular dysfunction plays key role in the pathogenesis of cardiovascular disorders in diabetes. The aim of this study was to compare vascular structure and function between control and diabetic rats. Furthermore, the treatment of tetrahydrobiopterin (BH4) has been investigated to restore the pathological changes of vascular structure and function in diabetic rats.

Otsuka Long-Evans Tokushima Fatty (OLETF) rats were divided into the vehicle- and BH4-treated groups. Long-Evans Tokushima Otsuka (LETO) rats were used as age-matched non-diabetic controls. Aorta and renal artery were dissected and isometric tensions of them were measured with phenylephrine (PE) and acetylcholine (Ach). Histological analysis and western blot analysis were performed to evaluate the related mechanism of vascular dysfunction in diabetic animal.

In the experiment of isometric tensions, PE-induced contraction was augmented and Ach-induced relaxation was attenuated in the aorta of OLETF compared to in those of LETO and BH4-treated group, which were restored by BH4. Interestingly, the renal artery from BH4-treated group exhibited a greater contraction and a lesser relaxation than the LETO and OLETF rats. In the histological study, the tunica media of aorta and of renal artery were significantly thicker in OLETF and in BH4-treated groups, respectively. The thickness of the collagen deposition in OLETF groups were increased than other groups. In western blot analysis, the ROCK2 protein levels in OLETF group were significantly increased compared with other groups and were restored to the level of control by BH4.

BH4 restores the impairment of contraction and relaxation in OLETF with restoration of vascular structure through regulation of ROCK signaling. This result suggests that BH4 has a potential usefulness as pharmacological management of DM-associated vascular dysfunction.

Key words: DM, Vascular dysfunction, Tetrahydrobiopterin, ROCK pathway.

Source of Funding
This work was supported by the National Research Foundation (NRF) of Korea and the funding was granted by the Ministry of Science, ICT & Future Planning of Korea (2011-0028925), by the Ministry of Education of Korea (2010-0020224).
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
The persisting pro-atherogenic effects of transient glucose exposure are equivalent to that observed in chronic hyperglycemia with diabetes

Abstract nr. 653
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Co-author(s) - Cooper, Mark
Co-author(s) - El-Osta, Assam
Co-author(s) - Thomas, Merlin
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Diabetes

Diabetes casts a long shadow over the lives of many people. It is now clear that even transient hyperglycemia can have long-lasting effects on the development and progression of diabetic complications. This ‘metabolic karma’ may explain why many patients with pre-diabetes manifest diabetic complications, including atherosclerosis, and why even brief periods of poor control in patients with diabetes may have a sustained adverse legacy. To explore this phenomenon, male C57Bl6 mice were randomised to receive four sequential injections of D-glucose (3g/kg IP) or an equivalent volume of saline or L-glucose delivered two hours apart. This protocol produces a sustained elevation in plasma glucose levels (15-25mM) for 8 hours, after which time no difference in plasma glucose levels is detectable between treated and control mice. Repetition of the transient hyperglycaemia protocol once every week for ten weeks in complications-prone apolipoprotein E KO mice led to the development of atherosclerosis comparable to that observed in streptozotocin induced diabetic mice. To explore the potential mechanism, aortae were isolated from mice one week after transient glucose exposure. These showed persistent up-regulation of ICAM-1, VCAM-1, MCP-1 and NFKB expression. Leukocyte recruitment was also increased and labelled human leucocytes showed increased adhesiveness to previously glucose-exposed aortae when exposed ex vivo. We have previously shown stable epigenetic modifications may be acquired through this protocol, so to explore the potential role of epigenetics in these functional changes, heterozygous mice for the methyltransferase SET7 were exposed to the same glucose load and notably failed to show induction of inflammation or increased adhesiveness. These experimental data support the hypothesis that intermittent glucose excursions, as occur in pre-diabetes, can have pro-atherogenic effects, potentially through the induction of stable epigenetic changes and support the call for their early treatment.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Unhealthy diet is associated with high prevalence of metabolic syndrome in Chilean adults: a cross sectional analysis from the NHS2009-2010

Abstract nr. 654
Author Attilio, Rigotti, Pontificia Universidad Católica de Chile, Santiago, Chile
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Co-author(s) - Echeverría, Guadalupe
Co-author(s) - Villarroel, Luis
Co-author(s) - Marin, Pedro Paulo
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Epidemiology,Nutrition,Prevention,Risk Factor

Introduction. The metabolic syndrome (MS) is a clustering of risk factors known to promote cardiovascular disease and diabetes. Genetic background, as well as environmental factors such as obesity, physical inactivity and unhealthy diet, plays a major role in the development of this condition. The aim of this study was to analyze the prevalence of MS and its components among Chilean adults and its association with food intake quality in this population, using data from the National Health Survey 2009-2010 (NHS2009-2010).

Methods. We analyzed data of 2,561 adults (≥ 18 years of age) included in NHS2009-2010 survey who had appropriate information to diagnose MS following ATP III guidelines. Consumption frequency of fish, whole grains, dairy, fruits and vegetables was also analyzed and associated with MS prevalence. By creating a Healthy Diet Score (HDS), we integrated the information available on food intake in NHS2009-2010 in order to describe overall diet quality and to further analyze its correlation with MS prevalence.

Results. The prevalence of MS in the Chilean adult population was 34.2%, being higher in women (36.9%) than men (30.2%). The most common components of MS were high blood pressure (48%) and low HDL cholesterol (47%). Among women, the most frequent criteria were abdominal obesity (56.9%) and low HDL cholesterol (54%). In men, the most common components were high blood pressure (54%) and high triglycerides (45%). Chilean adults showed low intake of all foods studied, particularly fish (only 11% consumed fish more than once a week). We also found an inverse association between whole grains and dairy intake with MS prevalence. HDS showed better diet quality among women and in subjects with increasing age and higher educational level. A HDS ≥ 3 points was significantly associated with lower prevalence of MS (Odds ratio HDS <3/HDS ≥3 = 2.794; p value = 0.003).

Conclusion. Based on the most recent nation health survey, Chilean adult population exhibits a high prevalence of MS linked to a poor diet quality. This situation predicts an increased risk of cardiovascular disease and diabetes that may be prevented by improving lifestyle, including food intake habits.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
DUSP4 deletion in bone marrow-derived cells reduces atherosclerosis in LDL receptor knockout mice.

Abstract nr. 655
Author Ren, Baoyan, Leiden Academic Centre for Drug Research, Leiden, Netherlands
Co-author(s) - Plevin, Robin
Co-author(s) - Van Eck, Miranda
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Inflammation

Dual specificity phosphatase 4 (DUSP4) controls the immune response by inactivating MAP kinases, including JNK and regulates cell proliferation and apoptosis. Until now, the role of DUSP4 in atherosclerosis is unknown. Microarray analysis showed that DUSP4 expression in the carotid artery is rapidly downregulated during collar-induced lesion development. In the later stages of lesion development a slight recovery is observed, possibly due to the infiltration of inflammatory cells. In order to investigate the role of DUSP4 in atherosclerosis, bone marrow from DUSP4 knockout (KO) mice and wildtype animals was transplanted into male LDL receptor knockout (LDLr KO) mice. DUSP4 deletion ablated JNK mRNA expression in peritoneal leukocytes and spleen after 12 weeks Western-type diet (WTD) feeding. In addition, the expressions of several chemokines in spleen, including CCL1, CCL2, CCL5, CXCL5, and CXCL9 were decreased. Hematological analysis showed no differences in circulating blood cells. However, in peritoneum neutrophils and lymphocytes were reduced. In spleen, also reduced amounts of neutrophils were detected (0.7-fold, p<0.05). No effects on absolute lymphocyte counts were found in spleen, but the percentages of anti-inflammatory CD4+CD25+FOXP3+ Tregs and CD4+GATA3+ Th2 cells were augmented (1.69-fold p<0.05 and 1.52-fold p<0.05, respectively). Importantly, DUSP4 KO transplanted mice showed a 34% reduction in atherosclerotic lesion size (p<0.01) after 12 weeks WTD challenge. In line with the decreased lesion size, the macrophage content was reduced (p<0.01). No difference in collagen content of the lesions was found, suggesting that DUSP4 deficiency in macrophages does not affect plaque stability. Notably, the observed reduction in lesion development in DUSP4 KO transplanted mice coincided with lower amounts of plasma cholesterol in VLDL and LDL (0.53-fold, p<0.05 and 0.74-fold, p<0.05). In conclusion, deletion of DUSP4 in bone marrow decreases atherosclerosis susceptibility of LDLr KO mice, probably due to lowered cholesterol in the circulation and suppression of inflammatory responses by less pro-inflammatory neutrophils, and more anti-inflammatory Tregs and Th2 cells. This work is supported by the Netherlands CardioVascular Research Initiative for the GENIUS project “Generating the best evidence-based pharmaceutical targets for atherosclerosis” (CVON2011-19) and the China Scholarship Council.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
High-density Lipoprotein Profile in Women with Polycystic Ovary Syndrome

Abstract nr. 656
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords HDL, Lipoproteins

Altered lipid profile is common in polycystic ovary syndrome (PCOS) with increased triglycerides and low high-density lipoprotein (HDL)-cholesterol levels. Recent findings indicate that these women also have altered lipoproteins, with increased atherogenic small dense low-density lipoproteins (sdLDL). However, their HDL subclass distribution is unknown. We therefore aimed to evaluate in this study the HDL profile in PCOS compared to non-PCOS.

Seventy-four women (49 with PCOS and 25 non-PCOS) were enrolled in the study. PCOS was diagnosed by ESHRE/ASRM criteria. Exclusion criteria included pregnancy, smoking, type-2 diabetes, uncontrolled hypertension and use of relevant medications (anti-hypertensives, lipid lowering, fish oil). Participants using hormonal (e.g. oral contraceptive pill) or insulin sensitizing medication were excluded unless willing to cease medication use 3 months prior the study.

Fasting plasma samples were collected for laboratory analyses, including 5 distinct HDL subclasses by gel electrophoresis. Statistical analysis was performed by Mann-Whitney test and the Spearman correlation.

In relation to non-PCOS, we found in PCOS women significantly higher body weight (p=0.004), waist circumference (p=0.001), body mass index (p=0.001), insulin (p=0.029), C-reactive protein (p=0.002), HOMA (p=0.046) and asymmetric dimethylarginine (p=0.019), while plasma lipids did not differ significantly. Regarding HDL subclass distribution, PCOS had decreased HDL2b (49.1±8.3 vs 53.4±8.1, p=0.041) and increased HDL3a particles (14.8±3.4 vs 12.5±3.4, p=0.005). In addition, HDL2b particles correlated negatively with triglycerides (r=-0.367, p=0.01), and positively with HDL-C (r=0.566, p=0.01), while HDL3a particles correlated negatively with HDL-C (r=-0.503, p=0.01).

Women with PCOS have decreased larger and increased smaller HDL particles even when there are no differences in the classic lipid profile. However, future prospective studies are required for the full investigation of altered lipoprotein profile and its relative contribution to CV risk.
Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Comparative ultrasonographic morphology of carotid artery between human newborn and Macaca nemestrina

Abstract nr. 657
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis

Purpose: The aims of the study was to compare the ultrasonographic morphology of carotid artery between human newborn and Macaca nemestrina.

Methods: This cross sectional study had been involved a human newborn subgroup and the Macaca subgroup. The assay of serum lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride) and B-mode USG of carotid intima-media had been done in both groups. Two of the Macacas were sacrificed for histologic examination. The common carotid artery was divided into 3 equal segments: proximal segment, mid segment and distal segment. Mid dorsum longitudinal cutting was done for each segment before fixation with buffer formalin. Serial longitudinal section and staining with Verhoef Van Gieson had been done.

Result: The mean total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride of the newborn subgroup were (45 ± 7.65) mg/dl, (21.15 ± 5.12) mg/dl, (17.85 ± 4.20) mg/dl and (24.95 ± 9.27) mg/dl . The mean total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride of the macaca subgroup were (90.3 ± 19.36) mg/dl, (45.70 ± 14.53) mg/dl, (36.90 ± 7.37) mg/dl, (35 ± 11.92) mg/dl.
The mean left common carotid artery and right common carotid artery diameter of the newborn subgroup were (2.92 ± 0.26) mm and (2.85 ± 0.28) mm. The mean left common carotid artery and right common carotid artery diameter of the macaca subgroup were (2.69 ± 0.25) mm and (2.51 ± 0.26) mm.
The ultrasonographic morphology of human newborn carotid artery was similar with Macaca nemestrina; it showed no intima media thickening (zero IMT). Histopathologic examination of Macaca’s carotid artery also showed the lamina elastica interna was covered by the endothelial cells.

Conclusions: The quality of human newborn carotid artery was similar with Macaca nemestrina carotid artery intern of no process of atherosclerosis with the level of LDL cholesterol < 50 mg/dl; and it's the golden quality of human blood vessel.

Subdivision 2. Translational Research
Presentation Preference Electronic poster presentation
Additional information
OxLDL-induced Apoptotic Dendritic Cells as a Novel Therapy for Atherosclerosis

Abstract nr. 658
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Immunity, Therapy

Modulation of immune responses may form a powerful approach to treat atherosclerosis. It has been shown that clearance of apoptotic cells by Dendritic Cells (DC) may results in immunological tolerance induction to the cleared antigens. This mechanisms appear impaired in atherosclerosis as antigen-specific tolerance is lacking. This lack of tolerance may partially result from reduced emigration of DCs from atherosclerotic lesions as a result of the high cholesterol environment. Nonetheless, already local induction of anti-inflammatory responses by apoptotic cell clearance may dampen atherosclerosis, as inhibition of apoptotic cell clearance was shown to worsen atherosclerosis. In this study we assessed whether intravenous administration of oxLDL-induced apoptotic (apop^OX^DCs), and as a control unpulsed apoptotic DCs (apop^ctrl^-DCs), could modulate atherosclerosis by inducing antigen specific tolerance.

Adoptive transfer of apop^OX^-DCs into LDLrKO mice either before or during Western-type diet resulted in increased numbers of CD103^+^ tolerogenic splenic DCs, with a concomitant increase in regulatory T cells (+78%, p<0.05). Interestingly, both types of apoptotic DCs induced an immediate 40% decrease in Ly-6C^hi^ monocyte numbers (P<0.05) and a 50% decrease in circulating CCL2 levels (p<0.05), but only apop^OX^-DCs were capable of sustaining the effects on monocytes and CCL2 levels for 9 weeks. While initial atherosclerotic lesion development was reduced by 40% in both treatment groups (p<0.05), only apop^OX^-DC-treatment were able to prevent lesion progression by 28% (P<0.05). Interestingly these lesions displayed enhanced stability, determined by a robust 45% increase in collagen content (p<0.05).

Our findings clearly show that apoptotic DC-treatment can significantly decreases atherosclerotic lesion development, but only apop^OX^-DCs can positively modulate atherosclerotic lesion progression and stability. Our findings may initiate future studies to assess the therapeutic potential of autologous oxLDL-induced apoptotic DC transfer for patients with established atherosclerotic disease.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Carotid intima-media thickness reduced after 8-month of liraglutide therapy in patients with type-2 diabetes and non-alcoholic fatty liver disease

Abstract nr. 659
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Diabetes, Therapy

There is a growing scientific interest in non-glycemic effects of liraglutide beyond its effects on glucose metabolism. Several its anti-atherogenic actions have been demonstrated including those on subclinical atherosclerosis. However, it is unknown about its potential benefits in subjects with non-alcoholic fatty liver disease (NAFLD).

This was an 8-month prospective study included 29 subjects with type-2 diabetes (T2DM) and NAFLD (16 men and 13 women, mean age: 61±10 years), who were matched for age and gender with 29 subjects with T2DM only (16 men and 13 women, mean age: 61±8 years). The NAFLD was diagnosed based on biochemical data and ultrasonography findings. All subjects were naïve to incretin-based therapies and treated with metformin only. Liraglutide was given, on top of metformin (0.6 mg/day for two weeks, followed by 1.2 mg/day for the rest of the study). At baseline and every 4 months fasting plasma samples were analyzed as well as carotid-intima media thickness (cIMT) was assessed by B-mode real-time ultrasound. Statistical analysis was performed by ANOVA and the Spearman correlation method.

From baseline to 4 and 8 months of liraglutide therapy significant reductions were found in HbA1c in both groups (from 8.9±1.5 to 6.6±1.2 to 6.5±1.1% in subjects with T2DM and NAFLD, and from 8.7±0.6 to 7.1±1.1 to 6.9±0.9% in subjects with T2DM only, p<0.0001 for both groups). On the other hand, changes in body weight, waist circumference, body mass index as well as in plasma lipids were not significant in both groups. It is possible that the small study groups could be reason for a failure to reach a statistical significance. cIMT reduced significantly only in the group of patients with T2DM and NAFLD (from 0.96±0.27 to 0.82±0.17 to 0.85±0.12 mm, p=0.0325). Correlation analysis revealed that changes in cIMT were not associated with changes in any other
evaluated parameter, including fasting glycemia or HbA1c. After 8 months of the treatment, liraglutide reduced significantly cIMT in patients with T2DM and NAFLD, independently of its benefits on glucose metabolism. Whether these findings may lead to a decreased overall cardiovascular risk, remains to be confirmed by larger studies. (Diabetologia 2014; 57 (Suppl1):S369)

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Mini-oral presentation
Additional information
Mesenchymal Stem Cells Reduce Murine Atherosclerosis Development

Abstract nr. 660
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Lipoproteins, Therapy

Apart from their regenerative properties, Mesenchymal stem cells (MSCs) have received increasing attention for their potent immunomodulatory capacities. As atherosclerosis is a lipid driven immune-mediated disease, we investigated whether adoptive transfer of MSCs could beneficially affect atherosclerotic lesion development.

Methods and Results: The immunomodulatory capacity of murine MSCs was first determined in vitro. In a co-culture of MSCs and dendritic cells (DCs), MSCs significantly reduced TNF-α (57% reduction, p<0.001), while increasing IL-10 production (45% increase, p<0.01) by DCs in response to Lipopolysaccharide. In addition, MSCs almost completely inhibited CD4+ and CD8+ T cell responses in a co-culture experiment and prevented the differentiation of naïve T cells.

Before assessing the effect of MSCs on atherosclerosis, we established the fate of fluorescently labelled MSCs after intravenous injection in atherosclerosis-prone low-density lipoprotein-receptor knockout (LDLr KO) mice on a cholesterol rich diet. After fifteen minutes, MSCs primarily accumulated in the lungs and subsequently redistributed to the liver, heart, mediastinal lymph nodes as well as the aorta. Next, LDLrKO mice were treated with three intravenous injections of MSCs (0.5 x 10^6 cells) every other day prior to induction of atherosclerosis by WTD feeding. Animals were fed a Western-type diet for eight weeks and we subsequently assessed atherosclerotic lesion development. MSC-treatment resulted in an almost immediate, yet short term, induction of regulatory T cells (51% increase, p<0.001), while later on overall numbers of differentiated T cells were reduced by MSC-treatment. Moreover, MSC-treated mice displayed a significant reduction in circulating monocytes (-33%, p<0.05) and serum CCL2 levels (-70%, p<0.01). Interestingly, MSC treatment significantly reduced serum cholesterol levels, mainly due to decreased VLDL production in the liver. Most importantly, these effect culminate in a significant 33% reduction (p<0.05) in aortic root lesion size and a 56% reduction in lesional macrophage content (p<0.01).

In conclusion, we show for the first time that MSC treatment significantly reduces atherosclerotic lesion development in mice by both affecting cholesterol homeostasis as well as inflammation. Our findings may initiate future studies to assess the therapeutic potential of MSCs in atherosclerosis.
Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
Human C-reactive Protein Metabolism is Linked to the Metabolism of ApoB in Triglyceride-rich Lipoproteins, and Atorvastatin Enhanced by Its Clearance

Abstract nr. 661
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins,Hypolipidemic Drugs,Inflammation,Metabolism

Elevated C-reactive protein (CRP) levels are associated with a greater risk of cardiovascular disease. Our goal was to study CRP metabolism, and to determine its relationship with lipoprotein metabolism, as well as to examine the effects of atorvastatin on CRP kinetics.

Eight subjects with combined hyperlipidemia underwent a 15-h primed-constant infusion with deuterated leucine after being on placebo or 80 mg/day of atorvastatin for 8 weeks. CRP was purified from the plasma density fraction greater than 1.21g/ml by affinity chromatography. Lipoprotein fractions were separated by sequential density ultracentrifugation. Isotopic enrichment was determined by gas chromatography/mass spectrometry.

On placebo, the subjects had mean CRP levels of 3.4 mg/L. The mean (SEM) CRP production rate (PR) was 0.050 (0.012) mg/kg/day and the mean CRP fractional catabolic rate (FCR) was 0.343 (0.056) pools/day (residence time 3.50 days). CRP pool size (PS) was significantly related to production (r=0.93; p<0.001), but not FCR. CRP PS was also related to body mass index (r=0.79; p=0.02). There was a significant association between CRP FCR and triglyceride rich lipoprotein (TRL) apoB-100 FCR (r=0.74, p=0.04), as well as between CRP PS and TRL apoB-48 FCR (r=-0.90, p=0.002), indicating linkage between CRP and TRL metabolism. Compared with placebo, atorvastatin did not affect CRP isoforms, decreased median CRP PS by 28.4% (13.31 vs 10.26 mg), increased median CRP FCR by 39.9% (0.343 vs 0.500 pools/day; p = 0.09), with no significant effect on median CRP PR (0.050 vs 0.049 mg/kg/day; p = 0.78).

We have the following conclusions: 1) Plasma CRP has a production rate of 0.050 mg/kg/day and a plasma residence time of 3.50 days; 2) Plasma CRP levels are strongly positively correlated with body mass index. 3) The main determinant of plasma CRP levels is its production rate. 4) There is a significant positive linkage between CRP fractional catabolism with both TRL apoB-100 and apoB-48 fractional catabolism, and 5) Maximal dose atorvastatin lowers plasma CRP levels by decreasing the CRP plasma residence time from 2.94 days to 2.0 days, with no effect on CRP production rate.
Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
Cerebral circulation, heart rate variability in patients with arterial hypertension and coronary artery disease receiving Ivabradine in combination with Perindopril

Abstract nr. 662
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Hypertension, Pharmacology

Objective: To investigate the effects of ivabradine in combination with perindopril on cerebral blood flow and heart rate variability (HRV) in patients with arterial hypertension (AH) and coronary artery disease (CAD).

Methods: 64 patients with AH and CAD were examined. Patients were divided into two groups: I (n=38) received ivabradine (mean dose 6.7±0.4 mg) in combination with perindopril (mean dose 4.1±0.8 mg), II (n=26) received metoprolol (mean dose 43.5±5.3 mg). Groups were matched for age, office blood pressure (143/83±2.0/1.8 mmHg in I and 139/85±2.4/1.5 mmHg in II), HR (85.6±1.0 bpm in I and 82.1±2.1 bpm in II). 24-hour monitoring of blood pressure and electrocardiography with HRV program, Doppler cerebral blood flow measurement were evaluated initially and after 8 weeks of therapy.

Results: At 8-week follow-up both groups showed a comparable decrease in blood pressure and HR and a decrease in peak systolic velocity of blood flow in the internal carotid arteries (73.8±4.4 vs 61.2±4.0 mm/s in I and 70.8±8.8 vs 63.1±6.1 mm/s in II (p<0.05). Group I had a reduction in pulsatility index (PI) 1.9±0.1 vs 0.7±0.1 and systolic/diastolic ratio (ISP) 2.5±0.1 vs 2.0±0.1 (p<0.05). In both groups there was noted that dynamic vagosympathetic interaction in heart rate regulation increased high-frequency component (HF) and time domain PNN50% as well as SDNNind and RMSSD in group II.

Conclusion: With similar effects on HRV, ivabradine in combination with perindopril compared to metoprolol has more favorable effects on blood flow velocity and resistance of cerebral vessels in patients with AH and CAD.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
Changes in glucose-dependent insulinotropic polypeptide (GIP) levels in metabolic syndrome.

Abstract nr. 663
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Inflammation, Metabolism, Obesity

Altered GIP secretion and action was recently linked to obesity-related metabolic disorders but the underlying mechanisms are not well understood. The objective of this study was to compare the pattern of GIP secretion and correlation with metabolic risk markers in obese subjects during fasting and postprandial states.

In obese and non-obese patients fasting and postprandial plasma GIP, glucose, insulin, lipids, glutathione peroxidase, IL-6, sE-selectin, MCP-1, leptin, adiponectin, visfatin were measured in five different time points during oral lipid and glucose tolerance tests.

A total of 114 obese patients and 37 control non-obese subjects, were randomized into the study. Fasting GIP levels differed between obese (32.22 pg/ml) and control (24.27 pg/ml) subjects (p < 0.05) and correlated with glucose, triglycerides, total- and LDL-cholesterol, as well as sE-selectin, MCP-1, visfatin and leptin/adiponectin ratio (p<0.05). The levels of GIP at 120 min after a high fat meal were significantly higher than those measured at 120 min after glucose ingestion both in obese (365.93 pg/ml vs 156.4 pg/ml, p<0.05) as well as control subjects (381.09 pg/ml vs 154.94 pg/ml, p<0.05). Enhanced postprandial GIP response to fat or glucose challenge (AUC) positively correlated with glucose AUC, TG AUC, FFA AUC and negatively with glutathione peroxidase activity (p<0.05). In patients with the highest fasting GIP concentrations (3rd tertile), increased sE-selectin (p<0.05) and MCP-1 blood levels (p<0.05) were observed. We suggest, that increased GIP levels may contribute to the development of atherosclerosis in patients with metabolic syndrome.

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Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Abstract nr. 664
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Endothelium, HDL, Inflammation

Atherosclerosis is associated with endothelial dysfunction and inflammation, and high-density lipoproteins (HDL) play critical roles in both. HDL transport a wide-variety of proteins, nucleic acids, and small molecules which likely confer many of HDL’s alternative functions, including its anti-inflammatory and anti-atherogenic properties. Using in vitro studies, we demonstrated that macrophages export miR-223 to HDL in vitro and subsequently, HDL delivers functional miR-223 to human coronary artery endothelial cells (HCAEC). Moreover, intracellular adhesion molecule 1 (ICAM-1), a miR-223 target, was found to be inhibited by HDL-miR-223 at a specific target site within ICAM-1’s 3’ untranslated region. These in vitro findings support the potential of a HDL-miRNA communication pathway that may contribute to atherogenesis. To establish the physiologically role of HDL-miRNA communication in vivo, bone-marrow transplant (BMT) studies were completed with Mir223−/− and C57B/6J (WT) mice. To demonstrate that functional HDL-miR-223 is delivered to recipient cells/tissues in vivo, BM from WT mice was transplanted into irradiated Mir223−/− mice. Strikingly, we found that HDL-miR-223 communication was restored after BMT, as evidenced by significantly increased miR-223 levels on HDL and in the liver, white adipose, aortic endothelium, and skeletal muscle. Conversely, HDL-miR-223 intercellular communication was depleted by transplanting BM from Mir223−/− into WT mice, and resulted in significant loss of miR-223 on HDL and in many tissues. In both BMT studies, miR-223 changes led to altered expression of many target genes in multiple tissues. Most importantly, these studies identified a novel miR-223 target gene which we experimentally validated, vesicle transport through interaction with t-SNAREs homolog 1A (VTI1A), which was decreased and increased in the aortic endothelium from each BMT study, respectively. Although defined as SNARE protein, VTI1A also contributes to cytokine secretion and membrane lipid dynamics. As such, HDL likely mediates an inflammatory cell-originating miR-223 communication pathway that suppresses inflammation and cytokine secretion in the aortic endothelium, thus antagonizing atherosclerosis.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Arterial stiffness, lipid profile and inflammatory markers in hypertensive patients with abdominal obesity

Abstract nr. 665
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Endothelium, Hypertension, Inflammation, Obesity

Objective: To study arterial stiffness, lipid profile and inflammatory markers in patients with arterial hypertensios (AH) and abdominal obesity (AO).

Methods: 115 patients were examined. 1 group included 72 subjects (mean age 47.4±1.6 years) with AH and AO. 2 group - 43 subjects (mean age 47.3±0.9 years) without metabolic disorders. The parameters of sphygmography and 24-hour blood pressure monitoring, lipid profile and inflammatory markers were estimated.

Results: In group 1 there were registered: significant increase pulse wave velocity (PWV), cardio-ankle vascular index, in mean 24-hour and mean daytime systolic blood pressure. In biochemical parameters significant increase in total cholesterol, low-density lipoprotein cholesterol, triglyceride level and in inflammatory markers - homocysteine and hsCRP level; decrease high-density lipoprotein cholesterol compared to the patients in group 2.

In group 1 there were registered positive correlation between inflammatory and lipid markers with parameters of sphygmography, parameters of 24-hour blood pressure monitoring.

It was shown that with an increase in total cholesterol level >5.0 mmol/l, the risk of high rate PWV in patients with AH and AO increased by 15 times. Besides we revealed that high levels of endothelin-1 and homocysteine were observed more frequently in patients with increased levels of total cholesterol, triglycerides, experience hypertension, body mass index and complex of intima-media in the right common carotid artery.

Conclusion: The relationship between the arterial stiffness, markers of inflammation, atherogenic lipids and vascular remodeling process factors indicates a high risk of cerebrovascular complications in patients with AH and AO.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Structural and functional parameters of vessel wall and biochemical parameters in hypertensive patients with abdominal obesity and dyslipidemia

Abstract nr. 666
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Dyslipidemia, Endothelium, Hypertension, Obesity

Objective: To study 24-h blood pressure (BP) profile, structural and functional parameters of vessel wall, biochemical parameters in patients with arterial hypertension (AH), abdominal obesity (AO) and dyslipidemia (DL).

Methods: 108 patients were examined. 70 subjects (mean age 44.8±0.9 years) with AH, AO and DL were involved in the group I. The comparison group II included 38 subjects (mean age 44.39±1.60 years) with AH without AO and DL. The parameters of 24-hour blood pressure (BP) monitoring, parameters of sphygmography, femoral arteries Doppler imaging, cerebral vessels ultrasound Doppler examination, parameters of flow-dependent vasodilation of the brachial artery, biochemical parameters – products of lipid peroxidation, lipids, endothelin–1 and nitrites were estimated.

Results: In I group there were registered: significant increase in mean 24-hour and mean daytime systolic BP, increase in night time systolic BP and diastolic BP variability, increase in cardio-ankle vascular index L-CAVI and index of cerebrovascular reactivity. In I group there were registered: tendency towards decreased flow-dependent vasodilation, increased intima-media complex of right common carotid artery, reduction of the diameter of right common femoral artery in systole and diastole. Besides we revealed in I group significant increase in malonic dialdehyde level, triglyceride level; decrease of catataze level and high-density lipoprotein cholesterol compared to the patients in II group.

Conclusion: The relationship between structural and functional parameters of vessel wall and biochemical parameter indicates a high risk of cardio and cerebrovascular complications in patients with AH, AO and DL.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
ADIPONECTIN PLASMA LEVELS CORRELATED WITH BRAIN ATROPHY IN TYPE 2 DIABETES

Abstract nr. 667
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes,Imaging

-Objective: To study the relationship between adiponectin plasma levels with gray matter brain volume
-Methods: We studied 25 type 2 diabetes patients aged 45-65 years (58 ± 5.1 years). Biochemical analysis and structural cerebral magnetic resonance imaging (MRI) including Voxel-Based Morphometry (VBM) were performed in each patient. Gray matter volumes changes were analyzed using the Statistical Parametric Mapping (SPM8) software.
-Results: Lower levels of adiponectin were correlated with lower gray matter volume in temporal regions (insula, hippocampal, parahippocampal, posterior cingulate, thalamus and caudate) [(p < 0.001), adjusted for age, gender, education, and the presence of at least one epsilon 4 allele for the apolipoprotein E (APOEε4 genotype)]
-Conclusions: Positive correlations between Adiponectin plasma levels and gray matter volume were found predominantly in temporal regions Alzheimer disease like.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Lipids baseline in patients with coronary atherosclerosis in China

Abstract nr. 668
Author Dai, Wen, Xiangya Second Hospital, Changsha, China
Co-author(s) - Zhao, shuiping
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Dyslipidemia, Epidemiology, Lipids

Objective: To determine the lipids baseline in patients of Hunan Province, China, with proven coronary atherosclerosis and compare it with those of patients from North America and European area.

Methods: 355 patients who were admitted to Xiangya second hospital, Hunan, China, proving to have coronary atherosclerosis by angiogram, with a mean age of 62±10 years were studied. Their lipids baseline including Total cholesterol(TC), High density lipoprotein cholesterol(HDL-C), Low density lipoprotein cholesterol(LDL-C), Triglyceride(TG), were compared to a control group of 215 individuals with no coronary atherosclerosis, mean age 58 ± 11 years. Comparison of lipids baseline with patients from North america and European area were also done.

Results: The mean(±standard deviation) plasma TC for the group with coronary atherosclerosis is 4.66±1.12 versus 4.29±0.91 mmol/l in the control group(p<0.01). LDL-C 2.88±0.97 versus 2.56±0.77 mmol/l (p<0.01). HDL-C 0.99±0.24 versus 1.03±0.28 mmol/l (p=0.57). TG level 2.08±2.01 versus 1.77±1.64 mmol/l(p=0.57). Lower TC, LDL-C and higher TG level in patients with coronary atherosclerosis in this study when compared with data from LIPIDS and ASTEROID study(p<0.05), and nonstatistically significant difference of the HDL levels are observed(p>0.05).

Conclusion: Chinese population(Hunan Province) with coronary atherosclerosis have lower level of TC and LDL-C compared with patients from North America and European area. Lipids lowering treatment is highly recommended for patients with ASCVD(Atherosclerosis cardiovascular disease). Maybe less aggressive lipid lowering treatment should be given to Chinese patients considering the lower level of serum lipids baseline, much more cost and side effect from lipid lowering drugs. However, intensive research need to be done for both lipid baseline study in which more people should be investigated and aggressive lipid treatment versus moderate treatment study in Chinese population.
Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
IL-35 amplify Foxp3 Treg-mediated immune suppression of atherosclerotic in apoE-/- mice

Abstract nr. 669
Author Tao, Linlin, Hefei, China
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Immunity

Objective: Accumulated study shows that the IL-35 is an anti-inflammatory cytokine, and may play a protective role in atherosclerosis. However, the exact role and etiology of IL-35 still remains incompletely understood. We investigated whether exogenous IL-35 can attenuate the atherosclerosis lesion in apoE-deficient mice, meanwhile, how to change of Foxp3 expression in peripheral blood and lesion in its progression. Methods: ApoE-/- mice were randomly divided into two groups respectively received basal diet (negative control group) and high-fat diet (HFD) for 4 weeks. Then HFD groups respectively received IL-35 or atorvastatin or no any treatment for 16 weeks. The plasma lipids were determined by diagnostic enzyme assay kits. HE staining, Immunohistochemistry, Flow cytometry were used to analysis the severity of atherosclerotic lesions in apoE-/- mice, as well as the expression of Foxp3 in plasma and atherosclerotic plaques. Results: The results showed that compared to negative control group plasma lipids were significantly increased and lesions were obviously formed in HFD groups. However, With the intervention of IL-35 and atorvastatin, the lesions were significantly narrowed, meanwhile, both in plasma and lesions, Foxp3 expression were up-regulated. Conclusions: IL-35 amplify Treg-mediated immune suppression of atherosclerotic in apoE-/- mice. IL-35 maybe a newly direction to treat atherosclerosis. Keyword IL-35; Foxp3; Treg; atherosclerosis

Acknowledgment This study was supported by the grants from the National Natural Science Foundation of China (No. 81300223), The Anhui Academic and Technology Leader Candidate Scientific Research Fund, and the Doctor Scientific Research Start Fund of the First Affiliated Hospital of Anhui Medical University.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Spotty Calcification as a Marker of Vulnerable Plaque in Survivors of Cardiac Arrest and Autopsied Patients of Sudden Cardiac Death

Abstract nr. 670
Author Pu, Jun, Shanghai, China
Co-author(s) - Zhang, Pei
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords ACS, Atherosclerosis, Imaging, Vulnerable Plaque

Backgrounds: We previously reported that spotty calcification detected by intravascular ultrasound (IVUS) was often associated with a histopathological fibroatheroma (J Am Coll Cardiol, 2014: 6321:2220-2233). However, the exact mechanisms underlying spotty calcification and the associated risk for ischemic events remain poorly understood. In the present study, we performed a multimodality imaging study on spotty calcification using IVUS, near-infrared spectroscopy (NIRS), and optical coherent tomography (OCT) in in vivo study in survivors of cardiac arrest and in vitro study in autopsied patients with sudden cardiac death.

Methods and Results: IVUS, NIRS, and OCT were performed in vivo in 62 vessels from 29 patients who experienced a documented sudden cardiac arrest but successfully resuscitated, and in vitro in 52 vessels from 31 cardiac sudden death patients at necropsy. IVUS and OCT detected spotty calcification in 88.5% survivors of cardiac arrest, 78.3% of them had spotty calcification in superficial location. About 83.3% superficial spotty calcification co-existed with echo-attenuated plaques on IVUS, 73.9% of them co-existed with thin-cap fibroatheroma or plaque rupture on OCT, and 88.8% of them contained lipid core plaque on NIRS (Figure 1). In spotty calcification, the arc of calcification was negatively correlated with the arc of echo-attenuation on IVUS (Spearman $\rho=-0.51$, $P=0.001$) and lipid core burden index on NIRS (Spearman $\rho=-0.40$, $P=0.03$), and positively correlated with the thickness of fibrous cap on OCT (Spearman $\rho=0.41$, $P=0.03$). In in vitro study, IVUS and OCT detected spotty calcification in 80.6% cardiac sudden death patients at necropsy, 84.0% of them was superficial in location. On pathological analyses, the arc of spotty calcification was negatively correlated with the arc of echo-attenuation on IVUS (Spearman $\rho=-0.51$, $P=0.001$) and lipid core burden index on NIRS (Spearman $\rho=-0.40$, $P=0.03$), and positively correlated with the thickness of fibrous cap on OCT (Spearman $\rho=0.41$, $P=0.03$). In in vitro study, IVUS and OCT detected spotty calcification in 80.6% cardiac sudden death patients at necropsy, 84.0% of them was superficial in location. On pathological analyses, the arc of spotty calcification was negatively correlated with i) inflammation of fibrous cap, ii) apoptosis by tunel staining, and iii) size of necrotic core. In addition, spotty calcification in superficial location was associated with larger size of necrotic core ($P=0.02$) and more thin-cap fibroatheroma ($P<0.001$) and plaque rupture ($P=0.006$) than that in deep location.

Conclusions: Spotty calcification, especially when superficial in location, was a marker of vulnerable plaque in survivors of cardiac arrest and autopsied patients of sudden cardiac death.
Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information

Figure 1. Example of Spotty Calcification in Survivors of Cardiac Arrest
Smoking is associated with higher resting energy expenditure

Abstract nr. 671
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Epidemiology,Lifestyle,Metabolism

Objective: Animal studies and human studies in small selected populations have shown a positive association between nicotine smoking and resting energy expenditure (REE), but data in large cohorts are lacking. We aimed to investigate the relation between smoking behaviour and REE in a large, population-based study.

Design: Population-based cross-sectional study.

Methods: In this cross-sectional analysis of baseline measurements from the Netherlands Epidemiology of Obesity (NEO) study (n=6,673), we included participants with REE measurement by indirect calorimetry who were not using lipid or glucose lowering drugs (n=1,189). We used weighted linear regression analysis to examine the association between REE per kilogram (kg) fat free mass (FFM) and smoking status (never-, former, occasional, current smoker) and smoking quantity (pack years), in a model adjusted for age, sex, ethnicity, educational level, physical activity, energy intake and body mass index (BMI).

Results: Mean (standard deviation, SD) age was 55.2 (5.9) years and BMI was 26.3 (4.4) kg/m². 57% of the participants were women. Mean (SD) REE/FFM (kcal/day/kg FFM) was for male never smokers 25.1 (2.0), male current smokers 26.4 (2.8), female never smokers 28.9 (2.5) and female current smokers 30.1 (3.7). After adjustment, only current smokers had a higher REE/FFM than never smokers (mean difference 1.28, 95% CI 0.64, 1.92). There was no association between pack years and REE/FFM in current smokers (mean difference -0.01, 95% CI -0.06, 0.04).

Conclusion: Current smoking is associated with higher resting energy expenditure in a large population based cohort.

Funding
The NEO study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Center, and by the Leiden University, Research Profile Area 'Vascular and Regenerative Medicine'. P.C.N. Rensen is an Established Investigator of the Dutch Heart Foundation (2009T038).
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
The patients with familial hypercholesterolemia (FH) are characterized by high LDL cholesterol levels in the blood and premature cardiovascular disease. FH is an inherited disorder, mainly caused by defects in low-density lipoprotein (LDL) receptor gene. Although most of heterozygous FH patients are usually treated with statin, ezetimibe and bile acid sequestrants, homozygous FH patients are resistant to drug therapy. Based on that, in Japan, many of homozygous FH patients used to be treated by LDL-apheresis. LDL-apheresis is a great procedure to remove LDL cholesterol from the blood and contribute to improve prognosis of homozygous FH patients. However, the effect of removing LDL cholesterol is temporary and still not enough. As a definitive therapy, liver transplantation is one of options to recover LDL receptor, but donor is always limited in Japan.

With the increase of the evidence about the safety of mesenchymal stem cells and percutaneous transhepatic portal approach in islet transplantation, we developed a cell transplantation therapy with allogeneic adipose tissue-derived multilineage progenitor cells (ADMPCs), as an alternative treatment instead of liver transplantation.

We have already demonstrated that xenogenic transplantation of human ADMPCs into Watanabe heritable hyperlipidemic (WHHL) rabbits via portal vein resulted in significant reduction in total cholesterol, and the reductions were observed within four weeks and maintained for 12 weeks. These results suggested that hADMPC transplantation could correct the metabolic defects and be a novel therapy for inherited liver diseases.

Subdivision 3. Clinical Studies
Presentation Preference Oral presentation
Additional information
SR-BI protects against the S1P/S1P2-mediated inflammatory response of human umbilical vein endothelial cells induced by oxLDL through SR-BI/PI3K/AKT pathway

Abstract nr. 673
Author Lu, Yan-Ju, University of South China, Hengyang, China
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Co-author(s) - Tang, Zhen-Li
Co-author(s) - Jiang, Yue
Co-author(s) - Yi, Guang-Hui
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Endothelium, HDL, Inflammation

**Purpose:** Endothelial dysfunction is a pivotal event in the development and progression of atherosclerosis. Oxidized low density lipoprotein (oxLDL) can induce vascular endothelial injury and inflammation in vessel wall. Scavenger receptor class B type I (SR-BI) is a high affinity receptor for high density lipoprotein (HDL) and mediates intracellular signal pathways, such as SR-BI/PI3K/AKT. Sphingosine-1-phosphate exerts a variety of biological action on different cells through its different receptors. In vascular endothelial cells, S1P possesses reciprocal effects for inflammatory response by receptors S1PR1/3 and S1P2. In this study we investigated the effects of SR-BI/PI3K/AKT pathway on S1P/S1PR2-mediated pro-inflammatory response induced by oxLDL in endothelial cells. **Methods:** Cultured human umbilical vein endothelial cells (HUVECs) were treated with 60 mg/ml of oxLDL, and then treated with different concentrations of S1P for 24h. The inhibitors of S1PR1/3 (VPC23019), S1PR2 (JTE-013), SR-BI (BLT-1), PI3K (LY294002) and Akt (MK2206), and transient transfection of SR-BI were applied to intervene the effects. The protein expression levels of cytokines TNFα, IL-1β and IL-10 were determined by Western blot and ELISA in cell and cell culture, respectively. The nuclear translocation of NF-κB were determined by immunofluorescence. **Results:** The protein expression levels of cytokines TNFα and IL-1β both in cell and in cell culture were significantly decreased, but IL-10 increased compared with control after treatment with S1P and apoA-1, or transfection with SR-BI. Nuclear translocation of NF-κB was also significantly reduced compared with control. The inhibitors BLT-1, LY294002 and MK2206 attenuated the antagonist effect of ApoA-1. **Conclusions:** SR-BI inhibits the pro-inflammatory effect of S1P/S1P2 on vascular endothelial cells, which may be associated with the SR-BI/PI3K/AKT signaling pathway.

**Key words:** Sphingosine-1-phosphate; Receptor; High density lipoprotein; Scavenger receptor class B type 1; Inflammation; Endothelial cell

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Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Liraglutide improves several metabolic parameters including carotid intima-media thickness in patients with the metabolic syndrome: a 18-month prospective study

Abstract nr. 674
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention

Keywords Cardiovascular Disease, Diabetes, Risk Factor, Therapy

Liraglutide exerts several beneficial non-glycemic effects in patients with type-2 diabetes (T2DM), such as those on blood pressure, plasma lipids and markers of inflammation. However, the effects of liraglutide on cardiovascular (CV) risk markers in subjects with the metabolic syndrome (MetS) are largely unknown.

One hundred-nine subjects (66 men and 43 women; mean age: 62±11 years) with the MetS as diagnosed by the AHA/NHLBI criteria, were enrolled in this 18-month prospective study. All subjects had T2DM, were naïve to incretin-based therapies, and were treated with metformin only. Exclusion criteria included the presence of a previous major CV event. Liraglutide (1.2 mg/day) was added to metformin (1500 mg/day). Fasting plasma samples were collected at baseline and every 6 months. Carotid-intima media thickness (cIMT) was assessed by B-mode real-time ultrasound. Statistical analysis was performed by ANOVA and the Spearman correlation.

Metabolic parameters significantly improved from baseline to 6, 12 and 18 months of liraglutide therapy as following: waist circumference from 109±15 to 105±11 to 105±13 to 101±18 cm (p=0.003), body mass index from 32±9 to 30±5 to 30±5 to 30±6 kg/m² (p=0.032), fasting glycemia from 9.5±3.7 to 7.5±2.4 to 7.1±2.1 to 7.2±3.0 mmol/l (p<0.0001), HbA1c from 8.8±0.9 to 6.8±1.3 to 6.6±1.2 % (p<0.0001), total cholesterol from 4.7±1.4 to 4.3±1.0 to 4.2±1.0 to 4.3±1.0 mmol/l (p=0.010), triglycerides from 1.9±1.2 to 1.6±0.7 to 1.5±0.7 to 1.5±0.7 mmol/l (p=0.008), LDL-cholesterol from 2.7±1.3 to 2.41±0.9 to 2.37±0.9 to 2.42±0.9 mmol/l (p=0.049), HDL-cholesterol from 1.13±0.3 to 1.18±0.3 to 1.19±0.3 to 1.17±0.3 mmol/l (p=0.484). Also, cIMT significantly reduced over the time from 0.97±0.17 to 0.97±0.13 to 0.83±0.12 to 0.82±0.16 mm (p=0.026). By correlation analysis, we searched for potential associations of changes in cIMT with
changes in all the evaluated metabolic parameters, and found a significant association between changes in cIMT and those in triglycerides ($r=0.261; p=0.026$) only.

Liraglutide improved several cardio-metabolic risk factors in subjects with the MetS without a previous CV event. The clinical role of these findings on CV prevention remains to be established by future studies.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Mini-oral presentation
Additional information
The effect of weight loss and adipokines on apolipoproteins after the adjustable gastric banding surgery in morbidly obese individuals

Abstract nr. 675

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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords HDL, Lipoproteins, Lp(a), Obesity

We evaluated the effects of weight loss after the laparoscopic adjustable gastric banding (LAGB) on serum lipoprotein (a) [Lp(a)], apolipoprotein A 1 [Apo A1], and apolipoprotein B [ApoB] levels and investigate whether the weight loss or adipokines may explain the changes in the apolipoprotein levels.

Methods: A total of 83 morbidly obese subjects with an average duration of 24.8±13.7 months of clinical obesity before LAGB were included in the analysis. Plasma apolipoprotein, leptin and adiponectin concentration levels at baseline and 12 months post-surgery was measured.

Results: The subjects demonstrated a significant reduction in body mass index of 46.9 kg/ m² at baseline to 40.08 kg/ m² (p<0.001), corresponding to 85.07 ± 22.29% of excess weight loss (EWL). The mean adiponectin level increased significantly from 10.72 to 14.72 µg/ml (p=0.008) as well as leptin level decreased from 36.88 to 26.90 ng/ml (p<0.001). Additionally, a significant improvement in plasma triglycerides (p<0.001), high - density lipoprotein cholesterol (p<0.001), apoA1 from 1.48 to 1.59 mmol/l (p<0.002), and rise of apoB 1.09 to 1.22 mmol/l were observed after LAGB. No significant difference was seen in plasma LDL cholesterol (p=0.683) and Lp (a) level (p=0.736) post surgery.

Finally, only the changes in ApoB levels closely correlated with EWL % (r=0.237 p<0.031) and the changes in leptine levels (r = 0.462 p < 0.0001).

Conclusions: weight loss in obese subjects may have beneficial effects on serum Apo A1 lipoprotein levels and adverse on serum ApoB. This effect on Apo B seems to be dependent on the leptin changes observed during weight loss.

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
HTE-DLP: An opportunity to improve the quality of prescribing of lipid-lowering therapy?

Abstract nr. 676
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, LDL, Lipids, Therapy

HTE-DLP is an artificial intelligence software (CDSS) that performs a sequence of clinical decisions including all lipids lowering therapy and shows specific recommendations for each patient using efficiency, safety and cost criteria. It is based on European Guidelines for the Management of Dyslipidaemia 2011. It is the first lipid-lowering therapy CDSS developed in Spain and the first to be validated in Europe.

Methods
It was a cluster-randomized trial comparing standard prescriptions with HTE-DLP assistance, conducted by 10 expert physicians (7 specialists and 3 general practitioners) in cardiovascular risk management from five different hospitals and primary care centers in Catalonia (Spain). Each physician was asked to recruit 10 patients. The physicians enrolled consecutive eligible patients with high cardiovascular risk aged >18 years old with LDL-cholesterol (LDL-C) >100 mg/dl...

Included patients were randomly distributed into the intervention or control group by a computer program. HTE-DLP was blocked automatically if a patient was assigned to the control group. Physicians used HTE-DLP in the "real-clinic-world". It was assessed the theoretical impact on the frequency of coronary artery disease with the CASSANDRA-REGICOR methodology. Researchers were asked to evaluate HTE-DLP with questionnaire QoE for applications in health.

Results
Use HTE-DLP meant additional lowering of LDL-C of 20.5%. When experts in vascular risk using HTA-DLP number of high vascular risk patients reaching lipid targets of LDL-C <70 mg/dl... increased by 4.4 times. In general practitioners would increase 5.8 times. Use of HTE-DLP reduced direct costs of lipid-lowering medication, 19% less per 1 mg of LDL-descended. The widespread use in Spain of HTE-DLP would mean in 2020 a decrease in coronary heart disease health costs between 4.7% and 6.4% (between 24 and 32 million Euros savings to the healthcare
system). Physicians expressed good agreement with the 1st HTE-DLP recommendation in 86.1% of cases and use was described as comfortable in 85% of cases. Assessing users HTE-DLP by Questionnaire QoE for applications in health was positive (3.89 / 5)

Conclusion

*Using in clinical practice a specific CDSS it is possible to improve the management of dyslipidemia with a decrease in coronary heart disease and lowering healthcare costs*

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Intestinal inflammation alters the expression of HDL genes in human and mouse cells by different mechanisms

Abstract nr. 677
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Apolipoproteins, HDL, Inflammation

Chronic inflammation is a hallmark in a range of clinical disorders including rheumatoid arthritis and inflammatory bowel diseases (IBD). Patients with IBD have low levels of High Density Lipoprotein (HDL), enhanced atherogenesis and increased levels of pro-inflammatory cytokines. The aim of the present study was to investigate changes in the expression of intestinal genes that could account for the above phenotypical characteristics of IBD. Caco-2 cells were used as an ex vivo model. Treatment of Caco-2 cells with TNFα resulted in a significant decrease in the mRNA levels of the APOA1, APOC3, APOA4 genes and Hepatocyte Nuclear Factor 4α (HNF4α). This was associated with reduced recruitment of HNF4α to the respective promoters. The mRNA levels of the nuclear receptor LXRα were decreased after a short treatment of Caco-2 with TNFα along with one of its major target genes, the ATP Binding Cassette Transporter A1 (ABCA1). Interestingly, TNFα treatment also reduced the binding of HNF4α to the LXRα promoter suggesting that these two nuclear receptors may operate in the same transcriptional network in enterocytes. We aimed to verify these findings in vivo by employing a widely-used animal model for acute colitis which requires treatment of wild-type C57BL/6 mice with 3.5% Dextran Sodium Sulfate (DSS) for 5 days. This treatment resulted in a dramatic increase in the expression of the inflammatory genes Il1β, Il6, Tnfa and Mcp1 in the large intestine. In agreement with results in Caco-2 cells, the expression of Hnf4α and its target genes Apoa1, Apoc3 and Apoa4 was significantly reduced and so was the expression of Lxra. Interestingly, the expression of the two main targets of Lxra, Abca1 and Abcg1, was not affected by the DSS treatment suggesting that different mechanisms regulate the expression of these two HDL genes in human and mouse intestinal cells. In conclusion, intestinal inflammation is associated with altered expression of many genes involved in HDL metabolism including key transcriptional regulators in human and mouse enterocytes by different mechanisms.

Funding: The work was funded by a grant from the General Secretariat for Research and Technology of Greece (ARISTEIA II Grant No 4220) to DK.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Perivascular adipose tissue modulates vascular reactivity in atherosclerosis of apolipoprotein E-deficient mice

Abstract nr. 678
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis

Perivascular adipose tissue (PVAT) surrounds the majority of blood vessels and until recently was not considered important in the pathogenesis of atherosclerosis. PVAT exerts anti-contractile effects that are abolished in disease states, these effects could potentially modulate atherosclerosis. The aims of this study were to determine the influence of PVAT, age, high fat diet (HFD) and associated progression of atherosclerosis on isolated arterial reactivity.

Male ApoE^{-/-} mice were fed a HFD or standard chow for 8, 16 or 26 weeks. Aortic atherosclerotic lesion area was analysed by en face quantification with Oil Red O. Contractile responses of thoracic aortae to cumulative doses of phenylephrine (1x10^{-10} - 3x10^{-5} M) or a maximal dose of serotonin (1X10^{-5} M) were measured in PVAT intact or denuded vessels using myography. Endothelial function was evaluated by relaxation to acetylcholine (1X10^{-5} M) on vessels pre-constricted with phenylephrine (1X10^{-5} M).

Atherosclerosis was accelerated by high fat feeding in the ApoE^{-/-} mice. PVAT significantly increased vasoconstrictor responses to phenylephrine at the 8 and 16 week time-points in HFD fed and age-matched controls (8 week chow: PVAT versus no PVAT P=0.006, n=12. 8 week HFD: PVAT versus no PVAT, P=0.03, n=10, 16 week chow: PVAT versus no PVAT P=0.0001, n=11. 16 week HFD: PVAT versus no PVAT, P=0.004, n=9). PVAT exerted anti-contractile effects in the 26 week controls (PVAT versus no PVAT P=0.02, n=7). PVAT had no effect on vessel contractility in the 26 week HFD group (HFD: PVAT versus no PVAT P=NS, n=9). Contractile responses to serotonin were unaffected by atherosclerotic progression or the presence of PVAT. Endothelial dysfunction was observed in the 26 week chow cohort however, this was not demonstrated in the HFD groups. PVAT had no effect on aortic relaxation.

In summary, PVAT modulated the vasoconstrictor responses of aortic segments during atherosclerotic disease progression in the ApoE^{-/-} mouse. However, relaxation in response to an endothelium-dependent dilator was not significantly altered by the presence of PVAT. These findings may have important implications in the pathogenesis of atherosclerosis.

This research was funded by the British Heart Foundation.
Endothelial function in ApoE-/- mice of PVAT intact or denuded aortic rings A) standard chow B) high fat diet One-way ANOVA n=6-8 *P<0.05, ** P<0.001

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
LP (a) LEVEL IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

Abstract nr. 679
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Familial Hypercholesterolemia, Lipids

Aim: to analyze Lp(a) in patients with familial hypercholesterolemia (FH).

Materials and methods: 81 patients (48 women) with definite FH were examined (FH was diagnosed by Dutch Lipid Clinic criteria). Mean age of patients was 39,1±0,4. We subdivided patients in two groups: 1) with raise level of Lp (a)>0,3 - 34 patients (41,9%), mean age 44,5±0,5; 2) with normal level of Lp (a)<0,3 g/l (47 patients (58,1%); mean age 37,5±0,3). Lipid levels: average total cholesterol in first group 8,2±0,4 mmol/l, in second – 7,9 ±0,3 mmol/l; LDL 5,2 ±0,1 mmol/l and 5,8±0,3 mmol/l relatively. Statistic analyze provide by «Byostat».

Results: The average level of Lp (a) in FH patients was 0,5±0,07, in FH patients older 18 - 1,2±0,05, in children of FH patients younger 5 years old - 0,03±0,01 and from 5 to 17 years - 0,5±0,07 g/l. In the first group it was 13 patients under the 40 years (39,3%), in the second – 14 (29,7%), p<0,05. Quantity of patients younger 18 in both groups was equal: 2 (4,5%) and 2 (5,8%) relatively. In the first group was 20 women (58,8%), in the second – 28 (59,6%). Frequency of other atherosclerosis risk factors: arterial hypertension was diagnosed in 17 (50,8%) in first group and 20 (42,6%) in second, p>0,05, obesity - in 12 (35,3%) and 15 (31,9%), smoking in 4 (11,8%) and 4 (8,5%) relatively. In FH patients with high Lp(a) level ischemic heart disease (IHD) was diagnosed in 32,4%, in the second group in 19,1%, p<0,05. Also frequency of myocardial infarction (MI) in first group was 23,5% in comparison with 8,5% in the second group, p<0,05. MI in patients under the 40 years were obtained only in the first group: one man (age 28 y. o.) and one woman (29 y. o.).

Conclusions: Above normal Lp(a) level was in 41,9% FH patients and correlated with age. In FH patients high Lp(a) level was associated with more frequency of IHD (1.7 times more) and MI (2.76 times more), while other atherosclerosis risk factors were the same in comparison with the FH patients with normal Lp(a).

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Effects of Supra-Physiological Levothyroxine Dosages on Lipids, Lipoproteins and Liver Parameters in Healthy Volunteers: A Randomized Controlled Crossover Study

Abstract nr. 680
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Lipids, Lipoproteins, Metabolism, Therapy

Background: In a recent study conducted in euthyroid patients with familial hypercholesterolemia, we observed that treatment with the liver specific thyroid hormone agonist eprotirome resulted in significant increases in liver parameters. Moreover, a modest decrease in atherogenic lipids and lipoproteins was observed. It is unknown whether the effects of eprotirome on liver parameters were due to either a drug specific effect or induced ‘local hyperthyroidism’.

Purpose: To study the effects of ‘local hyperthyroidism’ induced by supra-physiological levothyroxine dosages on liver parameters, plasma lipids and lipoproteins in healthy individuals.

Methods: We performed a post-hoc analysis of a single blind, randomized controlled crossover trial, comprising two studies. In both, healthy volunteers received levothyroxine or no medication for 14 days. In Study A, 16 individuals received 0.3 mg/day. In Study B, 12 individuals received 0.45 or 0.60 mg/day, depending on body weight. Lipids, lipoproteins and liver parameters were measured at baseline and follow-up. To compare the changes from baseline between both study periods, paired samples t-tests and Wilcoxon Signed Rank-tests were used where appropriate.

Results: After using levothyroxine, T3 levels increased from 1.99±0.31 to 2.61±0.49 nmol/L in all participants in study A, and in Study B T3 levels increased from 2.3±0.36 to 3.8±0.49 nmol/L. Decreases in total cholesterol (TC) levels (-11% and -15% in study A and B, respectively), low-density lipoprotein-cholesterol (LDL-C) (-13% and -17%) and apolipoprotein B (ApoB) levels (-8% and -16%) were observed after using levothyroxine, compared with the control period (change from baseline: TC +6% and -4%, for study A and B respectively; LDL-C +6% and -5%; ApoB +4% and -7%; p < 0.001 for all differences between study periods). Thyroid hormone excess did not have a clinically significant effect on aspartate aminotransferase, alanine aminotransferase, gamma glutamyltranspeptidase, alkaline phosphatase, total or conjugated bilirubin levels, in both studies.

Conclusion: Administration of Supra-physiological thyroid hormone doses in healthy volunteers does result in reductions in TC, LDL-C and ApoB levels but not in increased liver parameters. This may suggest that the effects of eprotirome on liver parameters as previously reported were
compound specific rather than thyroid hormone-dependent.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Myostatin underlies the association between abdominal aortic aneurysm development and impaired renal function

Abstract nr. 681
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model,Atherosclerosis,Chronic Kidney Disease,Pathogenesis

Background: Patients with large abdominal aortic aneurysms (AAAs) have a high prevalence of undiagnosed moderate chronic kidney disease (CKD). Thus, moderate renal dysfunction may contribute to atherosclerotic aortic wall deterioration and AAA formation. Myostatin (Mstn), a TGF-β family member, inhibits muscle growth and sustains proinflammatory and profibrotic processes. Mstn is increased in blood and skeletal muscle of CKD patients. To date, the role of Mstn on atherosclerosis and AAA development is unknown. We hypothesized that Mstn may represent a molecular link between CKD and AAA.

Methods and Results: The association between impaired renal function and AAA development was examined in ten-week-old, male C57BL/6 mice infused with angiotensin II (AngII; 1000 ng/kg/min via osmotic mini-pump) for 28 days. Forty percent of AngII-infused mice developed AAAs. Compared to untreated mice, Mstn immunopositivity was 8-fold higher in renal tubuli of AngII-infused mice than in controls (p<0.05). Conversely, immunostaining for the antifibrotic factor, KLF15, was 3 folds lower (p<0.05). AngII-treated aortas also exhibited higher Mstn protein positivity (3 folds; p<0.05).

In human aortic specimens, Mstn mRNA was 8- and 3-fold higher, respectively, in AAA (N=8) and non-aneurysmal advanced atherosclerosis lesions (NA-AA, N=3) than in areas of intimal thickening (N=3) (p<0.05). Staining for Mstn protein was 10-fold higher in AAA (N=8) and NA-AA (N=7) than normal aortas (N=2) (p<0.05). Mstn immunoreactivity co-localized with that for α-SMA, CD45, and CCR2, suggesting that Mstn is predominantly expressed in vascular smooth muscle cells (VSMCs) and leukocytes. Moreover, higher expression of Mstn was associated with reduced levels of Smoothelin, a marker of VSMC differentiation.

In A7R5 VSMCs, 48-hour incubation with serum from AAA patients upregulated Mstn protein by 3
folds compared to age-matched control serum (p<0.05). In THP-1 monocytes, AAA serum induced the differentiation into macrophages with 2-fold higher Mstn and α-SMA expression (p<0.01 vs. control)

Conclusion: The study on mice revealed that kidneys and aorta express Mstn early during AAA development. Human AAAs and NA-AA have increased Mstn at sites of leukocyte infiltration and VSMCs phenotype transition. Circulating factors in AAA induce Mstn overexpression in VSMCs, which lose their contractile function, and in macrophages, inducing a fibroblast-like differentiation.

Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
Evidence for a polygenic form of CAD manifesting with early cardiovascular events

Abstract nr. 682
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Co-author(s) - Natarajan, Madhu
Co-author(s) - Pare, Guillaume

Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Genetics, Risk Factor, Risk stratification

Background: Genome-wide association studies have identified common variants modestly associated with coronary artery disease (CAD), which can be combined into genetic risk scores. We hypothesized that some individuals with early, severe CAD would have scores at the high end (>99th percentile) of the distribution, thus identifying polygenic CAD as a novel inherited form of CAD.

Purpose: To determine the polygenic risk attributable to reported common variants in individuals with early, severe CAD and identify potential cases of polygenic CAD.

Methods: All individuals of European descent who underwent cardiac catheterization at the Hamilton Health Sciences Heart Investigation Unit between February and August 2014, had angiography proven CAD and were under the age of 40 for men and 45 for women were approached for participation in our study. Fourteen individuals consented to genetic analysis and were genotyped using the HumanCoreExome BeadChip (Illumina) with ensuing 1000 Genomes imputation. We calculated weighted genetic risk scores based on data from the CARDIoGRAMplusC4D Consortium, first with 45 single nucleotide polymorphisms (SNPs) reaching genome-wide significance (wGRS45) and subsequently with 144 SNPs associated with CAD at a false discovery rate of 5% (wGRS144). Genetic risk scores were compared to a distribution generated from the expected allele frequencies in the general population.

Findings: There was a significant enrichment in high wGRS45 and wGRS144 in the study population (p=0.0006 and 0.003, respectively). Two individuals had a wGRS45 above the 99th percentile of the expected distribution (p=0.008, Figure 1). The individual with the highest score had higher than three-fold and five-fold increases in risk of CAD according to wGRS45 and wGRS144 respectively. This male individual had a quadruple coronary artery bypass graft surgery at age 38 and presented with a graft occlusion 12 years later. He had none of the traditional risk factors for CAD before the first event. The genetic risk scores remained above the 99th percentile after excluding SNPs in loci associated with known risk factors.

Conclusions: These results suggest the existence of a polygenic form of CAD which could increase risk sufficiently to provoke premature events and emphasize the potential of genetic information for CAD risk assessment.
Figure 1: Genetic risk scores distribution

- wGRS45
- wGRS144

Odds ratio

General population

Patients
Figure 1
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Frameshift mutation in the APOA5 gene causing hypertriglyceridemia in a Pakistani family: management and considerations for cardiovascular risk

Abstract nr. 685
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Co-author(s) - Pare , Guillaume
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease,Dyslipidemia,Genetics,Triglycerides

Clinical problem: A 12-year-old boy of Pakistani origin was referred for hypertriglyceridemia (26 mmol/L) discovered during investigation for abdominal bloating. Eruptive xanthoma and lipemia retinalis were present on physical examination. Parents are consanguineous and there is a family history of premature cardiovascular disease in a paternal uncle who died at age 13 from sudden death, a paternal aunt who died at age 60 from myocardial infarction and had earlier coronary artery bypass graft surgery and a maternal uncle who had a myocardial infarction at age 50.

Purpose: Establish the genetic cause of the hypertriglyceridemia and determine optimal management concerning triglycerides and cardiovascular risk.

Methods: First degree relatives were screened with fasting lipid profiles and both parents and one sibling with hypertriglyceridemia were considered for genetic testing. Exome sequencing using the Ion Proton System (Life Technologies) and confirmation of findings with Sanger sequencing were performed in these four individuals. Additional lipid investigations including lipoprotein electrophoresis were performed for the index case.

Findings: A homozygous frameshift mutation in the apolipoprotein A5 (APOA5) gene was found in the index case (R143fs). Both parents and a hypertriglyceridemic sibling were heterozygous for the same mutation (Figure 1). Additional lipid investigations in the index case included lipoprotein(a) of < 50 mg/L and apolipoprotein B of 0.80 g/L. Lipoprotein electrophoresis showed type V hyperlipoproteinemia. The patient did not respond to Bezafibrate therapy at optimal dose (Table 1).

Conclusion: The cause of the hypertriglyceridemic phenotype in this family is a rare frameshift mutation in the APOA5 gene. Functional variants in this gene have previously been associated with hypertriglyceridemia and are thought to impair the abilities of APOA5 to enhance the catabolism of triglyceride-rich lipoproteins and to inhibit the rate of production of very low-density lipoproteins. One of these variants was shown to correlate with better response to fibrate therapy, which does not seem to apply to the described mutation. Interestingly, non-synonymous mutations in the APOA5 gene have also recently been associated with early myocardial infarction, the mechanism possibly involving the accumulation of triglyceride-rich lipoproteins. This poses
interesting questions for the management of the young man and his family.

Figure 1: Pedigree of the family (first-degree relatives of the index case)

18 y.o. TG = 2.27
17 y.o. TG = 7.67
15 y.o. TG = 2.11
12 y.o. TG = 26.54
10 y.o. TG = 0.93

- Homozygous for APOAS R143fs
- Heterozygous for APOAS R143fs
- TG: Triglycerides (mmol/L)

Figure 1

Table 1: Evolution of lipid and glucose parameters in the index case

<table>
<thead>
<tr>
<th>Timeline</th>
<th>Referral</th>
<th>2 months</th>
<th>7 months</th>
<th>10 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary advice</td>
<td>None</td>
<td>Low fat diet (30%)</td>
<td>Low fat diet (30%)</td>
<td>Very low fat diet (10%)</td>
</tr>
<tr>
<td>Pharmacological therapy</td>
<td>None</td>
<td>Bezafibrate 400 mg OD</td>
<td>Bezafibrate 400 mg SID</td>
<td>None</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>8.91</td>
<td>11.34</td>
<td>9.14</td>
<td>7.82</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>0.50</td>
<td>0.55</td>
<td>0.58</td>
<td>0.52</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>26.54</td>
<td>35.04</td>
<td>22.12</td>
<td>22.28</td>
</tr>
<tr>
<td>Apo B (g/L)</td>
<td>0.60</td>
<td>NA</td>
<td>0.86</td>
<td>NA</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>4.5</td>
<td>4.4</td>
<td>5.0</td>
<td>5.5</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; TG: Triglycerides; Apo B: Apolipoprotein B; FPG: Fasting plasma glucose; HbA1c: Glycated hemoglobin

Table 1

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Diagnosis and treatment of rare monogenic dyslipidaemias in children: case studies.

Abstract nr. 686
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia,Familial Hypercholesterolemia,Genetics,LDL

Some monogenic lipid disorders (homozygous familial hypercholesterolaemia (homo FH)), familial chylomicronaemia syndrome (FCS) and beta-sitosterolaemia are rarely diagnosed in routine Lipid Clinics and are difficult to treat. We present a 3 clinical cases with these rare dyslipidaemias.

Patient 1. S.I, 5 years old girl. Diagnosis: FCS, LPL deficiency? Patient had creamy serum from birth, initial total cholesterol (TC) level 10.2 and triglycerides (Tg) (mmol/l) 21.1 mmol/l and 3 episodes of acute pancreatitis before admission to lipid clinic in may 2012. DNA test revealed LPL defect (rs LPL: 118204061). Pts was treated with very low fat diet and nicotinic acid 2 gr, omega 3 fatty acids 2 gr and fenofibrate 145 mg a day. Over last 2 years lipids were well controlled with averaged TC 4,25 and Tg 2,8 mmol/l, treatment was safe and well tolerated. To date S.I had no episodes of pancreatitis.

Patient 2. I. V, 10 years old boy. Diagnosis:homo FH. On examination (2012): severe xanthomatosis, carotid stenosis 50 %, signs of aortic stenosis (ultrasound), regurgitation grade I-II in aortic valve. Pre-treatment lipids: (mmol/l): TC-20,8; Tg- 0,89;LDL-C- 19.9. DNA test: LDL-R mutation: C68F and C270X/ He takes rosuvastatin 40 mg+ ezetemibe 10 mg/d with mean on-treatment LDL-C of 12.3 mmol/l. Slight blanching of xanthomata was observed.

Patient 3. Ya. F, 8 years old boy. Diagnosis: Beta-sitosterolaemia. Hypercholesterolaemia since 2 year old, with maximal TC 30 mmol/l. No xanthomatosis and no family history of hypercholesterolaemia and CHD. Carotid ultrasound: stenosis 25% in right subclavial artery. Baseline lipids (mmol/l):TC 8.88, LDL-C-7.20; HDL-C-1.2,Tg- 0.7. Serum campesterol -204; sitosterol-344.8 mkmol/l (normal<3). DNA test: ABCG8: W361X (Prof Joep Defesche, AMC, the Netherlands).Treatment: ezetimibe 10 mg/day. Last lipid test (july 2014):TC 4.5 mmol/l, LDL-C 2.91; HDL-C -1.1,TG - 1.01 mmol/l.AST/ALTCK are normal.

Conclusion. Rare monogenic dyslipidaemias may be efficiently diagnosed in Lipid Clinics based on laboratory and clinical data, but DNA test is needed to confirm the diagnosis. Combination lipid-lowering therapy is indicated long-life to prevent pancreatitis (FCS) and atherosclerotic disease (homo FH and beta-sitosterolaemia).
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Cholesterol crystals activate the complement system to induce crystal phagocytosis and inflammation

Abstract nr. 687
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Inflammation

Introduction
Cholesterol crystals (CC) are a hallmark of atherosclerotic lesions, and associated with early atheroma development. Phagocytosis of CC leads to activation of intracellular immune receptors and cytokine secretion that may contribute to plaque inflammation (Duewell et al. 2010). However, important aspects of how phagocytes were induced to phagocytose CC, including the phagocytosis receptor for CC, were not known. Activated complement proteins are also present in atherosclerotic lesions (Vlaicu et al. 1985, Speidl et al. 2011), suggesting involvement in inflammatory responses in atherosclerosis.

Methods
Complement activation by CC was detected by ELISA, measuring total complement complex (TCC). Deposition of complement components TCC, C3c, C1q as well as IgM were measured on CC after incubation in human plasma by flow cytometry. Phagocytosis of CC and surface expression of complement receptor 3 (CR3) was measured by flow cytometry after incubation in human whole blood. CC-induced cytokine secretion in human whole blood was measured by multiplex.

Main findings
We found that CC were able to activate the complement system measured by an increase in TCC. Complement components and IgM were found deposited on the CC surface after incubation in human plasma. The deposition of complement components was repressed by complement inhibition. The same complement inhibition reduced phagocytosis of CC as well as CC-induced upregulation of CR3 on phagocytes and cytokine secretion.
Conclusions
We have investigated how CC activate complement (Samstad et al. 2014). The deposition of the complement components on CC indicates an important role of the classical pathway in the CC-induced complement activation. IgMs on CC after incubation in human plasma points toward the adsorption of IgMs as the initiator of this complement activation. We showed that inhibiting complement activation lead to decreased surface expression of the putative CC receptor CR3, as well as decreased phagocytosis and subsequent cytokine secretion. Manipulating the complement system may therefore be of interest in managing the inflammatory response in atherosclerotic plaques.

Key words: Inflammation, cholesterol crystals, phagocytosis, complement system.
Financed by the Research Council of Norway through its Centres of Excellence funding scheme and the Central Norway Regional Health Authority.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Lomitapide decreased serum triglyceride levels in patient with familial chylomicronemia

Abstract nr. 688  
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention  
Keywords Dyslipidemia, Therapy, Triglycerides

**Background:** Familial chylomicronemia (FC) is a rare genetic disorder due to a deficiency in lipoprotein lipase (LPL), resulting in severe hypertriglyceridemia and recurrent episodes of acute pancreatitis. Currently available therapies are ineffective in FC. Lomitapide, a microsomal triglyceride transfer protein inhibitor, limits secretion into blood of chylomicrons and VLDL particles, decreasing thus cholesterol and triglyceride levels. Lomitapide is approved for treatment of homozygous familial hypercholesterolemia, but recently it has also been reported to effectively reduce triglycerides in one patient with FC (Sacks, FM. JAMA 2014; 174: 443-446).

**Case report:** We report our experience of 9 months of lomitapide treatment in patient with FC. To our knowledge, this is a second report of lomitapide use in this disease. Severe hypertriglyceridemia has been discovered in this patient (now 56 year old) in his early adulthood. Diagnosis of LPL deficiency has been established by genotyping and measurement of LPL activity. He had four major attacks of acute pancreatitis and frequent episodes of abdominal pain. Currently available lipid lowering drugs had little effect. With dietary and lifestyle therapy, his triglyceride levels varied between 25 - 45 mmol/l. Lomitapide was started at the dose of 5 mg/d and it was well tolerated up to the dose of 20mg/d; higher dose was associated with diarrhea. With this dose, triglycerides decreased to 15 - 25 mmol/l (see figure). Episodes of abdominal pain disappeared during therapy. There was a mild increase in the liver tests (up to 3x ULN) during therapy, other safety test remained normal.

**Conclusions:** Lomitapide effectively reduced serum triglyceride levels in a patient with FC. Our results confirm the previously reported experience with lomitapide in this disease.

**Funding support:** Lomitapide was provided by Aegerion Pharmaceuticals, Inc. under compassionate use program.
Figure
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
Effects of LDL on T cell homeostasis and immune response

Abstract nr. 689
Author Mailer, Reiner, Karolinska Institute, Stockholm, Sweden
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Immunity, Inflammation, LDL, Metabolism

Atherosclerosis results from the inflammatory response against accumulated low density lipoprotein (LDL) particles in the arterial wall. Reinforcing the innate immune response, T cells are important mediators of the disease development through their recognition of ApoB100, the protein component of LDL. T cell differentiation largely depends on the microenvironment to determine their pro- or anti-inflammatory function. However, the effect of cholesterol and fatty acids delivered through LDL particles on T cell activation and differentiation has not been investigated so far.

We found that polyclonal activation induces LDL receptor expression on T cells and that T cell proliferation in serum-free culture medium is enhanced by supplementation with LDL particles in vitro. Moreover, we demonstrate that LDL particles decreased the in vitro differentiation towards pro-inflammatory IL-17 producing Th17 cells, whereas the anti-inflammatory regulatory T (Treg) cell population, assessed by the expression of the Treg-specific transcription factor FoxP3, was increased. The LDL-mediated FoxP3 up-regulation was comparable to the known Treg differentiation factor TGF-b and the combination of both, LDL particles as well as TGF-b, synergistically induced FoxP3 expression, thus indicating two distinct mechanisms. Furthermore, effects of LDL particles on T cell differentiation persisted in LDL receptor deficient T cells, demonstrating that T cell metabolism rather than the uptake of LDL particles affects the T cell differentiation program. To assess the effect of LDL in vivo, we analyzed T cell subsets in non-atherosclerosis prone mice strains that were fed either western diet or chow diet for 4 weeks. We observed enhanced FoxP3+ T cell populations in wildtype and FoxP3 reporter mice that received western diet compared to chow diet. Thus, in non-inflammatory settings LDL particles may contribute to increased Treg-mediated suppression due to the induction of FoxP3 in differentiating T cells.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
The effect of type 2 diabetes on recurrent major cardiovascular events for patients with symptomatic vascular disease at different locations

Abstract nr. 690
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Co-author(s) - Visseren, Frank
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Diabetes, Epidemiology, Risk Factor

Clinical problem: Patients with type 2 diabetes mellitus have a recently estimated loss of 6 life years, mainly due to a high incidence of vascular disease. In patients with clinically manifest vascular disease, type 2 diabetes increases the risk of recurrent major cardiovascular events (MCVE). It is unknown whether the risk increase caused by type 2 diabetes differs across patients with various locations of symptomatic vascular disease.

Purpose: Our aim is to quantify and compare the effect of type 2 diabetes on recurrent MCVE for patients with symptomatic vascular disease at different locations.

Methods: 6,841 patients from the prospective Secondary Manifestations of ARTerial disease (SMART) cohort study with clinically manifest vascular disease with (n=1155) and without (n=5686) type 2 diabetes were followed between 1996 - 2013. Patients with a single symptomatic vascular disease site were stratified according to disease location (cerebrovascular disease, peripheral artery disease, abdominal aortic aneurysm or coronary artery disease). Patients with symptomatic vascular disease at ≥2 vascular locations were classified as having polyvascular disease. The effect of type 2 diabetes on recurrent MCVE was analyzed with Cox proportional hazard models, stratified for location of vascular disease.

Main findings: Event rates were 9/100 person-years (PY) in cerebrovascular disease, 9/100 PY in peripheral artery disease, 20/100 PY in those with an abdominal aortic aneurysm, 7/100 PY in coronary artery disease and 21/100 PY in polyvascular disease. Type 2 diabetes increased the risk of recurrent MCVE in patients with coronary artery disease (HR 1.67; 95%CI 1.25-2.21) and seemed to increase the risk in patients with cerebrovascular disease (HR 1.36; 95%CI 0.90-2.07), while being no risk factor in patients with polyvascular disease (HR 1.12; 95%CI 0.83-1.50). Results for patients with peripheral artery disease (HR 1.42; 95%CI 0.79-2.56) or an abdominal aortic aneurysm (HR 0.93; 95%CI 0.23-3.68) were inconclusive.

Conclusions: Type 2 diabetes increases the risk of recurrent MCVE in patients with coronary artery disease, but there is no evidence for an association between type 2 diabetes and recurrent MCVE in other patients with symptomatic vascular disease.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Reduced Glomerular Filtration Rate Does Not Impair HDL Cholesterol Efflux Capacity: A Study in Chronic Kidney Disease Patients

Abstract nr. 691
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Co-author(s) - Hurt-Camejo , Eva
Co-author(s) - Lindholm , Bengt
Co-author(s) - Stenvinkel , Peter
Co-author(s) - Parini , Paolo

Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Chronic Kidney Disease, HDL, Lipoproteins, Reverse Cholesterol Transport

Background: Premature CVD is the main causes of death in patients with chronic kidney disease (CKD). The risk for cardiovascular mortality markedly increase once the patient reaches a glomerular filtration rate (GFR) ≤60 ml/min/1.73 m2. CKD subjects develop dyslipidemia characterized by low plasma levels of apo-AI and high density lipoprotein (HDL) cholesterol, decreased lecithin:cholesterol acyltransferase (LCAT) activity, and apparent HDL dysfunction.
Aim: To investigate HDL cholesterol efflux capacity (CEC) in CKD patients with a particular focus on the different GFR.
Study design and methods: Age- and sex-matched subjects were divided into 3 groups according to their GFR (ml/min/1.73 m2): a) >60 (n=20); b) ≤60 (n=10); c) ≤30 (n=49). All patients were naïve for dialysis. Whole and apoB-depleted sera were tested as cholesterol acceptors in human THP-1-derived macrophages (a model of total macrophage efflux), or in pathway-specific cell models for aqueous diffusion (AD), SR-BI-, ABCA1-, and ABCG1- mediated efflux. HDL subpopulations were characterized by 2D-gel electrophoresis.
Results: Total macrophage CEC of both whole and apoB-depleted serum was not changed among all the 3 groups. We also quantified the specific contribution of the mechanisms involved in cell cholesterol efflux. Serum CEC by AD was decreased in patients with GFR ≤30 compared to the other 2 groups. Serum CEC by SR-BI did not differ between the groups, while apoB-depleted serum from patients with GFR ≤30 had a decreased capacity to promote cholesterol efflux by SR-BI compared to the other CKD groups. CEC by ABCG1 was not different between the groups. Serum CEC by ABCA1 was increased in patients with GFR ≤30 compare to those with GFR>60. Compared to patient with GFR>60, the apoB-depleted serum from the other CKD patient group showed a higher capacity to accept cholesterol through ABCA1. In agreement with their CEC
profile, CKD patients with GFR ≤30 showed higher plasma levels of preβ-HDL. Conclusions: In CKD patients serum and HDL capacity to promote cholesterol efflux from macrophages does not seem to be compromised by the uremic milieu. This is possibly due to the greater CEC via ABCA1 of circulating smaller preβ-HDL, which are increased in CKD patients with low GFR.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Up-regulation of plasmalogens attenuates atherosclerosis: A new therapeutic strategy

Abstract nr. 692
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Lipids, Prevention, Therapy

Oxidative stress is a contributing factor to the progression of atherosclerosis. We observed that circulating levels of plasmalogens (phospholipids with anti-oxidant properties) were negatively associated with both stable and unstable coronary artery disease and with future cardiovascular events. The modulation of plasmalogen concentration by oral administration of alkylglycerols (precursor to plasmalogen synthesis) has been reported, but its effect on atherosclerosis has not been investigated. We hypothesised that increasing the concentration of plasmalogen will attenuate atherosclerosis progression. We aimed to assess the effect of plasmalogen enrichment on atherosclerosis progression in murine models of atherosclerosis with differing levels of oxidative stress.

Six-week old ApoE- and ApoE/GPx1-deficient mice (elevated oxidative stress model) were fed a high-fat diet (HFD) with or without 2% batyl alcohol (BA, 18:0-alkylglycerol) for 12 weeks. Lipids of plasma, lipoproteins, and heart were analysed using liquid chromatography electrospray ionisation-mass spectrometry. Atherosclerotic plaques in the aorta were quantified via the en face technique. Cross-sections of the aortic sinus were stained for plaques and immunostained for an inflammatory marker, VCAM-1 and oxidative stress marker, nitrotyrosine

Supplementation of BA resulted in significant increases in the total plasmalogen concentration in plasma, lipoproteins, and in heart (P<0.001 for both genotypes). Mice fed a HFD without BA developed extensive atherosclerotic plaques throughout the aorta. This was reduced by 70% (P<0.001) in the BA-treated mice for both genotypes. A significant reduction in plaque was also seen in the aortic sinus of the treated ApoE/GPx1-deficient mice (-40%, P<0.01) however, the reduction in the aortic sinus of the treated ApoE-deficient mice was not significant (-12%, P=0.18). Only the BA-treated ApoE/GPx1-deficient mice showed a significant decrease VCAM-1 expression in the aortic sinus (-28%, P<0.05) and in nitrotyrosine formation in the aorta (-78.3%, P<0.001).

Plasmalogen enrichment via BA supplementation attenuated atherosclerosis in ApoE- and ApoE/GPx1-deficient mice, with a greater effect in the latter group. Supplementation with BA may
exert its greatest protective effect in environments of elevated oxidative stress as seen in the ApoE/GPx1-deficient mice, particularly at atherosclerosis-prone sites (aortic sinus). Plasmalogen enrichment may represent a viable therapeutic strategy to prevent atherosclerosis and reduce cardiovascular disease risk.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Cholesterol Ester Storage Disease: A Frequently Missed Diagnosis In Patients With Familial Hypercholesterolemia of Unknown Genetic Cause?

Abstract nr. 693  
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Co-author(s) - Hovingh, Kees  
Co-author(s) - Joep, Defesche  
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins  
Keywords Dyslipidemia, Familial Hypercholesterolemia, Genetics

Background: Cholesteryl Ester Storage Disease (CESD) is caused by mutations in the LIPA gene, encoding lysosomal acid lipase. Although patients with CESD are typically characterized by hepatic cholesteryl ester accumulation, we recently identified homozygosity for LIPA mutations in 3 patients with a clinical diagnosis of familial hypercholesterolemia (FH).

Purpose: To establish the prevalence of LIPA mutations in patients with clinically defined FH without mutations in LDLR, APOB, PCSK9 or LDLRAP.

Methods: We selected 284 patients with phenotypic FH, who were referred to the national FH DNA diagnostic laboratory at the AMC, Amsterdam, the Netherlands and in whom no genetic basis for their phenotype was found after sequencing of the LDLR, APOB, PCSK9 and LDLRAP genes. In these patients, the LIPA gene was sequenced using Sanger sequencing. All variants were assessed for pathogenicity using a literature search in PubMed and in silico prediction models including SIFT, Polyphen2 and MutationTaster.

Findings: We included 217 adults and 67 children with a mean (± SD) age of 53.8±11.1 and 10.8±4.1, respectively. Mean LDL-C levels of adults and children were 7.8±1.3 mmol/L and 4.4±1.5 mmol/L. A total of 18 variants were identified of which 10 were previously described. Five patients were found to be heterozygous carriers of a potentially pathogenic mutation; 2 patients were carriers of c.683T>C in exon 7, encoding a phenylalanine to serine amino acid substitution (F228S) that was predicted to be pathogenic by all in silico prediction models used; 2 patients were carriers of the c.894G>A mutation in exon 8, and 1 patient was carrier of c.913T>A in exon 9, causing a phenylalanine to isoleucine substitution (F305I) that was predicted to be potentially pathogenic by polyphen2. No homozygous or compound heterozygous LIPA mutation carriers were found.

Conclusions: No homozygosity or compound heterozygosity for LIPA mutations was found in patients with FH of unknown origin, suggesting that CESD is not a frequently missed diagnosis in these patients.

Funding: This study was financially supported by Synageva BioPharma.

Subdivision 3. Clinical Studies
Presentation Preference Oral presentation
Additional information
Relationship of serum vaspin level to HDL subfractions and paraoxonase-1 activity in non-diabetic obese patients

Abstract nr. 695
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, HDL, Obesity

Background: Vaspin is a recently identified adipokine produced predominantly by the white adipose tissue. Human circulating vaspin was previously reported to possess potential insulin-sensitizing properties. Also, a positive and independent association was found between vaspin levels and carotid intima-media thickness; therefore, vaspin may have a potential role in the development of atherosclerosis. On the other hand, obese individuals are prone to develop atherosclerosis that is characterized by increased oxidative stress, in which HDL-associated paraoxonase (PON1) was demonstrated to be preventive by hydrolysing lipid peroxides and inhibiting LDL from oxidative modification. To date, the relationship between serum vaspin level and paraoxonase-1 (PON1) activity has not been clarified.

Patients and methods: Fifty non-diabetic obese (BMI: 41.96±8.6 kg/m²) and thirty-eight normal-weight healthy controls (BMI: 24.16±3.3 kg/m²) matched in age and gender were enrolled to our study. Serum vaspin level was measured by ELISA, PON1 arylesterase activity was determined spectrophotometrically. HDL subfractions were determined by the Lipoprint® System.

Results: Compared to controls, we detected the presence of low-grade inflammation (hsCRP: obese 8.24 [3.2-13.09] vs. control 1.4 [0.5-2.46] mg/l; p<0.001) and atherogenic dyslipidemia in the obese patients, while serum vaspin level and PON1 activity were similar in the two study groups. In the obese subjects, the proportion of the large HDL subfraction was significantly lower (p<0.001), while the proportion of small, dense HDL was significantly higher (p<0.001), compared to healthy controls. Serum vaspin concentration showed a significant positive correlation with the HDL-C level (r=0.3; p=0.039), with the proportion of small, dense HDL subfraction (r=0.37 p=0.03), and with PON1 activity (r=0.245 p=0.028), respectively. Multiple regression analysis (backward stepwise) indicated that PON1 activity is the independent predictor of vaspin level.

Conclusion: Vaspin was found to be related with HDL and HDL-associated antioxidant PON1 enzyme, indicating the protective anti-atherosclerotic effect of this adipokine. Therefore, vaspin may serve as a useful early cardiovascular biomarker in obese patients.

Acknowledgement: This work is supported by a grant from the Hungarian Scientific Research
Fund (OTKA 84196) and by the TÁMOP-4.2.2.A-11/1/KONV-2012-0031 project. The TÁMOP project is co-financed by the European Union and the European Social Fund.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Importance of non-synonymous genetic variation in WRN on risk of ischemic vascular disease and mortality in the general population

Abstract nr. 696
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Cardiovascular Disease, Epidemiology, Genetics

AIM: Twin studies have shown that longevity is moderately heritable with 25% of the variation in human lifespan being attributed to genetic factors. However, so far only the APOE gene has been consistently associated with longevity. Werner syndrome is a serious form of premature aging caused by severe truncating mutations in the WRN gene. Many clinical manifestations of the Werner Syndrome resemble normal physiological aging, and cardiovascular disease is a frequent cause of death in Werner syndrome patients. We hypothesized that non-synonymous genetic variation in the WRN gene is associated with risk of ischemic vascular disease and longevity in the general population.

METHODS: We genotyped all non-synonymous variants in WRN - which had previously been reported in Caucasian populations with minor allele frequencies at or above 0.5% - in 10,250 individuals from the Copenhagen City Heart Study (CCHS) (Figure 1). Of these, 2,946 developed ischemic vascular disease during up to 22 years of follow-up. Results were validated in the Copenhagen General Population Study (CGPS, n=48,034) with 3,366 ischemic vascular events during up to 10 years of follow-up.

RESULTS: A total of 11 non-synonymous and truncating genetic variants were identified in the CCHS. For 3 variants indications of an association with risk of ischemic vascular disease or longevity was seen in the CCHS or in the literature. None of these variants were however consistently associated with risk of ischemic vascular disease or mortality in the CGPS or in the combined study.

CONCLUSIONS: Non-synonymous genetic variation in WRN does not associate with clinically relevant effects on risk of ischemic vascular disease or mortality in the general population. Thus, although cardiovascular morbidity is one of the major clinical features in Werner syndrome patients, genetic variation in WRN does not seem to contribute significantly to development of cardiovascular disease in the general population.

FUNDING SOURCES: This study was funded by the Faculty of Health Sciences, University of Copenhagen; the Lundbeck Foundation; the Research Fund at Rigshospitalet; and the Danish Medical Research Council.
Figure 1. Study design
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Patients with familial hypercholesterolemia are characterized by presence of cardiovascular disease at time of death

Abstract nr. 697
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Familial Hypercholesterolemia

Aims: Familial hypercholesterolemia (FH) is associated with increased risk of premature atherosclerosis and cardiovascular disease (CVD). The aim of this study was to investigate the presence of CVD in a group of FH patients at time of death.

Methods and Results: We characterised the presence of CVD, lipid profile, medical treatment and cause of death from the medical records of 79 deceased FH patients, who died in the time period 1989-2010. Mean age at first CVD event was 44 years and time of death was 60 years. CVD was the registered cause of death in 50% of the deaths; however at time of death 89% of the FH patients had established CVD and 69% had experienced one or more myocardial infarction. The FH patients received statin treatment for an average of eight years prior to death. FH patients who died at a younger age (mean age 51 years) were significantly younger at first CVD event compared to FH patients who died at an older age (mean age 71 years, 40 versus 50 years, \(P<0.001\)). More FH patients who died at a younger age received statin treatment compared to that of the FH patients who died at an older age (98% versus 81%, \(P=0.038\)), however despite that their last measured level of total cholesterol and low density lipoprotein cholesterol was significantly higher in comparison with the older FH patients (7.3 versus 6.3 mmol/L, \(P=0.016\), and 5.3 versus 4.4 mmol/L, \(P=0.033\), respectively). There were more current smokers in the FH patient group who died at a younger age compared to the patients who died at an older age (55% versus 10%, \(P=0.001\)). Interestingly, we found no significant differences in CVD presence, age at first CVD event or age at time of death when dividing the group by gender.

Conclusion: The majority of FH patients are characterized by presence of CVD at time of death; this underscores the severity of the disease and the need for early diagnosis and treatment.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Regulation of lipid and carbohydrate metabolism by ligand-activated transcription factors during metabolic syndrome development in the hypertensive ISIAH rat strain

Abstract nr. 698
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Cardiovascular Disease, Diabetes, Dyslipidemia

It is known that the metabolic syndrome (MS) leads to serious cardiovascular disease which continues to be the number one cause of mortality in industrial countries. According to the National Cholesterol Education Program-Adult Treatment Panel III, the criteria of the metabolic syndrome include three or more impairments: increase in blood triglycerides, decrease in blood HDL cholesterol, hypertension, visceral obesity and increased blood glucose. The MS distribution is growing catastrophically, but molecular mechanisms responsible for development of complex impairments in MS still remain basically poorly investigated. A high percentage of MS morbidity determines much attention to MS modeling, mechanisms of MS development, and new approaches to MS treatment. The formation of complex MS symptoms suggests systemic impairments in lipid and carbohydrate metabolism; it appears that these impairments should have a common basis at the level of expression of appropriate genes. Expression of genes involved into lipid and carbohydrate metabolism is regulated by various transcription factors, including peroxisome proliferator-activated receptors (PPAR) (Lefebvre P. et all, 2006], liver X receptors (LXR) (Herzog B. et all, 2007), pregnane X receptors (PXR), and constitutive androstane receptors (CAR) (Moreau A. et all, 2008). In the hypertensive ISIAH rats compared with normotensive WAG rats the signs of the metabolic syndrome developing, correlating with the altered functional activity of ligand-activated transcription factors involved in lipid and carbohydrate metabolism were detected (Pivovarova E. N. et all, 2011). It was shown that fructose load (10 % fructose in the drinking water for 10 weeks) leads to the increase in the level of triglycerides in the blood serum of ISIAH rats. When comparing hypertensive ISIAH with two normotensive WAG and Wistar rat strains it was found higher content of glucose in the blood of ISIAH rats. It demonstrates relationship of high blood pressure, increased levels of glucose and increased levels of triglycerides. Complex studies of regulatory mechanisms, signaling pathways, and transcription targets for PPAR, LXR, PXR, and CAR may significantly help in better understanding of MS and provide valuable information for development of appropriate pharmacological approaches to MS therapy.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
Moroccan study among obese patients with or without metabolic syndrome: Nutritional survey and biological parameters

Abstract nr. 700
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Dyslipidemia, Nutrition, Obesity

Introduction: Mediterranean diet reflects a typical culture and lifestyle proper to the Mediterranean basin. However, Morocco has met an important nutritional transition for last years. To better become aware of these changes, we realized a dietary survey in obese Moroccan patients with or without metabolic syndrome (MS).

Patients and methods: We recruited 241 obese patients, mean-aged of 53.97± 10.50 years-old, and divided them into two groups: without MS (Ob without MS, n= 29 men and 92 women) and with MS (Ob with MS, n= 29 men and 91 women), matched for sex and age. MS has been defined in accordance with NCEP-ATP III criteria. We also assessed the relationship between lipid parameters, low grade inflammation and MS.

Results: Ob with MS’s diet was more caloric but poorer in polyunsaturated fatty acids (PUFA), in vitamins B9 and E. Both groups consume meals which macronutrient compositions were similar. The consumption of Retinol, Beta-carotene, Vitamin C and trace elements was higher in Ob with MS than in those without MS, whereas consumption of cholesterol and fibers were not significantly different.
In patients with MetS, lipoprotein profiles alterations and low grade inflammation were observed. Lipid ratios were better predictors of cardiovascular risk than lipids alone because of their relative associations with lipoproteins and apolipoproteins.

Conclusion: The present study showed that Moroccans have a rich diet, but poor in vitamins and trace elements, the overall translating a little knowledge of foods and theirs nutritional benefits.

Subdivision 3. Clinical Studies
Presentation Preference Electronic poster presentation
Additional information
Effect of 14 days experimental bed rest on plasma RBP4 and lipid profile in young and older subjects, PANGeA study.

Abstract nr. 701
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Co-author(s) - Passaro, Angela
Co-author(s) - PANGeA, Group
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Apolipoproteins, Elderly, Risk Factor, Triglycerides

Retinol binding protein 4 (RBP4) is a recently identified adipokines that may link obesity with insulin resistance, type 2 diabetes and lipid metabolism. Some studies investigated the possible role of exercise training on circulating RBP4, however nothing is known about the effect of physical inactivity on RBP4 concentration.

The PANGeA group set a 14-day horizontal Bed Rest (BR) model to evaluate the effect of bed confinement and following recovery (R) in young and older adults. In this study we evaluate the effect of BR on RBP4 concentration and lipid profile.

Methods: 23 healthy male subjects were enrolled. They were divided in “YOUNG” (n=7; 18-25 years) and “OLDERS” (n=16; 55-65 years). Blood samples for biochemical analysis were collected and body composition was assessed, by BIA, at baseline (BDC), after 14-day of bed rest (BR14) and after 14-day of recovery (R14). All subjects followed a diet supplying 1.2 times the estimated basal metabolic rate. RBP4 was measured with ELISA kit.

Results: At baseline (BDC) OLDERS and YOUNG showed similar BMI, glucose and insulin levels while RBP4 concentration was significantly higher in OLDERS. OLDERS also showed higher levels of cholesterol, LDL-cholesterol and triglycerides. At BDC RBP4 correlated with age, BMI, total cholesterol and triglycerides levels but not with glucose or insulin.

At BR14, a significant decrease of BMI was observed in both OLDERS and YOUNG. Moreover, OLDERS showed a significant cholesterol, LDL-cholesterol and tryglycerides reduction. Conversely, HDL-cholesterol was significantly reduced in YOUNG. After BR, RBP4 levels significantly decreased in OLDERS; this reduction was significantly associated with the reduction of cholesterol and triglycerides. In both groups, recovery was associated with a significant increase on RBP4 levels.

Conclusion: BR was associated with a reduction in BMI. This “catabolic state” was associated with
a significant decrease in RBP4, NEFA and triglycerides in OLDERS. Conversely YOUNG, who did not show a decrease in RBP4, did not show this apparent lipid profile improvement and actually showed decreased HDL-cholesterol. This data support a role of RBP4 in lipid metabolism and in bed rest metabolic adaptation, especially in the elderly.

This study was supported by the Crossborder Cooperation Programme Italy-Slovenia 2007-2013.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Specific clinics for follow-up in coronary artery disease

Abstract nr. 704
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Co-author(s) - Soner , Hatice.K
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Therapy

Introduction: The beneficial effects of statin treatment in the early stages and maintenance therapy of ST-elevation myocardial infarction (STEMI) has been shown in multiple studies. Recent guidelines emphasize the importance of strict LDL-C control for secondary prevention in coronary artery disease (CAD). Also, preventive guidelines focus on patient education and regular follow-up in order to achieve target the recommended LDL-C levels of ≤70mg/dL in CAD.

Purpose: The aim of this study is to evaluate if there is a difference in achieving LDL-C goals as recommended by guidelines ≤70mg/dL in STEMI patients in our tertiary center, by comparing results of standard outpatient clinic follow-up and results of a single physician strategy with a more constructed approach.

Methods: Consecutive 123 patients admitted between 2010–2014 with diagnosis of STEMI, treated with thrombolytic therapy were included. Group-A consisted of 64 patients followed by standard outpatient clinic and Group-B consisted of 59 patients, followed by a single physician. All patients had at least three visits and patients were educated on lifestyle changes for CAD.

Results: The mean age was 55±10 (77% male) and median follow-up was 6.2 months. Age, gender, diabetes, hypertension, hyperlipidemia, family history, renal failure, and LDL-C levels (Group-A 124±35 mg/dL, Group-B 129±37 mg/dL) were similar in both groups. There was a significant difference in last visit with regard to LDL-C levels and number of patients who reached treatment goal between groups (Group-A 105±35mg/dL vs. Group-B 92±41mg/dL, P<0.01, Group-A 6/64, Group-B 20/59, P<0.01 respectively). There was a significant difference in terms of change in the value of LDL-C between groups (Group A-10.2±34% vs. Group B -27±28%, P<0.01).

Discussion: Guidelines suggest intensive statin therapy in early and long-term follow-up of patient with CAD. In-hospital early initiated intensive statin therapy is important for long term patient’s compliance. However, interruption in drug use and difficulties in adapting to lifestyle changes are common problems. Regular follow-up to maintain target LDL-C levels and patient education have great importance. The importance of regular follow-up of CAD patients by a single physician with a more constructed approach including the education of the patients in achieving target LDL-C
levels are demonstrated in this study.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Medication compliance and lifestyle changes in secondary prevention of coronary artery disease

Abstract nr. 705
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Co-author(s) - Turkoglu, Cuneyt
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Prevention, Risk Factor, Risk stratification

Introduction: The incidence of CVD related deaths has decreased with the improvement of primary and secondary prevention and disease awareness in developed countries but it is not yet at the desired level. Life style changes and strict compliance to the treatment of risk factors are very important for a long and healthy life.

Purpose: The prospective evaluation of the compliance to treatment and the effect of lifestyle changes on CV risk factors including tobacco use, weight control, exercise, diet, blood lipid levels, blood pressure in patients with ST-elevated myocardial infarction (STEMI).

Method: Consecutive 67 patients admitted between 01/02/2013–01/12/2014 with diagnosis of STEMI who were treated with thrombolytic therapy were included. All patients underwent an education program and had regular follow-ups, since first month of diagnosis and every three months.

Results: Basal characteristics of the study population (mean age 55±10 years and 72% male, mean follow 9.5 months) are shown in the table. All patients were discharged with antiplatelet and statin therapy. Rate of dual antiplatelet and statin usage were 98.5% and 100% respectively in the first month and the ratio decreased to 95.3% for both in 6th month. There was a decrease in LDL-C levels in sixth month when compared to baseline levels (92±39mg/dL p<0.01). Arterial systolic and diastolic blood pressure was 120±17/72±13 mmHg at sixth month (p<0.01). At 6th month, 73% of patients stopped using tobacco and effective weight control, diet, and exercise were achieved in 27%, 43%, and 31%, respectively.

Discussion: Intensive treatment and education of patients’ have prime of importance in the early period including after discharge. This study showed that compliance to treatment was high in patients with STEMI after 6-month follow-up early after MI. However, despite intensive efforts for patient education, adaptation to lifestyle changes in the short term was not satisfactory. For the implementation of lifestyle changes, more constructed and intensive education and more frequent monitoring are needed.
### Basal characteristic of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Age (% male)</td>
<td>55 ± 10 (70%)</td>
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<tr>
<td>Diabetes mellitus %</td>
<td>18</td>
</tr>
<tr>
<td>Hypertension%</td>
<td>34</td>
</tr>
<tr>
<td>Hyperlipidemia%</td>
<td>18</td>
</tr>
<tr>
<td>Coronary artery disease%</td>
<td>12</td>
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<tr>
<td>Family history%</td>
<td>25</td>
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<tr>
<td>Smoker%</td>
<td>33</td>
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<tr>
<td>BMI %</td>
<td>27 ± 3.8</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>183 ± 41</td>
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<tr>
<td>LDL-C</td>
<td>126 ± 41</td>
</tr>
<tr>
<td>HDL-C</td>
<td>38 ± 10</td>
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<tr>
<td>Triglyceride (mg/dL)</td>
<td>185 ± 99</td>
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<td>SBP/DBP (mmHg)</td>
<td>135 ± 28 / 79 ± 15</td>
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<td>Hemoglobin (mg/dL)</td>
<td>14 ± 1.6</td>
</tr>
<tr>
<td>Hematocrit%</td>
<td>41.7 ± 5</td>
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<tr>
<td>Creatine (mg/dL)</td>
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</tr>
</tbody>
</table>

**Basal characteristic of the study population**

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
ATHEROGENIC INDEX OF PLASMA AND CARDIOVASCULAR RISK IN MALNOURISHED PATIENTS WITH CHRONIC KIDNEY DISEASE

Abstract nr. 706
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Chronic Kidney Disease, Dyslipidemia, Risk Factor

**Background:** Maintenance haemodialysis (HD) patients have a high prevalence of protein-energy malnutrition and dyslipidemia, which are strongly associated with cardiovascular disease (CVD) and accelerated atherosclerosis. Our study aimed to investigate the relationship between malnutrition and cardiovascular risk on chronic kidney disease through lipid ratios and atherogenic index of plasma (AIP) in Moroccan HD patients.

**Methods:** This cross-sectional study involved 126 patients were recruited at the department of Nephrology-dialysis-kidney transplantation UHC Ibn Rochd, Casablanca. Patients were divided in 3 groups: well nourished (group 1, n=26), moderately malnourished (group 2, n=60) and severely malnourished (group 3, n=41). Biochemical tests including albumin, prealbumin, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were performed. Then, we have assessed atherogenic indexes such as TG/HDL-C ratio, TC/HDL-C ratio, LDL-C/HDL-C ratio, non-HDL-C and AIP. The difference between each mean value of lipid levels was assessed by ANOVA tests. Linear regression analysis was performed to test associations between lipid ratios and predicted risk of developing a CVD.

**Results:** The mean age of our patients was 44.81±14.00 years-old, with a duration of treatment of 12.07±6.23 years. According to biological data, we found 68% of HD patients had hypoalbuminaemia and 70% had hypoprealbuminaemia. The most frequent lipid alteration recorded was increased non-HDL-C (88%), decreased HDL-C (60%) and hypertriglyceridaemia (35%). There was a significant difference in TG, HDL-C, LDL-C, and non-HDL-C and TC levels between malnourished and well nourished HD patients. Lipid ratios in group 2 were significantly higher than those in group 1, while group 3 presented significantly lower lipid ratios values compared to group 1. We observed that 74% of malnourished patients were at high CV risk with AIP>0.21 in comparison with well nourished (65%). Linear regression analyses shows that AIP had a strong association with serum albumin and prealbumin (P<0.001). AIP was positively associated with LDL-C, TC/HDL, LDL/HDL, non-HDL-C, non-HDL-C/HDL-C and TG/HDL (P<0.0001 for all), but negatively with HDL-C (P<0.001).

**Conclusion:** Our study suggests that lipid ratios, especially AIP, may be useful tools for risk of cardiovascular disease in malnourished HD patients.

Subdivision 3. Clinical Studies
Presentation Preference Electronic poster presentation
Additional information
Impact of fasting Ramadan on ambulatory Blood Pressure Measurement in hypertensive patient

Abstract nr. 707
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Keywords Blood pressure, Hypertension, Pharmacology

Introduction. Each year, millions of Muslims around the world do fasting, every day, during the month of Ramadan, from sunrise to sunset. The changes in sleep patterns, and changes in the medication schedule, may thus influence ambulatory blood pressure.

Methods. Prospective study includes 15 patients with hypertension (HT), who do fast for a month. A first measurement of ambulatory blood pressure ABPM1 was conducted between D29-D10 of Ramadan; a second ABPM2 between D20-D40 after Eid. This study was carried out by swapping the morning time of antihypertensive drug intake by the breaking time of fast (sunset) and the night intake by the shour (sunrise).

Results. Results are obtained from 15 patients (9 men and 6 women), mean age 48 ± 14 years, mean HT duration of 8 ± 4 years. HT is treated with monotherapy in two patients, dual combination therapy in 8 patients, combination of 3 drugs in two patients and 4 drugs in three patients. Four patients have diabetes. The number of drug and dosage is kept constant throughout the study. Mean office BP measurements performed before the implementation of the ABPM were 138±11/89±7 mmHg during Ramadan and 136±8/88±7 mmHg after Eid. 24h BP and daytime and nighttime systolic and diastolic BP were significantly higher during the month of Ramadan, except for systolic BP during the waking period that did not reach significance (Table 1). Systolic BP (mm Hg) diastolic BP (mm Hg) after Ramadan.

Discussion. 24h BP and nighttime systolic; and 24h BP, daytime and nighttime diastolic BP were significantly higher during the month of Ramadan. This can be explained by sleep disturbance and diet differences commonly seen in this month, with in particular more salt and calories consumption.

Conclusions. BP during the month of Ramadan is significantly higher compared to BP after the month of Ramadan for the same therapeutic prescription if one switches the morning intake by breaking fast time and the night intake by the shour (sunrise). This increase should be considered
in the therapeutic management of patients performing Ramadan.

<table>
<thead>
<tr>
<th>Period</th>
<th>Systolic BP (mm Hg)</th>
<th>Diastolic BP (mm Hg)</th>
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<tr>
<td></td>
<td>Ramadan (fast)</td>
<td>After Ramadan</td>
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<td>130,32</td>
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<tr>
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Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Plaque size is decreased but M1 macrophage polarization and rupture-related metalloproteinase expression are maintained after deleting Th1-lymphocytes in ApoE-null mice.

Abstract nr. 709
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Cardiovascular Disease,Immunity,Vulnerable Plaque

Background: Plaque rupture causes myocardial infarctions. We investigated whether deleting Thelper1 (Th1) lymphocytes reduces atherosclerotic plaque growth and vulnerability to rupture by inhibiting M1 macrophage activation causing reduced production of matrix degrading metalloproteinases (MMPs) and increased tissue inhibitor of MMPs, TIMP3.

Methods & Results: We compared male atherosclerosis prone Apolipoprotein-E (ApoE) knockout (EKO) mice with ApoE/T-bet double knockout (DKO) mice that lack Th1 lymphocytes. Mice were fed a high fat diet (HFD, 12 weeks) or a normal chow diet (ND, 35 weeks). Transcript levels of M1/M2 macrophage polarization markers, selected MMPs and TIMPs were measured by RT-qPCR in macrophages isolated from subcutaneous granulomas (produced after 4 weeks HFD) and in whole aortae (12 weeks HFD). Immunohistochemistry of aortic sinus (AS) and brachiocephalic artery (BCA) plaques was conducted to quantify protein expression of the same factors (after 12 weeks of HFD or 35weeks of ND).

Granuloma macrophages from DKO mice had less mRNA for the M1 marker iNOS than EKO mice (69.3±10.3 vs 32.8±11.1 copies/ngRNA n=6-8), but the same levels of M2 markers (CD206, arginase-1 and Ym-1), MMPs-2, 9, 12, 13, 14 and 19 or TIMPs-1 to 3. In whole aortae extracts (n=8-9), no differences were observed. After 12 weeks of HFD, AS and BCA plaques (n=10-18) were similarly sized between genotypes and had similar areas stained for iNOS, COX2, MMP12 and MMP14 protein levels. Interestingly, AS DKO plaques had more MMP-13, MMP14 and Arg-1 than EKO plaques (40.8±5.3 vs 20.4±2.6 % plaque area, n=11-12; 54.1±3.4 vs 42.4±3.6 %, n=11-15; 5.6±2.3 vs 0.9±0.5 %, n=7-10; respectively). After 35 weeks of ND, DKO mice had smaller AS and BCA plaques than EKO mice (in AS 97400±47600 vs 299000±30300 μm², n=10-11 and in BCA 18000±10400 vs 44900±11600 μm², n=10-11) but there were no differences in the areas of plaques stained for M1 or M2 markers, MMPs12, 13, 14, or TIMP3.

Conclusions: Deleting Th1 lymphocytes reduces plaque size but M1 polarization and expression of several MMPs are either maintained or increased, which translates to only limited protection from plaque rupture.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Cyclosporin A-mediated dyslipidemia in the LDLr knockout mouse

Abstract nr. 711
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia

Cyclosporin A (CsA) is an immunosuppressant drug commonly used in organ transplant recipients and auto-immune disorders. Long-term treatment with CsA is associated with hyperlipidemia and an increased risk of cardiovascular disease. The mechanism(s) by which CsA causes hyperlipidemia is unknown but are generally thought to be mediated via the Low Density Lipoprotein Receptor (LDLr). However, the data supporting a role for LDLr are inconclusive. To determine whether the LDLr plays a role in CsA-induced hyperlipidemia we examined the effect of CsA in LDLr knockout (LDLr-/-) and wild type C57Bl6/J mice.

Female mice fed a chow diet were treated with 20 mg/kg/day CsA administered subcutaneously by implantation of Alzet osmotic pumps for four weeks. Lipoprotein fractions were separated by FPLC. Lipid levels were measured using commercial enzymatic kits. Specific lipid species were determined by LC-MS/MS. Effect of CsA on hepatic genes involved in lipid metabolism were investigated by real time PCR and Western Blot analysis. Hepatic VLDL production rates were determined using Triton WR1339 and lipoprotein lipase (LPL) activity was determined before and after heparin injection using a commercially available kit.

CsA treatment increased plasma cholesterol and triglyceride levels 2- and 1.6-fold respectively in LDLr-/- mice, which was associated with increased plasma VLDL and LDL levels. No effect on plasma lipids was observed in C57Bl6/J mice. Analysis of specific lipid species suggested increased VLDL and LDL particle number. In addition small changes in some minor lipid species contained within VLDL and LDL were observed. Although mRNA levels of several genes involved in triglyceride synthesis were increased, protein levels of these genes were not altered. In vivo hepatic VLDL secretion studies indicated CsA did not affect hepatic VLDL production/secretion. CsA did however, inhibit plasma LPL activity in LDLr-/- mice.

In conclusion, CsA can induce hyperlipidemia independently of the LDLr. This is not mediated via increased hepatic VLDL production. Inhibition of plasma LPL activity by CsA is likely to contribute
to CsA-induced hyperlipidemia. These data also suggest the possibility that the hyperlipidemic effects of CsA may be more evident in patients with LDLr dysfunction.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Differential effects of gliclazide and glibenclamide on the development of atherosclerosis in a diabetic mouse model

Abstract nr. 712
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Diabetes, Endothelium

Recent studies suggest that several sulphonylureas (SU), but not gliclazide, are associated with increased cardiovascular risk compared to other hypoglycaemic drugs. Only gliclazide has antioxidant properties and other SU, such as glibenclamide, have a greater affinity for vascular potassium channels, potentially impairing vascular relaxation. We studied the effects of gliclazide and glibenclamide on the development of atherosclerosis in a diabetic atherosclerosis-prone mouse model. To evaluate the effects of SU exposure in endothelial cells we also investigated the effects of these drugs on gene transcription using microarray analyses.

Six-week old homozygous apo E-KO male mice were rendered diabetic using intraperitoneal streptozotocin, 60 mg/kg. Only animals with blood glucose >15 mmol/l were studied. Animals were randomized to receive either gliclazide 10mg/kg or glibenclamide 2mg/kg, added to drinking water. After 20 weeks, the animals were euthanased and blood was collected for biochemical analyses. Aortas were rapidly dissected and stained for measurement of plaque area. Photographs of the stained aortas were digitized and evaluated using an image analysis system. For the gene expression studies, primary HUVEC cells were exposed to 1µM of each compound for 24h. Gene expression profiles were analyzed using Affymetrix GeneChip arrays. Gene lists were generated using the following criteria: fold change >2 and p<0.05 (test vs. ctrl) and p >0.05 for the other drug. The effects on atherosclerosis are shown in the table.

Despite similar HbA1C and cholesterol levels, glibenclamide treated mice had significantly more aortic plaque area than untreated or gliclazide treated animals. A number of gene networks were identified for both drugs. Their functional categories related primarily to molecular transport and cell signalling. Analyses for upregulated genes showed an increased significance for gliclazide over glibenclamide for canonical pathways relating to nitric oxide signalling in the cardiovascular system.

We conclude that glibenclamide significantly accelerates diabetes associated atherosclerosis in this model, whereas gliclazide is neutral. The drugs also differ in their effects on endothelial cell gene expression. Given that both drugs are in widespread use in diabetic patients, the mechanisms underlying these differences warrant further evaluation.
* $p < 0.02$ vs. diabetic and vs. gliclazide

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
ABCA1 modulates SAA turnover

Abstract nr. 713
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Co-author(s) - Michikawa, Makoto
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Apolipoproteins, HDL, Inflammation

Background: Serum amyloid A (SAA) is a precursor protein of amyloid A, a component of amyloid fibrils in secondary amyloidosis. SAA is secreted from hepatocytes during inflammation and found as an HDL apolipoprotein in the plasma. ABCA1 is essential to maintain the plasma HDL concentration. ABCA1 KO mice plasma lacks HDL even in inflammation and SAA can be found in its VLDL/LDL fraction. ABCA1 KO mice hepatocytes secrete lipid-free SAA, which is about 50% of that in WT mice. However, both plasma SAA level and tissue amyloid deposition are much more lower in ABCA1 KO mice. We hypothesized that SAA turnover rate is increased in ABCA1 KO mice due to lacking in SAA lipidation.

Methods: WT and ABCA1 KO mice of both sexes, older than 4 months, were used in the experiments. Acute inflammation and proximal tubular injury were induced by intraperitoneal injection of LPS and Na maleate, respectively. Plasma and urine samples were obtained and analyzed.

Results and discussion: Inhibition of reabsorption in the proximal tubule by Na maleate was confirmed by the appearance of glucosuria in both WT and ABCA1 KO mice. Histological examination revealed that about 20-30% of proximal tubular cells were necrotic at 24 h after Na maleate administration. When acute inflammation was induced, SAA levels were increased in both genotype mice. However, the concentration of SAA in ABCA1 KO mice plasma was about 10% of that in WT mice, as was reported previously. Agarose gel electrophoresis of plasma lipoprotein detected induction of SAA-HDL in only WT mice. In this experimental condition, SAA in the urine was detected only after Na maleate injection. The concentration of urinary SAA was in the same range between WT and ABCA1 KO mice. These data indicated that Lower level of SAA in ABCA1 KO mice plasma is due to higher glomerular filtration rate of lipid-free HDL apolipoproteins. SAA protein in ABCA1 KO mice plasma is more readily excreted into urine than that in WT mice, then reabsorbed and degraded in the proximal tubular cells.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Reverse cholesterol transport assessed using 3H-cholesterol nanoparticles is reduced in subjects with ABCA1 and LCAT deficiency

Abstract nr. 714
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Co-author(s) - Mcparland, James
Co-author(s) - Billheimer, Jeffrey
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords HDL, Reverse Cholesterol Transport

Aim: The ability to remove cholesterol from macrophages and transport it back to the liver is one of the main atheroprotective functions of HDL; however no method exists to assess this HDL function in vivo in humans. We had previously developed a macrophage-specific method to assess reverse cholesterol transport (RCT) using nanoparticles containing radio-labeled cholesterol. We present the preliminary results of a validation study in subjects carrying mutations in ABCA1 and LCAT genes, encoding for key proteins of the RCT pathway.

Methods: Homozygous and heterozygous carriers and appropriately matched controls (1:2) received $^3$H-cholesterol nanoparticles ($^3$H-free cholesterol/albunin complexes) as i.v. bolus, followed by blood collection. Tracer counts were assessed in plasma and, where possible, in non-HDL, and HDL fractions.

Results: The figure shows the tracer as % of injected counts and as % cholesterol ester (CE) in plasma, in a patient with Tangier disease (A) and a patient with LCAT deficiency (B), and matched controls. Tracer counts over time were markedly reduced in both patients as compared with controls. Intermediate results were found in heterozygous subjects. Cholesterol esterification was grossly absent in the LCAT deficient subject, but apparently normal in the subject with Tangier disease.

Conclusions: ABCA1 and LCAT deficiency are associated with an impaired RCT. These preliminary data support the use of $^3$H-cholesterol nanoparticles to measure macrophage RCT in vivo in humans.
Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Predictor for mismatch in the disease extent of coronary artery disease between computed tomography coronary angiography and coronary angiography

Abstract nr. 715
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Imaging

Background and objectives: Computed tomography coronary angiography (CTA) is a useful tool for the identification of coronary artery disease. However, as other diagnostic tools, it might give us the information of overdiagnosed coronary artery disease or underdiagnosed one. The aim of this study was to identify predictor for mismatch in the disease extent of coronary artery disease between CTA and coronary angiography.

Subjects and Methods: Data from 109 consecutive patients who underwent CTA and coronary angiography with the suspicion for coronary artery disease were analyzed. The extent of coronary vascular disease was classified as insignificant, one, two and three-vessel disease in CTA and coronary angiography, respectively. Then, we investigated the predictor for mismatch between those classifications judged by CTA and coronary angiography.

Results: There were 54 patients whose CTA and coronary angiography showed discrepant results for the extent of coronary artery disease. Hypertension, dyslipidemia and smoking did not show different prevalence between discrepant result group and concordant result group. In addition, there were no differences in pulse wave velocity, ankle-brachial index and body mass index in two groups. However, diabetic patient group and nondiabetic patient group reveal the different discrepancy rate (70.5% and 35.4%, respectively : p<0.001). Conclusion: The diabetic patients are more likely to show discrepant result for the extent of coronary artery disease between CTA and coronary angiography. However, the other characteristics are less likely to show discrepancy.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
FMO3 Gene Loss-of-Function Variants Showed Lower HDL-C And Higher TG-rich HDL, But Lower Coronary Stenosis Indices in Japanese

Abstract nr. 716
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Co-author(s) - Inazu, Akihiro
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Co-author(s) - Mabuchi, Hiroshi

Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, HDL, Lipoproteins, Nutrition

High-fat foods is converted by intestinal bacteria and key enzyme flavin containing mono-oxygenase 3 (FMO3) to trimethylamine N-oxide (TMAO), harmful metabolite for cardiovascular disease (CVD). Recently, FMO3 has been recognized as a key molecule of reverse cholesterol transport system regulating LXR independent of TMAO in mice model, but no enough information in human. We investigated the effects of FMO3 loss-of-function variants in CVD high-risk population.

Methods: Coding regions of FMO3 gene were analyzed with high-resolution melting method followed by direct sequencing in total of 506 patients (255 males, mean age of 58±13) suspected CVD in our lipid clinic including 191 patients with genetically confirmed heterozygous familial hypercholesterolemia (FH). Coronary stenosis index (CSI) was estimated with angiography in 203 patients (118 males, mean age of 59 ± 12). Fasting lipid profiles without lipid-lowering drugs by ultracentrifugation, 75gOGTT, and other conventional coronary risk factors were analyzed.

Results: In FMO3 gene, 6% were heterozygotes of loss-of-function (LOF) variants (p.C197X, p.R205C, or p.R500X) that showed no activity or reduced activity with previous in vitro study. LOF carriers showed no differences in age, sex, BMI, TG, LDL-C, glucose tolerance, smoking, blood pressure, eGFR, except for lower HDL-C (50±16 vs. 43±12 mg/dL, p<0.05) and higher HDL-TG/apoAI ratio (0.12±0.06 vs. 0.16±0.10, p<0.01). In angiography, LOF carriers showed lower CSI (14.8±11 vs. 7.3±6, p<0.05). In subgroup, CSI was lower in non-FH group (14.0±11 vs. 5.8±5, p <0.05) even with lower HDL-C (51±17 vs. 42±11, p<0.05), but not significant in FH group. As HDL-C showed significant negative correlation with CSI in total study group, we interpreted that FMO3 LOF variants should be atheroprotective in CVD high-risk group independent of low HDL-C levels.
Relatively high-rate of FMO3 LOF variants can be a part of background of lower rate of CAD deaths in Japanese.  

**Conclusion:** FMO3 gene LOF variant carriers showed less coronary atherosclerosis in CVD high-risk group despite of low HDL-C levels. We propose FMO3 as a new candidate to fight atherosclerosis.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Dietary flaxseed reduces circulating cholesterol beyond the effects of cholesterol lowering medications in patients with peripheral artery disease.

Abstract nr. 717
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Hypolipidemic Drugs, Lipids, Nutrition

Nearly 67% of adults in the US with high cholesterol are unable to achieve normal levels despite current treatments and lifestyle modifications. Improved nutrition may assist in cholesterol lowering. Health Canada has released a Health Claim stating the cholesterol lowering benefits of consuming ground flaxseed (FX). However, the effects of FX when cholesterol lowering medications (CLMs) are also administered have not been investigated. Peripheral arterial disease (PAD) is a condition associated with elevated cholesterol that often leads to cardiovascular disease (CVD). The objective of our study was to determine if FX could lower circulating cholesterol levels beyond the effects provided by CLMs in patients with pre-existing CVD. We hypothesized that individuals consuming dietary FX would exhibit additional cholesterol lowering benefits beyond those observed with CLMs alone. A clinical population with PAD consumed food varieties containing either 30g of ground FX (N=58) or 30g of whole wheat placebo (PL, N=52) daily for one-year. The FLAX-PAD Trial was a single center, prospective, double-blinded, randomized controlled clinical trial (NCT00781950 at clinicaltrials.gov) involving 110 patients >40 years of age with PAD (ABI<0.9). At baseline, 74% of the patients were administered CLMs of which 90% of these were statins. Fasted blood samples were collected at 0-, 1-, 6- and 12-months and measured in the St. Boniface Hospital satellite laboratory. Following 12 months of consuming 30g/d FX+CLMs (N=36), LDL-cholesterol decreased by 8.5±3.0% compared to a 3.0±4.4% increase in the PL+CLM subgroup (N=26) (P=0.030). In another subgroup administered only FX (no CLMs) (n=11), preliminary findings showed a significant change after 6 months (-25.1±8.5%) compared to 1 month values (1.0±4.9%) in the same group (P=0.014). We conclude that patients adding 30g of ground FX to their diet does not interfere and can instead add to the cholesterol-lowering capacity of CLMs.

Supported by CIHR, Flax2015, Heart and Stroke Foundation of Canada, St. Boniface General Hospital Foundation, ARDI and the Canola Council of Canada.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation
Additional information
Impact of arterial stiffness on acute gain and late loss after percutaneous coronary intervention

Abstract nr. 718
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Co-author(s) - Kim, Sang-Hyun
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis

Background and objectives: Increased arterial stiffness is an accepted cardiovascular risk factor. However, the effect of arterial stiffness on the performance of percutaneous coronary intervention (PCI) is not well known. The aim of this study was to evaluate the impact of arterial stiffness measured by pulse wave velocity (PWV) on the relationship between acute gain and late loss after PCI. Subjects and Methods: Data from 242 consecutive patients who underwent PCI using drug eluting stents and pulse wave velocity study were analyzed. We divided the patients into two groups which were higher PWV group (121 patients) and low PWV group (121 patients). Results: Mean PWV, acute gain and late loss were 1675 ± 391 cm s(-1), 1.48 ± 0.55 mm and 0.13± 0.51mm. In higher PWV group, there was negative relationship between acute gain and late loss (correlation coefficient = -0.224 ; p=0.014). However, there was no relationship in lower PWV group between acute gain and late loss (correlation coefficient = -0.084 ; p=0.362) Conclusions: In the patients of increased arterial stiffness, the larger acute gain we get, the smaller late loss we encounter. However, this is not applicable to the patients of less arterial stiffness.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
PANCREATIC BETA-CELL DELETION OF ABCA1 AND ABCG1 PERTURBS GLUCOSE METABOLISM AND INCREASES ADIPOSITY IN MICE DUE TO SUBOPTIMAL INSULIN LEVELS

Abstract nr. 720
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Diabetes, Inflammation, Obesity

Evidence suggests that pancreatic lipid accumulation causes β-cell dysfunction. Mice with β-cell specific ABCA1 deletion and global knockout of ABCG1 have altered β-cell function. However, as mice with global deletion of ABCG1 also have low adipose tissue mass and do not become glucose intolerant or insulin resistant, that study did not provide an insight into the specific impact of β-cell dysfunction on glucose metabolism.

To investigate the effects of isolated β-cell-specific deletion of ABCA1 and ABCG1 on glucose metabolism in mice, we generated β-cell specific knockout mice. Control and knockout mice had comparable islet mass and insulin content. Knockout mice were markedly glucose intolerant (AUC 2649±230 vs 1539±189) and had reduced fasting insulin levels (0.67±0.18 vs 1.11±0.17 ng/mL). Insulin levels increased 1.5 fold in response to a glucose challenge in control animals, while no increase was observed in knockout animals. Both knockout and control animals were insulin sensitive. Despite similar weight and food intake, knockout mice had a 28% increase in adiposity and plasma IL-6 and MCP-1 levels were increased 3.5- and 5-fold, relative to the control animals. Adipose accumulation was attenuated when insulin levels in knockout mice were returned to normal by subcutaneously inserting osmotic pumps filled with Humulin R (31 U/mL) between 12 and 16 weeks of age (0.88±1.07% vs 4.03±1.09% increase in adiposity for insulin and PBS treated animals, respectively). β-cell specific deletion of ABCA1 and ABCG1 in mice reduces insulin secretion which in turn impairs glucose metabolism, alters body composition and increases systemic inflammation.

Subdivision 1. Basic Science
Presentation Preference Oral presentation

Additional information
Advanced atherosclerotic plaques in intracranial arteries from asymptomatic patients are scarce.

Abstract nr. 721
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Pathogenesis, Vulnerable Plaque

Rationale – Intracranial atherosclerosis is one of the main causes of ischemic stroke. However, the characteristics of intracranial arteries and atherosclerosis have rarely been studied. Therefore, we extensively studied the intracranial arterial vessel system, the so-called Circle of Willis (CoW) for the occurrence, location and morphology of atherosclerosis and its vessel characteristics.

Methods – Sixty-seven CoWs from asymptomatic patients (mean age, 67.3 ± 12.5 years) were collected at autopsy, of which a total of 1220 segments were collected from 22 distinct arterial sites. Atherosclerotic lesions were classified according to a modified Virmani classification. Hematoxilin-eosin and elastic von Gieson stainings were used to assess vessel and atherosclerotic plaque characteristics such as the presence of an external elastic lamina (EEL), elastin fibers in the media, and calcifications, which were analyzed semi-quantitatively.

Results – We observed that 81% (n = 989) of the segments had atherosclerotic plaques of which the majority were early lesions (66%, n = 808). Advanced plaques were mainly observed in large arteries such as the internal carotid, middle cerebral, basilar and vertebral artery. Only 1% (n =12) included complicated lesions of which 50% (n = 6) were intraplaque hemorrhages (IPH) and associated with patients that had cardiovascular events in their clinical history. From the four largest arteries were 77% (n = 85) of the vertebral artery (n = 111) segments most frequently associated with a continuous EEL and 56% (n = 62) with a high elastin fiber content in the media. Only 3% (n = 33) of the arteries contained calcifications, which were mostly observed in the VA 12% (n= 13).

Conclusion – Taken together, our study of atherosclerotic- and vessel characteristics of the CoW of asymptomatic patients shows that atherosclerosis in the CoW is mainly present in the 4 largest vessels and defined by plaques with an early and stable phenotype, and a low calcific burden. Furthermore, they frequently possess a continuous EEL and a high elastin content. In addition, IPH in complicated lesions is a rare event.

Clinical relevance - By studying multiple arterial sites in the CoW we improved the current understanding of intracranial atherosclerosis.
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Causes of Death in Homozygous and Heterozygous Familial Hypercholesterolemia Before and After Statin Period

Abstract nr. 722
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Epidemiology,Familial Hypercholesterolemia

Background and Purpose: Familial hypercholesterolemia (FH) is an autosomal dominant disease producing severe hyper-LDL-cholesterolemia, which leads to premature atherosclerotic cardiovascular disease (CVD). Worldwide the estimated prevalence of heterozygous FH (hetero-FH) is about 1/200 – 1/500 and approximately half of the untreated hetero-FH patients will develop CVD before 60 years of age. Statins are highly effective in decreasing LDL-C levels in hetero- and homo-FH patients and non-FH hyper-LDL-cholesterolemia. Our purpose is to estimate the effect of statin therapy on the prognosis of the hetero- and homo-FH before launching statins in 1991 (before statin period) and after 1992 (after statin period).

Methods: Clinical and genetic diagnostic criteria of hetero- and homo-FH were described in our previous articles. Final end point is the underlying causes of death estimated by ICD-10. The death information was obtained from their family members and home doctors. The design of this study has no control groups for ethical reasons.

Results: In our field of FH study, we collected 41 homo-FH and 1055 hetero-FH patients, and 14 homo-FH and 205 hetero-FH patients deceased during 1980-2014. In the homo-FH patients age at entry was 34.4±18.7 y.o., and age at death was 43.6±22.5 y.o. Before statin period one homo-FH died of leukemia, and after statin period one case died of leukemia, one case pancreas cancer and one case interstitial pneumonia. Other 11 cases died of CVD. Without LDL-apheresis treatment with statin alone improved lifespan of homo-FH patients from 28.4±12.7 to 58.7±20.0 yrs. For hetero-FH patients, 108 patients deceased before statin period and 93 hetero-FH patients after statin period. Among the deceased hetero-FH 67.3% died of CVD and 18 cases (8.8%) died of stroke. Their ages at entry before and after statin period were 59.5±12.6 and 57.4±12.4, respectively (n.s.) and the ages at death were 63.3±12.3 yrs and 75.9±12.4 yrs (p<0.0001). Death
of cancers was 13.0% before statin period, and 21.5% after statin period.

**Summary and Conclusions:** Causes of death in patients with hetero- and homo-FH were highly associated with atherosclerotic CVD and statin therapy reduced death due to CVD and improved life expectancy in both hetero- and homo-FH patients.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Assessment of Urinary Cotinine and lipid profile as risk factors of cardiovascular morbidity in pan masala containing tobacco (PMT) users

Abstract nr. 723
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Blood pressure,Lipoproteins,Risk Factor

Background: With the declining trends in the tobacco smoking, there is an increasing trend in the use of Pan Masala containing tobacco (PMT) in the Indian subcontinent. But PMT as an important cardiovascular (CV) risk factor has not been well studied. This study aims to find an association of CV risk factors between PMT users and control group by correlating urinary cotinine with CV morbidity.

Methods: On approval of the IERB we enrolled 130 PMT users and 70 non users as control group in this community based comparative cross sectional study done in Dharan Municipality of Nepal from July 2013 to June 2014. Participants of age groups 18-44 years who were using PMT for at least six months, willing to take part and not having any self- reported acute, chronic illness, alcoholics and any drug use were included in this study. Lipid-profile were measured by commercially available standardized kit in COBAS c311 and cotinine was measured by a solid phase competitive ELISA kits from Cal biotech, USA. Blood pressure, height and weight were measured using standard protocol. For descriptive statistics mean, SD, median, interquartile range, percentage were calculated. For inferential statistics, chi-square, Z-test was applied.

Results: Mean total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) were found to be significantly higher (p<0.001) in PMT users and high density lipoprotein (HDL) was lower than control group. Median cotinine level were found to be higher in PMT users than control group (p<0.001). Mean systolic, diastolic blood-pressure (SBP, DBP), lipid profile (TC, TG, LDL), quantity of PMT were positively correlated with cotinine (r=0.458, 0.480, 0.523, 0.297, 0.500, p<0.001). In multiple logistic regression model DBP, TC and Gender remained independently associated with higher cotinine level.

Conclusion: Urinary cotinine level was positively correlated with lipid-profile parameters except HDL-C and other risk-factors of CV-morbidity.

Subdivision 4. Not applicable. Abstract matches with track c
Presentation Preference
Electronic poster presentation
Additional information
The cholesterol content in pancreas correlates with insulin sensitivity

Abstract nr. 725
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Diabetes, Functionality, Reverse Cholesterol Transport

Type-2 diabetes mellitus (T2DM) and metabolic syndrome (MS) are among the five strongest risk factors for atherosclerosis and cardiovascular diseases. The ethiology of T2DM and MS is multifactorial and numerous triggers are described in the literature. No doubt, aberrations in glucose homeostasis play a central role with that respect. Genome wide association and candidate gene studies have identified more that 50 loci associated with T2DM and MS. Glucose stimulated insulin secretion (GSIS) is a primary mechanism of maintaining metabolic fuel homeostasis and impaired GSIS is essentially the key to ß-cell failure and T2DM. For the proper pancreatic ß-cell function the glucose sensor glucokinase (GK) is recognized to be rate limiting. In the present investigation we searched for micro-RNA’s (miR’s) affecting the activity of GK. In silico search actually revealed that miR-1/206 is a putative post-transcriptional regulator of GK. Notably, the miR1/206-GK binding site is highly conserved among different species. Luciferase reporter assays performed in COS-7 cells transfected with a p-MIR-REPORTER 3’UTR GK construct revealed that miR-206 might be functional in affecting GK expression. In subsequent experiments we investigated GSIS in control mice and in miR-206 knock-out mice and found that the latter not only had a significantly improved GSIS but also a significantly better glucose tolerance under normal chow – and even more so under high fat diet.

Since we found previously that miR-206 also affects LXR and the expression of ABC-transporters we hypothesized that pancreatic cholesterol homeostasis might be another possible cause for our observations. In fact we found that the ß-cell cholesterol content in miR206-KO mice was strikingly lower than in control mice. This decrease in ß-cell cholesterol correlated with the enzyme activity of GK.

We conclude that miR-206 knock-out has a positive effect on GISIS and on glucose tolerance. This is mediated by improved glucose sensing by GK and a lower cholesterol content of pancreatic islets particularly under high fat diet. If our studies can be confirmed to be operative in humans, suppression of miR-206 will be a potential target to combat atherosclerosis and cardiovascular diseases especially in cases that are triggered by T2DM and MS.

<table>
<thead>
<tr>
<th>CHOLESTEROL CONTENT OF PANCREATIC ISLETS</th>
<th>ANIMAL</th>
<th>CHOLESTEROL (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT mice</td>
<td>21.2 ± 6.0</td>
<td></td>
</tr>
<tr>
<td>miR-206 KO mice</td>
<td>11.2 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>ApoC-KO mice</td>
<td>68.7 ± 20.4</td>
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</table>

Subdivision 1. Basic Science
Presentation Preference Oral presentation
Additional information
Plasma Pentraxin 3 Levels and Severity of Coronary Artery Disease: an Angiographic Assessment

Abstract nr. 726
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Epidemiology

Background: Pentraxin 3 (PTX3) is an acute inflammatory protein in the same family as C-reactive proteins. We investigated the relationship between plasma PTX3 levels and the severity of coronary artery disease (CAD).

Methods and Results: 160 consecutive patients (male: 124, mean age: 67±11) with CAD symptoms were examined by coronary angiography (CAG). One who had > 50% luminal diameter stenosis of at least one major coronary artery was defined as CAD. The severity of CAD was defined as the sum of vessels (1vd, 2vds, 3vd). Ninety patients were diagnosed as acute coronary syndrome (ACS), 79 as myocardial infarction (MI), 11 as unstable angina pectoris (UAP). Seventy patients were diagnosed as non ACS, 28 as stable AP, and 42 as ischemic heart disease (IHD). Plasma PTX3 levels were significantly higher in the ACS group than in the non ACS group (3.27±2.57 ng/ml vs 2.18±1.30 ng/ml, p<0.001). And MI group had higher numeric value than IHD (3.32±2.39 ng/ml vs 2.09±1.31 ng/ml, p<0.005) and AP group (vs 2.31±1.29 ng/ml, p<0.005). However there was no correlation with the severity of CAD (see figures attached).

Conclusions: Plasma PTX3 levels were significantly higher in ACS group, however it was not associated with the severity of CAD assessed by angiography.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
APOA-I AS A VALUABLE INDICATOR OF CORONARY ARTERY DISEASE PROGRESS IN AZOREAN SUBJECTS

Abstract nr. 727
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Co-author(s) - Pavão, Maria Leonor
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Cardiovascular Disease, Dyslipidemia, Lipoproteins

In the Azores Islands (Portugal) mortality from coronary artery disease (CAD), which major cause is atherosclerosis (AT), doubles its rate comparatively to the rest of the country. For a deeper insight on the causes of AT in this archipelago, we carried out a study involving Azorean subjects, symptomatic and asymptomatic for CAD. Conventional risk factors for AT, as well as ApoA-I and ApoB levels were evaluated in order to study their behavior along the progression of the disease.

A group of patients constituted by 175 individuals with CAD symptoms (mean age 56 ± 9; 68% men) were submitted to coronary angiography and split into two groups: one formed by significant-CAD individuals (defined as the presence of ≥50% stenosis in at least one major coronary vessel) and the other by subjects with non-significant-CAD (less than 50% of narrowing of the coronary arteries). A third group of 152 asymptomatic subjects for CAD and with no other chronic diseases (mean age 50 ± 6; 62% men) constituted the controls. No significant differences among the 3 groups were found on the prevalence of obesity and smoking. Control subjects were younger (12%, P<0.001) and exhibited a significantly lower prevalence of both hypertension and dyslipidemia than patients. About 79% of significant-CAD and 60% of non-significant-CAD subjects, as well as 16% of controls were medicated for dyslipidemia. However, the concentrations of atherogenic lipids (LDL-C, non-HDL-C, and ApoB) were significantly higher in controls than in patients. The opposite was found regarding the levels of HDL-C and ApoA-I (P<0.001), which were both at the lower limit of the respective reference value in the significant-CAD group. No significant differences in ApoB/ApoA-I ratio were observed among the 3 groups, but ApoB/ApoA-I was 0.807 in controls. ApoA-I concentration decreased with the progression of disease, achieving its lowest value (126 ± 16 mg/dL) in significant-CAD subjects with 91-100% stenosis of coronary vessels. Results suggest that controls, albeit asymptomatic, are at a moderate risk of developing a cardiovascular event. In patients, medication had no effect in HDL-C improving. Finally, ApoA-I seems to be the only valuable parameter to monitor the progress of disease.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Probucol-induced HDL lowering impairs atherosclerotic plaque stabilization and induces plaque progression in APOE knockout mice post WT bone marrow transplantation

Abstract nr. 729
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model,Atherosclerosis,HDL

Probucol is an effective anti-oxidant and HDL-lowering compound. Despite its HDL reducing effects, probucol protects against the initiation of atherosclerosis, possibly by enhancing macrophage reverse cholesterol transport. However, the effects of probucol on existing atherosclerotic lesions are currently largely unknown. We aimed to study the probucol-induced remodeling of existing lesions under conditions of extensive lipid lowering. Hereto, 14 weeks-old female APOE knockout (KO) mice were transplanted with APOE positive wild type (WT) bone marrow and fed either chow (n=16) or chow plus 0.025% probucol (n=18) for 9 weeks. Reconstitution of APOE in bone marrow normalized hypercholesterolemia in both groups of transplanted APOE KO mice. The chow-fed animals displayed 3-fold (p<0.005) lower total cholesterol (TC) levels, while the values were 5-fold (p<0.005) lower in mice challenged with probucol. This difference could be explained by significantly lower HDL cholesterol levels upon probucol treatment (4.5-fold, p<0.05 vs chow). No progression of atherosclerotic lesions was observed in the chow group at 9 weeks after transplantation (1.2x10^5 µm² vs 1.0x10^5 µm² at baseline). The macrophage over collagen ratio was 3-fold (p<0.05) lower, indicative of more stable lesions. In contrast, the probucol-treated mice did develop augmented atherosclerotic lesion sizes [1.9-fold (p<0.005) vs baseline; 1.5-fold (p<0.01) vs chow]. Furthermore, the lesions displayed a less stable phenotype compared to chow-fed animals, as evidenced by a 2-fold (p<0.05) higher macrophage over collagen ratio. Lesion progression coincided with a 2-fold higher neutrophil over lymphocyte ratio (NLR) in blood, indicating a more pro-inflammatory status in the group treated with probucol. HDL is required for the steroidogenesis of immunosuppressive glucocorticoids like corticosterone. In line, corticosterone levels were 30% (p<0.05) lower in the probucol-treated mice versus chow-fed animals and explain the pro-inflammatory status.

In conclusion, although previous studies showed an athero-protective function for probucol, our findings indicate that probucol has detrimental effects on existing atherosclerosis upon bone marrow transplantation-induced normalization of hypercholesterolemia in APOE knockout mice.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
HDL and CER-001 Inverse-dose dependent inhibition of atherosclerotic plaque formation in apoE-/- mice: Evidence of ABCA1 down-regulation

Abstract nr. 730
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Atherosclerosis, HDL, Lipoproteins

CER-001 is a novel engineered HDL-mimetic comprised of recombinant human apoAI and charged phospholipids that was designed to mimic the beneficial properties of nascent pre-ß HDL. In this study, we have evaluated the dose-dependent regulation of ABCA1 expression in vitro and in vivo in the presence of CER-001 and native HDL (HDL3).

CER-001 induced cholesterol efflux from J774 macrophages in a dose-dependent manner similar to natural HDL. A strong down-regulation of the ATP-binding cassette A1 (ABCA1) transporter mRNA (- 50 %) as well as the ABCA1 membrane protein expression (- 50%) was observed at higher doses of CER-001 and HDL3 compared to non-lipidated apoA-I.

In vivo, in an apoE-/- mouse “flow cessation model,” the inhibition of atherosclerotic plaque burden progression in response to a dose-range of every-other-day CER-001 or HDL in the presence of a high-fat diet for two weeks was assessed. We observed a U-shaped dose-response curve: inhibition of the plaque total cholesterol content increased with increasing doses of CER-001 or HDL3 up to a maximum inhibition (- 51 %) at 5 mg/kg; however, as the dose was increased above this threshold, a progressively less pronounced inhibition of progression was observed, reaching a complete absence of inhibition of progression at doses of 20 mg/kg and over. ABCA1 protein expression in the same atherosclerotic plaque was decreased by -45 % and -68 % at 50 mg/kg for CER-001 and HDL respectively. Conversely, a -12% and 0 % decrease in ABCA1 protein expression was observed at the 5 mg/kg dose for CER-001 and HDL respectively.

These data demonstrate that high doses of HDL and CER-001 are less effective at slowing progression of atherosclerotic plaque in apoE-/- mice compared to lower doses, following a U-shaped dose-response curve. A potential mechanism for this phenomenon is supported by the observation that high doses of HDL and CER-001 induce a rapid and strong down-regulation of
ABCA1 both in vitro and in vivo. In conclusion, maximally efficient HDL- or CER-001-mediated cholesterol removal from atherosclerotic plaque is achieved by maximizing macrophage-mediated efflux from the plaque while minimizing dose-dependent down-regulation of ABCA1 expression.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are components of the worldwide growing epidemic of obesity. One of the approaches to treat such a disease is to control the associated hyperlipidemia. The use of statins as lipid-lowering agents through regulation of cholesterol-LDL, is still under evaluation and randomized clinical trials of adequate size and duration are required. Instead of attempting to reduce the LDL metabolic pathway, an alternative, and perhaps more fruitful, approach to NASH would be to increase elimination of cholesterol from the liver Reverse Lipid Transport (RLT), specifically by increasing HDL metabolism. A therapeutic strategy of increasing bile acid and cholesterol elimination in the liver through increased HDL trafficking had not previously been considered in the context of NAFLD and NASH pathophysiology. Indeed, we hypothesized that overall improvements in lipid elimination by the liver (as previously observed with CER-209 treatment) could favorably impact the fatty liver as well as the steato-hepatitis observed in patients.

We have recently described a new class of compounds (agonists of a hepatic protein-G-coupled receptor P2Y13 receptor), which by enhancing the number of small HDL particles in mice have a very strong impact in the development of the atherosclerotic plaque burden. The use of another atherosclerotic model, the high-cholesterol diet rabbit model, allowed us to both confirm the effect of CER-209 on atherosclerosis plaque but also by developing fatty liver to study the impact of the P2Y13r agonist on to the NAFLD/NASH. Upon treatment, there was significant regression of the atherosclerotic plaques in aorta (30 % decrease) of the agonist-treated animals as measured by the cholesterol content of the entire artery. The HDL content of the treated animals showed a very consistent increase of the small HDL particles, which leads to an increase of the efflux capacity of the plasma from the treated animals. After initiation of CER-209 treatment, a significant decrease in lipid content (cholesterol and triglycerides) of liver was observed. Liver histology further demonstrated a significant decrease of the steatosis in the treated animals resulting in a trend towards normalization of the liver physiology.
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Characteristics of Plaque Microstructures in Diabetic Patients Receiving Metformin: Frequency Domain Optical Coherence Tomography Analysis

Abstract nr. 732
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Co-author(s) - Nicholls , Stephen

Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis,Diabetes,Vulnerable Plaque

Background: Despite anti-atherosclerotic medical therapies, diabetic patients still exhibit an extensive and progressive atherosclerosis with a high prevalence of cardiovascular events. Metformin is currently recommended as first-line therapy for type 2 diabetes. It has been shown to not only lower glucose level but also improve dyslipidemia and modulate inflammation. However, the effect of metformin on atherosclerotic plaque has not been characterized. Frequency-optical coherence tomography (FD-OCT) provides high-resolutional images of atherosclerotic plaques in vivo. We sought to investigate features of non-culprit lipid plaques in patients treated with metformin.

Methods: We analyzed 120 non-culprit lipid plaques in 128 diabetic patients with coronary artery disease who underwent FD-OCT imaging within target vessel for percutaneous coronary intervention. Study subjects were stratified into two groups according to the use of metformin. FD-OCT derived plaque microstructures were compared.

Results: 34.6% (44/128) of study subjects received metformin. Patients treated with metformin were more likely to be male (83.4% vs. 61.3%, p=0.01) and have a history of myocardial infarction (55.5% vs. 23.5%, p=0.0009). FD-OCT demonstrated that patients receiving metformin exhibited smaller lipid arc (142±60° vs. 167±76°, p=0.04) and lipid burden index (740°mm vs. 1234°mm, p=0.03). No significant differences were observed with regard to fibrous cap thickness (108±57 vs. 93±47μm, p=0.16), thin-cap fibroatheroma (33 vs. 38%, p=0.62), plaque rupture (11.1% vs. 7.3%, p=0.52) and cholesterol crystals (25.0% vs. 30.8%, p=0.53). Multivariate analysis indicated metformin use as a significant factor associated with smaller lipid burden index (p=0.04).

Conclusions: Metformin use was associated with smaller size of lipid pool in diabetic patients with stable coronary artery disease. Our OCT findings might indicate the ability of metformin to stabilize plaque, potentially leading to the prevention of future cardiovascular events.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Comprehensive characterisation of the atheroprotective efficiency of modified dairy fats in the hyperlipidemic Hamster

Abstract nr. 733
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Metabolism, Nutrition, Prevention

We assessed the athero-modulating effects of modified dairy fats in conditions promoting the disease in the hamster model. A systems biology approach was implemented to reveal and quantify the dietary fat-related components of the disease.

Three modified dairy fats (40% energy) were prepared from a regular butter (RB) either by mixing with a plant oil mixture (VRB), by removing cholesterol alone and mixing with the plant oil mixture (VLCB), or by combining cholesterol removing and whole saturated fatty acids reduction (LCSB). A plant oil mixture (VM) along with the regular butter (RB) were used as control diets. The atherosclerosis status was assessed from aortic cholesteryl-ester levels. The biological status of each hamster at completion of the study was determined from a multiplatform analysis combining conventional blood clinical chemistry and fatty acids analyses, a wide atherosclerosis-related genes expression analysis in blood and liver, and untargeted metabolites profiling of biofluids.

The severity of atherosclerosis was higher in regular butter-fed hamsters compared with the other four groups ($P < 0.05$). Among the technologically transformed test-fats, the decholesterolized and de-saturazed dairy fat (LCSB) appeared more protective towards atherosclerosis development.

Eighty-seven of the 1666 variables measured were found strongly associated with the disease. To facilitate interpretation, the 87 variables were aggregated into 10 biological clusters and combined into a multivariate predictive equation (PLS method), explaining 81% of the disease variability, such as Predicted Atherogeneity = 0.108091*[dairy fat derived fatty acids] + 0.152669*[endogenous derived fatty acids] - 0.0340185*[mitochondrion function] + 0.173429*[regul. Lipid metab. Trans.] - 0.0751566*[vitamin E metabolism] - 0.153142*[hemostasis] - 0.110269*[amino acid metabolism] + 0.110269*[blood cholesterol related] + 0.231903*[inflammation] - 0.00456344*[miscellaneous & unidentified] + 4.06731. Using this equation, the biological cluster "regulation of lipid transport and metabolism" appeared central in atherogenic development in relationship to diets. The "vitamin E metabolism" cluster was the main driver of atheroprotection in the best performing transformed dairy fat (LCSB diet).

In conditions that promote atherosclerosis, the impact of dairy fats on atherogenesis can be
greatly ameliorated by technological modifications. Our modelling approach allowed identifying and quantifying the contribution of complex factors to atherogenic development upon each dietary set up.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Plaque Microstructures Under High-intensity Statin; Rosuvastatin versus Atorvastatin

Abstract nr. 734
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Co-author(s) - Puri, Rishi
Co-author(s) - Tuzcu, E.Murat
Co-author(s) - Nissen, Steven
Co-author(s) - Nicholls, Stephen
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Dyslipidemia, LDL, Vulnerable Plaque

Background: High-intensity statin therapy has been demonstrated to reduce cardiovascular events. Stain-mediated plaque stabilization is considered as one of important mechanisms contributing to better cardiovascular outcome. While high-dose rosuvastatin and atorvastatin are currently recommend as high-intensity statin in patients with atherosclerotic cardiovascular disease, the difference in their plaque stabilization effect remains to be fully evaluated. Frequency-optical coherence tomography (FD-OCT) is a novel intravascular imaging modality to visualize plaque microstructures in vivo. The current study compared FD-OCT derived plaque microstructures in patients treated with high-dose rosuvastatin and atorvastatin.

Methods: 117 non-culprit lipid plaques in 134 patients with coronary artery disease who received high-dose rosuvastatin or atorvastatin were analyzed. FD-OCT imaging was conducted within target vessel requiring percutaneous coronary intervention. Clinical demographics and FD-OCT derived plaque microstructures were compared.

Results: The average dose of rosuvastatin and atorvastatin was 21.5 and 68.7 mg, respectively. Patients treated with rosuvastatin were more likely to be obese (BMI; 34.4 vs. 29.5 kg/m², p=0.003), and have a history of hypertension (69 vs. 38%, p=0.03) and metabolic syndrome (91.6 vs. 48.2%, p=0.004). Lower levels of low-density lipoprotein cholesterol (75±17 vs. 91±35 mg/dl) and total cholesterol (144±28 vs. 160±33 mg/dl) were observed in rosuvastatin group although these comparisons failed to meet statistical significance (p=0.09 for both comparisons). On FD-OCT imaging analysis, non-culprit lipid plaques in patients treated with rosuvastatin were less likely to harbor microchannels (prevalence; 7 vs. 46%, p=0.02, multiple microchannels; 0 vs. 17%, p=0.10) Even after adjusted for clinical demographics, rosuvastatin use was still significantly associated with a lower prevalence of microchannels (p=0.03). While there was no significant difference in fibrous cap thickness (124±73 vs. 109±71um, p=0.49), the maximum arc of lipid was numerically smaller in patients treated with rosuvastatin (163±109 vs. 211±95°, p=0.08).

Conclusions: Two types of high-intensity statins had different effect on plaque microstructures at
non-culprit lesions on FD-OCT imaging. This finding might highlight more favourable plaque stabilization effect of high-dose rosuvastatin.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Distinguishing hepatic steatosis from steatohepatitis by magnetic resonance imaging enhanced with ultrasmall superparamagnetic particles of iron oxide

Abstract nr. 735
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Imaging, Obesity, Risk Factor

Introduction and aim: Where simple fatty liver disease is a relatively benign disorder, non-alcoholic steatohepatitis (NASH) is characterized by a high rate of progression towards cirrhosis. To date, non-invasive differentiation between simple steatosis and NASH is not possible. Superparamagnetic iron-oxide (SPIO) enhanced MRI was promising for this purpose, but SPIOs are no longer available. The aim of the present study was to 1) develop a protocol for the use of ultrasmall superparamagnetic particles of iron-oxide enhanced MRI (USPIO-MRI) to diagnose NASH, 2) evaluate whether hepatic USPIO uptake is decreased in NASH and 3) study the diagnostic accuracy of USPIO-MRI to differentiate NASH from simple steatosis.

Methods: This study was approved by the local institutional review board. Quantitative R2* MRI scans of the liver were performed at baseline and 72 hours after USPIO administration (3.6 mg/kg lean body mass ferumoxytol) in patients with biopsy-proven NASH (n=10), hepatic steatosis without NASH (heterozygous familial hypobetalipoproteinemia, n=7) and healthy controls (n=10). The hepatic USPIO uptake in the liver was quantified by the difference in R2* (ΔR2*) between the post-contrast scan and the baseline scan. The hepatic fat fraction was calculated using MRI.

Results: Subjects with NASH had a significantly lower ΔR2* 72 hours after USPIO administration compared to subjects with simple steatosis and healthy controls (respectively 39.3 ± 16.9 s⁻¹, 60.1 ±16.8 s⁻¹ and 72.2 ±22.0 s⁻¹). Hepatic fat fraction did not differ between subjects with NASH and simple steatosis (respectively 19.7 ± 9.3% and 20.3 ± 8.2%). The area under the receiver operating characteristics curve to distinguish NASH from simple steatosis was 0.84 (0.65 – 1.00).

Conclusion: USPIO -MRI is able to discriminate NASH from simple steatosis. Hence, USPIO -MRI can be a promising tool in the noninvasive evaluation of NAFLD, both for diagnostic purposes and the assessment of the efficacy of new treatment modalities in NASH.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Homocysteine level in patients with polycystic ovary syndrome

Abstract nr. 736
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Metabolism, Obesity

Polycystic ovary syndrome (PCOS) often is accompanied with metabolic and endocrine dysfunctions which can be associated with future comorbidities such as diabetes, cardiovascular disease, and endometrial cancer. Although a definitive link between PCOS and these chronic illnesses has not been demonstrated, there is significant overlap in the clinical characteristics of these disorders. Consequently, the issue of identifying and measuring potential conditions that may be associated with PCOS is a priority and should be the standard of practice in its management. Hiperhomocysteinemia has been shown as independent predictor of cardiovascular events in patients with atherosclerosis. The aim of our study was to determinate levels of homocysteine in woman with polycystic ovary syndrome compared with healthy woman. The prospective research included 47 patients diagnosed with PCOS using Rotterdam criteria. The control group was composed of 24 eumenorrheic women. The groups were matched according to age (23.5 ± 5.5 vs 25.5 ± 4.3; p=0, 5). Hormones concentration was measured and metabolic state was assessed by, lipid profile; body mass index (BMI) and homeostatic model assessment (HOMA). Blood samples were collected in early follicular phase. Total homocysteine was measured using fluorescent immunoassay. Statistically significant differences in serum concentration of homocysteine were observed between groups. Mean homocysteine level we found as (10.3 ± 2.9 vs. 7.0 ± 1.5) in PCOS and normal group respectively (p< 0.05). Women diagnosed with PCOS had significantly higher BMI (26,3±6,9 vs 23,5±4,9 kg/m2; p<0,01). For Macedonian population we found statistically significant increased homocysteine levels in woman with PCOS. Although the mean homocysteine levels are within normal limits, there are significant higher mean homocysteine concentrations between these two groups. Because an increased concentration of total homocysteine has been shown as an independent risk factor for cardiovascular alterations, it is essential in this group of woman are taken measures for early prevention.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Discordance analysis between LDL particle concentrations and LDL-C and ApoB values in type 2 diabetic subjects with atherogenic dyslipidemia

Abstract nr. 737
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Diabetes, Dyslipidemia, Lipoproteins

BACKGROUND: Type 2 diabetic subjects (T2DM) tend to present atherogenic dyslipidemia (AD), characterized by high triglycerides, low HDL cholesterol (HDL-C) levels, and a preponderance of small LDL particles. Moreover, both the number of LDL particles (LDL-P) assessed by NMR and the apolipoprotein B (ApoB) values have been suggested to be better predictors of cardiovascular risk than LDL cholesterol (LDL-C) in patients with high cardiometabolic risk.

METHODS: The associations between LDL-C, LDL-P, and ApoB values with carotid intima-media thickness (IMT), an indicator of anatomical atherosclerosis, were examined using a cohort of 323 with and without AD. The Liposcale test, a novel advanced lipoprotein test (ALT) based on 2D diffusion-ordered 1H NMR spectroscopy (DOSY), was used to determine the size and particle concentrations of the main lipoprotein classes, and the particle concentrations of nine subclasses. LDL-C and ApoB were measured using standard assays. Discordance analysis was used to study the cases when the LDL-C, LDL-P, and ApoB measures were discordant on the basis of population percentiles. Statistical models were adjusted for age, gender, hypertension, smoking status as well as for triglycerides and HDL concentrations.

RESULTS: VLDL particles were higher in T2DM subjects with AD, while medium HDL particles and total HDL-C were decreased. Despite we found no difference in LDL-C levels, mean levels of total LDL particles were higher in T2DM subjects with AD. We further analyzed the cases when the LDL-C, ApoB, and LDL-P measures were discordant on the basis of population percentiles, i.e., when LDL-C or ApoB were increased and LDL-P was normal and when LDL-P was increased but LDL-C or ApoB were normal. For those individuals with discordant LDL-C, ApoB, and LDL-P values, only LDL-P was associated with IMT (P=0.018 and P=0.038, respectively).

CONCLUSIONS: We used a novel NMR-based ALT that permitted a profound characterization of AD in T2DM subjects. For individuals with discordant LDL-C, ApoB, and LDL-P values, the LDL-attributable atherosclerotic risk was associated with LDL-P but not with LDL-C neither ApoB.
Discordance analysis between LDL-P and ApoB (left) and carotid IMT by tertiles of LDL-P (right).

Presentation Preference Oral presentation

Additional information
MicroRNA-200c plays a role in diabetes induced cardiac hypertrophy by modulating expression of MKP-1

Abstract nr. 738
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Animal model, Cardiovascular Disease, Diabetes, Pathogenesis

Introduction: The mitogen-activated protein kinase phosphatases (MKP) catalyze the inactivation of mitogen-activated protein kinases (MAPKs) and regulate their activity. MAPKs (ERK1/2, JNK, and p38) are shown to be upregulated in Diabetic Cardiomyopathy (DCM). MKP-1 has been reported to regulate activity of MAPKs in angiotensin II induced cardiac hypertrophy, however the role of MKP-1 in diabetes induced cardiac hypertrophy is not known. microRNAs are small non-coding RNA known to play a central role in regulating gene expression. However, little is known about the microRNAs regulating the expression of MKP-1 in cardiac hypertrophy. In present study, we investigated the role of microRNAs regulating MKP-1 in diabetes induced cardiac hypertrophy.

Methodology: Type 2 diabetes was induced in male Wistar rats by low streptozotocin-high fat diet combination. Cardiac hypertrophy was confirmed by increased cardiac expression of hypertrophic markers (ANP and β-MHC) and by histopathology. MAPK activity was measured by immunoblotting. MicroRNA targeting MKP-1 was identified using bioinformatics. Cardiac microRNA and MKP-1 expression was estimated in diabetic hearts and in hyperglycemia (HG) treated cardiomyocytes by qRT PCR. Effect of microRNA over expression and inhibition was examined on HG induced cardiac hypertrophy using synthetic miR mimic and inhibitor in HG treated cardiomyocytes.

Results: A significant increase in myocardial phosphorylated ERK, p38 and JNK was observed in diabetic hearts and in HG treated cardiomyocytes (p<0.05). Myocardial expression of MKP-1 was significantly decreased in diabetic group and in HG treated cardiomyocytes (p<0.05). miR-200c was identified as miRNA targeting MKP-1 and its expression was significantly increased in both diabetic rats and in HG treated rat cardiomyocytes (p<0.05). Inhibition of miR-200c induced increased expression of MKP-1 and decreased expression of phosphorylated ERK, p38 and JNK and attenuated cardiomyocyte hypertrophy in HG treated cardiomyocytes.

Conclusion: Our results suggest that miR-200c plays a role in diabetes induced cardiac hypertrophy by modulating expression of MKP-1.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
ADVERSE DRUG REACTIONS TO HYPOLIPIDEMIC TREATMENT IN OUTPATIENTS ADDRESSING A METABOLIC CENTER IN ITALY

Abstract nr. 740
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Dyslipemia, Hypolipidemic Drugs, Therapy

Introduction: It has been clearly established that lipid-lowering treatment reduce cardiovascular events in high-risk patient populations; both investigational trial and clinical practice showed that available drugs are generally well tolerated even if some safety concerns do exist with both mono- and combination therapy. Common adverse drug reactions (ADRs) to hypolipidemic therapy are relatively mild and often transient. Potentially more serious ADRs, partly dose-dependent, include myopathy and increasing transaminase plasma levels; they generally resolve with stopping treatment. Estimating ADR rates may result challenging in outpatients, because of differences from clinical trial population (age, comorbidity) and the variety of symptoms and signs.

Patients and methods: We evaluated hypolipidemic drug side effects in a wide population of dyslipidemic subjects (1932 individuals) which addressed our Metabolic Center in the last 24 months.

Results: Three hundred eighty-nine patients (20.1%) showed possible ADRs related to hypolipidemic drugs; 201 were female (53.7%, p = n.s.). Most of the ADRs were muscle-related (77.6%), ranging from asymptomatic elevation of CPK (24.7%) to simple myalgias (48.3%) and myositis (4.1%); there were also two cases of rhabdomyolisis (0.5%). Other less prevalent side effects were gastrointestinal complaints (4.4%), liver abnormalities (7.5%), neurologic (2.1%) and allergic reactions (4.9%). Side effects were more common with fat-soluble statin administration (46%). ADR patients were older (60.9 ± 10.7 vs 54.9 ± 14.0, p<0.001), had higher BMI (28.2 ± 4.7 vs 27.4 ± 4.7, p<0.01), more frequently diabetics (17.7% vs 14,3%, p<0.001), hypertensive (50.1% vs 34.1%, p<0,001), or affected by coronary heart disease (14.1% vs 7.3%, p<0,001), peripheral vascular disease (42.7% vs 24.5%, p<0,001), non-alcoholic fatty liver disease (18.8% vs 11.7%, p<0,001). Affected subjects showed higher cardiovascular risk, lower basal total cholesterol (227±
54 vs 235± 54 mg/dl, p<0.05) and LDL-cholesterol levels (138± 48 vs 146± 49 mg/dl, p<0.01).

**Conclusions:** Lipid lowering drugs, in particular HMG-CoA reductase inhibitors are rather well tolerated in common clinical practice, but ADRs related to their usage are not so uncommon. There is seemingly no difference between their prevalence in men and women but ADRs appear to be more common in older people at higher cardiovascular risk.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Mini-oral presentation

Additional information
Abstract nr. 741
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Obesity, Therapy, Thrombosis

Introduction: Platelet activation and aggregation play a crucial role after percutaneous coronary intervention. Clopidogrel is a thienopyridine derivative that inhibits platelet aggregation by blockade of platelet ADP P2Y12 receptor. Clopidogrel resistance can be described as the persistent activity of clopidogrel receptor despite an adequate antiplatelet regime. Leptin is the obesity gene product and its serum level increases with obesity. Platelets have leptin receptors on their surfaces. Hyperleptinemia may induce ADP-mediated platelet aggregation. Therefore, clopidogrel effect could be diminished by high serum leptin levels.

Aim: The aim of the study is to investigate relationship between clopidogrel resistance and obesity and serum leptin levels

Methods: 100 patients who have undergone percutaneous coronary intervention for coronary artery disease after their admission to hospital emergency department or cardiology clinic were included. Patients’ serum leptin levels were compared between clopidogrel resistant and responsive groups. Clopidogrel platelet inhibition level is measured by the Verify Now © P2Y12 system. Patients whose Platelet Reactivity Unit (PRU) level ≥ 240 were accepted resistant to clopidogrel, while PRU <240 ones were evaluated as responsive to clopidogrel. In addition, leptin levels ≥ 15 ng / ml was considered to be hyperleptinemia. Body mass indexes (BMI) of patients were also evaluated, BMI of 30 kg/m² or greater was accepted as obese.

Results: In our study, 37 % of patients are classified as clopidogrel resistant. Serum leptin levels are 8.03 ± 7.27 in patients with clopidogrel resistance, while 5.48 ± 5.82 are measured in clopidogrel responsive ones. However, this difference did not reach the level of statistical significance. (p = 0.116 ) Clopidogrel resistance is significantly more in hyperleptinemic patients. (72.7% vs 32.6%, p= 0.017) PRU value was also higher in in hyperleptinemic patients, statistically significant relationship was found. (292.18±70.11 vs 208.76±66.98, p= 0.001). Hyperleptinemia increases the likelihood of clopidogrel resistance 4.532 times. Similarly, in patients with BMI> 30, statistically significant greater number of clopidogrel resistance was observed. ( p=0.015 )

Conclusion: Clopidogrel resistance is seen more in obese and hyperleptinemic patients. Increasing the dose of clopidogrel should be considered in this group.
Table 3 Comparison of obesity and cut-off leptin level of 15 ng/ml between clopidogrel responsive and resistant groups

Table 1 Comparison of leptin level between clopidogrel responsive and resistant groups

Table 2 Comparison of PRU level according to cut-off leptin level of 15 ng/ml

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Trib1 deficient mice show increased insulin sensitivity and resistance to diet-induced obesity

Abstract nr. 742
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Metabolism, Obesity

Genome-wide association studies identified a novel locus near the gene TRIB1 to be associated with plasma lipids and the risk of CHD. We recently demonstrated that Trib1 deficiency (Trib1-/-) in mice on chow diet increases hepatic lipogenesis and VLDL production, leading to significant elevations of plasma cholesterol and triglycerides. In the present study, we aimed to investigate whether Trib1 is also involved in regulating energy homeostasis and might therefore fulfill additional functions in metabolism.

Trib1-/- mice and littermate controls were fed a high fat diet (60% kcal/fat) for 16 weeks with weekly recording of body weight. NMR measurements of body composition, intraperitoneal glucose and insulin tolerance tests were performed. Food intake, energy expenditure and activity were analyzed in metabolic cages and euglycemic-hyperinsulinemic clamp experiments were carried out. In addition, TRIB1 mRNA expression was determined in human white adipose tissue samples (n=627) and correlated with clinical phenotypes and metabolic markers.

Trib1-/- mice on high-fat diet remained significantly lighter and displayed a markedly lower body fat mass than controls. Concomitantly, we observed better glycemic control in Trib1-/- mice, as determined by glucose tolerance- and insulin tolerance assays. Trib1-/- mice also required a higher clamped glucose infusion rate to maintain an euglycemic state, corroborating an enhanced whole-body insulin action. While we did not observe differences in body temperature or less food intake, voluntary physical activity was decreased in Trib1-/- mice. Our findings in Trib1-/- mice correlated well with data obtained in human adipose tissue, where higher TRIB1 mRNA expression was associated with increased body weight and percentage of body fat, as well as increased HbA1c levels.

Our data strongly indicate that Trib1 contributes to multiple metabolic pathways involved in the regulation of whole body energy homeostasis in mice. Trib1 regulates, by yet unknown mechanisms, body weight as well as glucose and lipid metabolism in mice and humans. Further analyses are needed to reveal details on Trib1 mediated effects and to determine the underlying molecular mechanisms of Trib1 in energy metabolism.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Effect of quantity of physical inactivity of elderly people with hemiplegia on paralysis limb pulse wave velocity

Abstract nr. 743
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Elderly, Lifestyle, Prevention

Purpose:
Determine an association between physical quantity of inactivity estimate and pulse wave velocity by wearable camera in elderly people with hemiplegia.

Subject and method:
The subject was Elderly post stroke person who has received care with Long-Term Care Health Facility over three years or more (n=6). (Mean 82 years old, BW 47 kg). Participation criteria. Physical activity a day must maintain 1200-1400 Kcal. Independently standing. The research design was a crossing study.
The method measures posture and a working hour from the image which did capture in wearable camera (Lecora, Kingjimu, Japan). To measure the exercise intensity calibrated from posture and the combination of the working hour.
To extrapolate, it to the algorithm using this variable and infer physical active mass.
We measured pulse wave conduction velocity at the same time.
Analysis: We examined regression models as a response variable as a fixed factor in PIA and a sleep in paralysis limb brachial - ankle PWV.

Results:
Mean PA and standard deviation (Kcal) 1130+/-115. PWV paralysis side 1805+/-254, PWV non-paralysis side 1666+/-142.
ABI paralysis side 1.04+/-0.029, ABI non-paralysis side 1.01+/-0.16.
Predictive paralysis PWV = -0.7665 (β) + 2,665 (e). β (p=0.02), DW = 3.47, (R² = 0.99).
The PWV level of subjects of this study showed a high value in the paralysis side, and the paralysis side showed the results similar to many reports that it was to high level.
PWV of the Japanese healthy subject of the same age is considered to be 1,595+/-300 cm/s. There were no persons who surpassed this PWV.
It is important that we visualize physical active mass necessary to make bionomics maintained at the same time to protect a systemic blood vessel of the single paralytic.
It is important that arterial sclerosis index and association of the ADL were found.

Conclusions:
It was found that physical active mass by wearable camera in elderly people with hemiplegia and the brachial - ankle pulse wave velocity of the paralysis side had a negative straight line
In elderly people with hemiplegia, it is important that we secure physical active mass being aware of arterial sclerosis to prevent the exacerbation of the vascular function.

Relationship between predict PWV palsy and PA regression model

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation

Additional information
Two prevalent mutations (2542delG and Q378X) in the ATP-binding cassette transporters ABCC6 gene in Japanese patients with pseudoxanthoma elasticum (PXE)

Abstract nr. 744
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Cardiovascular Disease,Pathogenesis

Background: Pseudoxanthome elasticum (PXE), the cause of which is the mutations in the ABCC6 gene, is inherited connective tissue disease specific to skin, ocular-, and cardiovascular system. Little is known about the genetic background of Japanese patients with PXE due to rarity of this disease.

Methods: PXE was suspected in the patients with characteristic skin and ocular findings and the diagnosis of PXE was established by typical pathohistological findings on the biopsied skin specimen. Direct sequencing of all 31 exons and flanking introns of ABCC6 gene was performed after SSCP and MLPA screening in 23 Japanese patients (male: 7, mean age: 48 years old) with PXE.

Results: Eight different mutations were identified in 38 alleles out of total 46 alleles. The most common mutation was one base deletion in exon 19 (2542 delG), which was found in 16 out of 46 alleles (35%). Nonsense mutation in exon 9 (Q378X) was found in 8 out of 46 alleles (17%). Four other missense mutations were found in exon 10 (R419Q; 2 alleles), exon 26 (R1221C; 1 allele), R1235W; 2 alleles), and exon 29 (R1357W; 2 alleles) –of these R419Q was novel. Small deletions including exon 1 and 4 (6 alleles) and one entire deletion of ABCC6 gene (1 allele) were also found. We have found neither R1141X nor ABCC6_del23-29 mutations, which have been reported to be highly frequent in Caucasian population. No significant correlation could be established between the clinical manifestations and disease-causing mutations in our Japanese PXE patients.

Conclusions: We identified 8 different mutations in the ABCC6 gene, which could explain 82.6% in our PXE cohort. We have already reported plemimary results of two prevalent mutations (2542delG and Q378X) in 16 PXE patients in 2003 (Atherosclerosis Supplements 2003;4:138). This time we conducted the study in a larger cohort and confirm the previous result.Further investigations for a nation-wide survey of Japanese patients with PXE are needed to confirm our results.
Presentation Preference: Oral presentation
Additional information
A mathematical model of the beta-adrenergic signaling alterations in mouse ventricular myocytes in myocardial infarction

Abstract nr. 745
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Cardiovascular Disease

Coronary atherosclerosis has been suggested to cause myocardial infarction (MI) or arrhythmia. These diseases are associated with reduced cardiac contractility due to the alteration of β-adrenergic signaling.

The purpose of present study was to develop a mathematical model of mouse ventricular myocyte to understand β-adrenergic signaling pathway that underlie the altered electrophysiological properties associated with MI.

Our simulations show that the alterations of the Ca$^{2+}$-independent transient outward K current, the L-type Ca$^{2+}$ current and Na-K pump current could result in the change of the action potential duration, a well-known experimental finding. In addition, the model demonstrates that the slowed reactivation kinetics of these currents in MI myocytes can account for the alteration of the action potential duration, and that the decreased Na-K pump current results in a small depolarization in the resting membrane potential. Furthermore, these all changes were close related to the alteration of β-adrenergic signaling in MI and to contribute to abnormal intracellular Ca$^{2+}$ homeostasis.

Our simulation results provide novel information and integrative insights concerning plausible β-adrenergic signaling mechanisms for the observed changes in cardiac electrophysiology and excitation-contraction coupling in mouse ventricular myocytes in the setting of MI.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Diiodothyronines reduce triglyceride content and activate insulin signalling in human primary hepatocytes

Abstract nr. 746
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes, Dyslipidemia

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The liver is a central organ in the control of lipid homeostasis, and pathological changes in liver functionality contribute to the onset of metabolic diseases. Thyroid hormone metabolites, such as 3,5- and 3,3’-diiodothyronine (3,5-T2 and 3,3'-T2), have recently been recognised to affect specific aspects of lipid metabolism in rodents. No specific receptors have been identified for diiodothyronines yet, and their physiological significance and mechanism of action are still not fully understood. Currently thyrotoxic effects have been described for 3,5-T2 only in mice.

We aimed to investigate in human primary hepatocytes whether 3,5- and 3,3’-T2 are able to affect triglyceride metabolism and to activate insulin signalling. We also wanted to unravel the molecular mediators involved, focusing in particular on the second messenger pospatidylinositol-3-phosphate (PIP3) and on the key metabolic regulator mTORC2 complex and its main regulatory subunit Rictor.

Human primary hepatocytes were cultured on Matrigel. We quantified triglyceride content with a modified Folch method, while the signalling pathways were analysed by immunoblotting analyses. siRNA silencing of Rictor was used to verify the involvement of mTORC2 in the signalling cascade.

For the first time, we found in human primary hepatocytes that both diiodothyronines, at physiological plasma concentrations, reduced triglyceride content and activated insulin signalling through a PIP3/mTORC2 pathway. Our results suggest that diiodothyronines might be potentially useful to develop new therapeutic strategies for the treatment of metabolic diseases, and we propose Rictor as a potential key intracellular signalling target mediating the effect of the diiodothyronines.

Keywords: diiodothyronines; human primary hepatocytes; triglyceride metabolism; insulin signalling; PIP3/mTORC2 pathway; metabolic diseases.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
The assessment of left ventricular movement related to coronary atherosclerosis using computational 3D cardiac ventricle

Abstract nr. 748
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease

3D computational cardiac models have been studied to evaluate or predict cardiovascular diseases. To characterize the abnormal movements of left ventricle with coronary atherosclerosis (LVCA), we developed a 3D computational left ventricle model that reflect coronary artery governing region. Our model includes the coronary artery governing segments and biomechanical properties such as stream of blood flow, volume, pressure and stress in left ventricle. The simulation offers computational fluid dynamic analysis by ANSYS ICEM. Simulations show that the values of left ventricular volume and efficiency of cardiac output were lower in LVCA model than normal model. In addition, the left ventricle movement shows to be not rhythmical in LVCA model. These alterations of cardiac output, stroke volume and movements in LVCA model are well observed in the cardiac lesion by coronary atherosclerosis, which are well-known experimental finding. Our model could provide a useful understanding of the relationship between biomechanics and progress of cardiac diseases.

Keywords: Left ventricular model, Coronary atherosclerosis, coronary artery governing region, Myocardial infarction

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
Serum malondialdehyde concentration and its relation to cardiovascular risk markers and morbidity in Lithuanian population

Abstract nr. 750
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Risk Factor

The oxidative modification of low density lipoproteins (LDL) is one of the first steps in development of atherosclerosis. Malondialdehyde (MDA) is an endproduct of the oxidative damage of polyunsaturated fatty acids and therefore, it is frequently used as a biomarker of oxidative stress. The goal of our study was to evaluate the level of oxidative stress by measuring serum MDA in Lithuanian population and to assess its relation with other cardiovascular risk markers and morbidity.

Methods. 770 individuals (376 men and 394 women) aged 6-85 y. were randomly selected from 31 primary care centers representing Lithuania. The patients were interviewed using a specially created validated questionnaire comprising nutritional and lifestyle habits, family history, and health status. Serum MDA concentration was measured using ultra high performance liquid chromatography method, while other biochemical parameters were tested using standartized methods. Statistical software IBM SPSS (v.21) was used for statistical analysis.

Results. Mean serum MDA concentration in Lithuanian population was 93.3 ± 36.2 ng/ml. MDA concentration was significantly higher in men (96.0 ± 38.1 ng/ml) than in women (90.7 ± 34.1 ng/ml; p=0.04). MDA levels increased with age (Pearson correlation coefficient r=0.23; p<0.001). In adult population (>18 y., n=627) serum MDA concentration significantly correlated with total cholesterol (r=0.41; p<0.001), LDL cholesterol (r=0.26; p<0.001), apolipoprotein B (r=0.37; p<0.001) and fasting glucose (r=0.14; p<0.001). Adults with low HDL cholesterol level (<1.0 mmol/l, n=67) had significantly higher MDA levels (106.9 ± 40.6 ng/ml vs. 97.0 ± 35.6 ng/ml; p=0.03), while C-reactive protein concentration was not associated with MDA levels. Body mass index had no impact on serum MDA levels. Individuals with the history of cardiovascular disease had significantly higher MDA levels than healthy individuals (respectively, 105.9 ± 40.1 ng/ml vs. 94.5 ± 33.8 ng/ml; p<0.001).

Conclusions. Our results show that MDA concentration increases with age, is higher in men and is related to cardiovascular risk factors and morbidity.

The study was supported by LITGEN project (VP1-3.1-ŠMM-07-K-01-013) is funded by the European Social Fund under the Global Grant measure.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference
Electronic poster presentation
Additional information
FXR-agonist Px-102 mitigates LXR-agonist induced hepatic steatosis through cholesterol synthesis outcompeting de novo lipogenesis

Abstract nr. 752
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Hypolipidemic Drugs, Metabolism, Pharmacology

LXR-agonists promote reverse cholesterol transport and are therefore promising agents in the treatment of atherosclerosis. Its clinical utility is however severely hampered by its side effect: hepatic steatosis. We have shown previously that the FXR-agonist Px-102 is able to mitigate hepatic steatosis in C57/Bl6J-mice treated with the LXR-agonist T0901717 and that this coincides with a massive increase in fecal cholesterol production. Here we made use of a computational approach called Analysis of Dynamic Adaptations in Parameter Trajectories (ADAPT) to investigate whether the increase in fecal cholesterol production explains the mitigation of LXR-agonist induced hepatic steatosis observed upon co-treatment with an FXR-agonist. ADAPT is a computational approach that is able predict how parameters in a computational model must change through time in order to comply to a longitudinal experimental dataset. The model topology focuses on processes occurring in the liver and includes FFA-uptake, VLDL-production, de novo lipogenesis, cholesterol synthesis and bile acid synthesis. Notably, it connects de novo lipogenesis and cholesterol synthesis through a common precursor pool, cytosolic acetyl-CoA. To obtain adequate constraints for ADAPT we complemented measurements obtained from mice treated with LXR-agonist and FXR-agonist with measurements of VLDL-TG production and de novo lipogenesis in LXR-agonist treated C57/BL6J-mice. ADAPT was run on datasets from LXR-agonist treated mice and datasets from mice treated with both LXR-agonist and FXR-agonist. Subsequently, parameter predictions from the dataset of dual treated mice were compared with those predicted for mice treated with LXR-agonist alone. The ratio of parameter predictions between dual treated and LXR-agonist treated mice was used to modulate parameter predictions for the LXR-agonist group. By comparing model outputs for hepatic triglycerides using parameter trajectories from the LXR-agonist group as input along with a single parameter trajectory modulated then allows for determining which processes explain the mitigation of LXR-induced
hepatic steatosis best. Using this approach, we found that both a decrease in de novo lipogenesis and an increase in cholesterol production were major factors in the mitigating effect of FXR-agonists on LXR-agonist induced hepatic steatosis.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
THE EFFECTS OF ASPIRIN RESISTANCE ON CORONARY ARTERY DISEASE SEVERITY AND STENT THROMBOSIS

Abstract nr. 753
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Cardiovascular Disease, Therapy, Thrombosis

Introduction: Atherothrombosis, which is the primary cause of acute ischemic syndromes in vascular bed, comprises more than 35% of all the mortality in the world and is characterized by rupture of atherothrombotic lesion which is associated with thrombotic complications. Aspirin is an antithrombotic drug that is used in the primary and secondary protection against atherothrombotic diseases and their complications. Aspirin inhibits cyclooxygenase-1 enzyme by acetyling the serine residues and thus, prevents the synthesis of thromboxane-A2 and other eicosanoids from arachidonic acid. Despite all the positive effects of aspirin, it is known that aspirin using atherosclerosis patients experience recurrent vascular events in their long term follow ups. Studies show that, in patients with documented laboratory resistance to aspirin, there are increases in the incidence of myocardial infarction, stroke, cardiovascular mortality and reocclusion rate in patients with percutaneous coronary intervention.

Aim: To investigate the relationship between aspirin resistance and coronary artery disease severity and stent thrombosis related repetitive percutaneous coronary interventions.

Method: Our study included 100 patients who are followed with CAD diagnosis and under therapeutic dose aspirin treatment, among the patients, 70 are aspirin non resistant and 30 are aspirin resistant. In the study, the thrombocyte inhibition level of aspirin is measured with Verify Now© system, the patients with ARU>550 are defined as aspirin resistant and with ARU<550 are defined as aspirin non-resistant. Demographic variables, cardiovascular risk factors, body mass indexes, usage of drugs with the possible interaction with aspirin, biochemical parameters, coronary angiographic features are compared between the groups that are aspirin resistant and aspirin non-resistant. Results: In our study, the relation of coronary artery disease severity with aspirin resistance is found to be significant. The disease severity which is calculated with Gensini score is found 85,96±29,70 in aspirin resistant group and 55,28±29,70 in aspirin non-resistant group (p=0,001). Furthermore, in aspirin resistant group, the increase of stent thrombosis and repetitive percutaneous coronary interventions is also found to be significant (84,6% vs. 38,5%; p=0,016).

Conclusion: There is increased level of coronary artery disease severity and increased frequency of repetitive percutaneous coronary interventions in the patients with aspirin resistance.
Table 1: Comparison of stent thrombosis between aspirin responsive and resistant groups

Table 2: Comparison of Gensini score between aspirin responsive and resistant groups

Presentation Preference Electronic poster presentation

Additional information
Abstract nr. 754
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Diabetes

Aim: to evaluate if a supplementation with n-3 PUFAs at high doses could give a regression of the condition of impaired glycemia and also check their effects on fasting plasma insulin (FPI) and insulin resistance (HOMA-index).

Methods: we enrolled 281 patients with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT); 138 (49.11%) subjects were randomized to n-3 PUFAs group, 1 g three times a day, and 143 (50.89%) to placebo for 18 months. We assessed at baseline, and after 9, and 18 months these parameters: circumferences, body mass index (BMI), fasting plasma glucose (FPG), fasting plasma insulin (FPI), HOMA-index, lipid profile. Moreover, at baseline and at the end of the study, all patients underwent an oral glucose tolerance test (OGTT) with 75 g of glucose.

Results: we observed a decrease of glycemia and HOMA-IR with n-3 PUFAs, both compared to baseline (p < 0.05 at 9, and p < 0.01 at 18 months), and to placebo (p < 0.05 at 9, and p < 0.01 at 18 months). We recorded an increase of FPG and HOMA-IR after 18 months of placebo treatment (p < 0.05). Fasting plasma insulin decreased both compared to baseline after 9 (p < 0.05), and 18 months (p < 0.01) with n-3 PUFAs. Placebo increased FPI after 18 months (p < 0.05), moreover FPI recorded with n-3 PUFAs was lower compared to the one obtained with placebo after 18 months (p < 0.05). After OGTT performed at the end of the study, more patients returned to a condition of euglycemia with n-3 PUFAs compared to placebo. Total cholesterol, and LDL-C did not change during the study, while HDL-C increased after 18 months of n-3 PUFAs (p < 0.05). Triglycerides decreased after the introduction of n-3 PUFAs, both compared to baseline (p < 0.05 at 9, and p < 0.01 at 18 months), and to placebo (p < 0.05 at 9, and p < 0.01 at 18 months).

Conclusions: treatment with n-3 PUFAs was effective in reducing glycemia in patients affected by IFG or IGT and seems to be helpful to slow the development of type 2 diabetes mellitus.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Potential function of CD14high CD16 monocyte subsets in familial hypercholesterolemia

Abstract nr. 755
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia, Inflammation

Background: Monocytes and macrophages play important role in atherosclerosis progress of familial hypercholesterolemia (FH) patients. Different subsets of monocytes exert opposite effect as pro-inflammatory or anti-inflammatory, so the distribution of monocyte subsets is able to be a marker to measure the severity of atherosclerosis inflammatory response. Circulating monocytes are subdivided into CD14+CD16 inflammatory monocytes with a phenotype that resembles the original description of monocytes and CD14+CD16+ resident monocytes with a phenotype that resembles mature tissue resident macrophages. CD14+CD16+ monocytes are a heterogeneous population and consist of CD14\textsuperscript{high}CD16+ and CD14\textsuperscript{low}CD16+ subsets, according to the intensity of CD14 expression. It has been shown that the proportion of CD14\textsuperscript{+}CD16\textsuperscript{+} monocytes was significantly reduced in FH homozygotes compared with healthy persons. In this study, we try to do further exploration of the monocyte subsets distribution in heterozygous FH patients.

Objective: To investigate the characteristics and distribution of monocyte subsets of heterozygous familial hypercholesterolemia patients. In addition, to analyze the possible mechanisms of the different description of monocyte subsets and further understanding of the involving immune response in FH patients, thus in order to provide new methods to evaluate the progression and treatment efficacy of FH.

Method: Flow cytometry was used to detect monocyte subpopulations in peripheral blood from 54 heterozygous FH patients and 65 healthy persons by using monoclonal antibodies (CD14, CD16, and HLA-DR).

Results: There was no significant difference in the proportion of CD14\textsuperscript{+}CD16\textsuperscript{+} monocytes of the two groups (P>0.05). But further analysis in the two subpopulations of CD14\textsuperscript{+}CD16\textsuperscript{+} (CD14\textsuperscript{high} CD16\textsuperscript{+} and CD14\textsuperscript{low}CD16\textsuperscript{+} monocytes), compared with the normal control, the proportion of CD14\textsuperscript{high}CD16\textsuperscript{+} was down-regulated (2.75%±1.34% VS 3.36%±1.75% P<0.05).

Conclusions: Previous studies mainly focused on the distribution of CD16\textsuperscript{+} and CD16 monocyte subpopulations. But the CD14\textsuperscript{high}CD16\textsuperscript{+} subpopulation of CD16\textsuperscript{+} monocytes was the intermediate process that monocytes transform to macrophages. The decline of CD14\textsuperscript{high}CD16\textsuperscript{+} monocytes proportion showed, in FH patients, the transformation rate of inflammatory monocytes into macrophages was reduced and most of the monocytes showed the character of inflammatory
monocytes. So the proportion of CD14$^{\text{high}}$CD16$^+$ monocytes may be a more sensitive indicator to measure the inflammatory response in FH patients.

Subdivision 2. Translational Research

Presentation Preference Mini-oral presentation
Additional information
Gene Therapy for Lipoprotein Lipase Deficiency (LPLD): Learnings From the Clinical Development of Alipogene Tiparvovec, an AAV1 therapy for LPLD

Abstract nr. 756
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Genetics, Therapy, Triglyceride-Rich Proteins, Triglycerides

Introduction: The development program for alipogene tiparvovec started 10 years ago with the dosing of the first patient in 2005. Three interventional open label, prospective clinical trials and 2 retrospective studies have been completed in The Netherlands and Canada. EMA approval was achieved in 2012 (GlyberaTM).

Methods: 27 subjects received a single administration of 3 different doses of AAV1-LPLS447X at 1x10^11, 3x10^11, and 1x10^12 gene copies/kg via IM injections, with or without a 12-week immunesuppression regimen (ISR). Subjects with triglycerides >10 mmol/L, <20% of normal post-heparin LPL activity and confirmed LPL gene mutations were eligible. The duration of the 3 prospective clinical trials was 1 to 5 years. Retrospective studies completed after a median post-treatment follow up (PTFU) of 3 and 6 years included an analysis of hospitalizations and pre- vs post-treatment disease-related abdominal events (pancreatitis and acute abdominal pain events consistent with pancreatitis) by a blinded committee.

Results: Muscle expression of LPLS447X protein was confirmed via immunohistochemistry (N=17 subjects tested; PTFU range 10-52 weeks). Chylomicron (CM)-TG content and CM kinetics significantly improved. In 14 subjects tested at weeks 12 and 52, TG and cholesterol content decreased in a buoyant CM fraction, with reciprocal increases in a less buoyant, VLDL-rich fraction. Post-prandial CM (ppCM) clearance (as measured by AUC_24) improved by 93% and 68% at week 14 and week 52, respectively, vs baseline (N=5 and 3 subjects tested, respectively). Pooled analysis of all subjects’ events showed a decrease of 40% and 60% post-treatment in pancreatitis and acute abdominal pain events consistent with pancreatitis, respectively vs. an equivalent period pre-treatment, and parallel reductions in hospitalizations and ICU stays were documented after a median 6 years of PTFU. No trend was observed relating severe AEs to dose or ISR. Two deaths occurred, unrelated to gene therapy. No cellular or humoral immunological
response to the LPL$^{S447X}$ protein occurred.

**Conclusions:** A gene therapy approach to LPLD has shown sustained LPL protein expression, improvement in CM clearance, and long term reductions in disease-related abdominal events and hospitalizations. No significant safety concerns have emerged. Additional studies are planned to further extend biochemical and clinical observations.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation

Additional information
Statins in familial hypercholesterolemia - effect on cardiovascular disease free survival

Abstract nr. 757
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease,Familial Hypercholesterolemia,Hypolipidemic Drugs,Prevention

Introduction
Familial hypercholesterolemia (FH) is a monogenetic disorder characterized by severely increased levels of low-density lipoprotein cholesterol (LDL-C) and increased risk for premature cardiovascular disease (CVD). Statins are the first choice treatment for patients with FH. However, the effect size in terms of CVD prevention is unknown in FH since placebo-controlled trials are considered unethical and unavailable. The aim of our study is to quantify the effect of statins on CVD incidence in FH.

Methods
We performed a retrospective cohort study in the national Dutch FH registry. Eligible patients were heterozygous FH (HeFH) patients, identified between 1994 and 2014. Index patients and patients who experienced a CVD event before 1990 were excluded. We matched FH patients who started a statin in 1990 with non-users by age- and gender-adjusted LDL-C level, age, gender, type of FH mutation (LDLR or APOB), hypertension, diabetes, nicotine consumption and body mass index. We compared the incidence of CVD events between statin users and non-users by the Kaplan-Meier survival method, and estimated a hazard ratio (HR) for statin use by means of Cox proportional hazard modeling.

Results
Between 1994 and 2014, 27,210 HeFH patients were identified, of whom 21,097 were non-index patients without CVD before 1990. A statin was started in 1990 by 1,667 and 698 could be matched to 698 untreated FH patients. CVD free survival at 5, 10, 15 and 20 years was 97.8%, 95.2%, 92.5% and 90.2% in statin users compared to 95.2%, 90.2%, 84.3% and 80.6% in non-users (p<0.001). The number needed to treat was 38, 20, 12, and 10 for 5, 10, 15 and 20 years of statin treatment. A relative risk reduction of 58% was observed (HR 0.42; 95%CI: 0.28 to 0.62).

Conclusion
Statins significantly improve CVD free survival in FH patients, with acceptable numbers needed to treat. Due to the retrospective nature of the data and the accompanying indication bias, our results might be an underestimate of the effect size as might be obtained in placebo-controlled clinical trials.
CVD free survival in HeFH patients with and without statin treatment

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation

Additional information
Inhibition of indoleamine 2,3-dioxygenase (IDO) aggravates atherosclerosis in hypercholesterolemic mice

Abstract nr. 758
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Immunity, Inflammation, Therapy

**Background:** Indoleamine 2,3-dioxygenase (IDO), the first and rate-limiting enzyme of the kynurenine pathway of tryptophan (Trp) degradation, has been implicated in the control of inflammation and autoimmunity. Pharmacological inhibition of IDO with 1-methyl-tryptophan (1-MT) results in aggravation of disease in several models. However, the role of IDO and the endogenous degradation of tryptophan in atherosclerosis remain unknown.

**Aims:** In this study, we used the IDO inhibitor 1-methyl-Trp (1-MT) to determine the role of IDO-mediated Trp metabolism in vascular inflammation and atherosclerosis in hypercholesterolemic Apoe−/− mice.

**Methods and Results:** Apoe−/− mice were treated with 1-MT in the drinking water for 8 weeks. Systemic IDO inhibition led to significant increase in atherosclerotic lesions in the aortic root. 1-MT treatment resulted in upregulation of the aortic mRNA levels of TNF, MCP-1, and VCAM-1. In line with this results, immunohistochemical staining of aortic roots showed increased CD68+ macrophage infiltration, and VCAM-1 expression in plaques of 1-MT–treated mice. Notably, increased VCAM-1 expression was also found on smooth muscle cells (SMCs) of the tunica media. Additionally, we found that IDO-dependent Trp metabolism by SMCs regulates VCAM-1 expression, and that 1-MT-induced acceleration of atherosclerosis and vascular inflammation can be reversed by exogenous administration of the Trp metabolite 3-hydroxyanthranilic acid (3-HAA).

**Conclusions:** Our data establish that IDO-mediated Trp metabolism plays a major role in the regulation of vascular inflammation and atherosclerosis.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Results from the Long-Term Follow-Up of an Open Label Study (CT-AMT-011-01) of Alipogene tiparvovec (AAV1-LPLS447X) for Lipoprotein Lipase Deficiency (LPLD)

Abstract nr. 759
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Genetics, Therapy, Triglyceride-Rich Proteins, Triglycerides

Aim: LPL is a key enzyme in the metabolism of triglyceride (TG)-rich lipoproteins, namely chylomicrons (CM) and VLDL. In LPLD, both CM levels and risk of pancreatitis are increased. The aim was to study the long term safety and efficacy of alipogene tiparvovec in subjects with LPLD and history of pancreatitis.

Methods: A total of 14 subjects were divided into 3 sequential cohorts to receive a single treatment with alipogene tiparvovec at a dose of $3 \times 10^{11}$ genome copies/kg via multiple IM injections, without ($n=2$, cohort 1) or with ($n=4$, cohort 2) an immunosuppression regimen (ISR); cohort 3 ($n=8$) received $1 \times 10^{12}$ genome copies/kg with ISR. Study results from the first 2 years have been published (Gaudet et al., Gene Ther 2013;20:361-9). Long-term safety and efficacy up to 5 years are reported here.

Results: Pre-treatment median fasting plasma TG levels range was 13.0-65.5 mmol/L. TG levels at 5 years or to last observation available were variable; 10 subjects had reductions from baseline (range 4.4-63.7%), while 4 subjects had TG levels above baseline (range 2.2-12.0%). Mean pancreatitis rate declined from 0.16 episodes per year pre-treatment to 0.07 post-treatment. No humoral or cellular responses against the LPLS447X protein were observed. One death unrelated to treatment was reported 26 months after dosing.

Conclusion: No clear correlation between fasting plasma TG levels and efficacy was observed. This is in line with the notion that TG-lipoprotein characteristics and CM kinetics rather than plasma TG concentrations per se might be better markers of alipogene tiparvovec activity. Consistent with this concept are the following results: a) TG content in buoyant-CM and less buoyant-CM plus VLDL-rich fractions at weeks 14 and 52 post-treatment, suggesting a shift of lipid-lipoprotein distribution profiles (Gaudet et al., Gene Ther 2013;20:361-9) and, b) results from a subsequent study (CT-AMT-011-02) in 5 additional patients showing a marked decrease in CM-TG levels and improved CM metabolism at week 12 (Carpentier et al., J Clin Endocrinol Metab 2012;97:1635-44), and at week 52 post-treatment (unpublished). There was no trend relating
severe AEs to dose or ISR use. New studies evaluating CM metabolism kinetics are planned.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Modeling PCSK9 functions using hepatocyte-like cells (HLC) differentiated from urine-sample-derived human induced pluripotent stem cells (UhiPSC).

Abstract nr. 760
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia, LDL, PCSK9

Introduction. hiPSC are becoming a relevant model for the study of liver metabolic diseases once differentiated into HLC, and it has been shown that they can faithfully recapitulate autosomal dominant hypercholesterolemia (ADH). PCSK9 is a critical modulator of plasmatic low-density lipoprotein cholesterol (LDLc) uptake by the liver. While PCSK9 gain of function (GOF) mutations induce ADH, loss of function (LOF) mutations lead to low levels of circulating LDLc, thus making PCSK9 a hot target for ADH pharmacological treatment strategies. However, current models to study the role of PCSK9 in ADH or unknown functions are limited.

Hypothesis. Hepatic differentiation of patient-derived hiPS cells will provide an accurate and appropriate tool to model PCSK9-mediated ADH and enhance our understanding of PCSK9 functions.

Methods. We used urine samples as a source of somatic cells in order to obtain hiPSC upon episomal vectors-mediated reprogramming (UhiPSC). After characterization and validation, UhiPS control, carrying the GOF S127R mutation, which leads to an intracellular form of PCSK9 with unclear functions, and LOF R104C/V114A mutations, which lead to a default of PCSK9 secretion, were differentiated into HLC.

Results. Compare to control cells, HLC-S127R secreted 1.6 time (±0.8; p<0.05) less PCSK9, and had a dil-LDL uptake decrease of 3.5 fold (±0.17; p<0.01). A 24h pravastatin treatment at 10µM significantly enhanced LDLR and PCSK9 gene expression and PCSK9 secretion in both control and S127R HLC. Finally, while control HLC increased their dil-LDL uptake of a factor 1.38 (±0.49; p<0.01), the pravastatin treatment induced a 2.19 fold (±0.77; p<0.01) increase of dil-LDL uptake in HLC-S127R, which brought them to a level that was not significantly different from untreated control HLC (p=0.29) and was correlated to the original patient response. In another hand, our preliminary data showed that HLC-R104C/V114A displayed a 2.36 fold increase of LDL uptake.
compare to control cells, which could be partially inhibited by recombinant PCSK9.

**Conclusions.** Altogether, our study demonstrates that not only patient’s urine samples provide an attractive source of somatic cells for reprogramming and hepatocyte differentiation but also a powerful tool to further study PCSK9 functions.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Macrophage-specific knockout of prolyl-hydroxylase domain protein 2 (PHD2), the crucial cellular oxygen sensor, increased angiogenesis in mice. As angiogenesis is known to enhance atherosclerosis progression, we hypothesized that macrophage PHD2-/- would increase plaque size.

LysMCre-low density lipoprotein receptor knockout (LDLR-/-) and lysMCre PHD2^fl/fl^ LDLR-/- mice (PHD2-/-) mice were fed a high cholesterol diet (0.25% cholesterol) for 6&12 weeks to analyze early and advanced atherosclerosis. MAC3+ macrophage content, CD31+ microvessels, collagen content (Sirius red and collagen I) and percentage of αSMA+ smooth muscle cells (SMCs) were analyzed in plaques of the aortic root. Stimulation of collagen synthesis (Sirius red) was measured in SMCs incubated with PHD2-/- macrophage conditioned medium. Macrophage matrix degradation capacity of cell lysates and conditioned medium was determined using OmniMMP™ substrate.

Plaque size was increased after 6 (+60%, p<0.0002, H&E) and 12 weeks (+40%, p<0.0001, H&E) of diet in PHD2-/- mice, compared to control. Necrotic/lipid core content was similar. Although, advanced atherosclerotic plaques of PHD2-/- more frequently contained erythrocytes (+53% vs 17%, H&E, p<0.05), suggesting increased plaque angiogenesis, no difference in microvessel density (CD31) was found. Interestingly, total plaque collagen was increased in PHD2-/- vs control (+22%, p<0.0001, sirius red), suggestive of a more stable plaque phenotype. Specifically type I collagen was increased, in PHD2-/- mice (+30%, p<0.05, collagen I). The increase in collagen could not be attributed to an increase in SMC since the percentage of SMC was decreased by PHD2-/- (-35%, p<0.05, αSMA,). Similarly PHD2-/- reduced plaque macrophage content (-37%, Mac-3, p<0.01). Finally, body weight and hematopoiesis did not differ, whereas plasma cholesterol
was slightly lower in PHD-2⁻/⁻ mice (12 weeks -10%, p<0.05, no difference at 6 weeks). In vitro, the stimulation of collagen synthesis by PHD2⁻/⁻ macrophages could be excluded. However, PHD2⁻/⁻ macrophages displayed an impaired collagen degradation capacity (-12% p<0.01), possibly explaining the increase in fibrosis. Hematopoietic PHD2⁻/⁻ resulted in large fibrotic plaques with less inflammation. PHD2 inhibition in advanced atherosclerosis might therefore prevent plaque destabilization and could present a novel therapeutic target. Moreover, it is currently investigated how the increase in fibrosis by PHD2 deficiency is dedicated to impaired collagen degradation.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Genome-wide identification of microRNAs involved in the regulation of cholesterol metabolism and atherosclerosis development

Abstract nr. 762
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Pathogenesis, PCSK9

**Background.** MicroRNAs (miRNAs), a recently discovered class of noncoding RNAs, are involved in the post-transcriptional regulation of genes belonging to different biological processes, including cholesterol homeostasis. Despite recent advances in the characterization of these molecular switches, our understanding of the role of miRNAs in regulating lipid metabolism and atherosclerosis is limited. Technological progress facilitates the genome-wide identification and quantification of miRNAs, as well as the identification of differentially expressed (DE) miRNAs.

**Aim.** To identify DE miRNAs involved in the regulation of cholesterol metabolism and atherosclerosis development.

**Methods.** Six weeks old C57BL/6J mice as controls, hyperlipidemic/atherosclerosis-prone LdlrKO and hypolipidemic/atherosclerosis-resistant Pcsk9KO mice were fed standard (CHOW) or Western-type (WD) diet for 16 weeks.

At sacrifice, aorta, liver, small intestine, white adipose tissue, heart and brain were harvested. Small RNA was extracted, sequenced with an Illumina Genome Analyzer IIx and analyzed with state-of-the-art bioinformatics and statistical approaches.

**Results.** For each tissue, overall miRNA expression was comparable among different genotypes and dietary treatments, to the exception of liver and brain where LdlrKO mice fed WD showed higher and lower miRNA expression, respectively.

C57BL/6J and Pcsk9KO mice showed remarkable similarities in miRNA expression profiles, especially when fed WD. On the contrary, most comparisons of C57BL/6J and Pcsk9KO with LdlrKO mouse tissues in both dietary conditions revealed an elevated number of DE miRNAs.

Furthermore, C57BL/6J and Pcsk9KO mice shared most of the DE miRNAs when compared to LdlrKO mice.

Besides DE miRNAs already known to play a role in cholesterol metabolism, such as miR-33a/b, miR143/145, mir27a/b, miR-155, miR-128 and miR-370, many other DE miRNAs were identified. For example, 130 DE miRNAs were found by comparing C57BL/6J and LdlrKO aortas on CHOW
diet. The number of DE miRNAs was even higher in tissues from animals fed WD, with brain and liver showing the highest (~ 300 in each comparison, 217 shared between C57BL/6J and Pcsk9 KO vs Ldlr KO).

Conclusions.
MiRNomic analysis found many DE miRNAs in addition to the ones with known ties with cholesterol metabolism. Predicted targets will be evaluated and the highest ranking ones chosen for subsequent in vitro analysis, which may disclose novel regulatory circuits.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Effect of Resistance Training on Plasma Nitric Oxide and Asymmetric Dimethylarginine Concentrations in Type I Diabetic Rats

Abstract nr. 763
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Cardiovascular Disease, Diabetes, Lifestyle

Background: Asymmetric dimethylarginine (ADMA) has a predominant role in progression of some cardiovascular diseases, including diabetes. It interferes with Larginine in production of nitric oxide (NO) by inhibition of NO synthase. The purpose of this study was to evaluate the effect of resistance training on plasma NO and ADMA concentrations in type 1 diabetic male rats.

Methods: Thirty-six male wistar rats were randomly divided into four groups: (1) control; (2) diabetic; (3) diabetic trained, and (4) control trained (n = 9 each). In the trained groups, the animals undertook one training session per day, 3 days/week, for 4 weeks. At the end of experiment, blood samples were taken and the concentrations of plasma glucose, insulin, lipid profile, NO and ADMA concentrations were determined.

Results: Plasma ADMA concentration showed a significant increase in diabetic rats compared to control group (0.73 ± 0.07 vs. 0.62 ± 0.04 μmol/l; P < 0.05). The plasma ADMA level in the trained diabetic and control were lower than the sedentary groups, although it was not statistically significant. Plasma NO concentration in diabetic group was lower than control (P < 0.05). Resistance training significantly increased plasma NO concentration in diabetic animals (P < 0.05).

Conclusion: Elevated ADMA level in diabetic animals can normalize during resistance exercise. Reduced ADMA level and increased NO level following resistance training might improve cardiovascular risk in diabetic subjects.

Keywords: Asymmetric dimethylarginine, diabetes, nitric oxide, resistance training

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Background. Familial hypercholesterolemia (FH) is an autosomal dominant genetic disorder associated with significantly elevated levels of low-density lipoprotein cholesterol, resulting in a dramatically increased lifetime risk of premature atherosclerotic cardiovascular disease. Given low awareness on prevalence of FH in the Russian Federation, low percentage of cases are diagnosed and treated. The main aim of the present study is to evaluate the extent to which FH is underdiagnosed and undertreated in the Russian Federation in order to reduce the atherosclerotic cardiovascular disease risk in the country. Such knowledge is urgently needed to guide appropriate screening and treatment strategies.

METHODS AND DESIGN. The Russian FH Registry (RuFH) is a multicenter, national registry of patients diagnosed with FH from Russian federal and outpatient practices. During 2014-2015, approximately 1000 patients with total cholesterol ≥ 7.5 mmol/L (290 mg/dL), or LDL-C ≥ 4.9 mmol/L (190 mg/dL) will pass a non-invasive clinical examination, including patient demographics, past medical history, family history of coronary disease and hypercholesterolemia, physical findings, current lipid-lowering therapy, blood tests, genetic analysis, sonographic Achilles tendon characteristics, echocardiography, carotid duplex ultrasound, and exercise stress myocardial perfusion test. We will ascertain definite, probable, and possible FH cases based on the Dutch Lipid Clinic Network and Simon Broome Registry criteria. As an expected outcome, this program will raise awareness and increase appropriate assessment and treatment of FH patients, leading to a timely detection of the disease and therapy initiation. There is an unmet need to validate the clinical criteria for the FH diagnosis in the Russian population.

CONCLUSION. Epidemiological data suggest that the high rate of cardiovascular morbidity and mortality in Russia are partly due to an underestimation of the significance of hypercholesterolemia, including the high prevalence of FH. Timely detection of FH will help to initiate treatment not only in the indexed case, but also to motivate relatives for a clinical and genetic screening, thus increasing the number of promptly diagnosed individuals. The RuFH Registry is supported by Russian National Atherosclerosis Society, International Atherosclerosis Society and Pfizer (11532493), Amgen Inc, AstraZeneca, INVITRO Laboratory.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
On the Analysis of Sport’s Effect and Physical Activities on the Cooperation of Nurses in the Clinical Decision-Making

Abstract nr. 766
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Co-author(s) - MousaviNafchi, Sayed Morteza
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Epidemiology, Intervention, Lifestyle

(A Case Study in Alzahra Hospital, Isfahan)
Introduction: clinical decision making is an essential part of nurses’ professional function which differentiate professional nurses from the normal and non-expertise caring personnel. in comparison with the other factors, clinical decision making has a great effect on the quality of taking care of the patients, Clinical decision making process is to identify the patient’s need requires and determine the best nursing action.
Method: The objective of the present survey is to analyses of relationship between exercising and physical activities with nurses’ clinical decision making on 2014. The method of this survey is descriptive-correlation and of survey research; its statistical population include all of the nurses who work in AL Zahra hospital in Isfahan with the population of 1125 people. The data collection tools includes two questioners of clinical decision making (PDAQ) and the researcher-made questionnaire of physical activities which its content validity and face validity has been confirmed by the eight experts of nursing fields, clinical cares and physical science. Also its the reliability is earned through Cronbach’s Coefficient Alpha as 0/90 and 0/81.
Findings: The results show that there is a significant relationship between exercising, physical activities and taking part in nurses` clinical decision making. (R = 0/476, P value = 0/001). Also, multiple regression’s results show that group sports such as (Volleyball, Basketball, Badminton and Futsal) with Beta Correlation as (B = 0/398, P value = 0/034) are significant.
Results: The results show the great and positive effect of sport on clinical decision making and its factors (include problem identification) to choose the best solution to analyze the suggested solutions in a way that a nurses could make the right decision and select proper solution by exercising.
Keywords: Sport and physical activities, Decision making, Nurses

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
PAF receptor interacts with LOX-1 and promotes oxidized LDL-induced cellular responses

Abstract nr. 767
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Endothelium

**Background:** Endothelial dysfunction is implicated in initiation and development of atherosclerosis. LOX-1 is the major receptor for oxidized LDL (oxLDL) in endothelial cells and LOX-1-mediated oxLDL actions induce endothelial dysfunction, while some reports have shown that PAF receptor (PAFR) might mediate a part of the action of oxLDL.

**Aim:** Here, we investigated the potential synergism between LOX-1 and PAFR in signal transduction in response to oxLDL.

**Methods and results:** Cellular responses to oxLDL were examined with COS-7 cells transfected with LOX-1 and PAFR and cultured human aortic endothelial cells (HAEC). oxLDL-induced cell responses, such as ERK phosphorylation and NF-κB activation, were promoted by additional expression of PAFR compared with cells expressing solely LOX-1 or PAFR. In this condition, the binding of oxLDL to the cells expressing both LOX-1 and PAFR was not significantly different from the cells expressing solely LOX-1 or PAFR. In addition, PAFR antagonists, ABT-491, suppressed oxLDL-induced ERK phosphorylation in HAEC. Furthermore, immunoprecipitation analysis showed that LOX-1 was co-immunoprecipitated with PAFR from the cells transfected with both PAFR and LOX-1, while dectin-1, the nearest C-type lectin-like protein family member of LOX-1, was not. These results suggested that LOX-1 and PAFR expressed physically proximal to each other in cell surface might cooperatively strengthen the signal of oxLDL action.

**Conclusion:** Coupling of LOX-1 and G-protein coupled receptors such as PAFR might be of importance in oxLDL-induced pathological vascular reaction.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Glucocorticoid receptor alpha is overexpressed in patients with coronary artery disease - evidence for increased glucocorticoid sensitivity

Abstract nr. 768
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Inflammation, Pathogenesis

Hypothalamic-pituitary-adrenal axis dysfunction has been associated with chronic inflammatory conditions. Coronary artery disease (CAD) is characterized by low-grade chronic inflammation including increased levels of leukocyte-derived matrix metalloproteinase (MMP)-9 and its tissue inhibitors (TIMPs). Underlying mechanisms are not clarified but may involve a dysfunctional glucocorticoid regulation of MMP-9 and TIMPs. In the present study, we hypothesized that increased expression of MMP-9 and TIMPs in peripheral blood mononuclear cells (PBMCs) was associated with reduced glucocorticoid sensitivity in CAD patients.

Blood sampling was performed between 6 and 18 months after myocardial infarction in 55 patients (median age 67 y). Thirty clinically healthy subjects were included as controls (median age 66 y). Gene expression of glucocorticoid receptor (GR)-α, GR-β, MMP-9, TIMP-1 and TIMP-2 was analyzed in freshly isolated PBMCs. We further compared ex vivo the effects of dexamethasone (a synthetic glucocorticoid) on mRNA and protein levels of GR-α, GR-β, MMP-9, TIMP-1 and TIMP-2 in PBMCs from patients and controls.

In CAD patients, mRNA levels of MMP-9, TIMP-1 and TIMP-2 were significantly higher than in controls. Also, GR-α mRNA levels were markedly increased in CAD patients, 0.50 (0.38-0.59) vs 0.26 (0.18-0.37), p < 0.001, whereas GR-β mRNA levels did not differ. Ex vivo, dexamethasone efficiently suppressed mRNA and protein expression of MMP-9 and TIMPs. Sensitivity to dexamethasone was equal to or slightly higher in patients compared with controls. GR-α mRNA expression in vivo correlated with dexamethasone-induced suppression ex vivo. Moreover, dexamethasone treatment induced a significant reduction of GR-α mRNA in PBMCs. Circulating PBMCs in CAD patients exhibited overexpression of MMP-9, TIMP-1 and TIMP-2. The concomitant overexpression of GR-α strongly indicated an increased sensitivity to glucocorticoids. It may be suggested that increased levels of leukocyte-derived MMP-9 and TIMPs are associated with a state of relative hypocortisolism in CAD patients, thus contributing to a systemic low-grade inflammation.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
HIGH BILE ACID SYNTHESIS IN MORBID OBESITY IS RAPIDLY NORMALIZED BY A LOW CALORIC DIET INDEPENDENT OF FGF19

Abstract nr. 769
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Lipids, Nutrition, Obesity, PCSK9

Background and aim
Bile acid (BA) synthesis is regulated by feedback inhibition by BAs in the liver and presumably from an intestinal-liver axis by fibroblast growth factor 19 (FGF19). BA synthesis is increased in morbid obesity of unclear reasons. To gain insights in the latter, we analyzed plasma samples from a study where a preoperative low caloric diet (LCD) was given to evaluate the order of liver size reduction achievable prior to gastric bypass surgery.

Materials and Methods
Ten women (means 43 yr, 114 kg, and BMI 42 kg/m²) were examined on day 0, 3, 7, 14 and 28 after commencing a LCD (800-1100 kcal/day). Serum was collected at each visit when liver volume and intrahepatic fat content were measured by magnetic resonance imaging.

Results
After 3 days, plasma LDL cholesterol and BAs were significantly increased by 10% and 3% respectively, and plasma levels of lathosterol (cholesterol synthesis marker) and PCSK9 decreased by 20% and 15% respectively. BA synthesis, monitored by the plasma marker C4, decreased progressively 50-60% during the entire LCD period. However, there were no significant changes of plasma levels of the BA synthesis suppressor FGF19. As previously reported, the LCD reduced liver volume by 18% within the first 2 weeks of treatment. Intrahepatic fat decreased continuously over 28 days.

Conclusions
Our results suggest that a LCD to morbidly obese subjects strongly reduces fecal loss of BAs due to a severe reduction of BA-binding food contents in the intestine. Consequently BAs immediately pile up and strongly shut down hepatic BA synthesis as seen from reduced C4 levels. This presumably occurs by a direct BA-induced hepatic FXR response since FGF19 plasma levels did not increase. Consequently, hepatic cholesterol levels increase thereby reducing cholesterol synthesis (lathosterol) and plasma PCSK9 levels. The results suggest that high BA synthesis in
morbid obesity is linked to the level of food intake in this condition.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
INDIVIDUALS WITH ABCA1 AND LCAT MUTATIONS ARE CHARACTERIZED BY INCREASED ARTERIAL STIFFNESS AND ARTERIAL OUTWARD REMODELLING

Abstract nr. 771
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, HDL, Imaging

Background. Carriers of mutations in ATP-binding cassette transporter A1 (ABCA1) and Lecithin-Cholesterol AcylTransferase (LCAT) are characterized by low plasma HDL-C levels, increased arterial wall thickness and increased risk for cardiovascular disease. In this way, ABCA1 and LCAT mutation carriers can be considered as a model for genetically determined early vascular aging. The aim of the present study was to assess arterial remodelling by MRI and arterial stiffness in ABCA1 and LCAT mutation carriers and healthy controls and test the association between arterial remodelling and arterial stiffness.

Methods and Results. We measured carotid-femoral pulse wave velocity (PWV), carotid artery dimensions by MRI and tested the association between PWV and carotid artery dimensions in 64 subjects with genetically low HDL (24 ABCA1 and 40 LCAT mutation carriers) and 48 matched controls. Mutations in ABCA1 and LCAT were associated with outward remodelling of the carotid artery as evidenced by an increase in outer wall area (OWA, 0.52±0.12 in mutation carriers vs 0.46±0.09 mm² for controls, p=0.003) without a change in lumen area (LA, 0.33±0.06 in mutation carriers vs 0.32±0.05 mm² in controls, p=0.12). Outward remodelling was accelerated by approximately 10 years compared to controls. PWV was increased in ABCA1 and LCAT mutation carriers (8.3±2.2 vs 7.3±1.5 m/s, p=0.005) and PWV correlated significantly with outward remodelling (OWA r=0.53, p<0.001; LA r=0.29 p=0.002).

Conclusion. ABCA1 and LCAT mutation carriers are characterized by increased arterial stiffness and outward remodelling of large arteries and these parameters are strongly correlated. The association with structural arterial wall changes, resulting in outward remodelling, underlines the clinical impact of PWV measurements as a non-invasive easy to use method to assess CVD risk.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Angptl4 mediates shuttling of lipid fuel to brown adipose tissue during cold exposure

Abstract nr. 772
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Lipoproteins, Metabolism, Triglycerides

Brown adipose tissue (BAT) activation via cold exposure is increasingly scrutinized as a potential approach to ameliorate cardiometabolic risk. The transition to a cold environmental temperature requires major changes in the partitioning of energy substrates, re-routing fatty acids to BAT to fuel non-shivering thermogenesis. However, the mechanisms behind the re-distribution of energy substrates to BAT remain largely unknown. Angiopoietin-like 4 (Angptl4), a protein that inhibits lipoprotein lipase (LPL), is highly expressed in BAT and white adipose tissue (WAT). The function of Angptl4 in BAT is, however, unknown. To examine the role of Angptl4 in BAT, Angptl4 knock-out, wild-type and transgenic mice were placed at 4 degrees or at thermo-neutrality for 10 days. Upon cold exposure, LPL activity and uptake of fatty acids from circulating triglyceride-rich lipoproteins were dramatically increased in BAT. In accordance with a function for Angptl4 as a repressor of LPL activity in BAT, increased LPL activity and uptake of fatty acids were accompanied by marked down-regulation of Angptl4 mRNA levels and protein in BAT of wild-type mice. This reduction in Angptl4 expression was mediated via activation of AMPK in BAT. Opposite to the changes seen in BAT, Angptl4 mRNA and protein levels were induced in WAT during cold, suppressing the activity of LPL in WAT. Induction of Angptl4 in WAT was mediated via activation of the β-adrenergic signalling pathway. Taken together, these data suggest that regulation of Angptl4 facilitates the pronounced increase in fatty acids uptake by BAT upon cold exposure, by directly acting upon the activity of LPL in both BAT and WAT. The opposite regulation of LPL activity in these tissues by Angptl4 suggests that Angptl4 is a key regulator of plasma lipid partitioning during cold.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Association between social class and metabolic syndrome in a Tunisian population

Abstract nr. 773
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Diabetes, Dyslipidemia, Epidemiology, Lifestyle

Background and aims: In Tunisia, demographic changes and social transition lead to many changement within the tunisian population. The relationship between social class and metabolic syndrome has received little attention in recent years. In this study we sought to evaluate the consequences of these transitions in a sample of Tunisian subjects with metabolic syndrome.

Methods: Three hundred ninety three of the general population, aged between 18-75 years, participated in this study. Education level, occupation, monthly income, age, body weight, body height, waist circumference, blood pressure were collected. glycaemia, triglycerides, total cholesterol and HDL-cholesterol were measured. Participants were classified into two groups according to the health state: healthy (Group 1, n=105) and patient having metabolic syndrome according to the recent diagnostic criteria of the International Diabetes Federation and the American Heart Association/National Heart, Lung, and Blood Institute (2009) (Group 2, n=288).

Results: Individuals with metabolic syndrome are older than healthy group (55.1±15.4 years vs 42.2±13.8 years), the most common in the healthy group are manufacturing and liberal profession while in patient group are retired or unemployed participants. Within the whole population 40% had primary level of study. Illiteracy is more frequent in group 2 than group 1 (34,1% vs 22,5%). Smoking behavior and alcohol drinking are similar in both group. Additionally, our results show that the highest quintile of income (Q5) was recorded in group 1 with 15,9% against 1,4% in group 2.

Conclusion: The current study strengthens that healthy participants are younger and more educated than patients. Lower education and monthly income level are associated with higher risk of metabolic syndrome among the Tunisian individuals.

Key words: Social class, metabolic syndrome.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Tribbles Homolog-1 (Trib1) deficiency causes increased hyperlipidemia and atherosclerosis in LDL-receptor knockout mice

Abstract
Trib1-/- mice were crossed onto the Ldlr-/- background to generate double knockout mice (Trib1-/-Ldlr-/-) and fed a semisynthetic, modified AIN76 diet (0.02% cholesterol). At 20 weeks of age, Trib1-/-Ldlr-/- mice exhibited 7.4-fold larger atherosclerotic lesions at the aortic root as compared to Trib1+/+Ldlr-/- littermate controls (457763 vs. 62127 µm², p<0.0001). Further, we noted significantly elevated plasma total cholesterol (TC) and triglyceride (TG) levels in Trib1-/-Ldlr-/- mice (TC: 1024 vs. 609mg/dL, p<0.0001; TG: 360 vs. 136mg/dL, p<0.0001). Analysis of plasma lipoproteins revealed that higher levels of TC and TG were mainly due to increased VLDL-cholesterol (5.0-fold, p<0.0001) and increased VLDL-triglycerides (4.1-fold, p<0.0001) in Trib1-/-Ldlr-/- mice. To explore potential underlying mechanisms, we generated hepatic mRNA expression profiles and observed profound up-regulation of key genes associated with lipoprotein synthesis and assembly in Trib1-/-Ldlr-/- mice. In addition, we identified a significant enrichment of binding motifs for C/EBP transcription factors, which are known to regulate lipid homeostasis, in promoter regions of genes up-regulated in Trib1-/-Ldlr-/- mice. Concomitantly, nuclear abundance of C/EBPα protein was increased in livers of Trib1-/-Ldlr-/- mice, suggesting a role of C/EBPα in mediating Trib1 downstream effects.

In conclusion, we provide experimental evidence for the first time that the novel lipid candidate gene Trib1 modulates atherosclerosis. In Ldlr-/- mice, Trib1–deficiency increases plasma VLDL-C and VLDL-TG levels through a mechanism potentially involving C/EBP transcription factors and severely aggravates atherosclerotic lesion formation. Additional functional studies are necessary to unravel the precise molecular mechanisms by which Trib1 regulates lipoprotein metabolism.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
RISK OF CARDIOVASCULAR DISEASE IN PATIENTS WITH OSTEOARTHRITIS: RESULTS FROM THE MUST-HEART STUDY

Abstract nr. 775
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Risk Factor, Risk stratification

Purpose: Controversies exist regarding whether patients with osteoarthritis (OA) have an increased risk of cardiovascular (CV) disease. Our aim was to evaluate the CV risk and presence of established CV disease in a population-based OA cohort.

Methods: The Musculoskeletal pain in Ullensaker STudy (MUST) is a cross-sectional investigation comprising a thorough clinical examination, recording of CV risk factors in addition to radiographic evaluation of persons with self-reported OA. Of the 604 persons examined, 438 fulfilled the American College of Rheumatology classification criteria for OA. CV risk was calculated by the Systematic Coronary Risk Evaluation (SCORE) algorithm for persons without CV disease, not using lipid lowering and/or antihypertensive medication (OA n=200 and non-OA n=87). An estimated CV risk <5% for experiencing a fatal myocardial infarction coming 10 years is defined as low to medium risk, while ≥5% is the cut off for initiation of CV preventive pharmacotherapy.

Results: The median CV risk for patients with OA [1.40 (IQR 0.65, 2.92)] was significantly higher compared to non-OA [0.99 (IQR 0.52, 1.92)] (p=0.02). The difference in the estimated CV risk was related to higher age (p<0.001), but not to total cholesterol (p=0.07), systolic blood pressure (p=0.13) or to the OA diagnosis. Only 17/200 (8.5%) of the OA patients and 3/87 (3.4%) of the non-OA persons had a CV risk ≥5% (p=0.12). The presence of established CV disease was comparable for those with (16.8%) and without OA (21.1%) (p=0.23). Inflammatory biomarkers were in the normal range for the whole study population, with no difference between OA and non-OA (p=0.30 and 0.10).

Conclusions: Inhabitants with OA in a Norwegian municipality had an overall low risk of CV disease and did not have higher prevalence of established CV disease compared to non-OA.

Subdivision 4. Not applicable. Abstract matches with track c
Presentation Preference: Oral presentation

Additional information
FAMILIAL HYPERCHOLESTEROLEMIA IN KARELIA: SPREADING, CLINICAL AND GENETIC PECULIARITIES, THERAPY (10 YEARS MANAGEMENT EXPERIENCE)

Abstract nr. 777
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Familial Hypercholesterolemia, Genetics, Lipids

Aim: to study the genetic and clinic peculiarities of FH in Karelia. Totally 196 patients with FH from 124 families out of Karelia were examined (69 % was female). Average age was 48 ± 2,3 years old. Genetic tests were performed in 109 patients (55,6 %). Clinical diagnosis of FH was set according to Simon Broom criteria.

Results: For analyze spreading of FH in Karelia we research 28.225 history of patients with therapy pathology in Petrozavodsk city hospital for 10 years period. We looking for patients who conform Simoon Brooom criteria and we exam them for exclude secondary dyslipidemia (84 patients). Occurrence FH in Karelia is about 1:330.
Main clinical features of FH in Karelia: FH patients frequently had additional non lipid risk factors (arterial hypertension in 64,5 %, smoking in 34 %, obesity in 48 %). The presence of FH stigmata was not frequent (corneal arcus in 26 %, tendon xantomas in 17,3 %, xantelasma in 34 %). Atherosclerosis was diagnosed in 117 patients (59,7 %), among them – ischemic heart disease in 27,5 % (more than half of them had myocardial infarction), atherosclerosis of brain vessels – 26,5 % (stroke in 6 %), atherosclerosis of lower extremities in 4,6 %, multifocal atherosclerosis in 21 %. Manifestation of ischemic heart disease became in 45 years, myocardial infarction – in 45,4 years, brain atherosclerosis in 56 years.
Special genetic features: no evident “founder” effect in Karelian FH was demonstrated; specific Finnish mutations were not typical in Karelia, new LDL receptor mutations were found, namely c.192del110/ins8, c.195_196insT, c.2191delG, S206R; R3500Q mutation of the APOB gene was not found in Karelain sample of 109 persons at all. We estimated 10- years period follow-up monitoring for FH people. There are 78% of patients receive statin therapy. The aim lipid level was obtained in 27 % patients with FH, who had received statins. The causes of non-achievement of aim lipid level were: strong native hyperlipidemia, transaminites, myalgia and social problem (46%).

Conclusion: In FH patients from Karelia some clinic and genetic specific features were obtained. The aim lipid level was obtained in 27 % patients with FH.
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
CARDIOVASCULAR PREVENTIVE MEDICATION AND TREATMENT TARGETS IN PATIENTS WITH OSTEOARTHRITIS: RESULTS FROM THE MUST-HEART STUDY

Abstract nr. 779
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Blood pressure, Cardiovascular Disease, Lipids, Therapy

Purpose: Guidelines recommend cardiovascular (CV) preventive pharmacotherapy when the CV risk evaluated by SCORE is ≥5%, and after diagnosed CV disease. Undertreatment and poor goal attainment of blood pressure (BP) and lipid goals in both primary and secondary prevention has been reported in the general population. Our aim was to evaluate if patients with osteoarthritis (OA) received appropriate CV preventive treatment and if treatment targets were achieved in the Musculoskeletal pain in Ullensaker STudy (MUST). Methods: The MUST is a population-based postal survey and a comprehensive clinical examination of persons with self-reported OA (n=630), of which 438 fulfilled the American College of Rheumatology criteria for OA. In the MUST-Heart study, usage of CV preventive medication as lipid lowering agents (LLA), anti-hypertensive medication (a-HT) and anti-thrombotic medication (AT) recorded. Guideline recommended BP goal is ≤140/90 mmHg, and low density lipoprotein cholesterol (LDL-c) goals for primary/secondary prevention are ≤2.5/≤1.8 mmol/L, respectively.

Results: Secondary or primary CV prevention was indicated in 72 and 26 patients, respectively. The female/male ratios 45/27 and 5/21 and the median (IQR) age was 68.5 (65.0, 75.8) and 66.5 (65.0, 73.8) years. Total Cholesterol (TChol) was: 5.17 (1.25) (SD) mmol/L/5.97 (1.19) mmol/L, LDL-c: 2.97 (1.06) mmol/L/3.82 (1.06) mmol/L, BP was 140.5 (18.7)/82.4 (8.3) mmHg/155.7 (14.5) mmHg/87.2/10.1 mmHg, for the secondary/primary prevention groups. Of the 72 patients with diagnosed CV disease, 38 (52.8%) were using LLA, 47 (65.3%) a-HT medication and 25 (34.7%) were on AT medication. Of the 125 patients (without CV disease) who had hypertension, 57 (45.6%) used a-HT medication. Of the 26 patients with a SCORE ≥5%, 2 (7.7%) used LLA. Of the patients using a-HT medication, BP goal attainment was 20/47 (42.6%) and 0/57 (0%) for patients in the secondary and primary prevention groups. Of all patients using LLA, patients with CV disease achieved goals for TChol were 31.6% and LDL-c: 23.7%. Conclusions: There was a substantial underuse and poor goal attainment of with regard to cardio-protective drugs in patients with OA in the MUST-Heart study. The goal achievement of BP and lipids in patients with OA is even lower than reported for the general population.
Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Lipoprotein(a) and risk of myocardial infarction in major ethnic groups

Abstract nr. 780
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords ACS,Dyslipidemia,Lp(a),Risk stratification

Background: Lipoprotein(a) (Lp(a)) levels predict risk of cardiovascular disease, yet are not routinely measured. Lack of assay standardization, differences in average concentrations between populations, reported heterogeneity of association across ethnic groups and uncertainty regarding role of isoform size may be responsible for the modest use of Lp(a) measurements in clinical practice.

Methods: To address these issues we studied a total of 4,052 first myocardial infarctions (MI) matched to 4,450 controls of the same ethnic groups from the INTERHEART study. There were 775 Africans, 1,352 Arabs, 1,856 Europeans, 1,469 Latin Americans, 1,829 South Asians and 1,221 Southeast Asians. We measured Lp(a) concentration in each participant using an assay insensitive to isoform size and assessed isoform size using real-time PCR.

Results: Differences in Lp(a) concentrations and isoform size were observed between populations, with Africans having markedly higher concentrations and Europeans having larger isoforms. High Lp(a) concentrations (>300 mg/L) were associated with an increased risk of myocardial infarction (OR=1.29; 95%CI 1.16-1.42, P=1.3 x 10^{-6}) in the overall study. However, heterogeneity in risk estimates was observed (P=0.04), which was due to a non-significant but directionally inverse association of Lp(a) concentration with MI in Africans (OR=0.82; 95%CI 0.60-1.12). No interaction with other MI risk factors was observed. Categorizing individuals according to quintiles of Lp(a) concentration and isoform size, there was no association between isoform size and MI overall (OR=0.89; 95% CI 0.78-1.02) but significant heterogeneity was noted (P_{heterogeneity} =0.004), even after adjustment for quintiles of Lp(a) concentration (P_{heterogeneity} =4.3 x 10^{-4}). Indeed, isoform size was inversely associated with MI in Latin Americans, positively associated in South Asians and Southeast Asians, but no relationship was present in other populations including Europeans.

Discussion: Our results demonstrate significant differences in Lp(a) concentration and isoform size between ethnic groups. Higher Lp(a) concentrations were associated with increased risk of MI in all ethnic groups, except among Africans. The relationship between isoform size and risk of MI is complex and appears to be specific with respect to ethnic groups, indicating that...
Recommendations for isoform size interpretation may have to be tailored by ethnicity.

**Association of quintiles of Lp(a) concentration and isoform size with MI. Base model includes adjustment for age, sex and clinical risk factors.**

**Subdivision 3. Clinical Studies**

**Presentation Preference** Oral presentation

**Additional information**
Specific Lipoprotein(a) Apheresis for Coronary and Carotid Atherosclerosis Regression in Stable Ischemic Heart Disease Patients

Abstract nr. 783
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Intervention, Lp(a), Pathogenesis

Background: Lipoprotein(a) (Lp(a)) is an independent cardiovascular risk factor and each fifth person in population has its elevated level. The main purpose of this single-center, partially-blind study was to evaluate the effect of 18-month course of specific Lp(a) apheresis on coronary and carotid atherosclerosis dynamics in patients with stable ischemic heart disease (SIHD) and elevated Lp(a) levels.

Methods: A total of 30 SIHD subjects (mean age 53.5 ± 8.3 years, 70% male) with clinically indicated coronary angiography, Lp(a) > 50 mg/dL, and low density lipoprotein cholesterol < 2.5 mmol/L on atorvastatin therapy were allocated into two equal groups: 1) specific weekly Lp(a) apheresis (Lp(a) Lipopak® columns, POCARD Ltd., Russia), 2) the control group. Quantitative coronary angiography with intravascular ultrasound and duplex scanning of carotid arteries were performed at the baseline and after the 18-month treatment period. The end-point measurement indicated the absolute change of the percentage of diameter stenosis of coronary artery, minimal lumen diameter, total coronary atheroma volume (TAV), and carotid intima-media thickness (CIMT).

Results: Immediately after the apheresis procedure, Lp(a) level decreased by an average of 73 ± 12% from 110 ± 22 to 29 ± 16 mg/dL whereas other lipid values did not change significantly throughout the study in both groups. The final change in Lp(a) level in the apheresis group was -31.7 ± 22.3 mg/dL, as compared with 4.8 ± 10.8 mg/dL in the control group (P < 0.0001). Under apheresis treatment we obtained a significant improvement of evaluated instrumental parameters characterized coronary and carotid atherosclerosis: percent of diameter stenosis decreased by 5 ± 12%, minimal lumen diameter increased by 0.20 ± 0.39 mm, TAV diminishing was 7.2 ± 8.8 mm³, and CIMT reduced by 0.07 ± 0.15 mm (p<0.05 for all). There were no significant changes in atherosclerotic surrogate end-points in the control group.

Conclusion: Sustained and substantial decreasing of Lp(a) level with specific Lp(a) apheresis
during 18 months resulted in a significant regression of coronary and carotid atherosclerosis in patients with stable ischemic heart disease and Lp(a) excess. These data suggest that future studies with hard end-points and agents that specifically affect Lp(a) could demonstrate an improvement of prognosis of high-risk subjects.

The study was supported with the research grant 8/3-284n-10 by the Moscow State Government.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Angiopoietin-like Protein 3 Antisense Oligonucleotide Improves Mixed Dyslipidemia, Insulin Sensitivity and Hepatic Steatosis in Murine Models of Metabolic Syndrome

Abstract nr. 784
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes, Dyslipidemia, Triglyceride-Rich Proteins, Triglycerides

Metabolic syndrome (MS) represents a spectrum of disorders that includes mixed dyslipidemia and insulin resistance. MS has been shown to be an important risk factor for the development of cardiovascular disease, type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), and decreased lifespan. Recent evidence indicates that angiopoietin-like protein 3 (ANGPTL3) plays an important role in various dyslipidemias and hepatic steatosis. Homozygous carriers of ANGPTL3 loss of function alleles, referred to as familial hypobetalipoproteinemia 2 (FHBL2), are characterized by extremely low levels of apoB containing lipoproteins without increased hepatic steatosis. To determine if inhibition of ANGPTL3 would improve some of the characteristics of MS, second generation antisense oligonucleotides (ASO) to murine ANGPTL3 were developed and administered (50 mg/kg/wk) to diet induced obesity (DIO) mouse models (n=7-12/group) for a period of six weeks. In DIO mice, administration of an ANGPTL3 ASO significantly reduced hepatic ANGPTL3 mRNA expression, plasma triglyceride (TG), LDL-C, and hepatic TG when compared to mice administered a control ASO. In addition, ANGPTL3 ASO administration significantly reduced fed plasma glucose, insulin, and leptin concentrations, suggesting improved insulin sensitivity. To further define the effects of ANGPTL3 inhibition on dyslipidemia, ANGPTL3 ASOs were administered to human apolipoprotein C-III (APOC3) transgenic mice. APOC3 is yet another gene that has been shown, through both preclinical and loss-of-function mutations, to be important in regulation of plasma TG levels. Administration of ANGPTL3 ASOs to human APOC3 transgenic mice, which exhibit severe hypertriglyceridemia (>1000 mg/dl), significantly reduced plasma apoC-III (by -45%) and plasma TG (by -68%). Conversely, administration of an ANGPTL3 ASO to western diet fed apoC3 -/-, Ldlr -/- mice significantly reduced plasma TG (-78%), indicating that ANGPTL3 modulates plasma TG independently of apoC-III. Additional preclinical studies have demonstrated that inhibition of ANGPTL3 lowers plasma lipids by both reducing hepatic TG secretion and enhancing clearance. These results suggest that inhibition of ANGPTL3 may
represent an important therapeutic intervention for the treatment of dysregulated metabolic states, including mixed dyslipidemia and NAFLD.

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Fasting and postprandial triglyceridemia is associated with an increased arterial stiffness in postmenopausal women

Abstract nr. 785
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Endothelium, Lipoproteins, Triglyceride-Rich Proteins

An increased and prolonged postprandial lipemia is believed to be associated with higher risk of cardiovascular disease. However, there is not so much information how triglyceride (TG) and/or postprandial TG concentration affect an endothelial function. To address such a question, we analyzed the relationship between triglyceridemia and postprandial triglyceridemia induced by a fat load and arterial stiffness and endothelial dysfunction.

Seventeen postmenopausal women (age 59 ± 3 years, BMI 26.3 ± 5.1 kg/m², cholesterol 5.8 ± 0.9 mmol/l) were given meal containing 75 g of fat (cream) + 25 g glucose. The blood for determination of total cholesterol (TC), TG, LDL-C, HDL-C, glucose, insulin and triglyceride-rich lipoproteins (TRL) by ultracentrifugation was drawn before meal and 4 hours later. Endothelial function was assessed using EndoPat™ and pulse wave velocity (PWV) was measured. Triglyceridemia rose from 1.25 ± 0.68 to 2.22 ± 1.12 mmol/l, TRL-TG from 0.77 ± 0.59 to 1.59 ± 1.01 mmol/l, and TRL-C from 0.34 ± 0.30 to 0.44 ± 0.31 mmol/l four hours after test meal. The reactive hyperemia index (RHI) measured by EndoPat™ was 2.32 ± 0.55 and PWV 8.70 ± 1.84 m/s. There was no relationship between total cholesterol, LDL-C, and HDL-C and both PWV and RHI values. On the other hand, statistically significant association was found between fasting and postprandial TG, TRL-TG and TRL-C and PWV, not with RHI.

It can be concluded that both fasting and postprandial TG, TRL-TG and TRL-C are associated with deteriorated arterial stiffness in postmenopausal women.
Supported by grant No. NT 14027-3/2013 from IGA MH CR.
### Table

The correlation coefficients between arterial stiffness (PWV) and endothelial function assessed as reactive hyperemia index (RHI) with various metabolic markers.

<table>
<thead>
<tr>
<th></th>
<th>chol 0 h</th>
<th>LDL-C 0 h</th>
<th>HDL-C 0 h</th>
<th>Total 0 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV</td>
<td>-0.030</td>
<td>-0.140</td>
<td>-0.274</td>
<td>0.620</td>
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<tr>
<td>RHI</td>
<td>-0.075</td>
<td>-0.159</td>
<td>0.065</td>
<td>-0.05</td>
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</table>
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
C-REACTIVE PROTEIN AND CARDIOVASCULAR MORTALITY IN COMMUNITY DWELLING FRAIL ELDERLY

Abstract nr. 786
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Elderly, Inflammation, Risk Factor

INTRODUCTION: Cardiovascular mortality is the leading cause of death in elderly people. Aging is associated with activation of the entire inflammatory cascade. It is possible that biomarkers of inflammation are more strongly related to vascular risk in aged people than measures of plasma lipids, or others established risk factors.

OBJECTIVE: Objective of this study was to examine the importance of high sensitivity C-reactive protein (hsCRP) as a predictor of cardiovascular mortality in frail elderly people.

METHODS: Participants of the study were 253 community dwelling elderly aged 65 to 99 years. It was a prospective study with 32 months follow-up period. Patients were divided into four main groups according to the baseline level of hsCRP: GROUP A- Patients with hsCRP<1,0mg/L; GROUP B- hsCRP 1-3 mg/L; GROUP C- hsCRP 3-10; GROUP D- hsCRP ≥10mg/L. We investigated traditional risk factors and hsCRP (high sensitivity CRP) as inflammatory marker. The associations between mortality risk and different biomarkers were assessed using logistic regression analysis. The results are presented as odds ratios (OR) and 95% confidence intervals (95%CI). Statistically significant differences were p<0.05.

RESULTS: The baseline mean age of participants was 82 years (78.3% women); 53.8% had prior major cardiovascular event (MACE). During the study 109 patients (43.1%) died from cardiovascular cause of death. In our patients there was no statistically significant difference between four hsCRP groups in age, gender, functional ability, smoking habits and presence of hypertension and MACE. Between lipid risk factors only HDL-cholesterol was statistically different between groups (higher in group A comparing with group D 1.58 vs. 1.31; p<0.001). Nevertheless, Group D has five time higher risk for cardiovascular mortality comparing with group A (OR 5.391; 95%CI: 2.372-12.254; p<0.001). In our participants presence of prior MACE (OR 3.173; 95%CI: 1.693-5.946; p<0.001), low level of serum albumin (OR 0.898; 95%CI: 0.835-0.966; p<0.01), and low diastolic blood pressure (OR 0.954; 95%CI: 0.914-0.997; p<0.05) raise risk for cardiovascular mortality.

CONCLUSION: In our study hsCRP was predictive for cardiovascular mortality in frail elderly regardless of traditional cardiovascular risk factors. Traditional cardiovascular risk factors were not predictive for cardiovascular mortality in our study group.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Serum PCSK9 and Incident Cardiovascular Disease in the Swedish 60-year-olds Cohort

Abstract nr. 787
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, LDL, Lipoproteins, PCSK9

**Importance:** Protein proprotein convertase subtilisin/kexin type 9 (PCSK9) is a promising new therapeutic target for lowering of plasma low-density lipoprotein cholesterol (LDL-C) and prevention of cardiovascular events. PCSK9 is present in serum and plasma but its relationship to incident cardiovascular disease (CVD) in the general population is so far unknown. **Objective:** To investigate if serum PCSK9 concentrations are associated with incident CVD and if a potential association is explained by correlation with established CVD risk factors. **Design:** Serum PCSK9 concentrations were analysed as predictor for first-time CVD in a prospective cohort study. **Participants:** A total of 4,232 men and women from Stockholm, aged 60 at recruitment and selected as representative sample for the general population in the Greater Stockholm area, who were followed for incident CVD for 15 years using Swedish national registries. After 15 years of follow-up, a total number of 491 incident events (fatal and non-fatal myocardial infarctions, unstable angina, deaths from coronary heart disease, fatal and non-fatal ischemic strokes) were recorded. **Exposure(s):** Serum PCSK9 concentration, established CVD risk factors. **Main Outcome(s) and Measure(s):** Incident CVD, including fatal and non-fatal myocardial infarctions, unstable angina, death from CHD, fatal and non-fatal ischemic strokes. **Results:** Baseline serum PCSK9 concentrations predicted incident CVD; concentrations in the 4th quartile (>122.3 ng/ml), as compared to quartiles 1-3 (≤73.1 ng/ml to ≤122.3 ng/ml), were associated with a HR of 1.46 (95% CI 1.18-1.79). Adjustment for sex, LDL-C, high-density lipoprotein cholesterol, Lipoprotein (a) and triglycerides resulted in a decrease of the HR (HR 1.38 95% CI 1.12-1.71). The HR was further reduced to 1.31 (95% CI 1.05- 1.63) after additional adjustment for hypertension, diabetes, smoking, obesity and physical inactivity. Conversely, PCSK9 concentrations in the lowest quartile (≤73.1 ng/ml) of the distribution were, as compared to quartiles 2-4 (>73.1 ng/ml to >122.3 ng/ml), significantly associated with a reduced risk of suffering a CVD event: HR 0.79 (95% CI 0.63-0.99) after full adjustment. **Conclusions and Relevance:** Serum PCSK9 concentrations are
associated with future risk of CVD and add prognostic information over and above established CVD risk factors. Further studies are needed to confirm this observation.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Asymptomatic carotid plaques in patients with rheumatoid arthritis are associated with increased high density lipoprotein cholesterol function

Abstract nr. 788
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Atherosclerosis, HDL, Reverse Cholesterol Transport

Purpose
Reverse cholesterol transport (RCT) is a major anti-atherogenic function of high density lipoprotein cholesterol (HDL) and has been shown to be related to disease activity in patients with rheumatoid arthritis (RA). Our aim was to evaluate if atherosclerosis affects HDL-function differently in RA patients compared to controls.

Methods
RA patients without cardiovascular (CV) disease and not using statins or biologic disease modifying anti-rheumatic drugs were included. Healthy community controls were selected by Statistics Norway. RCT was measured as plasma induced 14C-cholesterol efflux from 14C-cholesterol loaded human THP1 macrophages. Apolipoprotein (Apo) A1 and paraoxonase-1 (PON-1) activity were measured.

Results
RA patients, 10 with and 10 without carotid plaques (CP), and 10 controls were age and gender matched. Traditional CV risk factors were comparable in RA patients with and without CP and controls; smoking: p=0.55, systolic blood pressure: p=0.77, total cholesterol: p=0.48, low density lipoprotein cholesterol p=0.31, HDL: p= 0.89, triglycerides: p=0.85. None had diabetes. Untraditional biomarkers of CV disease such as C-reactive protein (CRP) and erythrocyte sedimentation rate were also comparable across the 3 groups; p=0.53, p=0.86 and p=0.45, respectively. RA disease factors such as disease duration, rheumatoid factor, anti-CCP and disease activity score using 28 joints (DAS28) were comparable between RA patients with and without CP (p=0.81, p=0.34, p=0.34 and p=0.94). Efflux capacity was significantly increased in RA patients with CP compared both to controls without CP (p=0.03) and controls with CP (p=0.01)(Table 2). Likewise, both ApoA1 and PON-1 activity were increased in RA patients with CP compared to controls (p=0.02 and p=0.05, respectively). Further, APOA1 and PON-1 activity were comparable between RA patients without CP and controls (p=0.58 and p=0.69, respectively).

Conclusion
The cholesterol efflux capacity was increased in RA patients with early atherosclerosis compared
to controls, independent of HDL level and CRP. Our findings indicate an association between atherosclerosis and upgraded HDL function in patients with RA having low disease activity, possibly as a compensatory mechanism to the atherosclerotic process. This study is hypothesis generating and larger studies are warranted to verify these findings.

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Atorvastatin inhibits human T cell activation induced by oxLDL-treated dendritic cells by suppressing dendritic cell maturation

Abstract nr. 789
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Immunity, LDL, Pathogenesis, Therapy

Background: Atherosclerosis is an inflammatory condition, and a major cause of cardiovascular disease. Dendritic cells (DCs) and activated T cells are abundant in the atherosclerotic plaques, especially in association with plaque rupture. Low-density lipoprotein (LDL) is an important risk factor and modified and/or oxidized forms of LDL (oxLDL) can promote inflammation and cell death and are accumulated in foam cells. Statin (here atorvastatin), HMG-CoA reductase inhibitors are widely used to lower LDL levels, but they also have been reported to have anti-inflammatory, pleiotropic properties. Here we focus on the statin effects on human DCs and T cells.

Methods and Results: Human DCs were differentiated from peripheral blood monocytes and stimulated with oxLDL. Naïve autologous T cells were co-cultured with pre-treated DCs. The effect of atorvastatin was tested on DCs and T cells. Atorvastatin suppressed the maturation and production of TNF-α and IL-1β of oxLDL-treated DCs. T cells produced IFN-γ, IL-6 and IL-17 in response to oxLDL-treated DCs. Atorvastatin-treated DCs inhibited Th1 and Th17 polarization, while induced T regulatory cells with IL-10 production. Mevalonate abrogated the effect of atorvastatin. Experiments on T cells derived from carotid atherosclerotic plaques gave similar results. In addition we studied the effect of atorvastatin on human coronary artery smooth muscle cells (SMCs). Atorvastatin induced apoptosis of oxLDL-treated SMCs. Interestingly DCs pre-exposed to the statin-treated SMCs reduced significantly TNF-α and IL-6, but increased IL-10 production in response to oxLDL.

Conclusions: We demonstrate that atorvastatin suppresses oxLDL-induced dendritic cell maturation and inhibits the subsequent T cell activation. Further, atorvastatin promotes an anti-inflammatory T cell response and induction of T regulatory cells. The specificity of the reactions is supported by addition of mevalonate which restores oxLDL effects. Our finding shows a novel beneficial immunological effect of statins, especially in relation to inflammation and plaque rupture-prone lesions.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
PHD1 deficiency promotes an atheroprotective metabolic phenotype

Abstract nr. 790
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis,Dyslipidemia,Metabolism

Knockout of the oxygen-sensor HIF prolyl hydroxylase 1 (PHD1) was shown to switch metabolism towards glycolysis and reduced cellular oxygen consumption. As we recently showed a pro-atherosclerotic effect of plaque hypoxia, we hypothesised that reduced cellular oxygen consumption in PHD1 knockout mice alleviates atherosclerosis development. Indeed, atherogenesis was attenuated in LDLr-/-PHD1-/- mice fed a western type diet (WTD, 0.25% cholesterol), with reduced aortic root plaque size (-35%, p=0.008, H&E) and necrotic core content (-38%, p=0.004, H&E) compared to LDLr-/- mice (n=17/10). Plaque hypoxia was reduced in LDLr-/-PHD1-/- mice (-32%, p=0.02, pimonidazole), despite unchanged MAC3 macrophage content, suggesting reduced macrophage oxygen consumption. PHD1-/- lowered plasma cholesterol (-21%, p<0.001) and triglyceride levels (-22%, p<0.001), despite similar food intake and weight gain. Flow cytometry of whole blood showed normalized leucocyte count in LDLr-/-PHD1-/- (-39% CD45+ leucocytes, p=0.001), affecting all leucocyte subsets, including Ly6Chigh monocytes (-62%, p=0.03). Haematopoietic PHD1-/- did not affect plasma cholesterol levels or atherogenesis compared to wildtype bone marrow transplantation into LDLr-/-.

Cholesterol lowering was mainly seen in the VLDL-cholesterol fraction. Mechanistically, in LDLr-/-PHD1-/- mice, trans-intestinal cholesterol efflux was markedly elevated (+3.8 fold p<0.01), while biliary cholesterol excretion was slightly reduced (-27% p<0.01), as measured by flux calculations based on oral D5-cholesterol and i.v. D7-cholesterol distribution. Also, hepatic $^{14}$C-labelled VLDL-remnant uptake was elevated in LDLr-/-PHD1-/-, (+14%, p<0.05), pointing towards enhanced cholesterol clearance in LDLr-/-PHD1-/-.
Neutral sterol excretion in the faeces was enhanced on chow diet in LDLr-/-PHD1-/- (38%, p=0.0127). Enhanced cholesterol absorption in LDLr-/-PHD1-/- mice (+42%, p<0.05) obscured this effect on WTD. $^{13}$C-acetate incorporation into cholesterol, representing whole body cholesterol synthesis and hepatic cholesterol synthesis, was unaltered,
as confirmed by equal hepatic HMG-CoA reductase expression and acetyl-CoA content. Hepatic cholesterol and triglyceride content (µg lipid/mg protein) was unaffected by the PHD1−/−, while liver weight decreased (-12%, p=0.004). Hepatic triglyceride re-entry into the circulation was similar in both genotypes (n=6/group, 3 weeks WTD), based on i.p. poloxamer 407 administration (1000mg/kg).

In conclusion, PHD1 deficiency promoted an atheroprotective metabolic phenotype, by improving cholesterol efflux. Additional studies will elucidate the exact mechanism and potentially point towards hitherto overlooked regulators of lipid metabolism.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Streptococcal Serum Opacity Factor Promotes Reverse Cholesterol Transport

Abstract nr. 791
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Co-author(s) - Gotto, Antonio
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Functionality,HDL,Hypolipidemic Drugs,Reverse Cholesterol Transport

Although plasma concentrations of HDL-cholesterol (C) negatively correlate with atherosclerotic cardiovascular disease, this correlation is not axiomatic; some HDL are dysfunctional and atherogenic. Interventions that raise HDL-C have not been uniformly successful suggesting that mechanisms by which HDL-C is increased determine its anti atherogenic potency. Additionally, mice overexpressing SR-BI have lower plasma HDL-C levels and less atherosclerosis. Thus, new strategies that reduce HDL-C while promoting reverse cholesterol transport (RCT) are needed. Serum opacity factor (SOF) disrupts HDL and forms three products, including a cholesteryl ester-rich microemulsion (CERM) containing apo E and the CE of ~400,000 HDL particles. CE uptake in Huh7 hepatocytes is faster when delivered by CERM than by HDL, and cleared both in vitro and in vivo. We investigated the therapeutic potential of SOF in promoting RCT by comparing the final RCT steps, hepatic uptake, CE metabolism to cholesterol and bile salts, and secretion, of $^{14}$C-CE-HDL, -CERM and –LDL. Cells were pulse-labeled for 2 h with 20-50 mg/mL HDL protein (6-17 mg/mL $^{14}$C-CE), or the CE-equivalent as CERM or LDL, and chased for 0, 2 or 6 h. Cells, pulse and chase media were analyzed for sterols by b-counting, TLC and solvent partitioning. $^{14}$C]CE uptake from LDL was greater than from CERM (2-4X) and HDL (5-10X). Half-times for $^{14}$C]CE hydrolysis were 3.0 + 0.2, 4.4 + 0.6 and 5.4 + 0.7 h respectively for HDL, CERM and LDL-CE. Bile acids in cells after uptake from HDL or CERM were low, <0.2% of total sterol but higher in cells after uptake from LDL, 4.2 + 2.2 % of total sterol ($p = 0.03$) at the 2 h chase time. The fraction of sterols secreted as bile acids was comparable for all three particles. After the 2 and 6 h respectively, ~40 and 50% of the secreted sterols were bile acids. These ratios were the same for cells treated with $^{14}$C-CE-HDL, CERM or LDL. Thus, the rates of hepatic metabolism of CERM-CE to free cholesterol, to bile acids and secretion are intermediate between those for HDL- and LDL-CE, supporting the therapeutic potential of SOF as a promoter of RCT.

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation
Additional information
DOSE-DEPENDENT EFFECT OF ATORVASTATIN ON KIDNEY FUNCTION AND ASSOCIATED CARDIOVASCULAR OUTCOMES

Abstract nr. 792
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Intervention, Lipids, Renal function

Aim:
Kidney function usually declines over time and the slope of this change is powerful for predicting both renal and cardiovascular (CV) outcome. We studied whether patients randomized to atorvastatin are protected from decline of kidney function and whether this preservation is dose-dependent. Moreover, we assessed the association between kidney function slopes and cardiovascular outcome.

Methods:
Slopes of reciprocals of serum creatinine were studied in 6 randomized controlled trials (30,527 patients). All trials were long-term CV outcome trials (follow-up: 1-4.9 yrs). Studies were eligible for inclusion if follow-up was ≥12 months, participants were >18 yr, and >2 serum creatinine values were measured. Studies in patients with predefined renal disease were excluded. Based on treatment arms, three poolings were formed: placebo (n=10,001), atorvastatin 10 mg daily (n=12,720), and 80 mg daily (n=7,806). Mixed model included treatment, time of assessment and adjustments for study.

Result:
The slope of reciprocal of serum creatinine level displayed linear improvement over time in all three groups. Placebo, atorvastatin 10 mg, and 80 mg had slopes (mean (SE)) of 0.008 (0.0006), 0.011 (0.0008), and 0.014 (0.0011)(mg/dL)^{-1}/yr, respectively. Head-to-head comparison (10 mg vs. 80 mg atorvastatin) indicated a highly significant dose dependency (p=0.0009; data from TNT (n=10,001)). Cox proportional hazard model showed a highly significant (p<0.0001), negative association with risk of CV events (HR (95% CI) per 1 SD of slope: 0.80 (0.75-0.86); 0.76 (0.71-0.81); and 0.78 (0.73-0.83), respectively).

Conclusion:
In patients at risk or with CVD, atorvastatin improves kidney function over time in a dose-dependent manner. Kidney function improvement is strongly associated with lower CV risk.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
LDL apheresis in children with homozygous familial hypercholesterolemia: a case series

Abstract nr. 793
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords LDL, Lipoproteins

**Objectives** Homozygous familial hypercholesterolemia (HoFH) is a rare genetic disorder, clinically characterized by markedly elevated low-density lipoprotein (LDL-C) levels, extensive xanthomas, and premature and progressive atherosclerotic cardiovascular disease. Extracorporeal removal of LDL-C through LDL-apheresis is an effective way to lower circulating LDL-C levels. Several LDL-apheresis methods are available. The aim of this study was to evaluate the effect of LDL-apheresis, utilizing the DSA (dextran sulphate adsorption) technology, on LDL-C levels, xanthomas and carotid intima-media thickness (c-IMT) in pediatric patients with HoFH.

**Methods** In this case series, we selected three pediatric HoFH patients for whom maximal statin therapy failed to reduce their LDL-C levels sufficiently. In these patients, weekly LDL-apheresis was started using Kaneka liposorber 15 with dextran sulphate cellulose columns to selectively remove LDL-C from the plasma. Levels of LDL-C were measured prior to and after each procedure. C-IMT measurements were performed by one experienced sonographer before the start of apheresis and approximately 1.5 year after performing apheresis. Medical photographs were made before and one year after apheresis.

**Results** Three HoFH patients aged 6 (girl), 10 (boy) and 11 (girl) years were included. Their LDL-C levels before (and after) maximum statin therapy were 20.8 (13.3), 16.9 (9.3), and 15.5 (11.2) mmol/L, respectively. All patients had extensive cutaneous xanthomas (knee, elbow). Mean plasmapheresis dosage was 50 cc plasma/ kg during sessions of 60-85 minutes. The mean acute reductions in LDL-C after a single LDL-apheresis session were 77%, 74% and 69%, respectively. The mean LDL-C levels during a stable period of on average 9 LDL-apheresis sessions were 4.1, 4.7 and 4.0 mmol/dL, respectively. Furthermore, a significant regression of cutaneous xanthomas was observed in all patients (Figure 1). In one patient, c-IMT decreased substantially (0.420 mm to 0.391 mm); c-IMT results for the other two patients will shortly be available. LDL-apheresis was well tolerated; only mild side effects in 1 patient during the first 5 shifts (dizziness, shivering, mild nausea) were reported.

**Conclusions** Our findings suggest that LDL-apheresis (DSA) in children with homozygous FH is a safe and effective method to lower LDL-C levels, decrease c-IMT and to significantly reduce xanthoma size.
**Figure 1a.** Before start apheresis. This boy was hardly able to walk anymore due to extremely filled eruptive xanthomas on and around his knees.

**Figure 2a.** After one year of apheresis. The cholesterol deposits completely disappeared and he can walk normally again.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Reduction of atherosclerosis and macrophage infiltration in apoE/lymphotoxin beta-receptor double-deficient mice

Abstract nr. 794
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation

Lymphotoxin (LT) α1β2 heterotrimers belong to the tumor necrosis factor superfamily and signal through the LTβ receptor (LTβR) which is critically involved in the development and homeostasis of lymphatic tissues. LTβR is constitutively expressed on stromal cells, myeloid cells and on activated macrophages. While for many inflammatory disorders contribution of Lymphotoxin (LT) α1β2/LTβR signalling is well known, the possible involvement in atherosclerosis remains currently unknown.

Therefore, aim of the present study was to study the role of LTβR during atherogenesis and –progression.

Male 8-week-old apolipoprotein E/LTβR-double-deficient mice (apoE−/−/LTβR−/−) and apoE−/− littermate controls were fed a Western-type diet for 4 and 15 weeks, respectively. After 15 weeks, apoE−/−/LTβR−/− mice showed reduced atherosclerotic plaque burden in the aorta as quantified by Oil Red O staining. Focussing on the underlying mechanisms, immune cell influx into the developing lesions was studied. After 4 weeks of feeding decreased invasion of macrophages could be detected by Mac-2 staining of the aortic root in apoE−/−/LTβR−/− mice compared to their apoE−/− littermates. In order to identify the responsible cell type for the reduced atherosclerotic plaque burden in apoE−/−/LTβR−/− mice, bone marrow transfers were performed pointing towards a major role of hematopoietic cells. Analysis of circulating blood cells revealed elevated numbers of lymphocytes as well as strong increases in absolute cell numbers of monocytes in apoE−/−/LTβR−/− mice, in particular based upon elevated numbers of CD115+Ly6clow monocytes. Specific labelling of CD115+Ly6clow monocytes showed that these cells invade atherosclerotic lesions to a lesser extent in apoE−/−/LTβR−/− mice compared to apoE−/− mice. Gene array analysis and in vitro
experiments using isolated monocytes pointed to a key role of the CCL5/CCR5 pathway for LTβR-mediated monocyte migration.

In conclusion, the present results strongly suggest that signalling by the LTβR on monocytes is critically involved in the development of atherosclerosis probably by supporting the CCL5/CCR5-mediated cell invasion into the developing lesion.

This work was supported in part by a research grant from the Forschungskommission der Heinrich-Heine-Universität Düsseldorf and in part by the Deutsche Forschungsgemeinschaft (IRTG 1902, S.H., J.W.F.).

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Adiposity in Children and early sub-clinical CVD risk: ApoB48 has a stronger association with central fat than classic lipid markers.

Abstract nr. 795
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Epidemiology, Obesity, Triglyceride-Rich Proteins

Problem
Atherosclerotic Vascular Disease (ASVD) begins in childhood and obesity is an important risk factor. The obesity epidemic in children from developed countries may exacerbate risk and lead to premature ASVD. Traditional lipid markers (total cholesterol, LDL-C, HDL-C, TG) are often normal in overweight/obese children. There is emerging evidence that elevated fasting apolipoprotein (apo-) B48 concentration (remnant dyslipidemia) is a key independent risk factor for ASVD that may predispose children to subclinical atherosclerosis.

Purpose
To determine if apoB48 vs. traditional lipid markers are elevated with increasing central adiposity in a cohort of Canadian children with a family history of obesity; and to assess if the relationship varies by central adiposity status.

Methods
Data were drawn from the prospective cohort of 630 Caucasian families in Quebec, Canada (QUALITY cohort). Children aged 8-10 years underwent examination at baseline and at 2-year follow-up. Trunk fat mass was determined by dual energy x-ray absorptiometry. Central fat mass index was calculated as CFMI=trunk fat mass/height^2 (kg/m^2). Three groupings were created (CFMI <1.5; 1.5-<3.0; ≥3.0 kg/m^2) to indicate low, moderate or high central adiposity. Changes over time in outcomes (apoB48, total, LDL-C and HDL-C, TG) were compared using paired t-test and multiple regression that adjusted for age, sex, and Tanner stage.

Results
ApoB48 increased with increasing central adiposity and most dramatically (37%) in children who transitioned from low to moderate levels of central adiposity (delta apoB48=1.5). Conversely, worsening of classic lipids was observed only among children who transitioned from moderate to high levels of central adiposity. Interestingly, for every 1 kg/m^2 increase in central adiposity over the 2-year period, an apoB48 increase was 30-fold greater among children with lower baseline...
central adiposity, compared with higher central fat. In contrast, this relationship did not exist for classic lipid markers.

Conclusions
Changes in apoB48 were more consistent with changes in central adiposity over time than classic lipid markers. ApoB48 may be sensitive to changes in adiposity at lower levels of central fat (early periods of risk), whereas traditional lipid markers may detect increased risk at higher levels of central adiposity.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Decreased cardiovascular events in familial hypercholesterolemic patients treated with mipomersen, an antisense inhibitor of apolipoprotein B translation

Abstract nr. 796
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Familial Hypercholesterolemia, Hypolipidemic Drugs, Lipoproteins

Background: Familial hypercholesterolemia (FH) is associated with a 10-20 fold increase in cardiovascular (CV) events. Mipomersen has been shown to significantly lower levels of atherogenic lipoproteins in plasma. Previous safety analysis of all patients in phase 3 trials found no meaningful imbalance in CV events between placebo and mipomersen arms.

Hypothesis: Treatment with mipomersen for at least 1 year will reduce CV events in FH patients taking maximally tolerated lipid-lowering therapy.

Methods: Rates of major adverse CV events (MACE) during 2 years prior to mipomersen treatment were compared to MACE after treatment in 104 FH patients who participated in one of three phase 3 blinded randomized placebo-controlled 6-month trials and an open-label extension study (NCT00607373, NCT00706849, NCT00794664, NCT00694109). One third of patients (n=34) received placebo for the initial 6 months followed by mipomersen for at least 1 year. Two thirds (n=70) received blinded mipomersen for 6 months followed by at least 6 months in open label treatment. MACE were defined as non-fatal MI, stroke, unstable angina, and revascularization procedures (PCI/CABG). MACE occurring before randomization were identified in medical history. On-study MACE, including those for placebo-treated patients, were adjudicated post-hoc by an independent committee.

Findings: MACE were identified in 62% of patients (64 patients with 146 events [39 MI, 99 PCI/CABG, 5 UA, 3 stroke]) during 24 months prior to mipomersen treatment, and 9% of patients (9 patients with 12 events [2 UA+MI, 6 PCI/CABG, 4 UA]) during a mean of 24.4 months after initiation of mipomersen treatment (MACE rate 25.7/1000 patient-months vs 3.6/1000 patient-months, OR = 0.035 [95% CI 0.009 - 0.144], p<0.0001 by exact McNemar’s test). The marked reduction in MACE coincided with the absolute mean reductions in LDL cholesterol levels (-49 to -
113 mg/dL) reported for the phase 3 FH clinical trials.
Summary: MACE rates were significantly lower during mipomersen treatment compared with 24 months prior to mipomersen (p<0.0001).
Conclusions: Results from this limited analysis suggest that treatment with mipomersen may reduce cardiovascular events in patients with FH.
Funding: Genzyme Corporation, a Sanofi Company
Encore abstract: AHA Scientific Sessions 2014, Circulation 2014; 130: A16531

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Loss-of-function PCSK9 p.R46L genetic variant does not alter glucose homeostasis

Abstract nr. 797
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes, Genetics, PCSK9

Objective
PCSK9 (Proprotein Convertase Subtilisin Kexin type 9) is a critical regulator of cholesterol homeostasis. There are conflicting data in the literature regarding the consequences of PCSK9 deficiency on glucose homeostasis in mouse models. Here, we analyzed in humans, the association between PCSK9 p.R46L loss-of-function variant and i) glucose homeostasis parameters in a cross-sectional analysis, ii) diabetes status in a case-control analysis, and iii) the risk of 9-year incident type 2 diabetes (T2DM).

Research Design & Methods
PCSK9 p.R46L was genotyped in two French studies: Data from the Epidemiological Study on the Insulin Resistance Syndrome [D.E.S.I.R.] (n=4,618, including 299 T2DM) and Corbeil study (1,342 T2DM).

Results
In the D.E.S.I.R study, significant associations were found between the p.R46L variant and lower total cholesterol (-0.394 mmol/l), LDL-cholesterol (-0.393 mmol/l) and apolipoprotein B concentrations (-0.09 g/l). However, no significant association was observed between p.R46L and markers of glucose homeostasis, such as fasting plasma glucose, HbA1C or HOMA-IR, HOMA-B, nor with the presence of T2DM in the case-control study (P=0.261). Finally, no significant association between p.R46L variant and risk of incident T2DM was observed in D.E.S.I.R. (Hazard Ratio [95% CI] = 0.34 [0.11; 1.07]; P = 0.065).

Conclusion
The PCSK9 p.R46L LOF variant was not associated with impaired glucose homeostasis in humans. These data are reassuring regarding the safety of PCSK9 inhibitors.

Subdivision 3. Clinical Studies
Presentation Preference Oral presentation
Additional information
Emerging cardiovascular disease biomarkers and incident diabetes risk in statin-treated patients with coronary artery disease

Abstract nr. 798
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Diabetes, Inflammation, Intervention, Risk Factor

Objectives: To determine whether a panel of 19 biomarkers previously associated with the risk of cardiovascular disease also predicts incident diabetes in statin-treated patients with coronary artery disease (CAD).

Research Design and Methods: Substudy in the TNT (Treating to New Targets) study population, a randomized trial that compared the efficacy of high (80mg) vs. low (10mg) dose atorvastatin for the secondary prevention of coronary heart disease events. Fasting plasma levels of standard lipids and of 19 emerging CAD risk biomarkers were obtained after an 8-week run-in period on atorvastatin 10mg in a random sample of 1424 patients. After exclusion of patients with diabetes at baseline (n=253), 101 patients developed diabetes during the median follow-up of 4.9 years. Incident diabetes was defined prospectively as at least 2 post-baseline FBG measurements >126 mg/dl and at least 1 post-baseline FBG >36 mg/dl above baseline. We also included patients for whom incident diabetes was identified through adverse event reporting.

Results: Among standard lipids, after adjusting for age, sex and treatment arm, total cholesterol and triglyceride levels as well as the total to high-density lipoprotein (HDL) cholesterol ratio were positively associated with incident diabetes risk while HDL cholesterol levels were negatively associated with incident diabetes risk. The hazard ratios per 50% decrease in emerging CAD risk biomarkers for incident diabetes (after adjusting for age, sex, treatment arm, smoking, hypertension, body mass index and HDL cholesterol and triglyceride levels) are presented in the Figure.

Conclusions: Results of this study suggest that plasma lipids and some emerging CAD risk biomarkers such as adiponectin and Lp-PLA2 may be useful for predicting incident diabetes in statin-treated patients with stable CAD. Additional studies validating the clinical usefulness of these biomarkers in the prediction of incident diabetes will be required.
Figure. Hazard ratios (95% confidence intervals) per 50% decrease in emerging CAD risk biomarkers for incident diabetes. Hazard ratios are adjusted for

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Smoking and lipid lowering drugs as main determinants of cholesterol trends in Catalonia in the 1990's.

Abstract nr. 799
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Epidemiology,Hypolipidemic Drugs,Lipids,Risk Factor

Total serum cholesterol (TSC) is been regarded as the major determinant of coronary heart disease (CHD) mortality differences across populations at least since the Seven Countries Study. Spain has one the lowest CHD mortality rates and relatively low cholesterol levels. The purpose of this study was to identify determinants of TSC secular trends in the MONICA-Catalonia study population.

METHODS: Three independent MONICA surveys in independent random samples of the general population in men and women aged 25 to 64 years (n=2571, 2934 and 3485) carried out in in 1986-88, 1990-92, and 1994-96. TSC was measured in 12-hour fasting venous samples and analyzed by automatic enzymatic methods. Multivariate linear regression analysis was used to analyze the determinants of TSC temporal trend separately by sex over a ten-year period, using SPSS. Studied independent variables were age, BMI, current cigarette smoking, leisure time physical activity, educational level, diet for high cholesterol and lipid lowering drug use.

RESULTS: A significant decline of 2% in TSC over ten years was observed in both sexes from 5.7 mmol/l in men and 5.5 mmol/l in women. The decline was observed in all age groups except in women aged 35-44. Smoking (β=-0.238, 95%CI -0.383 to -0.093), BMI (β=0.0296, 0.009 to 0.051) and lipid lowering drug use (β=-0.317, -0.592 to -0.042) were the major significant determinants explaining the trends in TSC in men. In women none of the studied factors was able to explain the decline in TSC.

CONCLUSIONS: A real shift in population STC occurred in Catalonia during the MONICA period which was driven mainly by a fall in smoking and an increase in lipid lowering drug use, but only in men. Unknown factors should explain the fall in middle aged women. These findings highlight the importance of both types of prevention, and the need for continued research on determinants and risk factors in women.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Delivery of infused apoAI mimetic ‘CER-001’ into atherosclerotic plaques in patients depends on local plaque characteristics

Abstract nr. 800
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, HDL, Imaging, Reverse Cholesterol Transport

Introduction
Therapeutic targeting of reverse cholesterol transport (RCT) in trials by infusion of apolipoprotein A1 mimetics has provided us with equivocal results. The discrepant response rates may relate, among others, to heterogeneous permeability of atherosclerotic plaques in patients preventing apoA1 from gaining access to intraplaque lipid-rich foam cells. Here, we evaluated local delivery of the apoA1 mimetic CER-001 in carotid plaques in patients.

Methods and results
CER-001 was covalently linked to the radioisotope Zirconium-89. Eight patients with atherosclerotic carotid artery stenosis (>50%) received an infusion of CER-001 (3 mg/kg) and 89 Zr-CER-001 (10 mg-18 Mbq). After infusion, mean plasma apoA1 increased by 9.9 mg/dL (p=0.026), with a concomitant relative increase in plasma cholesterol efflux capacity of 13.8% (p=0.001). Positron emission tomography/computed tomography (PET/CT) demonstrated a significant increase in Target-To-Background Ratio (TBRmax) at 24, 48 and 72 hours compared to directly after infusion (TBRmax t=0: 1.0; t=24h: 1.25 (p<0.001); t=48h: 1.26 (p<0.001); t=72: 1.29 (p=0.023)). Using (Dynamic Contrast-Enhanced) MRI, we will report on plaque permeability (k trans) and wall shear stress to predict CER-001 permeation in advanced plaques.

Conclusions
Infusion of the apoA1 mimetic CER-001 leads to enhanced cholesterol efflux capacity of plasma, whereas infused CER-001 also enters advanced atherosclerotic plaques in patients. We will report on whether this is dependent on local plaque permeability. These data imply that CER-001 is suitable as an RCT enhancer in patients. Moreover, DCE-MRI might serve as a tool to select
patients most suitable for effective CER-001 infusion.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
The effect of weight loss therapy on some CVD risk factors

Abstract nr. 801
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Lipids, Obesity, Visceral Fat

Background: Obesity and body fat distribution plays an important role in determining CVD risk and metabolic aberrations. Adipose tissue secretes cytokines, which play an important role in the pathogenesis of atherosclerosis.

Methods: The study group consisted of 104 obese subjects (30 ≤ BMI < 35), both sexes, (71 woman and 33 man), 30-50 years old, non-smokers, not under any pharmacological therapy. Recruited subjects participated in a 3-month dietary intervention program based on recommendations of The National Food and Nutrition Institute. Dietary compliance regarding energy and nutrient intake before weight loss program were monitored by 3-day food records. Body composition and VAT volume (1-18 scores) was measured by TANITA BIA MC-180MA. Plasma lipids, glucose, and insulin concentrations were determined by routine laboratory methods, while insulin resistance index (IR-HOMA) was calculated using the following formula: insulin(µU/ml)x fasting blood glucose (mg/dl)/ 405. Adiponectin level was measured by ELISA assay.

Results: Total energy of diet (1665,72±717kcal) before weight loss program consisted: 17,8% of energy as proteins, 33,5% as fat and 48,7% as carbohydrates (vs 20% energy as a protein, <30% energy as fat and >50% energy as carbohydrates during weight loss program).The main VAT value before (9,54 ± 3,31) and after (7,63±2,42) therapy was significantly (p<0,05) higher in men (13,48±2,73 vs 10,66±2,46) than in women (7,66±1,3 vs 6,97±1,56). Comparison of plasma lipid levels before and after dietary program showed significant improvement: 22,2% reduction of tryglicerides, about 6% reduction of total and LDL cholesterol. There was a significant correlations between VAT: and HDL cholesterol level, triglicerides, and IR-HOMA [r = -0,39, r = 4,2, r = 4,2 (all p<0,07)] and also between adiponectin and HDL and LDL cholesterol (r= 0,39, r = - 0,3, p<0,05).

Conclusions: Dietary intervention reduce the risk of CVD via VAT and plasma lipids reduction. Adiponectin is associated with changes of HDL and LDL cholesterol.
This study was supported by The Polish National Science Centre (No 2011/01/N/NZ7/04559)
Key words: Obesity, CVD factors, VAT, diet, plasma lipids, adiponectin

Subdivision 3. Clinical Studies
Presentation Preference
Electronic poster presentation
Additional information
The effects of a novel apoA-I transcriptional regulator (RVX-208) on whole plasma and HDL lipidomes

Abstract nr. 803
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Diabetes, HDL, Lipids

High-density lipoprotein (HDL) displays altered lipid composition and becomes dysfunctional under conditions of metabolic disease. This study aimed to determine the effects of a novel, first-in-class BET inhibitor with apolipoprotein A-I (apoA-I) inducing effects, RVX-208 on whole plasma and HDL lipidomes.

Twenty unmedicated males (38-69 years) with prediabetes received 100mg b.i.d RVX-208 and placebo each for 29-33 days separated by a wash-out period of 21-35 days in a randomised, cross-over design. Lipoprotein particle concentration was measured by nuclear magnetic resonance (NMR) analysis (LipoScience). Whole plasma and HDL lipid profiles (24 lipid classes containing 342 individual lipid species) were measured in fasting blood samples from 18 out of the 20 participants collected before and after both treatment periods using electrospray-ionisation tandem mass spectrometry.

RVX-208 treatment elicited no significant changes in apoA-I and HDL-C or other conventional lipid measures. The concentration of medium HDL particles increased ($p=0.01$) and small HDL particles decreased ($p=0.04$) after RVX-208 treatment. There was no effect of RVX-208 on whole plasma lipid classes or individual lipid species. However, RVX-208 treatment increased the concentration of 8 lipid classes in the HDL lipidome, including ceramides (Cer: RVX-208 vs placebo 21±29%; mean % of change±SD), monohexosylceramides (MHC: 18±23%), trihexosylceramides (THC: 21±30%), GM3 gangliosides (GM3: 23±23%), alkylphosphatidylcholines (PC(O): 19±25%), alkenylphosphatidylcholines (PC(P): 18±24%), lysophosphatidylcholines (LPC: 16±22%) and lysophosphatidylethanolamines (LPE: 23±34%) (all $p<0.05$; repeated Measures ANOVA with Benjamini–Hochberg correction for multiple comparisons).
In metabolic syndrome, total PC(P) with potential anti-oxidative properties and THC in the HDL lipidome are lower compared to healthy controls (Khan & Meikle, unpublished data). In our population with prediabetes, RVX-208 treatment increased these surface sphingolipid and phospholipid classes towards concentrations observed in healthy controls. Four weeks of treatment with RVX-208 in individuals with prediabetes induces changes in the HDL lipidome without changing the plasma concentrations of either HDL cholesterol or apoA-I. This study was supported by the National Health & Medical Research Council of Australia (APP1065462), Resverlogix Corp. and in part by the Victorian Government’s Operational Infrastructure Support (OIS) Program.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
HDL-microRNA-92a Intercellular Communication Underlies Endothelial Dysfunction Associated with Atherosclerosis and Chronic Kidney Disease

Abstract nr. 804
Author Vickers, Kasey, Vanderbilt Univ. School of Medicine, Nashville, U.S.A.
Co-author(s) - Wiese, Carrie
Co-author(s) - Toth, Cynthia
Co-author(s) - Landstreet, Stuart
Co-author(s) - Kon, Valentina
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Atherosclerosis, Chronic Kidney Disease, HDL

Chronic kidney disease (CKD) is associated with endothelial dysfunction and atherosclerosis. High-density lipoproteins (HDL) serve as general cargo carriers for a wide-variety of proteins, small molecules, and nucleic acids, namely microRNAs (miRNA), which likely confer many of HDL’s alternative functions. Here, we report that HDL-miR-92a communication to endothelial cells likely promotes atherosclerosis in CKD. miR-92a was found to be significantly increased 8.5-fold on HDL from CKD subjects compared to healthy controls. In addition, HDL-miR-92a levels were found to be increased 20-fold in mouse models of CKD (5/6 nephrectomy). HDL-miR-92 levels are likely originating from inflammatory cells, as macrophages were found to export miR-92a to HDL. Using photoactivatable-ribonucleoside-crosslinked-immunoprecipitation-high-throughput-sequencing, we were able to trace HDL-miR-92a from macrophages to Argonaute2 RNA-induced silencing complexes in recipient human coronary artery endothelial cells (HCAECs). HDL transfer of functional miR-92a to HCAECs is likely through scavenger receptor BI (SR-BI), as HDL-miR-92a delivery was inhibited using SR-BI blocking antibodies in HCAECs. Using whole-genome arrays, we found that HDL alters the expression of many genes in HCAECs, including the significant down-regulation of 18 putative miR-92a mRNA targets. Aortic endothelial miR-92a levels were also found to be significantly decreased (50%) in apolipoprotein E-deficient (Apoe^{-/-}) mice compared to wild-type controls, which may be a function of the absence of HDL in these mice. Nevertheless, we found that miR-92a levels are dramatically increased in aortic endothelium from wild-type mice with 5/6 nephrectomy. HDL-complexed to locked nucleic acid (LNA) inhibitors of miR-92a (20mg/kg LNA-92a + 4mg HDL) were intravenously-injected into Apoe^{-/-} mice with 5/6 nephrectomy (7 weeks), a model of CKD-associated atherosclerosis. Strikingly, we found significant reduction in aortic endothelium miR-92a levels after 7 days. Most interestingly, we found a significant 21% reduction in atherosclerotic lesion area after only 7 days with a single injection, compared to HDL alone. We also found a marked ~30% regression of atherosclerotic lesions after 7 days. Collectively, HDL mediates a macrophage-to-endothelial communication pathway that is increased in CKD and likely contributes to atherosclerosis in these subjects; however, HDL-miRNA based therapies have great potential to reduce the atherosclerotic burden in all subjects.
Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Allelic Effects of APOA1 and ABCA1 Gene Variants on HDL-c Concentration

Abstract nr. 806
Author Mohd Mokhsin, Nurul Atiqah, Faculty of Medicine, UiTM, Sungai Buloh, Malaysia
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Co-author(s) - Sakri, Farah Hanis
Co-author(s) - Rahman, Thuhairah
Co-author(s) - Hoh, Boon Peng
Co-author(s) - Nawawi, Hapizah
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Genetics, HDL

Background: Coronary artery disease (CAD) is the major cause of death in Malaysia. It has been well established that high density lipoprotein cholesterol (HDL-c) concentration is inversely correlated with risk of CAD. Molecular defects that lead to reduction in HDL-c concentration have been identified in several candidate genes including Apolipoprotein A1 (APOA1) and ATP–binding cassette transporter A1 (ABCA1). Thus, the aim of this study is to investigate the effects of APOA1 and ABCA1 variants on HDL-c concentration.

Methodology: Two candidate genes, namely APOA1 and ABCA1 were amplified by polymerase chain reaction (PCR) and sequenced among 70 Malaysian subjects (mean age±SD=46.1±10.8 years) with low HDL-c concentration (HDL-c≤0.6 mmol/L and HDL-c≤0.7 mmol/L in males and females respectively) and 140 age-, gender-, ethnicity-, diabetes- and hypertension-match controls (mean age±SD=43.6±12.2 years, HDL-c≥1.0 mmol/L and HDL-c≥1.3 mmol/L in males and females respectively). Confirmation of the APOA1 and ABCA1 gene variants were analysed using Mega 5.1. Mean comparison of HDL-c concentration between two allele of each gene was analysed by using T-test.

Results: Four APOA1 (rs12718465, rs2070665, rs5072 and rs7116797) and two ABCA1 (rs4149337 and rs2066881) gene variants were significantly associated with low concentration of HDL-c (p <0.05). Of the 4 variants in APOA1 gene, rs12718465 was shown to significantly reduce the most HDL-c concentration by 51% (p<0.05) in the subjects (p = 0.02). For ABCA1 gene, rs4149337 significantly reduced the HDL-c concentration of the subjects by 8% (p=0.05).
Combination of the minor alleles for these two variants further reduce 55% of the HDL-c concentration amongst patients carrying minor alleles A and C in APOA1 and ABCA1 (p = 0.015) gene respectively.

Conclusion: This study confirms that genetic variations of APOA1 and ABCA1 play an important role in HDL-c deficiency amongst Malaysians.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Correlation and Association of Calcium Score with Area of Calcification and Degree of Stenosis in Sudden Death

Abstract nr. 807
Author Chainchel Singh, Mansharan Kaur, Universiti Teknologi Mara (UiTM), Selangor, Malaysia
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Co-author(s) - Mohd Noor, Norizal
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Co-author(s) - Omar, Effat Dr
Co-author(s) - Mahmood, Mohd Shah Dr
Co-author(s) - Abdullah, Nurliza Dr
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Co-author(s) - Nawawi, Hapizah Prof. Dr.

Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Imaging

Aim: Computed Tomography (CT) has been used to detect calcification of coronary arteries, thus indicating presence of atherosclerosis. However, data on Post Mortem Computed Tomography calcium score (PMCT CS) with histopathological correlation of coronary artery atherosclerosis is scarce. Thus, this study aims to determine: (a) correlation between PMCT CS and histological area of calcification and degree of occlusion of the Left Anterior Descending Artery (LAD 1) and c) whether calcified area or degree of occlusion of the same artery is an independent predictor for PMCT CS.

Method: A total of 101 autopsy cases with a mean age ± SD=38.9±11.6 years comprising 87 males and 14 females were recruited. 36 / 101 cases (35.6%) were sudden death cases, 52/101 cases (51.5%) died due to motor vehicle accident and 13 / 101 cases (12.9%) were homicide or suicide cases. The presence or absence of CAD was obtained from autopsy findings and histopathological examination. CT images of the heart were acquired using a multislice CT machine (Toshiba Aquilion 64 TSX-101A, Japan) and total calcium score was calculated using Cardio Scoring software (CSCS -001A-Agatson's, Korea) on the Infinit Monitor (PACS version 3091, Korea). The area of calcification and the percentage of stenosis were calculated by morphometry.

Results: PMCT CS of LAD1 showed a positive correlation with area of calcification on microscopy (r=-0.95, p<<em>0.001). Calcium score was higher in minimal degree compared to moderate, [mean ± SEM Calcium score (1.50 ± 0.82 vs. 124.4 ± 47.9, p=0.003)] and severe degree of occlusion [mean ± SEM Calcium score (1.50 ± 0.82 vs. 134.4 ± 57.4, p=0.006)]. Chi square analysis showed quartiles of PMCT calcium score to be positively associated with the quartiles of both area of calcification and degree of occlusion (p<0.001). However, PMCT CS is not an independent predictor for neither calcified area nor degree of occlusion (p>0.05) after correcting for age, gender, ethnicity, waist circumference and cause of sudden death.
**Conclusion:** PMCT calcium score is a non-invasive, potentially useful tool for predicting the area of calcification and degree of stenosis of LAD1.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
Disruption of Myeloid Adiponectin Receptor 2 Leads to Aberrant Macrophage Inflammatory and Metabolic Response and Increased Atherosclerosis

Abstract nr. 808
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Metabolism, Obesity

**Rationale:** Adiponectin (APN), a beneficial adipokine that exerts anti-inflammatory and anti-atherogenic effects in vascular cells, especially macrophages, is an important therapeutic target to control inflammation and atherosclerosis. APN pleiotropic actions mediated by its receptors AdipoRs (AdipoR1 and AdipoR2) in macrophages are regulated by the activation phenotype. The role of macrophage AdipoR2 in vascular inflammation and atherosclerosis has not been investigated.

**Objective:** To investigate the role of macrophage AdipoR2 expression in the control of inflammatory and metabolic functions and atherosclerosis.

**Methods and Results:** To determine the role of macrophage AdipoR2 expression in atherosclerosis, we used bone marrow transplantation (BMT) to selectively eliminate macrophage AdipoR2 expression in the atherogenic LDLR<sup>−/−</sup> mice. Transplantation of AdipoR2<sup>−/−</sup> bone marrow leading to the loss of macrophage AdipoR2 in LDLR<sup>−/−</sup> mice significantly increased diet-induced atherosclerosis without affecting plasma lipid levels. Analysis of systemic cytokine levels by multiplex ELISA demonstrated a marked increase in inflammatory cytokines (TNF-α, IL-6, IL-12, MCP-1 and RANTES) in AdipoR2<sup>−/−</sup>;LDLR<sup>−/−</sup> mice compared to those in AdipoR2<sup>+/+</sup>;LDLR<sup>−/−</sup> mice. Immunohistochemical analysis of lesion composition revealed increased macrophage content in AdipoR2<sup>−/−</sup>;LDLR<sup>−/−</sup> mice. To identify molecular mechanisms involved in AdipoR2 regulation of macrophage functions, we performed RNA-seq analysis to compare AdipoR2<sup>−/−</sup> and AdipoR2<sup>+/+</sup> macrophages. Our transcriptome analysis on genome-wide scale revealed several novel macrophage genes regulated by AdipoR2. The most enriched pathways affected by macrophage AdipoR2 deficiency were inflammation, signaling, metabolism and atherosclerosis. Interestingly, qRT-PCR analysis revealed that loss of AdipoR2 function in macrophages substantially upregulated inflammatory genes (IL-6, MCP-1, CCR2, IL-12), scavenger receptors...
(SRA-1, CD36, LOX-1), and metabolic genes (SREBP, HMGCoA reductase, FAS). Consistent with scavenger receptor upregulation, peritoneal macrophages from AdipoR2−/− mice exhibited substantially increased Ox-LDL uptake both under basal and LPS-induced conditions. Interestingly, our studies revealed that APN failed to induce cholesterol transporter, ABCA1 in AdipoR2−/− macrophages. Overall, these results suggest that AdipoR2 is a critical endogenous regulator of macrophage inflammatory and metabolic functions and provides atheroprotection. **Conclusion:** Our studies identify AdipoR2 as an endogenous regulator of macrophage inflammatory and metabolic functions demonstrating that macrophage AdipoR2 activity is critical in the control of macrophage homeostatic functions and protection against inflammation and cardio-metabolic disease.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Modified (desialylated) low-density lipoprotein measured in serum by lectin-sorbent assay.

Abstract nr. 809
Author Melnichenko, Alexandra, Institute of General Pathology and Pathophysiology, Moscow, Russian Federation
Co-author(s) - Sobenin, Igor
Co-author(s) - Elizova, Nataliya
Co-author(s) - Orekhova, Varvara
Co-author(s) - Myasoedova, Veronica
Co-author(s) - Orekhov, Alexander
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Lipoproteins

Modified low-density lipoprotein (LDL) with a low sialic acid content was found in the blood of patients with coronary atherosclerosis. This desialylated lipoprotein causes lipid accumulation in arterial smooth-muscle cells and stimulates cell proliferation and production of the extracellular matrix, i.e., induces all atherogenic manifestations at the cellular level. We have developed a lectin-sorbent assay for the determination of desialylated LDL in sera. The assay is based on the binding of desialylated LDL by immobilized Ricinus communis agglutinin with subsequent measurement of lipoprotein through use of anti-apolipoprotein (apo) B antibody. The assay is sensitive to desialylated apo B concentrations as low as 5 micrograms/L. The intraassay and interassay CVs were 4.8% and 11.3%, respectively. Comparison between the lectin-sorbent assay and a lectin chromatographic technique showed a good correlation. This determination of modified desialylated LDL in human serum with high accuracy and reproducibility may help establish the diagnostic value of this lipoprotein as a risk factor of atherosclerosis.

Supported by Russian Ministry of Education and Science (RFMEFI61614X0010).

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
USP2 antagonizes the LXR-IDOL-LDLR axis

Abstract nr. 810
Author Nelson, Jessica, Academic Medical Center / University of Amsterdam, Amsterdam, Netherlands
Co-author(s) - Zelcer, Noam
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, LDL

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1 Department of Medical Biochemistry, Academic Medical Center of the University of Amsterdam, The Netherlands, 2 Department of Cellular and Molecular Physiology, University of Liverpool, United Kingdom

Cardiovascular diseases are the leading cause of morbidity and mortality in Western countries, with elevated levels of circulating LDL cholesterol representing a major risk factor. The low-density lipoprotein (LDL) receptor (LDLR) is a central determinant of circulating level of LDL-cholesterol, and as such subject to tight regulation. We have recently identified the Inducible Degrader Of the LDLR (IDOL) as an E3 ubiquitin ligase (E3) that specifically promotes ubiquitylation and subsequent lysosomal degradation of the LDLR. IDOL itself is subject to direct transcriptional regulation by the sterol-sensing transcription factors Liver X Receptors (LXR). Therefore, the LXR-IDOL-LDLR axis represents a potent ubiquitylation-dependent mechanism to acutely limit lipoprotein-derived cholesterol uptake into cells.

Given the reversible nature of ubiquitylation, we hypothesized that deubiquitination enzymes (DUBs) may counteract IDOL action on the LDLR. Herein, using a genetic screening approach we identify the ubiquitin-specific protease 2 (USP2) as a novel regulator of IDOL-mediated LDLR degradation. We demonstrate that the deubiquitylase USP2 interacts with IDOL and promotes its de-ubiquitylation and stabilization. Paradoxically, this also prevents IDOL from degrading the LDLR and from attenuating LDL uptake. Conversely, loss of USP2 reduces LDLR protein in an IDOL-dependent manner and limits LDL uptake. These findings associate USP2 as a novel regulator of lipoprotein clearance owing to its ability to control ubiquitylation-dependent activity of IDOL, and implicate USP2 as a potential regulator of cholesterol metabolism.

This work is supported by a VIDI grant from The Netherlands Organization of Scientific Research (NWO) and by an ERC Consolidator grant from the EU.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation

Additional information
CLINICAL IMPACT OF CONVENTIONAL THERAPY (CT) IN CHILDREN WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

Abstract nr. 812
Author ARAUJO, MARIA BEATRIZ, CASTELAR, Argentina
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia, Genetics, Therapy

HFHO is a rare, autosomal dominant disease with a prevalence of 1/360,000-1/1,000,000, characterized by very high LDL-C (650 to 1,000mg/dl), xantomas and death in the first decades of life due to severe atherosclerosis. Usually, the primary defect is a mutation in the R-LDL gene. Conventional HFHO therapy (CT) consists of a lipid-lowering diet, pharmacotherapy (PT) since diagnosis (statins at highest-tolerable dose, absorption inhibitors e.g. ezetimibe), and LDL-apheresis or, in defect, plasmapheresis (PA).

Objective: To present the clinical and anthropometric evolution of 4 HFHO patients with CT.
MM: Descriptive, retrospective study. Variables: Initial LDL-C, LDL on PT, mean LDL-C on plasmapheresis (PA), Hb, albumin, vitamins ADE, essential fatty acids, height and BMI Z-score.
Results: 173 PA procedures. All patients received CT that was well tolerated, with normal growth and no evident side effects. table1
Conclusions: LDL-apheresis is not feasible all centers. CT with PA was well tolerated and effective.
<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at Diagnosis (Years)</strong></td>
<td>4</td>
<td>0.3</td>
<td>6.9</td>
<td>7</td>
</tr>
<tr>
<td><strong>Age at PT Initiation (Years)</strong></td>
<td>10</td>
<td>6.5</td>
<td>7</td>
<td>7</td>
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<tr>
<td><strong>Age at PA (Years)</strong></td>
<td>12.5</td>
<td>9.6</td>
<td>8.2</td>
<td>8</td>
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<tr>
<td><strong>Total PA Time (Years)</strong></td>
<td>5.7</td>
<td>4.4</td>
<td>1.3</td>
<td>1</td>
</tr>
<tr>
<td><strong>Procedures</strong></td>
<td>70</td>
<td>53</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td><strong>Frequency PA</strong></td>
<td>1/month</td>
<td>1/month</td>
<td>2/month</td>
<td>2/month</td>
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<tr>
<td><strong>Initial LDL-C (mg/dL)</strong></td>
<td>410</td>
<td>408</td>
<td>482</td>
<td>464</td>
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<tr>
<td><strong>LDL-C on PT (mg/dL)</strong></td>
<td>336</td>
<td>361</td>
<td>315</td>
<td>304</td>
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<tr>
<td><strong>Mean LDL-C on PT+PA (mg/dL)</strong></td>
<td>198.7</td>
<td>213.3</td>
<td>143.5</td>
<td>168</td>
</tr>
<tr>
<td><strong>Hemoglobin (g/dL)</strong></td>
<td>13.1</td>
<td>14.8</td>
<td>11.9</td>
<td>12.5</td>
</tr>
<tr>
<td><strong>Vitamin ADE</strong></td>
<td>Normal</td>
<td>Normal</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Essential Fatty Acids</strong></td>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Initial Height SD</strong></td>
<td>+0.77</td>
<td>+0.51</td>
<td>+1.44</td>
<td>+0.8</td>
</tr>
<tr>
<td><strong>Height SD on PT+PA</strong></td>
<td>-0.08</td>
<td>+0.95</td>
<td>+1.21</td>
<td>+0.85</td>
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</tbody>
</table>
Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Ten years of combined pharmacotherapy in children and adolescents with heterozygous familial hypercholesterolemia

Abstract nr. 813
Author ARAUJO, MARIA BEATRIZ, CASTELAR, Argentina
Co-author(s) - Pacce, maria
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Dyslipidemia,Familial Hypercholesterolemia, Therapy

Atherosclerosis begins in childhood and depends on various risk factors. Familial hypercholesterolemia is the best demonstration of how LDL is one of them. A precocious treatment in this population may improve the final outcome of the disease


Treatment scheme: all patients >10y and LDL>190mg/dl or >160 mg/dl with 1 CVRF or >130mg/dl with 2 or more CVRF; and those patients <10 y and LDL>240mg/dl or familial antecedent of heart attack before 40y, were medicated. 3 to 6moths before PT all patients received indication of lipid-lowering-diet (LLD). Then all patients began with ezetimibe 10mg/d if were >10y or 5mg/d if were <10y. Only the patients who did not achieved therapeutic goal were indicated statins at the lower necessary dosis.

Variables: Age, age of beginning PT, age of beginning statins, time of PT, LDL initial (after LLD was seted), medium LDL during ezetimibe monodrug, medium LDL during combined PT (ezetimibe+statins), delta LDL and percentage of LDL decrease.

Results
N: 80p with intention to treat, 70p went to PT, 51p currently continue to follow, 19p were sent to adults specialists at 18-20y. No growth and development problems were found. 3p had side effects with statins, nobody with ezetimibe monodrug

conclusions
Some pediatrics patients FHHE and high risk of cardiovascular disease could begin PT precociously with good tolerance and without evident side effects. Ezetimibe was effective and safe drug in this pediatric population, used as monodrug and as combined therapy with statins too
<table>
<thead>
<tr>
<th>LDL-C mg/dl</th>
<th>Initial LDL (Then 3 to 6 months of lipids lowering diet) (n:70)</th>
<th>LDL with esetimibe monodroga (n:70)</th>
<th>LDL with esetimibe + statin (n:18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL decrease n(%)</td>
<td>235 (153-439)</td>
<td>166 (97-422)</td>
<td>150 (98 - 257)</td>
</tr>
<tr>
<td></td>
<td>-68 (28%)</td>
<td>-37 (12%)</td>
<td></td>
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<tr>
<td>Characteristics</td>
<td>Media</td>
<td>Range</td>
<td></td>
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<td>------------------------------------------------------</td>
<td>-------</td>
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<td></td>
</tr>
<tr>
<td>N: 70</td>
<td></td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>11,8</td>
<td>4,2 – 20</td>
<td></td>
</tr>
<tr>
<td>Age of beginning</td>
<td>9,3</td>
<td>2 – 17,5</td>
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<tr>
<td>Pharmacotherapy (years)</td>
<td></td>
<td></td>
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<tr>
<td>Patients who began pharmacos 6 – 10 years n(%)</td>
<td>22 (26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients who began pharmacos before 6 years n(%)</td>
<td>17 (25)</td>
<td></td>
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<tr>
<td>LDL-C initial mg/dl</td>
<td>235</td>
<td>153-439</td>
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<tr>
<td>Time of pharmacologic therapy</td>
<td>2,5</td>
<td>0,5 – 7,7</td>
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Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
ABCA1 preferentially mediates cholesterol efflux to small dense HDL

High-density lipoprotein (HDL) is a heterogeneous population of particles and it is possible that differences in the capacities of HDL subfractions to remove cellular cholesterol may explain variable correlations between HDL-cholesterol and cardiovascular risk. The ATP binding cassette transporter A1 (ABCA1) facilitates cholesterol efflux to lipid-free apolipoprotein A-I (apoA-I), but the majority of apoA-I in the circulation is transported in a lipidated state and ABCA1-dependent efflux to individual HDL subfractions has not been systematically studied.

Our aims were to determine which HDL particle subfractions are most efficient in mediating cellular cholesterol efflux from foam cell macrophages, and to identify the cellular cholesterol transporters involved in this process.

We used reconstituted HDL particles of defined size and composition, isolated subfractions of human plasma HDL, cell lines stably expressing ABCA1 or ABCG1 and mouse and human macrophages in which ABCA1 or ABCG1 expression was deleted. We found that ABCA1 is the major mediator of macrophage cholesterol efflux to HDL, demonstrating most marked efficiency with small, dense HDL subfractions (HDL3b and HDL3c). ABCG1 has a lesser role in cholesterol efflux and a negligible role in efflux to HDL3b and HDL3c subfractions.

We conclude that small, dense HDL subfractions are the most efficient mediators of cholesterol efflux and that ABCA1 mediates cholesterol efflux to small dense HDL as well as to lipid-free apoA-I. HDL-directed therapies should target increasing the concentrations or the cholesterol efflux capacity of small dense HDL species in vivo.
Pharmacological inhibition of dynamin II reduces constitutive protein secretion from primary human macrophages

Abstract nr. 816
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Co-author(s) - Xue, Jing
Co-author(s) - Nawara, Diana
Co-author(s) - Katharina, Gaus
Co-author(s) - Jessup, Wendy
Co-author(s) - Robinson, phillip
Co-author(s) - Kritharides, Leonard
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins

Dynamin II belongs to a large family of GTPases which mediate vesicle fission processes and are crucial in endocytic and exocytic membrane events. Different classes of pharmacological inhibitors have been developed to target various diseases. These studies investigate whether these therapeutics regulates protein secretion from primary human macrophages in particular the secretion of anti-atherogenic apolipoprotein E (apoE).

Primary human macrophages were exposed to three different classes of dynamin inhibitors. Cellular and secreted proteins were determined by Western blotting and mRNA levels were determined by quantitative real time-PCR. The role of dynamin was confirmed using siRNA oligo’s and dynamin triple knockout (TKO) fibroblasts. Degradation and secretion rates were determined using $^{35}$S methionine-pulse chase labeling. Processing of apoE was analysed by 2D gel electrophoresis. Intracellular localization and microtubule effects were determined using confocal microscopy. Live cell imaging was performed after transfection with apoE-GFP on a Zeiss LSM confocal microscope.

Inhibitors that target recruitment of dynamin to membranes (MiTMABs) or directly target the GTPase domain (Dyngo™ or Dynole™), dose- and time- dependently reduced the secretion of apoE. siRNA oligo’s confirmed the involvement of dynamin II. Inhibition of secretion was not mediated via effects on mRNA or protein synthesis and 2D-gel electrophoresis indicated that inhibition occurred after apoE was processed and glycosylated in the Golgi. Live cell imaging showed that inhibited secretion was associated with reduced post-Golgi movement of apoE-GFP-containing vesicles. Inhibitory effects on protein secretion were also observed in liver and TKO cells indicating that the effect was not restricted to macrophages. Although dynamins regulate tubulin dynamics, analysis of tubulin stability showed that the inhibition of protein secretion was not associated with effects on microtubules. Inhibition of dynamin also altered the constitutive
secretion of other proteins, decreasing the secretion of fibronectin, matrix metalloproteinase 9, Chitinase-3-like protein 1 and lysozyme but unexpectedly increasing the secretion of the inflammatory mediator cyclophilin A. We conclude that pharmacological inhibitors of dynamin II modulate the constitutive secretion from macrophages as a class effect, and that their capacity to modulate protein secretion may affect the biology of diseases involving macrophage infiltration including atherosclerosis.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Ezetimibe prevents atherogenesis through increased catabolism and excretion of LDL-cholesterol and reduced plaque inflammation in ApoE-/- fed a Paigen diet

Abstract nr. 817
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Hypolipidemic Drugs, Imaging, Inflammation

Background - Intestinal cholesterol absorption inhibitor ezetimibe added to a statin therapy has recently demonstrated clinical benefits in the IMPROVE-IT trial by further reducing LDL-cholesterol levels than statin therapy alone. Here we investigated the mechanisms by which inhibition of intestinal cholesterol absorption could contribute to cardiovascular events reduction in apolipoprotein E-/- (ApoE-/-) mice.

Methods - ApoE-/- mice were fed a Paigen diet (1.25% cholesterol, 0.5% cholic acid and 15% fat) supplemented or not with ezetimibe (7mg/kg/day) for 6 weeks. To evaluate the effects of ezetimibe on LDL-cholesterol metabolism and excretion, a first set of mice (n=14) was injected intravenously with 3H-cholesteryl oleate labeled human LDL. A second set of mice (n=20) was used to evaluate the expression of the inflammatory marker Vascular Cell Adhesion Molecule-1 (VCAM-1) in vivo in the atherosclerotic lesions using the imaging agent 99mTc-cAbVCAM1-5. The same mice were then sacrificed for autoradiography and histology of aortic atherosclerotic plaques.

Results – Compared with control mice, mice treated with ezetimibe showed a significant 41% and 65% reduction in plasma total cholesterol levels and atherosclerotic plaque area, respectively. After injection of 3H-cholesteryl oleate labeled LDL, mice treated with ezetimibe showed a 173% higher LDL-cholesteryl ester catabolism (p<0.001 vs. control). At time 96 hours after radiolabeled LDL injection, 3H-tracer hepatic recovery was reduced by 61% in mice treated with ezetimibe (p<0.001). Meanwhile, LDL-derived 3H-tracer excretion in the feces was increased by 107% in the fecal cholesterol fraction (p<0.001). Similar trends were observed for hepatic cholesterol levels and fecal cholesterol mass excretion, with a 75% reduction and 99% increase with ezetimibe, respectively (both p<0.001).

The anti-atherogenic effect of ezetimibe was successfully monitored by 99mTc-cAbVCAM1-5 SPECT imaging with a 53% reduction in aortic tracer uptake (0.9 ± 1.3 vs. 1.9 ± 0.4 %ID/cm3,
respectively, P<0.01). These in vivo results were further confirmed by ex vivo biodistribution and autoradiographic imaging (both p<0.01).

**Conclusion** – Inhibition of intestinal cholesterol absorption with ezetimibe promotes anti-atherosclerotic effects through increased LDL-cholesterol catabolism and LDL-derived cholesterol fecal excretion, and reduced inflamed atherosclerotic plaques. These mechanisms may contribute to the benefits of adding ezetimibe to a statin therapy.

*Representative 99mTc-cAbVCAM1-5 transversal views selected at the level of the aortic roots (arrow) of Paigen-group (top) and Eze-group (bottom) mice*

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
School-based prevention interventions on childhood overweight and obesity in a developing country

Abstract nr. 818
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Intervention, Lifestyle, Obesity, Prevention

Introduction: The development in middle-income countries has been changed food habits, energy dense foods consumption increases and physical activity reduces. The carbohydrate intake, in populations with limited resources for its low cost, is contributing to increase prevalence of obesity in children. The childhood obesity are influenced by environmental factors like home where they live and the school, so interventions involving the participation of family and school are the best to controlling obesity and overweight.

Objective: To determine the effectiveness of educational interventions on nutrition, health and physical activity at school including children, educators and parents to prevent childhood overweight and obesity.

Methodology: Quasi-experimental non-equivalent group pre-posttest design. We included 64 primary school children attending 4th-5th grade of elementary school (37 in both intervention and control group) and their teachers who received educational interventions (nutrition, health and physical activities in intervention group and nutrition in control group); parents received nutrition educational intervention. The intervention was around 4 months. Primary outcomes were changes in body mass index (BMI), waist and hip circumferences. Study-measured child height and weight were used to calculate BMI z scores. For repeated measures ANCOVA analysis of covariance was used.

Results: Pre-intervention measures by BMI, hip and waist circumference were 19.92 (±3.67), 79.01 (±7.74) and 67.73 (±9.28) respectively (control group) and 21.06 (±4.13), 80.65 (±8.44) and 70.5 (±10.23) respectively (intervention group). A One-way ANCOVA was conducted, there was a significant effect of intervention on the BMI (post intervention) after controlling for the effect of basal BMI (F (2, 71) = 1372, p<0.001). The educational-intervention reduced around 40% the probability to increase BMI in the intervention group (OR=0.59, CI95%: 0.45-0.78, p<0.001); there were no effect on the hip and waist circumference (OR=0.61, CI95%: 0.24-1.55, p=0.31 and OR=0.48, CI95%: 0.13-1.74, p=0.27, respectively)

Conclusion: There is an urgent need on effective obesity prevention interventions for children in developing countries. We found a positive effect of educational-intervention on reduction childhood body mass index; our findings suggest intervention effects are promising. Further studies with
longer term follow up are required.
Table 1. Mean of overweight and obesity pre and post educational intervention according BMI z score

| Subdivision | Group | Pre Intervention | | Post Intervention | |
|-------------|-------|-----------------||-------------------|---|
|              | Female (n=25) | Male (n=25) | Female (n=15) | Male (n=15) | Female (n=10) | Male (n=10) |
| Overweight   | 32.0% | 26.9% | 40.0% | 37.0% | 40.2% | 37.0% |
| Obesity      | 21.2% | 21.2% | 21.2% | 21.2% | 21.2% | 21.2% |

*BMI was calculated using child’s weight and height, then used to find the corresponding BMI-for-age percentile for child’s age and sex.

Table 1. Mean of overweight and obesity pre and post educational intervention according BMI z score

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Electronic poster presentation

Additional information
A systems biology approach identifies fatty acid-binding protein 4 as a potential prognostic and diagnostic biomarker for coronary artery disease

Abstract nr. 819
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Risk stratification, Vulnerable Plaque

Background: Blood-borne biomarkers reflecting atherosclerotic plaque burden have great potential to improve clinical management of atherosclerotic coronary artery disease (CAD) and acute coronary syndromes (ACS).

Methods and results: Using data integration from transcriptomic analysis of coronary thrombi and proteomic analysis of atherosclerotic plaques-derived secretomes we identified fatty acid-binding protein 4 (FABP4) as a biomarker candidate for CAD. The diagnostic and prognostic performance of FABP4 was validated in three different clinical settings: 1) in a cross-sectional cohort of patients with CAD, ACS and healthy individuals (clinical cohort; n=820), 2) in a nested case-control cohort of patients with ACS with 30-day follow-up (prospective clinical cohort; n=200), and 3) in a population-based nested case-control cohort of healthy individuals with 5-year follow-up (prospective population-based cohort; n=414). In the clinical cohort, circulating FABP4 was only marginally higher in patients with ST-elevation myocardial infarction compared with asymptomatic controls. However, elevated FABP4 was associated independently of age, sex, BMI and renal function with incidence of adverse secondary cardio- or cerebrovascular events during 30-day follow-up after primary ACS. It also predicted death or myocardial infarction with similar prognostic performance as the GRACE in-hospital risk score. In the prospective population-based cohort, no significant difference between baseline FABP4 was found in healthy individuals with or without coronary events during follow-up.

Conclusions: FAPB4 has very limited potential as diagnostic biomarker for ACS or predictive risk factor in the asymptomatic population. However, FABP4 may prove useful as a prognostic biomarker in risk stratification of patients with ACS.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Atypical sphingolipids predict cardiovascular events and new-onset of diabetes independently from conventional risk factors in patients undergoing coronary angiography

Abstract nr. 820
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Diabetes, Lipids

Background: Serine palmitoyltransferase (SPT) catalyzes the condensation of serine and palmitoyl-CoA, the first step in the de-novosphingolipids synthesis. Apart from these canonical substrates SPT can also metabolize alanine and other acyl-CoAs. We here investigated in cross-sectional and prospective studies their association with diabetes mellitus and coronary heart disease.

Methods: 349 subjects, who underwent coronary angiography for the evaluation of established or suspected stable CAD, were enrolled in the study at baseline and followed up for cardiovascular events and new onset diabetes over a period of 8 years (median 7.7 years). The levels of the different sphingoid bases (sphinganine (SA) and sphingosines (SO)) in the extracted plasma sphingolipids were determined by LC/MS after acid-base hydrolysis.

Results: 1-deoxysphingolipids (1-deoxSLs) were found to be significantly elevated in plasmas of patients with MetS, impaired fasting glucose and T2DM. Patients who developed T2DM during the follow-up period (n = 32) showed significantly higher 1-deoxySL levels at baseline compared to those who did not develop T2DM until the end of the study (n = 70). 1-deoxySO levels were independent predictors for T2DM even after adjusting for HbA1c (standardized adjusted OR = 2.1, CI 95% [1.19-3.71]; p = 0.010) or MetS ((standardized adjusted OR = 1.97, CI 95% [1.13-3.43]; p = 0.017) and other risk factors such as (age, sex, BMI and lipid lowering drugs). Similar results were observed for the 1-deoxySA levels. Plasma levels of C18SAdiene were found to be significantly lower in CAD patients at baseline, while the levels for C16SA, C16SO, C17SO, C18SA, C18SO, and C19SO and 1-deoxy sphingoid bases were not different. In the prospective analysis C20SO significantly predicted cardiovascular events (standardized adjusted HR = 1.20, CI 95% [1.03-1.41]; p = 0.022) after adjusting for traditional risk factors, the use of lipid-lowering drugs and angiographically-determined CAD at baseline.

Conclusion: Plasma C20SO levels are independent predictive biomarkers for cardiovascular events, even after adjusting for the traditional risk factors including coronary stenosis. Moreover and in agreement with their beta-cell-toxic properties 1-deoxySL are independent predictive
biomarkers for the development of T2DM. These associations of atypical SL with CAD and T2DM need confirmation in independent studies.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
MICROPARTICLES CONTAINING MICRO DNA FROM ARCHAEA AND MMP9 ARE RELATED TO PLAQUE VULNERABILITY

Abstract nr. 821
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Pathogenesis, Vulnerable Plaque

Background – Microparticles (MPs) are associated to several chronic inflammatory diseases, including atherosclerosis. Plaque vulnerability has been related to the vessel inflammation, collagen degradation, and rising in the amount of MMP9 collagenase. Moreover, archaea (Arch) are microorganisms with morphology of microparticle, often producing collagenases.

Objective – The present work hypothesizes that vulnerable plaques (VP) are related to MPs containing microDNA from Arch and MMP9 collagenase.

Methods – MPs were compared in 3 groups of coronary arteries: VP (n=13, obtained from coronary atherectomy), stable plaques (SP) (n=7, obtained of ischemic heart disease receptors) and normal vessels (NV) (n=7, obtained from dilated cardiomyopathy receptors). Immunohistochemistry and in situ hybridization at electron microscopy were used to quantify MMP9 antigens and Arch DNA inside and outside MPS. Double staining with colloidal gold particles of different sizes and the counting of these particles at electron microscopy made possible to co-locate them simultaneously.

Results – MPs in VP and SP were positive for Arch DNA, but not in NV group. MPs and MMP9 were present in larger numbers in VP than in SP. There was a positive correlation between MMP9 and Arch DNA inside and outside MPs at VP (r=0.41, P=0.07 and r=0.49, P=0.05) and at SP only inside MPs (r=0.83, P=0.02), without correlation outside MPs (r=0.08, P=0.86). Sizes in µm² are demonstrated at the table.

Conclusions – Atheromas are rich in MPs containing Arch DNA. In VPs, MPs seem to release MMP9 collagenase, contributing to degeneration of collagen. Also in VPs, arch DNA are released to the extracellular, in correlation with free MMP-9.
Immunohistochemistry and in situ hybridization at electron microscopy analysis to evaluate number of MPs and presence of MMP9 and Arch DNA.

Immuno electron microscopy with double staining: colloidal gold 5nm anti-MMP9 (thin arrow) and 10nm Arch DNA (thick arrow)

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Different hydrogen sulfide donors provide cardioprotection in infarcted mice through distinct mechanisms

Abstract nr. 822
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords ACS, Animal model, Cardiovascular Disease, Pharmacology

We and others have previously shown that Hydrogen sulfide (H$_2$S) protects the heart from ischemia/reperfusion (I/R) injury. Administration of H$_2$S donors improves cardiac function and reduces necrosis in a nitric oxide-dependent manner, while mice lacking cystathionine γ-lyase exhibit elevated oxidative stress and exacerbated myocardial I/R. In the majority of studies evaluating cardioprotection, ultra fast H$_2$S-releasing donors (salts) have been used. Herein, we investigated the ability of slow releasing and mitochondrial-targeted H$_2$S donors to reduce infarct size and compared them to the inorganic salt Na$_2$S.

Anesthetized male mice were subjected to 30min regional myocardial ischemia by LAD ligation, followed by 2hr of reperfusion. The necrotic area was evaluated by thiphenyl tetrazolium staining.

Animals were randomized into 5 groups as follows: 1) control, no further intervention, 2) Na$_2$S (1mg/Kg), 3) thiovaline (4μmol/Kg), 4) GYY4137 (26.6μmol/Kg) and 5) AP39 (250nmol/Kg). All drugs were administered as i.v. bolus at the 20th min of reperfusion. None of the treatments affected blood pressure and all of the groups had similar risk/all areas.

Infarct to risk area (I/R) for the control group was 52.7+9.5%. Na$_2$S and GYY4137, an ultra slow releasing H$_2$S donor, reduced infarct size to a similar extent (17.5+3.9% vs 16.6+2.2 for Na$_2$S and GYY4137, respectively). Similarly, we observed that thiovaline reduced infarct size to 14.0%+3.9% and AP39, the mitochondrial H$_2$S donor, reduced I/R to 21.1%+4.3%. Na$_2$S and GYY4137 enhanced eNOS phosphorylation in the ischemic area, while AP39 failed to do so. The infarct size-reducing effects of Na$_2$S and GYY4137 were reversed by inhibition of cGMP-dependent protein kinase I (PKG-I) inhibition. In contrast, the beneficial effect of AP39 was not reversed by the PKG-I inhibitor DT2, suggesting that AP39 affords cardioprotection in a NO/cGMP–independent manner.

This observation is in agreement with the finding that AP39 fails to increase cGMP levels and VASP phosphorylation.
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Do all statins exert dysglycemic effects? Pitavastatin favours normalisation of Diabetogenic plasma lipidome in metabolic syndrome with Insulin resistance

Abstract nr. 823
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Diabetes, Dyslipidemia, Hypolipidemic Drugs, Triglycerides

Background: Statin treatment favours normalisation of the dyslipidemia of Metabolic Syndrome (MetS), a prediabetic state, but may increase risk of type 2 diabetes. Statins are distinct in their pharmacology; do all statins exert dysglycemic effects? We analysed changes in the plasma lipidome mediated by pitavastatin treatment in MetS subjects which might negatively or positively impact such risk.

Methods and Results: Twelve phenotyped, dyslipidemic, insulin-resistant males with MetS were treated with pitavastatin (4 mg/day) for 180 days in the CAPITAIN study; healthy, normolipidemic, age-matched males (n=12) constituted the control group. Pitavastatin treatment (4 mg/day) reduced non-HDL-C (-39%), triglycerides (-41%) and remnant cholesterol (-55%), and increased HDL-C (+4%). Mass spectrometric analysis of 330 plasma lipid species across glycerolipid, phospholipid, sphingolipid and sterol classes identified 138 lipid species that were increased and two that were decreased (relative to non-HDL-C) in response to treatment; these included species of sphingomyelin and glycosphingolipids as well as alkyl- and alkenylphospholipids (plasmalogens). Hypergeometric analysis was applied to compare statin-mediated changes in the plasma lipidome in MetS to the lipidomic profile characteristic of type 2 diabetes as determined from two large, independent cohort studies. Plasma lipid species prominently associated with type 2 diabetes and present at elevated levels in the diabetic lipidome were enriched among those that were normalized by pitavastatin treatment.

Conclusions: Pitavastatin treatment of MetS patients favoured normalisation of biologically-active lipids in the diabetogenic plasma lipidome, consistent with its neutral effect on glucose homeostasis.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
FAMILY HISTORY OF PREMATURE CARDIOVASCULAR EVENTS AND TOTAL PLAQUE AREA

Abstract nr. 824
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Genetics, Pathogenesis, Risk Factor

Background and Objectives: Although a family history of premature cardiovascular events (FHPCVE) has been considered a putative risk factor for decades, it has not been incorporated in cardiovascular risk evaluation in daily clinical practice along with other established risk factors such as hyperlipidemia, hypertension, and smoking. The objective of this study was to investigate whether FHPCVE is associated with a higher atherosclerotic burden, measured as carotid total plaque area (TPA), in a population with no traditional risk factors and no personal history of CVE.

Methods: Using the Blossom DMO primary prevention database (Argentina) consisting of 4351 patients, 34 individuals with FHPCVE were identified after applying exclusion for traditional risk factors and previous CVE. The definition of the Framingham study (family history of CAD<55 years in men and <65 years in women, first degree relatives) was used to consider a patient with FHPCVE. A control group (n=56) matched for age, sex, blood pressure, and BMI was identified. A secondary analysis was also performed to include only hypertensive patients. An experimental group of 32 participants with FHPCVE and an age, sex, blood pressure, and BMI-matched control group of 44 participants was produced. TPA was measured by Duplex ultrasound.

Results: FHPCVE was associated with higher TPA. Mean TPA was 29.4±6.6mm² for patients with FHPCVE and 15.6±2.9mm² among controls; i.e. in the absence of traditional risk factors TPA was approximately 88% higher in patients with FHPCVE (p<0.05). In our secondary analysis, mean TPA was 69.7±12.1mm² vs. 39.4±5.3mm², (p<0.05) for hypertensive patients with and without FHPCVE, respectively. Thus, in hypertensive patients in the absence of all other traditional risk factors, TPA was approximately 77% higher in patients with FHPCVE, confirming our original analysis. Using a Generalized linear model with TPA as the variable response suggests that in FHPCVE patients TPA progresses faster than in controls. The effect of FHPCVE was absent only when a BMI>30Kg/cm² was present.

Conclusions: FHPCVE was associated with increased subclinical atherosclerosis in the absence of other CVD risk factors. This supports the notion that appropriate ultrasound screening in patients with FHPCVE can detect high risk patients who may benefit from early intervention.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Long Term Survival in the Oslo Diet and Antismoking Trial

Abstract nr. 825
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Guidelines, Nutrition

Introduction: The role of reducing saturated fat in the diet to promote human health is continually debated. In the field of nutrition, however, very few randomized controlled intervention trials (RCT) have had enough statistical power to report on outcome of cardio vascular (CVD) mortality and all-cause mortality. Background: In 1972-3 a 5-year RCT was performed in healthy high-risk middle aged men, comparing the effects on CVD incidence of diet and antismoking advice versus control. A significant reduction (47 %) of incidence of first myocardial infarction was found. We followed the mortality up to 40 years.

Material and methods: At screening 16,203 (63 % of invited) men 40-49 years participated. Included were 1,232 men with total cholesterol 6.9-8.9 mmol/L (80 % smokers) in the trial. The dietary intervention consisted mainly to decrease intake of of saturated fats and sugar, and increase in fish and vegetable products intake, and weight reduction in overweight subjects. Smokers were urged to stop smoking.

Results: Few stopped smoking completely in both groups. The intervention group showed a sustained reduced risk of dying at first myocardial infarction, HR=0.71 (0.51-1.00; P=0.049), compared to controls. During follow up the beneficial effect developed proportionally up to about 15 years. Later, the curves were parallel until end of follow-up. All cause mortality decreased statistically significant during the period from 8 to 20 years after randomization but the curves overlapped the last 20 years. Mean survival time increased with 0.5 years (restricted due to censorship) in intervention versus control and 0.9 years if an exponential distribution of survival times was modeled for the rest of the lifetime in the survivors.

Conclusion: This RCT show that advice on diet and healthy life style during a 5 year period lead to statistically significant reduced mortality and life long benefits.
Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
The single nucleotide polymorphism (SNP) of the HL gene promoter (LIPC) -250G/A (rs2070895) reduces the activity of HL and increases HDL-C levels, but its effect on CVD is controversial. This study was conducted to identify the relationships between this SNP, carotid atherosclerosis and reverse cholesterol transport (RCT) parameters. A total of 285 asymptomatic and normolipidemic volunteers participated in the study. SNP detection was performed in the TaqMan® OpenArray® Real Time PCR Plataform. Carotid intima-media thickness (cIMT) and the presence of atherosclerotic plaques were determined by β-mode ultrasound. Genotype frequencies were equal to 44%, 41% and 15% for GG, GA and AA individuals, respectively. No variations were observed for cIMT, but the proportion of individuals with plaques to without plaques was 3.5 times higher in AA than in GG (p≤0.05). Multivariate linear regression analysis showed positive associations of AA genotype with HDL-C, HDL size and activity of lipoprotein lipase (LPL) and an inverse relationship with HL activity. The logistic regression analysis showed an increased risk of development of plaques in individuals with A allele as compared with G (OR=3.9; 95%CI=1.54-10.33; p≤0.004) despite the increases in HDL-C, HDL size and apo A-I. HL and endogenous lecithin: cholesterol acyltransferase (LCAT) were reduced by 38 and 19% respectively, and LPL increased by 30% (AA x GG). We speculate that the positive association with CVD could be a consequence of the impairment of RCT, explained by the reduction of LCAT and HL; the higher LPL activity, pro-RCT, could be impacted by this reduction. As a result reduction of LCAT activity would impair HDL maturation and delay the cholesterol efflux. An increase in HDL-C, apo A-I and HDL size would happen, representing the HDL$_2$ not transformed into HDL$_3$ by HL. Also a lower action of HL in the hepatic uptake of cholesteryl esters and in the generation of HDL immature particles would occur impairing the initiation of another RCT cycle. With HDL$_2$ accumulation in the circulation enrichment of triglycerides with reduction in cholesterol esters would occur because of the action
of cholesteryl ester transfer protein, which could make HDL dysfunctional and less atheroprotective.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Use of dynamic metabolic modeling to study heterogeneity in development of metabolic syndrome in APOE*3-Leiden.CETP mice on a high-fat diet

Abstract nr. 827
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Dyslipidemia, Metabolism, Triglycerides

Metabolic syndrome is characterized by obesity, insulin resistance and dyslipidemia. Clear risk factors in the development of metabolic syndrome are sedentary lifestyles and a 'western' diet. Though these factors are well established, there is a marked heterogeneity within the human population in the response to these risk factors, with some more prone to develop the characteristics of metabolic syndrome than others. To identify protective factors against the development of metabolic syndrome in humans, we studied development of metabolic syndrome in an animal model with a 'humanized' lipid profile, APOE*3-Leiden.CETP mice. The APOE*3-Leiden transgene reduces clearance of lipoprotein remnants, causing high levels of plasma non-HDL-triglyceride and cholesterol, and the CETP transgene further increases non-HDL. Interestingly, APOE*3-Leiden.CETP mice show large heterogeneity in response to high-fat diet in developing these characteristics, allowing distinction between 'responders' and 'non-responders'. This makes this mouse strain a good proxy to study heterogeneity in development of metabolic syndrome in humans.

Here we make use of a computational approach called Analysis of Dynamic Adaptations in Parameter Trajectories (ADAPT), to delineate the differences between 'responders' and 'non-responders'. ADAPT integrates a longitudinal dataset with a model of ordinary differential equations (ODE model) and predicts how the parameters in the ODE model must change through time to comply to the longitudinal dataset. In this way, ADAPT is able to predict how processes not measured must change through time. Since ADAPT necessarily takes into account all modeled processes at the same time, this may lead to insights that cannot be achieved with reasoning alone.

ADAPT was applied on an ODE model that focused on how the interplay between liver and peripheral tissues leads to dyslipidemia. In addition, the ODE model accounts for energy intake and expenditure. The dataset integrated with the ODE model consisted of plasma lipids (free fatty acids, triglycerides, non-HDL-cholesterol, HDL-cholesterol), liver (triglycerides, cholesterol), fluxes
(hepatic de novo lipogenesis, cholesterol synthesis), energy balance (food intake, indirect calorimetry) and cholesterol balance (cholesterol intake, fecal cholesterol excretion). ADAPT shows that 'non-responders' have a higher energy expenditure than 'responders'. Validation of this finding in a larger study is currently underway.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Autoantibodies against oxidized low-density lipoproteins: relationship with the severity of coronary atherosclerosis.

Abstract nr. 828
Author Shogenova, Maryana, Russian Cardiology Research Center, Moscow, Russian Federation
Co-author(s) - Karpov, Alexandr
Co-author(s) - Zhetisheva, Radima
Co-author(s) - Masenko, Valery
Co-author(s) - Naumov, Vladimir
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Immunity, LDL, Pathogenesis

**Background:** The available data on the role of autoantibodies against OxLDL (anti-OxLDL) in atherogenesis are contradictory.

**Purpose:** To determine anti-OxLDL role in immuno-inflammatory process in patients (pts) with coronary atherosclerosis.

**Methods:** Three groups of male pts aged from 28 to 68 years were included in study: healthy pts group without coronary heart disease (n = 10); a group with angiographically documented severe coronary stenosis (> 50%, n = 50); and group with initial coronary arteries atherosclerotic lesions (<50%, n = 20). Serum anti-OxLDL were identified by immune-enzyme analysis using OLAB IgG Biomedica commercial kits.

**Results:** Anti-OxLDL titers in healthy patients group were significantly higher compared to the patients with severe coronary stenosis [p = 0.029]. SYNTAX Score was used to assess the severity of coronary lesions in group of patients with severe coronary atherosclerosis. The anti-OxLDL level was significantly higher in patients with SYNTAX index below the average median [p = 0.03].

**Conclusions:** Anti-OxLDL titers in healthy patients group were higher than in pts with severe coronary atherosclerosis. Among the pts with severe coronary atherosclerosis, a higher level of anti-OxLDL was observed in patients with a lower coronary lesions severity.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
New data on the intima and media protein composition of the thoracic aorta atherosclerotic lesions using proteomic technologies.

Abstract nr. 829
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Co-author(s) - Shogenova, Maryana
Co-author(s) - Kovalev, Leonid
Co-author(s) - Naumov, Vladimir
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Cardiovascular Disease, Lipids, Pathogenesis

Background. Proteomic studies may broaden our understanding about main proteins involved in the pathogenesis of atherosclerosis.

Purpose. Layerwise thoracic aorta research (intima, media) with human proteomic technologies in normal and atherosclerotic changes. Identification the most pronounced protein changes in atherosclerosis. Adding a complex multi-level database of the medial layer proteins of aorta.

Methods. Proteins examined in autopsy specimens from patients with advanced atherosclerosis (including coronary arteries) died at the age of 45 to 75 years with autolysis period not exceeding 48 hours (n = 14) in pairs normal tissue and with atherosclerotic changes. To study the protein composition we used complex proteomic technologies (two-dimensional electrophoresis by O'Farrell, modifications NEPHGE, IEF, time-of-flight spectrometry and MALDI-TOF tandem mass spectrometry et al.). Image analysis was performed using the MELANIE (6,7).

Results. According to comparative analysis of the thoracic aorta layers we detected the following protein fractions specific to the intima: β and γ fibrinogen, lamin A/C and proteoglycan-prolargin. For the medial layer were identified as specific electrophoretic isoforms of smooth muscle calponin and transgelins, smooth muscle myosin light chain and protein S100-A11, which reflect the presence of significant smooth muscle cells number in this layer.

A comparative study of the protein composition of the thoracic aorta layers in a number norm - lipid stain/band-lipofibrotic plaque was revealed a dynamic increase in the number of several proteins: apolipoprotein A-1, atypical isoforms: β-fibrinogen and cathepsin D, as well as macrophage capping protein-1. In addition, electrophoretic properties changes of glyceraldehyde-3-phosphate dehydrogenase isofrom 2 were detected, which is possible due to its modification and changes in functional properties in the process of glycolysis in the affected area. In all cases studied, the amount of non-regulatory proteins was more represented in the intima tissue, and to a lesser degree in the media, which apparently reflects the lesion depth of the aortic wall.

Conclusions. Previously developed a multi-level computer database of the medial layer proteins of human aorta (ef.mp.inbl.ras.ru) included 29 identified proteins. At present, we have verified additional 47 proteins and, thus, greatly expanded database could be useful for further...
atherosclerosis pathogenesis research.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Oxidized low density lipoproteins in patients with coronary atherosclerosis and healthy volunteers.

Abstract nr. 830
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Co-author(s) - Masenko, Valery
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, LDL, Lipoproteins, Pathogenesis

Background: Oxidized low-density lipoproteins (OxLDL) are considered to be one of the autoimmune-inflammatory triggers in atherogenesis.
Purpose: To determine OxLDL role in immuno-inflammatory process in patients (pts) with coronary atherosclerosis.
Methods: Two groups of male pts aged from 28 to 68 years were included in study: pts with angiographically documented coronary atherosclerosis (n=70) and healthy volunteers group without coronary heart disease (n = 10). Serum OxLDL were identified by immune-enzyme analysis using MDA-oxLDL Biomedica commercial kits. All investigated defined level of lipid profile (total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein, triglycerides) and inflammatory markers (CRP, IL-6).
Results: Although the difference in levels of lipid profile (total cholesterol [p=0.02], LDL [p=0.008]) and inflammatory markers (CRP[p=0.02], IL-6 [p=0.01]) in a healthy pts and a pts with coronary atherosclerosis was statistically significant, there were not significant differences in OxLDL titers between these groups of pts [p=0.3].
Conclusions: We didn't found significant evidence of association between OxLDL titers and coronary atherosclerosis in our clinical study.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Intimal and medial layer's autoantigens of the thoracic aorta in atherosclerotic lesions.

Abstract nr. 831
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Co-author(s) - Kovalev , Leonid
Co-author(s) - Naumov , Vladimir
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Pathogenesis

Background. The role of autoimmune reactions in the pathogenesis of atherosclerosis is not detailed enough currently.

Purpose. To detect and identify autoantigens among intimal and medial layer proteins of thoracic aorta using proteomic technologies.

Methods. The material of the study were serum samples obtained from patients with advanced atherosclerosis, including coronary arteries (n = 10). The first phase was fractionation of thoracic aorta proteins by O.Farrell two-dimensional electrophoresis in polyacrylamide gel density gradient. The second stage, to identify autoantigens in proteins of intima and medial layers of aorta, immunoblotting was performed in a sandwich embodiment, using as the first antibodies the patient's serum with advanced main arteries atherosclerosis (including coronary arteries). The second antibodies were goat's peroxidase conjugate to human IgG. Then with time-of-flight spectrometry and tandem mass spectrometry identification of proteins, which gave immune response to serum was carried out, separately for intimal and medial layer of aorta from 2DE gels used for electrotransfer to nitrocellulose replica.

Results. According to the results serum's immunoblotting, 3 of 10 patients were given a significant immune response, all of which were obtained from patients with severe widespread atherosclerosis. Response in the intima was identified in three cases to lamin A / C, prolargin, laktadherin and α-enolase (one marked further reaction with apolipoprotein A1). In the medial layer in one of these cases also highlighted the reaction to one of the transgelin transcriptional isoforms, which indicates a greater severity of the autoimmune process.

Conclusions. Proteomic analysis of aortic tissue in all cases showed a marked accumulation of heavy and light chain immunoglobulins, what may indicate involvement autoimmune processes in the pathogenesis of atherosclerosis.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Antiinflammatory Effects of MCS-18 on Dendritic Cells and Endothelial Cells - Impact on Advanced Atherosclerosis in ApoE-deficient Mice

Abstract nr. 832
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Immunity, Therapy

Atherosclerosis progression is characterized by leukocyte recruitment and cell adhesion to the endothelium. Mediating both processes, dendritic cells (DC) play a crucial role in plaque formation. The herbal substance MCS-18 is able to inhibit essential DC-functions and has shown protective effects in murine atherosclerosis onset. The aim of our project was, to investigate the impact of MCS-18 on murine plaque progression in advanced atherosclerosis and on proatherogenic processes in vitro.

ApoE-deficient mice were fed Western-type diet for twelve weeks, followed by normal chow and i.p. injections of MCS-18 (500µg, n=12) or saline (n=12) twice a week for the following twelve weeks. Lipid content of the aortic root was quantified by magnetic resonance imaging (MRI). By histology, plaque size was determined in the aortic root and thoracoabdominal aorta. In addition, human DCs were maturated together with MCS-18 (50-200µg/ml) for three days, whereupon DC migration and adhesion to an endothelial monolayer under different patterns of shear stress was analyzed. Additionally, WST-8 assay served to investigate potential effects of MCS-18 on the proliferation rate of THP-1 cells.

In mice, plaque size was significantly lower following administration of MCS-18, which was associated with reduced lipid content (839125±266370 µm² vs 664219±206956 µm², p=0.012) and a lower number of apoptotic cells (196±41 cells vs 88±38 cells, p=0.02) in the atherosclerotic lesions. Concentration of the proatherogenic cytokines IL-6, IL-18 and IL-22 was decreased in serum of MCS-18-treated animals. In vitro analyses showed reduced DC migration (13.88±2.40 vs 6.54±0.065 RMI, p<0.05) and lower adhesion (24.44±5.29 cells vs 10.79±2.82 cells, p<0.05) of MCS-treated compared to saline-treated DCs to the endothelial monolayer in regions of non-uniform shear stress. C-type lectin CD209 was significantly decreased in DCs, while HUVECs showed lower levels of ICAM-1 and NFKB-p65. In THP-1 cells, incubation with MCS-18 caused a
significant down-regulation of cell proliferation (100±0.85 vs 67.98±0.54 %; p<0.05).
MCS-18 exhibits protective effects when applied in advanced murine atherosclerosis. The
observed resultssuggest its antiatherogenic impact to be associated with a suppression of DC
adhesion to endothelial cells, possibly caused by the down regulation of the c-type lectin CD209
and ICAM-1 signaling.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Influence of adherence to lipid-lowering therapy after CABG on the progression of coronary atherosclerosis according to repeated angiography

Abstract nr. 833
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Cardiovascular Disease, Dyslipidemia, Pharmacology

Aim. Evaluate the influence of adherence to atorvastatin therapy on progression of coronary atherosclerosis at patients with coronary heart disease (CHD) and diabetes mellitus (DM) type 2 after coronary artery bypass grafting (CABG) during one year follow-up.

Materials and method. The examination, including coronary angiography (CA), was performed before intervention and after 12 months. All patients received complex therapy, which included atorvastatin 40 mg. Retrospectively the patients were divided into three groups according to adherence to the treatment. The first group included patients who took treatment continuously, the second with an average compliance, the third group – who stopped receiving therapy.

Results. Fifteen percent of patients didn’t not follow recommendations for lipid-lowering therapy, 54 % patients adhered to some degree, 31 % were highly compliant. Adherence to treatment with atorvastatin at a dose of 20–40 mg was associated with optimization of lipid profile and lowering of coronary atherosclerosis progression according to the CA data. Progression of coronary atherosclerosis was registered in 100 % of non-compliant patients, against 76 % who were moderately compliant and 42 % – with high compliance. Revealed changes prove the necessity of adherence to treatment to reduce the risk of cardiovascular complications in patients with CHD and DM type 2 after CABG.

Conclusion. Our results demonstrate the need strict compliance with treatment to reduce risk of cardiovascular complications at patients with coronary heart disease and DM type 2 after coronary artery bypass grafting

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation

Additional information
High-mannose and complex/hybrid N-glycans are associated with hypercholesterolemic rabbits and human

Abstract nr. 835
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Cardiovascular Disease, Dyslipidemia, Lipoproteins

Glycans are the most abundant and structurally diverse class of molecules in nature, playing an important physiological and pathological role in organisms. Clinically, glycans have been considered to be as a key diagnosis marker for many metabolic diseases. However, the expression profiling of N-glycans in the plasma of hypercholesterolemia animals or patients are unclear. In the current study, Japanese white rabbits were fed with high cholesterol diet (HCD) for 14 weeks. Plasma cholesterol and low-density lipoprotein cholesterol (LDL-C) were significantly increased in HCD fed rabbits. Atherosclerotic lesions were also seriously developed in rabbits fed with HCD. The expression profile of N-glycans in rabbit’s plasma before or after HCD treatment was analyzed by electrospray ionization mass spectrometry (ESI-MS). We found that the expression of high-mannose, mainly including 1257, 1419, 1581, 1743 and 1905 dramatically increased in the plasma of rabbits fed with HCD. The level of complex, mainly undecorated and sialylated N-glycans showed the increased trend in cholesterol fed rabbits. The expression profile of N-glycans in plasma of healthy persons and hypercholesterolemia patients was detected by ESI-MS. Consistently, the expression of high-mannose, including 933, 1095, 1257, 1273, 1280, 1435, 1581, 1760, and 1921 remarkably increased in the plasma of hypercholesterolemia patients compared with that of healthy persons. Compared with healthy persons, the level of complex/hybrid N-glycans, mainly undecorated and sialylated were also remarkably increased in the plasma of hypercholesterolemia patients. Interestingly, we found that fucosylated (996 and 1444), or fucosylated and sialylated modified N-glycans (1365 and 1511) in plasma of hypercholesterolemia patients showed much higher expression than healthy controls. These data demonstrate that N-glycans play a pivotal role in hypercholesterolemia.

Subdivision 2. Translational Research

Presentation Preference Electronic poster presentation
Additional information
Dietary cocoa powder improves hyperlipidemia and reduces atherosclerosis in Apo-E deficient mice through inhibition of hepatic endoplasmic reticulum stress

Abstract nr. 836
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Animal model, Atherosclerosis, Lifestyle, Prevention

Cocoa powder is rich in flavonoids which have many beneficial effects on human health, such as anti-oxidative and anti-inflammatory effects. To investigate whether intake of cocoa powder has any influence on hyperlipidemia and atherosclerosis and examine the underlying molecular mechanisms, we fed apoE knock-out mice with a Western diet supplemented with either 0.2% (low group) or 2% (high group) cocoa powder for 12 weeks. Dietary cocoa powder led to significant reduction of both plasma cholesterol levels and aortic atherosclerosis compared to the control group. Analysis of mRNA profiling of aortic atherosclerotic lesions revealed that several genes related to apoptosis, lipid metabolism, and inflammation were significantly reduced while anti-apoptotic gene, BCL2 was significantly increased in the cocoa powder group compared to the control. RT-PCR analysis along with Western blotting showed that cocoa powder feeding inhibited hepatic endoplasmic reticulum stress expression. Our data suggest that cocoa powder intake improves hyperlipidemia and atherosclerosis and such beneficial effects are possibly mediated through the suppression of hepatic endoplasmic reticulum stress.
PROTEASOMAL DEGRADATION MEDIATES THE REDUCTION OF MACROPHAGE ABCA1 PROTEIN LEVEL ELICITED BY ADVANCED GLYCATION

Abstract nr. 837
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Diabetes,Lipids,Reverse Cholesterol Transport

Advanced glycation endproducts (AGE) disturb macrophage reverse cholesterol transport promoting intracellular accumulation of sterols. This is due to the reduction of ABCA1 that occurs independently of changes in its mRNA. ABCA1 protein content is mainly dictated by its degradation by surface calpains and proteasomal system. We investigated how ABCA1 protein degradation is affected in macrophages treated with AGE-albumin. J774-macrophages were treated with C or AGE-albumin for 18h with LXR-agonist (T0901317) and in the presence or absence of the proteasomal inhibitor (MG132) or calpain inhibitor (calpeptin). To determine the ABCA1 protein decay rate (DR), cells were incubated for 12h with T0901317. After washing, cells were added simultaneously with C or AGE-albumin, cycloheximide and MG-132. Proteins were analyzed by immunoblot. The ABCA1 DR was calculated by the slope of the linear regression. Results were compared by ANOVA or Student t test. The ABCA1 DR was faster in macrophages incubated with AGE-albumin (slope = -0.064) in comparison to those treated with C-albumin (slope = -0.032;p=0.035). Calpeptin enhanced ABCA1 in cells treated with C-albumin (68%) which was not observed in cells exposed to AGE-albumin. In comparison to C-albumin, AGE-albumin reduced 32% ABCA1 protein level in plasma membrane, which was not rescued by the inhibition of calpains by calpeptin. MG132 increased the amount of ABCA1 in macrophages treated with C-albumin (72%) as well as with AGE-albumin (129%). In addition, in the presence of MG132 the ABCA1 DR rate was similar between macrophages treated with C and AGE-albumin. The reduction of ABCA1 protein level elicited by advanced glycated albumin is mediated by proteasomal degradation independently of the action of surface calpains.

Funding: FAPESP(#2012/12088-7, Brazil) and MEXT (S1201007, 24614018, Japan)

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
GLUCAGON-LIKE PEPTIDE-1 INFLUENCES PLATELET RESPONSE: EFFECTS ON THE AGONIST-INDUCED ACTIVATION OF PI3K AND MAPK PATHWAYS AND OXIDATIVE STRESS

Abstract nr. 838
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Cardiovascular Disease, Diabetes, Therapy, Thrombosis

Background and aims: Glucagon-like peptide-1 (GLP-1), an incretin hormone secreted by intestine after meal, exerts important metabolic effects justifying the GLP-1-based therapy in diabetes and acts also on cardiovascular system with anti-atherogenic effects: its influence on platelets, cells deeply involved in the pathogenesis of vascular complications, however, is unknown.

Aim of this study is to investigate the GLP-1 influence on the arachidonic acid-induced activation of the signalling pathways PI3-Kinase (PI3K) and MAP-Kinase (MAPK) and of oxidative stress in platelets.

Materials and Methods: In washed platelets from 24 healthy subjects (M/F 13/11; age 25.6±5.9 years, BMI 22.5±2.4 kg/m²) we measured the influence of a 15 min pre-incubation with the native form GLP-1(7-36)(100nmol/l) on the effects of Na-arachidonate (NaA) (0.5 mmol/l) on: i) phosphorylation of Akt and Erk-1/2, molecules of the PI3K and MAPK pathways, respectively (WB); ii) ROS production (DCF-DA assay). Experiments were repeated in the presence the Erk-1/2 inhibitor U0126 (40 micromol/l) and the GLP-1 receptor (GLP-1R) antagonist exendin (9-39) (100 nmol/l).

Results: GLP-1 reduced platelet signalling induced by NaA. In particular: i) the fold increase on basal values with NaA alone and Na+GLP-1 (7-36) was 11.2±2.1, 3.1±0.8 respectively (p<0.0001 vs NaA alone) for pAKT, and 14.6±2.5, 3.9±1.0 respectively (p<0.0001 vs NaA alone) for pERK-2; ii) the fold increase on basal values of ROS with NaA alone and NaA+GLP-1 (7-36) was 8.2±1.1, 5.6±2.0, respectively (p=0.001 vs NaA alone); iii) the Erk-1/2 inhibitor U0126 reduced the NaA-induced activation of ROS (p<0.0005 vs NaA alone). In the presence of GLP-1R antagonist exendin (9-39) the effects of GLP-1 were not modified.

Conclusions: In human platelets, GLP-1, independently of GLP-1R, reduces the NaA-induced activation of PI3K and MAPK pathways and of oxidative stress. Because MAPK activation is involved in the NaA-induced increase of oxidative stress, the inhibiting effects of GLP-1 on MAPK activation can account for its ability to attenuate the NaA-induced increase of oxidative stress. Besides its metabolic effects, these results suggest a beneficial role of GLP-1 on platelet response.
modulation.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
A simple risk score to detect rural Asian Indian (Bangladeshi) adults at high risk for type 2 diabetes

Abstract nr. 840
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Diabetes, Risk stratification

Aims/Introduction: To develop and evaluate a simple non-invasive diabetes risk score for detecting individuals at high risk for type 2 diabetes in rural Bangladesh.

Materials and Methods: Data from 2,293 randomly selected individuals aged ≥20 years from a cross-sectional study in a rural community of Bangladesh (2009 Chandra Rural Study) was used for model development. The validity of the model was assessed in another rural cross-sectional study (2009 Thakurgaon Rural Study). The logistic regression model used included age, sex, body mass index, waist hip ratio and hypertension status to predict individuals who were at high risk for type 2 diabetes.

Results: On applying the developed model to both cohorts, the area under the receiver-operating characteristic (ROC) curve was 0.70 (95% confidence interval (CI) 0.68–0.72) for the Chandra cohort and 0.71 (95% CI 0.68–0.74) for the Thakurgaon cohort. The Risk Score of >9 was shown to have the optimal cut-point to detect diabetes. This score had a sensitivity of 62.4 and 75.7% and specificity of 67.4 and 61.6% in the two cohorts, respectively. This risk score had shown to have improved sensitivity and specificity to detect type 2 diabetes cases compared to the Thai, Indian, Omani, UK, Dutch, Portuguese and Pakistani diabetes risk scores.

Conclusions: This simple non-invasive risk score can be used to detect individuals at high risk for type 2 diabetes in rural Bangladesh. Subjects with a score of 9 or above (out of 15) should undergo oral glucose tolerance test (OGTT) for definitive diagnosis of diabetes.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
The role of chemokine CCL17 and T-cell receptor CCR4 in atherogenesis

Abstract nr. 841
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Pathogenesis

Background: Peripheral blood mononuclear cells, such as macrophages and dendritic cells express CCL17 chemokine. CCL17 interacts with CCR4 receptor on the surface of T-helper cells (CD3+CD4+) and induces their chemotaxis and activation. In recent years, some studies demonstrated involvement of dendritic cells, expressing chemokine CCL17 in the development of atherosclerosis.

Objective: To study the association between blood chemokine CCL17 and T-helper cells, expressing CCR4 receptor, with the severity of coronary atherosclerosis.

Methods: The study included 53 males aged 24 to 80 years, divided in 2 groups. The main group included patients with CHD with revealed hemodynamically significant coronary artery stenosis (n = 34) on the results of coronary angiography. The control group was composed of patients without hemodynamically significant coronary artery stenosis (n = 19). All patients underwent determination of total CD3+CD4+ cells number and T-helper cells, expressing CCR4 receptor (CD4+CCR4+) on their surface by color flow cytofluorometry; chemokine CCL17 by the quantitative sandwich enzyme immunoassay. Severity of coronary lesions was evaluated using Gensini score.

Results: The study showed that patients with stenotic coronary atherosclerosis, compared with control group had significantly higher blood levels of CD3+CD4+ (0.866x10^9/l and 0.595x10^9/l, respectively, p = 0.003) and CD4+CCR4+ (0.276x10^9/l and 0.17x10^9/L respectively, p = 0.0003). CCL17 chemokine titre in the control group was significantly higher than in the study group (441.27 pg/ml, and 231.04 pg/ml, respectively, p = 0.01). The average Gensini score in study group was 0.89 (0-5.5), and 58.65 (4-171) (p = 0.0001 in the control group.

Conclusions: We found a direct correlation between coronary atherosclerosis severity and CD3+CD4+ blood levels, as well as with CCR4 expression intensity. Patients with coronary atherosclerosis had lower CCL17 levels, which may be due to the binding of a chemokine to more receptors.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Functional characterization of the first homozygous FH patient due two novel PCSK9 alterations

Abstract nr. 842
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia, Functionality, Genetics, PCSK9

Purpose: Familial hypercholesterolemia (FH) is a genetic condition characterized by a high cholesterol concentration in plasma since birth. The most frequent causes of FH (>90%) are inherited defects in the Low Density Lipoprotein Receptor gene (LDLR) but, in less than 10%, mutations in the apolipoprotein B gene (APOB) and in the propotein convertase subtilisin/kexin type 9 gene (PCSK9) are also responsible for FH.

In this report we present the functional characterization of two novel PCSK9 gene alterations found in a compound heterozygous patient of a large Portuguese kindred.

Methods: The Portuguese FH study performs the genetic diagnosis of FH in different phases: 1 analysis of LDLR and APOB (most frequent mutations) by PCR and Sanger sequencing, phase 2 is MLPA analysis for LDLR; phase 3 the study of PCSK9 by PCR and Sanger sequencing in patients where a mutation was not found in previous phases. Functional characterization of PCSK9 variants was analyzed by quantification of the relative effect on the LDLR expression as well as LDL uptake in transiently transfected HepG2 cells by Flow Cytometry. Lymphocytes from the homozygous proband were used to confirm the normal function of the LDLR and the response to PCSK9 in the presence or absence of statins.

Results: A total of 348 individuals were negative in phase 1 and 2 at the time of this study. In phase 3 apart from the already described PCSK9 Portuguese mutation (D374H), two novel alterations were identified in a child (A62N and P465A). Functional characterization showed that both missense alterations were pathogenic and therefore the child has homozygous FH. The child, 11 years old, presented a more severe phenotype than her heterozygous parents, with an LDL of 234 mg/dl.

Conclusions: This is the first PCSK9 homozygous patient functionally characterized so far. Functional characterization of the alterations found in FH patients is essential to avoid misdiagnosis of FH and to determine the severity of the disease. The proband is a child and homozygous for FH so, she needs to receive appropriate counselling and treatment, as well as
dietary and lifestyle advice, in order to reduce her elevated cardiovascular risk.

Subdivision 2. Translational Research

Presentation Preference Oral presentation
Additional information
Complete regression of xanthomas and decreased of intima-media thickness with LDL apheresis in a severe homozygous familial hypercholesterolemia patient

Abstract nr. 843
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Familial Hypercholesterolemia

Homozygous familial hypercholesterolemia (FH) is a very uncommon, life-threatening condition, with a prevalence of 1 in 1,000,000, characterized by very high levels of low-density lipoprotein cholesterol (LDL-C) and premature cardiovascular disease. Without treatment, overt atherosclerosis develops before the age of 20 years.

Material and Methods
We describe a patient with homozygous familial hypercholesterolemia (FH) with an extremely elevated LDL-C and very prominent xanthomas, which regressed completely with oral treatment and LDL apheresis.

Results
A 13 years-old male with homozygous FH is presented. Diagnosis was based on generalized xanthomas that appeared at 10 months of age (see figure 1, aged 5) and the lipid profile [total cholesterol: 1081 mg/dL; LDL-C: 980.6 mg/dL; HDL-C: 67 mg/dL; triglycerides: 167 mg/dL; lipoprotein (a) (Lp(a)): 177 mg/dL]. Genotyping showed two mutant alleles in the LDLR gene [[1197_2205del/ 1197_2205del]. Pravastatin 10mg/d was initiated at two years of age and treatment has been increased progressively, up to the current 80 mg of atorvastatin, 10 mg of ezetimibe, 3250 mg of colesevelam and armolipid plus. Weekly LDL apheresis with double filtration plasmapheresis was started at 5 years and 11 months of age. The LDL-C and Lp (a) levels before starting LDL apheresis were 380 mg/dL and 112 mg/dL, respectively, and decreased below 190 mg/dL and 51 mg/dL. The average reduction rate of LDL-C and Lp(a) per session were 48.4 ± 7.0% (average ±SD) and 50.5 ±9.5%, respectively.
Although treatment has not been able to control cholesterol levels in this patient, the xanthomas had completely regressed at the age of 10 years (see figure 2, aged 13). Moreover, the intima-media thickness in the carotid arteries, evaluated by ultrasound measurement, decreased from 0.9 mm in 2010 to 0.6 mm in 2014.

Conclusion
These results demonstrate that LDL apheresis is a useful therapeutic tool in a patient with a very severe form of FH, where it has led to a reduction of cholesterol deposits in subcutaneous tissue
and arteries.

*Very severe homozygous familial hypercholesterolemia: a total regression of xanthomas and decreased of intima-media thickness with LDL apheresis*

*Figures 1 and 2: Pictures before (fig. 1) and after LDL-apheresis therapy (fig. 2)*

Presentation Preference Electronic poster presentation

Additional information
Cardiometabolic risk associated with excess of body fat could be explained by adipose tissue derived palmitoleic acid

Abstract nr. 844
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Co-author(s) - Masana, Luis
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Dyslipidemia, Triglycerides, Visceral Fat

Aim: Increasing evidence suggests that palmitoleic acid (POA) could act as a lipokine and impact on metabolic disturbances. Since human studies on this topic are scarce, we aimed to assess whether POA plasma concentration (as a surrogate of adipose tissue release) relates to cardiometabolic risk.

Methods: This cross-sectional study included 299 participants aged 30 to 65-y at high-cardiovascular risk. Physical examination, demographic, anthropometric, carotid intima media thickness (IMT) and biochemical parameters were obtained. The concentrations of POA in plasma non esterified fatty acid fraction were analyzed by gas chromatography. The main outcome was the association between POA concentrations and prevalence of Metabolic Syndrome (MS) and its components, non-alcoholic fatty liver disease (NAFLD) by fatty liver index (FLI ≥ 60) and subclinical atherosclerosis.

Results: POA was higher in women than in men (mean ± SD, 1.25 ± 1.12 vs. 0.81 ± 0.80 mmol/L, P<0.001). In women, POA was associated to higher prevalence of MS (B: 0.052; 95%CI: 0.007; 0.098) and two of its components (impaired glucose metabolism [B: 0.052; 95%CI: 0.005; 0.099] and high blood pressure [B: 0.042; 95%CI: 0.002; 0.106]). For men, POA values were directly associated to abdominal obesity (B: 0.010; 95%CI: 0.002; 0.087). POA was associated to increased prevalence of NAFLD in both men (B: 0.046; 95%CI: 0.009; 0.083) and women (B: 0.117; 95%CI: 0.066; 0.169) IMT was not associated with POA.

Conclusions: Our observational data supports that increased POA plasma levels are associated to MS and NAFLD.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Identification and management of statin-intolerance: a survey of clinicians from 13 countries

Abstract nr. 845
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Co-author(s) - Stroes, Erik
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease, Dyslipidemia, Epidemiology, Prevention

Introduction: More than 40% of patients using statins discontinue therapy due to the onset of side effects (also termed ‘statin intolerance’). Discontinuation of therapy increases the risk of cardiovascular morbidity and mortality. Clinical guidelines lack consistent criteria for diagnosis and management of ‘statin intolerance’ (SI).

Purpose: To understand how patients with SI are identified and managed in an outpatient setting.

Methods: A web-based survey was conducted in Australia, Brazil, Canada, France, Germany, Italy, Japan, the Netherlands, Poland, Spain, Sweden, the UK, and the US. Pre-specified quotas targeted 60 clinicians (specialists and general/family physicians [GPs], 2:1 ratio) per country (90 in US). Eligible specialists (cardiologists, internists) were to have treated 75 patients and GPs had to have treated 50 patients with hypercholesterolemia, and at least 5 patients with SI in the previous 12 months. Clinicians answered pre-defined questions about the diagnostic criteria, the estimated rate and their choice of treatment in SI. All participants provided informed consent.

Results: Overall, 810 clinicians (78% cardiologists) completed the survey. Clinicians reported that of the suspected SI complaints, an average 72% of patients with suspected SI presented with muscle-related symptoms (range across countries [RAC] 50–87%). In these patients, clinicians took a range of steps to establish SI, including 1) discontinuation of statin (average 59%; RAC 48–67%); 2) statin re-challenge (average 74%; RAC 60–85%); and 3) modification of statin regimen (average 76%; RAC 65–85%); some clinicians reported trying a combination of above steps. An average of 38% of clinicians (RAC 32–46%) performed all three steps prior to diagnosing SI. Eventually, 6% of hypercholesterolemia-patients qualified as statin intolerant (RAC 2–12%). On average 52% of ‘confirmed’ SI patients continued to receive low-dose statin, usually with other lipid-lowering therapies (LLT). Of the remaining 49%, 75% received alternative LLT only. An average of 11% of patients with confirmed SI received no LLT.

Conclusion: Current clinical practice in patients with statin intolerance lacks consistency for diagnosis and management. A structured work-up to identify SI patients, followed by a defined
therapeutic algorithm, is expected to more satisfactorily address CV risk management in these patients.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Mortality among patients with familial hypercholesterolemia. A registry-based study in Norway 1992-2010

Abstract nr. 846
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Co-author(s) - Ose, Leiv
Co-author(s) - Iversen, Per Ole
Co-author(s) - Borgan, Jens-Kristian
Co-author(s) - Veierød, Marit B
Co-author(s) - Leren, Trond P
Co-author(s) - Retterstøl, Kjetil
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease,Epidemiology,Familial Hypercholesterolemia,Risk stratification

Introduction: Untreated patients with familial hypercholesterolemia (FH) are at increased risk of premature cardiovascular death. The primary aim of this study was to investigate if this also is the case in the statin era. Methods: In this registry-based study, 4 688 male and female Norwegian patients from Unit for Cardiac and Cardiovascular Genetics (UCCG) Registry with verified molecular genetic diagnosis of FH in the period 1992-2010 were linked to the Norwegian Cause of Death Registry. Standardized mortality ratios (SMRs) and 95% confidence intervals (CIs) were estimated. Results: There were 113 deaths. Mean age of death was 61.1 years. Cardiovascular disease (CVD) was the most common cause of death (46.0%), followed by cancer (30.1%). Compared to the Norwegian population, CVD mortality was significantly higher in the UCCG Registry in all age groups below 70 years: SMR= 2.29,95% CI(1.65-3.19)) in men and women combined, SMR= 2.00 (1.32-3.04) in men, and SMR=3.03 (1.76-5.21) in women. No significant differences were found in all-cause mortality or cancer mortality. Conclusions: Despite prescription of lipid lowering drugs, FH patients still had significantly increased CVD mortality compared to the general Norwegian population.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Regulation of lipid and carbohydrate metabolism by ligand-activated transcription factors during metabolic syndrome development in the hypertensive ISIAH rat strain

Abstract nr. 848
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Animal model, Atherosclerosis, Blood pressure, Cardiovascular Disease

It is known that the metabolic syndrome (MS) leads to serious cardiovascular disease which continues to be the number one cause of mortality in industrial countries. According to the National Cholesterol Education Program-Adult Treatment Panel III, the criteria of the metabolic syndrome include three or more impairments: increase in blood triglycerides, decrease in blood HDL cholesterol, hypertension, visceral obesity and increased blood glucose. The MS distribution is growing catastrophically, but molecular mechanisms responsible for development of complex impairments in MS still remain basically poorly investigated. A high percentage of MS morbidity determines much attention to MS modeling, mechanisms of MS development, and new approaches to MS treatment. The formation of complex MS symptoms suggests systemic impairments in lipid and carbohydrate metabolism; it appears that these impairments should have a common basis at the level of expression of appropriate genes. Expression of genes involved into lipid and carbohydrate metabolism is regulated by various transcription factors, including peroxisome proliferator-activated receptors (PPAR) (Lefebvre P. et all, 2006), liver X receptors (LXR) (Herzog B. et all, 2007), pregnane X receptors (PXR), and constitutive androstane receptors (CAR) (Moreau A. et all, 2008). In the hypertensive ISIAH rats compared with normotensive WAG rats the signs of the metabolic syndrome developing, correlating with the altered functional activity of ligand-activated transcription factors involved in lipid and carbohydrate metabolism were detected (Pivovarova E. N. et all, 2011). It was shown that fructose load (10 % fructose in the drinking water for 10 weeks) leads to the increase in the level of triglycerides in the blood serum of ISIAH rats. When comparing hypertensive ISIAH with two normotensive WAG and Wistar rat strains it was found higher content of glucose in the blood of ISIAH rats. It demonstrates relationship of high blood pressure, increased levels of glucose and increased levels of triglycerides. Complex studies of regulatory mechanisms, signaling pathways, and transcription targets for PPAR, LXR, PXR, and CAR may significantly help in better understanding of MS and provide valuable information for development of appropriate pharmacological approaches to MS therapy.
The content of total cholesterol (a), HDL cholesterol (b), and triglycerides (c) in blood serum of normotensive WAG and hypertensive ISIAH rats

The DNA-binding activity of the transcription factors PPAR and CAR in liver nuclear extracts of normotensive WAG and hypertensive ISIAH rats

Subdivision 1. Basic Science

Presentation Preference Mini-oral presentation

Additional information
HDL-c as a residual risk factor for vascular events and all-cause mortality in patients with type 2 diabetes

Abstract nr. 849
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Co-author(s) - Westerink, Jan
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Diabetes, HDL

Background: Despite the clear benefit in reducing cardiovascular risk in patients with type 2 diabetes (DM2) by lowering LDL-cholesterol (LDL-c), a significant residual risk remains. Low HDL-cholesterol (HDL-c), a hallmark of DM2 dyslipidemia, is associated with an increased risk for cardiovascular events, independent of LDL-c.

Objectives: To evaluate the relation between HDL-c levels and risk for cardiovascular events and all-cause mortality in patients with DM2 in different strata of lipid-lowering therapy intensity and LDL-c levels

Methods: Prospective cohort study of 1759 patients with DM2 included in the SMART cohort. Cox proportional hazard models were used to evaluate the risk of HDL-c on the composite of major cardiovascular events (myocardial infarction (MI), stroke, vascular death) and all-cause mortality. Analyses were performed in strata of LDL-c levels and lipid-lowering therapy intensity and were adjusted for age, gender, BMI, smoking, alcohol, LDL-c, TG, systolic blood pressure, eGFR, Glucose and HbA1c.

Results: 369 new cardiovascular events (MI, ischemic stroke, vascular death) were observed and 353 deaths occurred during a median follow-up of 6.7 years (IQR 3.4-10.0). In patients with DM2, no relation was found between HDL-c and cardiovascular events or all-cause mortality (HR 0.97, 95%CI 0.93-1.01, HR 1.00, 95%CI 0.96-1.04). However in patients on intensive lipid-lowering therapy, higher HDL-c was related with a higher risk for all-cause mortality (HR 1.13, 95%CI 1.01-1.22). A similar relation was found in patients with LDL-c levels <2.0 (All-cause mortality HR 1.15, 95%CI 1.08-1.22). Higher HDL-c was related to higher risk for cardiovascular events in patients with LDL-c levels <2.0, in contrast to patients with LDL-c levels between 2.0-2.5 mmol/l where HDL-c was related to lower risk (HR 0.86, 95%CI 0.76-0.97). In patients with LDL-c levels >2.5 mmol/l, HDL-c is related to a lower risk for cardiovascular events (HR 0.95, 95%CI 0.91-1.00) and all-cause mortality (HR 0.95, 95%CI 0.91-1.00).

Conclusion: In patients with DM2 on intensive lipid-lowering therapy or LDL-c levels <2.0 mmol/L, HDL-c is related with a higher risk for all-cause mortality. Higher HDL-c was related with a higher risk for cardiovascular events in patients with LDL-c levels <2.0 mmol/L.
Risk of HDL-c on cardiovascular events and all-cause mortality according to lipid lowering therapy and LDL-c levels

A. according to lipid lowering therapy intensity

<table>
<thead>
<tr>
<th>Hazard ratio*</th>
<th>All-cause mortality</th>
<th>Cardiovascular events</th>
<th>Composite of major CV events</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>10</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>1.0</td>
<td>25</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>1.5</td>
<td>50</td>
<td>25</td>
<td>50</td>
</tr>
</tbody>
</table>

B. according to LDL-c levels

<table>
<thead>
<tr>
<th>Hazard ratio*</th>
<th>% of sample</th>
<th>&lt;0.5 mmol/L</th>
<th>0.5-1.5 mmol/L</th>
<th>&gt;1.5 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>50</td>
<td>250</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

*Adjusted for age, gender, BMI, smoking, alcohol, LDL-c, TG, systolic blood pressure, eGFR, Glucose, HbA1c

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Cholesterol Efflux Capacities and HDL Subfractions after Single Ascending Doses of MDCO-216 (apoA1-Milano/POPC) in Human Volunteers and Stable CAD patients

Abstract nr. 850
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords HDL, Lipoproteins, Pharmacology, Reverse Cholesterol Transport

Introduction: MDCO-216, a complex of dimeric recombinant apolipoprotein A-1 Milano (ApoA-1M) and a phospholipid (POPC), is currently under development to improve cardiovascular outcomes by reducing plaque burden in patients with atherosclerotic disease. The purpose of this study was to assess dose-response (DR) relations of basal and ABCA1-mediated cholesterol efflux capacities from J774 macrophages and of various HDL subfractions after a single administration of MDCO-216 in healthy volunteers (HV) and coronary artery disease patients (CAD).

Methods: 16 HV and 16 CAD received a single 2 hour IV infusion of MDCO-216 (5, 10, 20, 30 or 40 mg/kg). Basal and ABCA1-mediated cholesterol efflux capacities, levels of HDL-subfractions (by 2-D electrophoresis), and of prebeta-1 HDL (by ELISA) were used as pharmacodynamic biomarkers. Responses were calculated as areas-under-the-effect-curve (AUEC) for these biomarkers over 0-24 h.

Results AUECs of basal and ABCA1-mediated efflux capacities increased approximately linearly over the entire dose range in both HV and CAD with no difference between the groups. AUEC for prebeta-1 HDL ELISA also increased linearly but were higher in CAD than for HV (Fig.1). AUEC for alpha-1 HDL increased linearly for HV but for CAD the curve reached plateau at 20 mg/kg and was below HV at 40 mg/kg. AUECs for alpha-3 HDL decreased curvilinear-downward in both HV and CAD reaching nadir at 20 mg/kg while DR-curves for alpha-4 were U-shaped with nadir reached at 20 (in CAD) or 30 (HV) mg/kg (Fig.2). The upward leg of the alpha-4 HDL is due to unreacted MDCO-216 having the same mobility.

Conclusions: The results support the hypothesis that MDCO-216 reacts with small (alpha-3 and alpha-4) HDL creating new alpha-1, alpha-2 and prebeta-1 HDL. This conversion of MDCO-216 was incomplete at higher doses at the end of the infusion, with CAD patients showing saturation at a lower dose than HV. The linear increases in AUECs of efflux capacities seem to be mediated by increased levels of prebeta-1 HDL, alpha-1 and alpha-2 HDL and also by unreacted MDCO-216 at the higher doses.
Cholesterol efflux capacities and prebeta-1 HDL (ELISA)

alpha-1-4 HDL
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
The Best Cut-Off Point For Triglyceride/High-Density Lipoprotein Cholesterol Concentration Ratio to Predict Cardiovascular Disease Outcome Among Iranian Population

Abstract nr. 852
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Epidemiology, Lipids, Lipoproteins

Sarrafzadegan

Background: The ability to identify high-risk individuals with elevated Triglyceride (TG) and low HDL before the development of cardiovascular disease would be substantial clinical benefit as this cut-off point varies among different nations so this study aimed to determine the best-cut point of TG/HDL in predicting cardiovascular events among Iranian population.

Method: Isfahan Cohort Study was a longitudinal population-based study that was conducted on adults aged 35 years or older, living in urban and rural areas of three districts in central Iran. After 10 years of follow-up, 3255 participants were re-evaluated using a standard protocol similar to the baseline. At both measurements, participants underwent medical interview, physical examination, and fasting blood measurements. The discrimination power of indices was assessed using receiver operating characteristic (ROC) analysis and the best cut-off value for each index was derived.

Results: TG/HDL-C ratio at a threshold 3.68 can be used to screen for cardiovascular events among study population. Subjects were divided into two groups (“low” and “high” risk) according to the TG/HDL-C concentration ratio at the baseline. A slightly greater number of “high” risk individuals were identified European cut points (505, 14.35%) in compare with ICS cut point (376, 16.2%). Multivariate model demonstrated TG/HDL able to predict cardiovascular events even after adjustment for demographic variables and traditional coronary risk factors including diabetes. The increased risk seems to be confined to the TG/HDL-C women with a TG/HDL-C ratio of 3.76 and 4.42 for men.

Conclusion: Threshold of TG/HDL >3.68 is the best predictor for cardiovascular events among Iranian population. These cut-off values 3.68 should be advocated and used in Iranian population.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Mini-oral presentation

Additional information
Pre-hypertension or pre-diabetes: which is better for predicting cardiovascular events?

Abstract nr. 853
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Co-author(s) - Sadeghi, Masoumeh
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Hypertension

**Background:** Despite partially demonstrated role of pre-hypertension and pre-diabetes to progress atherosclerotic events; it remained questioned which of these factors can predict cardiovascular events more effectively. The present study aimed to assess the value of these factors to predict cardiovascular events including myocardial infarction, brain stroke, and sudden cardiac death in general population.

**Methods:** A population-based, cross-sectional survey was conducted representing a great sample of the general Iranian population, aged 18 years and older, from the Isfahan Province and determined using a random, multistage cluster sampling scheme. The three endpoints considered as study outcome were acute occurrence of myocardial infarction, brain stroke, and sudden cardiac death.

**Results:** Of the 5398 studied subjects scheduled for assessing diabetes state, 536 were diabetics and 623 were pre-diabetics and other were non-diabetics. Also, of 6323 participants who scheduling for assessment of blood pressure abnormalities, 506 had hypertension, 461 had pre-hypertension, and other ones were normotensive. Adjusted for gender and age variables, pre-diabetes status could effectively predict occurrence of myocardial infarction (OR = 1.965, 95%CI: 1.135-3.401, P = 0.016), but did not predict appearance of brain stroke or sudden cardiac death. In the same logistic models, pre-hypertension status could not predict any of these events after adjustment for gender and age.

**Conclusion:** Our data provide valuable evidences on triggering role of pre-diabetes on appearance and progression of acute ischemic events even in healthy individuals. In this line, the value of pre-diabetes for predicting acute myocardial infarction is clearly superior to pre-hypertension state.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
Metabolic syndrome and its association with left ventricular dysfunction in patients with left bundle branch block

Abstract nr. 854
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Co-author(s) - Nilforoush, Peyman
Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease

Background: The present study and for the first time hypothesizes that the patients with left bundle branch block (LBBB) suffer considerably from metabolic syndrome (MetS) and this metabolic phenomenon can be associated with cardiac dysfunction status such as ventricular dilation and reduced left ventricular ejection fraction (LVEF) in these patients.

Methods: A retrospective study was conducted on 220 consecutive patients with diagnosed LBBB. MetS status was diagnosed using the Adult Treatment Panel III of the National Cholesterol Education Program criteria. Systolic function state was assessed using two-dimensional echocardiography.

Results: The overall prevalence of MetS among studied LBBB patients was 16.8%. Regarding left ventricular functional status in the two groups, the mean LVEF in the groups with and without MetS was 37.03 ± 9.09% and 43.43 ± 15.62% with a significant difference (p = 0.017). However, left ventricular dilation was similarly detected in both groups with and without MetS (21.6% versus 30.6%, p = 0.273). Multivariable linear regression model showed subjects with MetS had lower LVEF in the presence of confounders (Beta = 6.915, p = 0.039).

Conclusion: A notable number of LBBB patients suffered from MetS and this metabolic phenomenon is significantly associated with lowering left ventricular function in LBBB patients.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
From clinical to genetic diagnosis in Bulgarian familial hypercholesterolemic patients

Abstract nr. 855
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Epidemiology,Familial Hypercholesterolemia,LDL,Risk stratification

According to recent studies Familial Hypercholesterolemia (FH) is more frequent than we have imaged and diagnosed surprisingly not enough. There is barely enough data about the Bulgarian population for FH. Aim of this study is the screening of small population with severe HH for clinical diagnosis of FH (Dutch Lipid Clinic Network- DLCN) and molecular-genetic analysis (MBA) in them. Material and method: We investigated 1300 patients with severe HH (≥7.5 mmol/l, LDL-C ≥ 4.9 mmol/l). 120 of them were diagnosed with clinical FH, in according of the DLCN. We found 4 patients with a “definitive” diagnosis FH (>8 subject score), “probable” (6-8 subject score) - 65, “possible” (3-6 subject score) – 37, “unlikely” (< 3 subject score) – 14. The patients were separated into two groups: carriers – 22, and non-carriers – 98. In all of them MBA was performed – mutation of R3500Q in Apo-B gene and point mutation and polymorphism of LDL-R gene. We investigated many atherogenic biomarkers: total cholesterol, HDL-C, triglycerides, LDL-C, Apo-B, Apo-A1; markers of endothelial dysfunction – asymmetric dimethylarginine and total homocystein; markers of endothelial activation – cell adhesion molecules – sICAM, sVCAM, P-selectine, E-selectine. Results: A mutation of R3500Q in Apo-B-100 receptor binding domane was not found. Out of four patients with tendon xanthomas 3 were with LDL-R mutation in exon 11, and one of them with polymorphism and mutation in the same exon. The four patients were with mutation in exon 9. We found 18.33 % sensitivity of clinical criterias of FH and 81.67 % false positive. The laboratory parameters of the carriers of the LDL-R defect did not differ significantly from the group of HH non-carriers (p>0.05) in expect of the atherogenic index Apo-B/Apo-A1 (carriers – 3.32±0.61; non-carriers – 2.48±0.56; p<0.001), even after standardization of age. Conclusion: This is one of the first studies of FH in Bulgaria. Our data shows that FH is more frequent, than we think in this high risk population of coronary heart disease.

Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Lack of Nox4 promotes an atherogenic phenotype in mouse aortic smooth muscle cells

Abstract nr. 857
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Co-author(s) - Szyndralewiez, Cedric
Co-author(s) - Schmidt, Harald HHW
Co-author(s) - Jandeleit-Dahm, Karin
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis,Pathogenesis

NADPH-derived reactive oxygen species (ROS) are important mediators of cellular damage and signalling pathways that drive atherosclerosis development and progression. Recently, the NADPH oxidase subunit isoform Nox4 was identified to play an important role in maintaining the differentiated vascular smooth muscle cell (VSMC) phenotype. De-differentiated VSMCs elaborate proinflammatory cytokines and ROS to drive atherosclerotic plaque formation.

The aim of this study was to investigate the role of Nox4-derived ROS in proinflammatory pathways in mouse aortic smooth muscle cells (MASMs).

Whole aortas from ApoE KO and Nox4/ApoE DKO mice were snap frozen or enzymatically digested for MASM cell isolation. Gene and protein expression was assessed by RT-PCR and western blot, respectively. In culture, MASM cells were treated with platelet-derived growth factor (PDGF)bb (10ng/ml) for 4 hours. Superoxide production was measured via L012 chemiluminescence in the presence or absence of the Nox1/Nox4-dual inhibitor, GKT137831 (1µM).

Nox4/ApoE DKO aortas showed decreased αSMA and increased PDGF gene expression. MASMs isolated from Nox4-deficient mice also showed decreased αSMA gene and calponin protein expression levels as well as increased expression of proinflammatory molecules PDGF, monocyte chemotactrant protein (MCP)-1 and interleukin 6 compared to ApoE KO MASM cells. Nox4-deficient cells displayed increased Nox1 gene expression to a similar level as PDGF-treated ApoE KO cells. Consistent with these findings, Nox4-deficient demonstrated increased superoxide production which was abrogated by GKT137831.

Nox4/ApoE DKO MASM cells showed decreased expression of contractility markers, increased proinflammatory molecule expression and enhanced ROS generation indicative of the atherogenic VSMC phenotype. Treatment of DKO cells with GKT137831 abrogated the observed increase in superoxide generation implicating Nox1 as the source of ROS in these cells. In this study we identify a role for Nox4 in the regulation of VSMC responses which may involve compensatory feedback via Nox1. These findings suggest a protective role for Nox4 in atherogenesis with respect to VSMC pathophysiology and highlight the importance of cell type and Nox isoform specific intervention strategies in vascular disease.
Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Associations between self-reported anxiety and serum lipid profile in hypertensive patients

Abstract nr. 858
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Co-author(s) - Valeanu, Madalina
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Blood pressure, Dyslipidemia

Abstract
Objective
Observational studies indicate that psychologic factors strongly influence the course of coronary artery disease. Several studies demonstrated an association between dyslipidemia and psychiatric disorder. The current study aims to assess levels of serum lipid profile in hypertensive patients with high and low anxiety.

Methods
A total of 105 hypertensive subjects (64% women, 36% men), mean age 57.23 years, have been submitted to physical measurements and laboratory tests. The diagnosis of hypertension was defined according to the ESH 2013 (European Society of Hypertension) criteria. Lipid profile was defined according to NCEP/ATPIII criteria (National Cholesterol Education Program, Adult Treatment Panel III). All participants were asked to complete the Spielberger’s self-reported state-trait anxiety inventory (STAI). Participants with scores higher than 46 and lower than 34 were included in high anxiety group and low anxiety group, respectively. Levels of fasting serum lipids, including total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides were compared between the two groups. Statistical analysis of the collected data was performed.

Results
According to the Spielberger’s STAI, of a total of 105 hypertensive subjects, 19 had high anxiety, 44 had low anxiety and 42 had mild anxiety. At women, mean anxiety scores (14.23±8.12) were higher in comparison with men (8.54±6.1)(p=0.000). The levels of total cholesterol and LDL cholesterol were significantly higher in the high anxiety group (P<0.004). There were no significant differences in the levels of triglycerides, HDL cholesterol.

Conclusion
High anxiety increases total cholesterol and LDL cholesterol in hypertensive patients. Diagnosis of anxiety in hypertensive patients and early therapeutic interventions may decrease the risk of subsequent cardiac events in this population.
Keywords: anxiety, total cholesterol, LDL cholesterol.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Ezetimibe is more effective on apolipoprotein B48 metabolism compared to statins in impaired glucose tolerance and diabetes patients.

Abstract nr. 859
Author Inagaki, Kyoko, Nippon Medical school, Tokyo, Japan
Co-author(s) - Sudo, Mariko
Co-author(s) - Oikawa, Shinichi
Co-author(s) - Sugihara, Hitoshi
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Diabetes, Lipoproteins, Triglyceride-Rich Proteins

Background: The randomized controlled trials provided the evidence that statin reduces atherosclerotic cardiovascular disease (ASCVD) event. Statin is recommended to prevent ASCVD in high ASCVD risk subjects. However, whether other lipid lowering drugs reduce atherosclerosis and cardiovascular events is not established. Recently IMPROVE-IT: Improved Reduction of Outcomes: Vytrin (ezetimibe/simvastatin tablet) Efficacy Interventional Trial showed the combination drug (ezetimibe/simvastatin) reduce cardiovascular event in diabetic patients. It is not clear whether the effect on chylomicron metabolism is different between statins and ezatimibe in impaired glucose metabolism patients. Apolipoprotein B48(apoB48) is the characteristic protein of chylomicron and the level of apoB48 reflect chylomicron remnant levels.

Object: To investigate the effect of statin and ezetimibe on chylomicron remnant in impaired glucose tolerance (IGT) and diabetes patients (DM).

Method: Subjects are the out patients who have high LDL-C and IGT or DM. (n=93) Subjectes were divided to three groups, diet group (n=33), statin group (n=15), and ezetimibe group (n=45). They were received each therapy for 3 months and fasting blood samples were took at each month. Lipid profiles and apoB48 levels were compared among groups. The JMP 11 software was used for statistical analysis. Written informed consent was obtained from the participants included in the study. The study had the approval of the Ethics Committees from Nippon Medical School.

Result: Age, Body mass index, fasting blood glucose, and HbA1c were not different among groups. After 3 month therapy, apoB48 levels were decreased in ezetimibe group (9.1±1.2ug/m to 5.9±1.0ug/ml (mean±SD), p=0.03), but not decreased in diet group (6.1±1.4ug/m to 7.5±1.3ug/ml, p=0.07)and statin group (9.5±2.1ug/m to 7.1±1.9ug/ml, p=0.29).

Conclusion: Ezetimibe is more effective on chylomicron metabolism and reduces more chylomicron remnant in IGT and DM than statins.

Subdivision 3. Clinical Studies

Presentation Preference Mini-oral presentation
Additional information
SOLE EPA REGIMEN INCREASES EPA, BUT DECREASES DHA LEVEL IN ERYTHROCYTE MEMBRANE IN PATIENTS WITH DYSLIPIDEMIA

Abstract nr. 860
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Lipids, Pharmacology, Therapy, Triglycerides

To treat patients with hypertriglyceremia n-3 polyunsaturated fatty acids (PUFA) from fish eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are usually administered in combination (EPA+DHA). However, sole EPA regimen is available in Japan as a registered drug and as supplements all over the world. Japanese mega trial for secondary prevention adding sole EPA on top of statin, JELIS, reported that EPA treatment to 8,321 participants increased their serum EPA by 69% but decreased DHA by 14%. Although EPA functions to reduce ischemic events by its actions on platelets, favorable effects of DHA on cardiac function to prevent heart failure and arrhythmia and on central nervous system to slow progression of dementia are assumed due to its action in tissue cell membrane and cytoplasm. We thus tried to assess changes in EPA and DHA levels of erythrocyte membrane which are reported to reflect those of tissue cell membrane in humans after giving EPA and EPA+DHA to dyslipidemic patients. Patients were administered sole EPA 1,800 mg regimen (n=55) or EPA+DHA (DHA 1,500 mg and EPA 1,860 mg, n=50) daily for 4 months in an open-label, randomized, single-blinded design, and in a crossover manner for another 4 months. After 8-hour fasting blood was drawn for serum and erythrocyte analyses. Serum and erythrocyte PUFA levels were analyzed by HPLC. Sole EPA and EPA+DHA decreased serum triglyceride and RLP-C by 7.7 mg/dl (n.s.), 2.50 mg/dl (p<0.01), and 53.1 mg/dl (p<0.001), 6.25 mg/dl (p<0.001), respectively. Serum EPA was elevated by both sole EPA and EPA+DHA by 1.50 and 1.73 %vol, respectively (both p<0.001), but serum DHA was decreased by sole EPA by 0.71 %vol and increase by EPA+DHA by 1.38 vol% (both p<0.001). Similarly, in erythrocyte membrane EPA was increased by both sole EPA and EPA+DHA by 1.29 vol% and 1.17 vol%, respectively (both p<0.001); however, DHA was decreased by sole EPA by 1.54 vol% (p<0.001) and increased by EPA+DHA by 1.41 vol% (p<0.001).

In conclusion sole EPA regimen may be less efficacious compared with EPA+DHA in that the former may deteriorate cardiac functions and cognition by decreasing DHA in tissue cell membrane and cytoplasm.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Familial hypercholesterolemia: classification of mutation severity according to percentile LDL-cholesterol useful for predicting coronary artery disease risk.

Abstract nr. 861
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Familial Hypercholesterolemia, LDL, Risk stratification

Objective
The large clinical variability in patients diagnosed with familial hypercholesterolemia (FH) is largely related to severity of the underlying mutation. Historically, mutations are classified according to mutation class, ranging from receptor deficient class 1 mutations defined as most severe to potential functional mutations. This classification might ignore the association with the phenotype, and we therefore set out to classify the mutations according to their effect on LDL-C levels, the pathological substrate in FH.

Methods
We used the FH cascade screening cohort in the Netherlands to classify LDLR mutations. We calculated the mean age and gender corrected percentile of LDL-C of all untreated carriers of each specific FH mutation and classified the mutations in tertiles using the 90th and 95th percentile as boundaries. We also classified according to the mutation class. We calculated coronary artery event (CAD) free survival, censoring in 1990 (introduction of statins in the Netherlands), and evaluated the risk of CAD (HR 95%CI interval) in mutation carriers compared to unaffected relatives tested for the same mutations, using cox regression.

Results
34197 persons were tested for 456 different pathogenic LDLR mutations, identified in the index patient of their family, between 1994 and 2010. Of these 12245 (36%) were found carriers. 4588 FH patients carried mutations associated with a mean untreated LDL-C above the 95th percentile and these patients from the highest tertile had a CAD risk of 8.4 (6.2-11.5). The FH patients in the middle and lowest tertile of hypercholesterolemia had lower CAD risk, of 4.5 (3.0 to 6.7) and 3.3 (2.5 to 4.6), respectively. The 4197 patients carrying a class 1 mutation had a 7.2 (5.4-9.7) higher risk of CAD than their unaffected relatives. The patients carrying other class mutation - i.e. 2A, 2B, 3 and 5- had a variable risk of CAD, on average 4.1 (3.2 to 5.3) higher than unaffected relatives.

Conclusion
Mutation severity classification based on the effect on LDL-C holds greater value for CAD risk prediction compared to the traditional classification. This approach might offer advantages in clinical use particularly regarding mutation severity for non-class 1 mutations.
Hazard ratio of coronary artery disease in FH patients with different mutation classifications

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation

Additional information
The collagen binding protein fibromodulin contributes to atherosclerotic plaque inflammation and cerebrovascular events

Abstract nr. 862
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Co-author(s) - Bengtsson, Eva
Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Pathogenesis, Vulnerable Plaque

Aim: We have recently shown that mice lacking the collagen binding protein fibromodulin develop atherosclerotic plaques with altered collagen fibril structures. These plaques were also smaller in size and contained less lipids in comparison to plaques from mice expressing fibromodulin. The results suggest that fibromodulin has a significant impact on the development of atherosclerotic lesions. The aim of the present study was to analyze the importance of fibromodulin in human atherosclerotic plaques.

Methods and Results: The amount of fibromodulin, lipids and smooth muscle cells in carotid plaques (152 patients) was analyzed on stained sections which were scanned and digitalized. Positively stained areas were quantified using BioPix iQ software. Cytokines and vascular endothelial growth factor were analyzed in homogenates (multiplex immunoassay). A total of 75 plaques were symptomatic, e.g. they were obtained from patients that had experienced a cerebrovascular event prior to surgery. Fibromodulin expression was higher in symptomatic plaques (p=0.0001). Fifty-one plaques were from diabetic patients and these had also higher levels of fibromodulin than plaques from non-diabetics (p=0.001). A high plaque content of fibromodulin correlated with increased lipids (r=0.18; p=0.033) and with low content of smooth muscle cells (r=-0.17; p=0.048). Fibromodulin expression in plaques was also associated with increased concentration of CD40L (r=0.19; p=0.044), macrophage inflammatory protein-1b (r=0.20; p=0.032) and vascular endothelial growth factor (r=0.22; p=0.018) and with low concentrations of the anti-inflammatory cytokine interleukin-10 (r=-0.29; p=0.002).

Conclusions: Our observations provide clinical support for previous experimental findings of a role of fibromodulin in atherosclerosis and suggest that fibromodulin is associated with plaque inflammation and cerebrovascular events.

Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
Bone marrow specific caspase-1/11 deficiency inhibits atherosclerosis development in Ldlr-/- mice

Abstract nr. 863
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Animal model, Atherosclerosis, Cardiovascular Disease, Inflammation

Background: Recent investigations have suggested that the inflammasome plays an important role during atherosclerosis. Upon activation, the inflammasome induces the processing and release of pro-inflammatory cytokines IL-1β and IL-18 via activation of caspase-1/11. Previously, it was described that complete caspase-1 deficiency is protective against atherosclerosis development. However, while macrophages are the main inflammatory cells involved in atherosclerosis, the exact role of macrophage-specific caspase-1/11 activation during the development of cardiovascular disease has never been investigated. We hypothesized that hematopoietic caspase-1/11 deficiency leads to reduced atherosclerosis development.

Approach and results: To investigate the specific contribution of hematopoietic caspase-1/11 activation to atherosclerosis development, Ldlr-/- mice were transplanted (tp) with wild-type (Wt) or caspase-1/11-/- bone marrow and fed a high-fat, high-cholesterol (HFC) diet for 12 weeks. Our results showed an increase in anti-inflammatory blood leukocyte profile in caspase-1/11-/--tp mice compared to Wt-tp mice as indicated by decreased Ly6C^high and increased Ly6C^low monocytes. In line with our hypothesis, hematopoietic deletion of caspase-1/11 resulted in a strong reduction in atherosclerotic plaque size. Furthermore, necrotic core content was dramatically decreased in caspase-1/11-/--tp mice.

Conclusions: Our data indicate that hematopoietic caspase-1/11 activation is involved in vascular inflammation and atherosclerosis, and suggest that besides inflammation also pyroptosis plays an important role in cardiovascular disease progression.

This research was supported by the Dutch Heart Foundation, Dutch Diabetes Research Foundation, Dutch Kidney Foundation, Maag Lever Darm Stichting (MLDS) and Vidi.
Subdivision 1. Basic Science

Presentation Preference Electronic poster presentation
Additional information
The estimation of nephroprotective effect of combined lipid-lowering therapy in decompensated type 2 diabetes

Abstract nr. 864
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Diabetes,Hypolipidemic Drugs,Renal function

Aim – to estimate the comparative nephroprotective influence of combined and mono kinds of lipid-lowering therapy at patients with type 2 diabetes in decompensated stage.

Materials and methods. In study was included 68 patients with decompensated type 2 diabetes, not receiving lipid-lowering therapy. All patients were randomized on three comparable groups. The patients of the first group (26 persons) was admitted simvastatin 40 mg daily. The patients of the second group (18 persons) received the combined lipid-lowering therapy with simvastatin (40 mg daily) and ezetimibe (10 mg daily). In 24 patients of third group lipid-lowering therapy was not performed. In all patients the lipid panel, biochemical indicators of liver function, glomerular filtration rate and the intensity of microalbuminuria was determined before and after six months of treatment period.

Results. The improvement of indicators of lipid metabolism as well as during mono and combined lipid-lowering therapy was shown on patients with decompensated type 2 diabetes. It was marked the decrease in level of the general cholesterol, low density lipoproteins cholesterol, triglycerides, atherogenic index and insignificant increase in level of high density lipoproteins cholesterol. The marked decrease of low density lipoproteins cholesterol from 4,42±0,27 to 3,01±0,23 mmol/l at combined therapy in compare with from 3,58±0,29 to 3,14±0,16 mmol/l on simvastatin only was determined. The level of triglycerides decreased by 15,4% at simvastatin therapy in compare with 30,6% on combined therapy. The lipid-lowering therapy was accompanied by the increasing of glomerular filtration rate from 91,61±7,74 to 107,18±8,35 ml/min/1,73 m² and the reduction r treatment. All kinds of therapy lead to the reduction of microalbuminuria to 32,4% at combined versus 17,6% at monotherapy. The assessment of a functional condition of a liver after lipid-lowering therapy in patients with decompensated diabetes showed their safety.

Conclusion. At patients with type 2 diabetes the beginning of lipid-lowering therapy in decompensated stage leads to the normalization of indicators of a lipid metabolism and renal function more expressive at combined therapy. The initiation of treatment in a decompensated stage is safe and doesn't influence on indicators of a liver function.

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Multi-pathway screening approach for selecting anti-atherosclerotic compounds: in vitro studies

Abstract nr. 865
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Atherosclerosis, Intervention, Pharmacology

Background
Liposomal delivery of drug compounds to plaque macrophages is an attractive strategy for treatment of atherosclerosis. However, despite its anti-inflammatory properties, we have previously found that local delivery of liposomal prednisolone lacked efficacy in humans, underlining the need to take a wide array of macrophage-related processes into account. The aim of this study was to develop a dedicated screening procedure to identify compounds likely to exert anti-atherogenic effects on monocyte/macrophages in a lipid-rich, atherogenic environment, for further development in a liposomal drug delivery system.

Methods and results
We developed an in vitro screening assessment for potential drug compounds of relevant pathways for atherosclerosis: inflammation, oxidative stress, lipid handling, endoplasmic reticulum (ER) stress, efferocytosis and proliferation. Subsequently, representative candidates – namely prednisolone, methotrexate, LXR-agonist T0901317, pterostilbene, 6-mercaptopurine, simvastatin and rapamycin - of anti-atherosclerotic pathways were examined and scored for their impact on macrophage tests.

Conclusion
Based on overall performance, pterostilbene, simvastatin and the LXR-agonist T09 appear to be the most promising candidates for plaque-targeted liposomal delivery. Further studies are warranted to determine the predictive value of our screening approach for the anti-atherosclerotic potency of liposomal compound delivery in experimental and clinical atherosclerotic settings.
**IN VITRO COMPOUND SCREENING**

**INFLAMMATION**
- Nrf2 activity
- TNF-α
- IL-6

**OXIDATIVE STRESS**
- ROS
- NO production

**LIPID HANDLING**
- oxLDL uptake
- CD36
- Chol. efflux
- ABCA1
- FABP4
- IRE1
- PERK

**ER STRESS**

**EFFECRYSIS**

**PROLIFERATION**
- BrdU

**SCORE**
- 2
- 2
- 12
- 13
- 2
- 11
- 5

2-fold change +2 Anti-atherosclerotic
Stat. sign. change +1
Stat. sign. change 0 No change
2-fold change -2 Pro-atherosclerotic

**Figure 1. In vitro compound screening results.**
Graphical representation of the effects of the selected compounds on relevant pathways in atherosclerosis. The potential anti- or pro-atherosclerotic effects were scored unweighted to give an indication of overall performance of the compounds in the different macrophage-related assays.

**In vitro screening compound screening results**
Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
Clinical characteristics, lipid-lowering treatment and LDL-C achievement rates in 506 FH patients: pooled results from 4 lipid clinics.

Abstract nr. 867
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Co-author(s) - Kuznetsova, Tatiana
Co-author(s) - Mandelshtam, Michail
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Cardiovascular Disease, Dyslipidemia, Familial Hypercholesterolemia, Hypolipidemic Drugs

Aim of the study was to analyze records of FH patient (pts) underwent clinical care in 4 Lipid Clinics (LC). Final data set included records of 506 patients with phenotypic diagnosis of definite FH (Dutch Lipid Clinic Network Score ≥ 8).

Table 1. Main results of the Study (n=506).
Initial= data at first visit, **on Rx- on lipid-lowering treatment

In 106 FH pts with CHD (+) on LLT 24 FH pts (22.6%) were treated with initial doses of statins, 35 pts (33%) took moderate doses, 22 (20.8%) pts received LLT with high doses of statins. Combination LLT with initial doses of statins+ezetemibe were taken by 3.8% of pts, high doses of statins and ezetemibe therapy -6.6% pts. Only 6.6% FH pts with CHD (+) on LLT achieved LDL-C < 1.8 mmol/l, and 17.3% FH pts CHD (-) achieved LDL-C < 2.6 mmol/l.

Conclusion: Most index pts (58%) with definite FH and CHD (+) attending 4 lipid clinics are at very high CVD risk or recurrent events at the age of 50. Lipid–lowering therapy in FH pts with and without CHD is sub-optimal and need to be intensified.
Table 1. 

<table>
<thead>
<tr>
<th>Parameter</th>
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<tbody>
<tr>
<td>Age</td>
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<tr>
<td>Males/females</td>
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<tr>
<td>Index population</td>
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<tr>
<td>FH pts with</td>
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<tr>
<td>Current smokers</td>
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<tr>
<td>Hypertension</td>
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<tr>
<td>Mean LDL</td>
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<td>All FH pts</td>
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</tbody>
</table>
Table 1. Main results of the Study (n=506).

Subdivision 3. Clinical Studies

Presentation Preference Oral presentation
Additional information
Dietary patterns and mortality from cardiovascular disease: Isfahan Cohort Study

Abstract nr. 868
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Cardiovascular Disease,Epidemiology,Nutrition,Prevention

Background/ Objectives: We are aware of no prospective study reporting the association between dietary patterns and cardiovascular mortality from Middle-eastern countries. This study was performed to examine the association between major dietary patterns, identified by factor analysis, and CVD mortality in Iranian adults.

Subjects/Methods: This population-based prospective cohort study was done among 4834 randomly selected participants aged ≥35 years in urban and rural areas of central Iran (2001-2009) in Isfahan Cohort Study (ICS). Dietary intakes were assessed using a food frequency questionnaire and major dietary patterns were identified by means of exploratory factor analysis. Subjects or their next of kin were interviewed biannually looking for possible occurrence of events. Cardiovascular mortality was defined as mortality from fatal myocardial infarction (MI) and other ischemic heart disease, fatal stroke and sudden cardiac death.

Results: During the median follow-up of 9.0 years and 50282 person-years, we found a total of 118 CVD mortalities. Four major dietary patterns were identified: "Western", "Mediterranean", "Animal fat" and "Fast food" dietary patterns. Adherence to the Mediterranean dietary pattern was protectively associated with CVD mortality; such that those in the highest quartile were 46% (HR: 0.54; 95% CI: 0.32-0.91; P for trend=0.03) less likely to have incident CVD mortality than those in the lowest quartile. Further adjustment for potential confounders, strengthened this association (HR:0.42; 95% CI: 0.19-0.96; P for trend=0.02). We found no significant association between adherence to the Western, animal fat and fast food dietary patterns and CVD mortality.

Conclusions: We concluded that even in the setting of a developing country, consumption of a Mediterranean dietary pattern was associated with reduced risk of cardiovascular mortality.

Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Electronic poster presentation
Additional information
Safety and efficacy of polyprenols and coenzyme Q10 for statin-induced myopathy: the first results of a pilot study

Abstract nr. 869
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Therapy

Introduction. Statin-induced myopathy is a rare side effect partially attributed to ubiquinone deficiency. Supplementation with plant polyprenols and ubiquinone (or coenzyme Q10, CoQ10) may offset this deficiency and reduce symptoms of myopathy.

Purpose. We aimed to study efficacy and safety of plant polyprenols and CoQ10 in treatment of statin-induced myopathy.

Methods. This was an open-label, one-center pilot study. Five patients (2 women and 3 men) matching strict statin-induced myopathy criteria received conifer-tree needle polyprenols (4mg/day) and CoQ10 (100mg/day) for 4 months. Myopathy was evaluated subjectively according to symptom severity score (rating scale 0-10 for pain, weakness, cramps), creatine kinase (CK) levels, exercise performance on veloergometry, and dynamometry. For safety evaluation, we performed complete blood count test, clinical biochemistry and electrocardiography.

Results. Three patients (60%) reported improvement of at least one symptom at 2 months of follow-up. Of these, two patients had further improvement of symptoms over the following 2 months. Among 4 patients with muscle pain at baseline symptom severity score decreased from average 6.0 [3.5-7.75] to 3.5 [0.25-6.75] (p=0.109; n=4). Muscle pain at rest disappeared in one out of three patients. Among 4 patients with muscle weakness at baseline, it mildly decreased in one case and resolved completely in another case. Muscle weakness severity score changed from 6.5 [5.0-8.0] to 5.5 [1.25-7.5] (p=0.180; n=4). One of two patients with muscle cramps reported improvement of the symptom. No significant change in maximum workload during exercise test, nor arm muscle strength measured with dynamometry was observed. CK levels did not change significantly, although a trend of increase was noticeable (from 134±80 to 147±53 U/l; p=0.662).

Conclusions. Conifer tree polyprenols and CoQ10 treatment may reduce symptom severity in some patients with statin-induced myopathy. The findings support conduction of a larger scale, placebo-controlled study to confirm the efficacy and safety of conifer tree poliprenols and CoQ10 in combination and separately to alleviate statin-induced myopathy.
Subdivision 3. Clinical Studies

Presentation Preference Electronic poster presentation
Additional information
Underestimation of LDL-C<70 mg/dL by Friedewald, Hopkins and ‘Direct’ assay Compared to Preparative Ultracentrifugation and Overestimation of LDL-C Reduction

Abstract nr. 870
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Co-author(s) - Stein, Evan
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords LDL

Background: Calculated LDL cholesterol (LDL-C) by the Friedewald formula (LDL-\(C_F\)) has been the basis for clinical and regulatory decision making for >40 years. As clinical guidelines and new therapeutic agents reduce LDL-C levels to below levels originally validated by LDL-\(C_F\), studies show substantial underestimation and an alternative formula, Hopkins (LDL-\(C_H\)), or ‘direct/homogeneous’ (LDL-\(C_D\)) assays have been proposed. We compare LDL-C by these methods to the ‘gold standard’, preparative ultracentrifugation (LDL-\(C_P\)), in 1,299 samples with 961 ≤70 mg/dL.

Methods: Patient samples were analyzed in a central laboratory that was CDC-NHLBI Part 3 Standardized for lipid measurements. LDL-\(C_F\), LDL-\(C_H\), LDL-\(C_D\) and LDL-\(C_P\) were compared including at clinically important cutpoints of 100, 70, 50 and 25 mg/dL and within each cutpoint by triglyceride (TG) levels.

Results: See Table. While the difference between LDL-C methods were significant at nearly all levels the differences increased and became clinically meaningful as LDL-C decreased <70 mg/dL and further deteriorated ≤50 and 25 mg/dL, especially for LDL-\(C_F\) and LDL-\(C_H\). Below 70mg/dL, for each 100 mg/dL TG increase >100 mg/dL these differences increased irrespective of LDL-C method.

Conclusion: Traditional or novel formulas for calculating LDL-C, and ‘direct’ LDL-C measurement show significant and clinically meaningful differences when true LDL-C is <70 mg/dL; and even moderate TG increases have major consequences. As measurements of LDL-C by these commonly used methods underestimates lower LDL-C they result in substantial underestimation of post-treatment LDL-C which in turn overestimates the reduction in LDL-C with new more effective agents.
### Summary Statistics of LDL-CF, LDL-CH, LDL-CD and LDL-CP by Friedewald categories

#### Subdivision 3. Clinical Studies

**Presentation Preference** Oral presentation

**Additional information**

<table>
<thead>
<tr>
<th>Friedewald (mg/dL)</th>
<th>N</th>
<th>LDL-CF (mg/dL)</th>
<th>LDL-CH (mg/dL)</th>
<th>LDL-CD (mg/dL)</th>
<th>LDL-CP (mg/dL)</th>
<th>Mean (SD)</th>
<th>Mean (SD)</th>
<th>Mean (SD)</th>
<th>Mean (SD)</th>
<th>Percent Differencea (%)</th>
<th>Percent Differenceb (%)</th>
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<tr>
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<td>22.6 (7.6)</td>
<td>22.3 (6.9)</td>
<td>3.2 (1.4)</td>
<td>13.1 (8.4)</td>
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<td>-9.4 (5.1)</td>
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<td>-3.5 (13.4)</td>
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**Legend:**

- **a:** Percent difference = 100*(LDL-CF - LDL-CH)/LDL-CH
- **b:** Percent difference = 100*(LDL-CD - LDL-CP)/LDL-CP
- **c:** P-values are from a one sample t-test performed on percent difference

**Note:** Overall N=1239 for LDL-CF, and N=289 for other parameters.
Novel Dual Pathway Enhancement of Cholesterol Efflux by Heat Shock Protein 27

Abstract nr. 871
Author O'Brien, Edward, University of Calgary / Libin CV Institute, Calgary, Canada
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Co-author(s) - Shi, Chunhua
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Atherosclerosis, Dyslipidemia, HDL, Reverse Cholesterol Transport

**Introduction:** Previously we reported that elevated serum levels of Heat Shock Protein 27 (HSP27) are predictive of reduced cardiovascular clinical events (MI, CVA, death). Moreover, attenuation of experimental atherogenesis, characterized by reduced cholesterol accumulation in the artery wall and serum, can be achieved by augmenting serum HSP27 levels. While the reduced influx of cholesterol into lesions may occur because HSP27 binds to and reduces the expression of Scavenger Receptor-A, this incompletely explains the stabilization/regression of experimental atherosclerosis that we have also observed.

**Hypothesis:** Exogenous HSP27 enhances cholesterol efflux from lesions by enhancing a) conventional and b) novel (exosomal) reverse cholesterol transport pathways.

**Methods / Results:**
- a) THP-1 macrophages pre-treated with oxLDL and incubated with rHSP27 for 24h showed enhanced mRNA and protein expression levels for the reverse cholesterol proteins ABCA1 and ABCG1; whereas treatment with a truncated (inactive C-terminus) form of HSP27 (rC1) did not. As well, when THP-1 macrophages were labeled with NBD-cholesterol, treated with rHSP27 for 24h then incubated with 40 ug/mL apoA1 or 100 ug/mL HDL for 1h there was a marked increase in cholesterol efflux to apoA-1 and HDL.
- b) NBD-cholesterol and the exosome biomarker CD81 co-localized in exosome particles (as detected by FPLC and flow cytometry). In separate studies we demonstrated that HSP27 autoantibodies are generated in vivo and potentiate its signaling. Hence, we added the HSP27 / autoantibody complex to macrophages pre-treated with NBD cholesterol and found a 30% increase of secreted cholesterol-NBD into the medium (p<0.0001). Moreover, using an exosome capture ELISA we show that exosome secretion is promoted (~95%; p<0.001) after treatment with the HSP27 / autoantibody complex. Finally, compared to the same concentration of HSP27 without antibodies, the HSP27-autoantibody complex increased the abundance of cholesterol-NBD positive exosomal particles by approximately 70%, as measured by flow cytometry.

**Conclusion:** HSP27 has a dual action on cholesterol efflux. First, it promotes the upregulated expression of proteins participating in reverse cholesterol and is associated with increased HDL formation. Second, HSP27 enhances cholesterol efflux via a novel exosomal pathway. Taken together, these data provide potential mechanistic explanations for the athero-regressive effects of HSP27.
Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Importance of apolipoprotein D in the interaction of lipoproteins and transfer of lipids

Abstract nr. 872
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Co-author(s) - Paulie, Staffan
Co-author(s) - Kumagai-Braesch, Makiko
Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Functionality, HDL, LDL

Background and aim: Apolipoprotein (apo) D is an intriguing protein produced by many tissues and necessary for development and repair of the brain and in the protection from oxidative stress. ApoD is a lipocalin mainly associated to HDL3 particles in human plasma and several findings have suggested it to be important for the transport of lipids and lipid hormones. Even though apoD has been shown to be involved in several physiological and pathophysiological processes, its exact role has not been elucidated.

We have recently showed that apoD mediates binding of HDL to LDL and to growing T24 carcinoma cells. By using detergent-free ELISAs and biosensor assays we could prove that both purified apoD and HDL3 very efficiently bind LDL and that apoD increases the binding of HDL to actively growing (but not to confluent) T24 cells. These recently publish data emphasizes the relevance of apoD in lipid metabolism and suggests that apoD could be of importance for lipid transfer. The aim with the current study was to investigate the influence of apoD and another lipocalin apoM on lipid transfer between lipoproteins. We also studied if apoM is promoting the interaction between lipoproteins, in a similar way as apoD.

Methods: Interactions between apoM and lipoproteins were investigated in human plasma from healthy individuals and in purified lipoproteins using monoclonal antibodies (Mabs) (from Mabtech) in ELISAs and biosensor assays. In-vitro studies were carried out in T24 and HepG2 cell-lines. ApoD, apoM and cholesterol ester transfer protein (CETP) were knocked out in T24 and HepG2 cells using CRISPR gRNA technology. Knock out responses were confirmed with Mabs and ELISAs for respectively protein and lipid transfer were investigated using CETP activity assays.

Results: By using detergent-free ELISAs we could show that immobilized Mabs to apoM could bind LDL and VLDL from fresh plasma. The findings indicate that apoM is mediating interactions of lipoproteins in a similar way as apoD, although they partly resides in different lipoprotein particles.

Conclusion: The lipocalins ApoD and apoM have unique properties as important mediators of lipoprotein interactions, emphasizing their relevance in lipid metabolism.

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Paradoxical HDL fall and its genetic determinants in atorvastatin treated South Asian Population.

Abstract nr. 873
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Co-author(s) - K.V , Vinod
Co-author(s) - Satheesh , Santhosh
Co-author(s) - Chandrasekaran , Adithan
Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Dyslipidemia, Genetics, HDL, Hypolipidemic Drugs

India leads the world with highest number of deaths due to CAD. Statins reduce CAD events by 50%. Low serum High Density Lipoprotein Cholesterol (HDL-C) is one of the factors contributing to the residual risk of CAD irrespective of Low Density Lipoprotein Cholesterol (LDL-C) levels. Statins protect against CAD by lowering LDL-C and additional mechanisms such as maintaining plaque stability and increasing HDL-C levels. All statins decrease Total cholesterol, LDL-C, and triglycerides and increase HDL-C by 5-10% but there interindividual differences in statin response with genetic factors playing a role. The aim of the study was to find the genetic variants associated with lipid lowering response to atorvastatin, being the widely used statin.

**Study subjects and methodology:** 286 newly diagnosed dyslipidemic patients (LDL-C >100 mg/dl diabetics and/or CAD patients, LDL-C > 130 mg/dl others) were recruited from the outpatient departments of medicine and cardiology of JIPMER. Patients who had discontinued atorvastatin use- for reasons other than adverse reaction/intolerance for atleast 1 month prior to the study were also included in the study and were initiated on atorvastatin therapy 10-40 mg for 6 weeks.

**Results:** There was a significant (p<0.0001) reduction in serum total cholesterol, triglycerides and LDL-C following atorvastatin treatment. A majority (66%) of the patients had an unexpected decrease in HDL-C levels following 6 weeks of atorvastatin. HDL-C increased only in 34% of the patients.

Logistic regression analysis found that atorvastatin dose of 20 mg, baseline HDL-C, Multidrug resistance protein -1 (MDR1)3435 CT genotype, Apolipoprotein E -APOE *2 CT genotype predicted HDL non responder state.

**Conclusion:** Atorvastatin treatment in dyslipidemic SouthAsian patients is associated with significant fall in HDL-C cholesterol in a majority of the patients. Atorvastatin dose, baseline HDL-C and genetic factors predicted HDL-C non responder state in our study. Whether this decrease in HDL-C will negate the benefit of atorvastatin therapy in a population vulnerable to developing CAD has to evaluated before drawing conclusion on this unexpected outcome of atorvastatin use.
Funding
This study was supported by the grant from Department of Biotechnology –Govt of India.

Key words:
South Asians, dyslipidemia, atorvastatin, HDL-C, genetics, CAD

Subdivision 2. Translational Research

Presentation Preference Oral presentation

Additional information
PROJECT OF ACTIVE SEARCH FOR PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA IN THE CZECH REPUBLIC

Abstract nr. 875
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Familial Hypercholesterolemia, Genetics

Familial hypercholesterolemia (FH) is the most common inherited metabolic disease, characterized by high LDL-cholesterol levels and premature clinical manifestation of atherosclerosis. Most of FH patients remain undiagnosed and untreated, although diagnosis based mainly on familial history and lipid examination is easily available and the efficient therapy exists. A project of active search for FH patients (MedPed) was initiated in the Czech Republic (CR) in 1998.

A network of 2 complex national centres, 15 regional adult centres, 10 regional paediatric centres and additional 35 cooperating physicians has been established. Patients' data have been inserted into the national database. An emphasis has been put on early diagnostics, FH screening in families and molecular analysis. Up to now, more than 6,100 patients fulfilling clinical criteria of FH have been evidenced, which represents 31 % of all FH patients expected to live in CR (considering FH prevalence 1:500). Pathogenic mutation in apolipoprotein B or LDL-cholesterol gene has been detected in almost 1200 out of 3250 analysed patients (36 %).

The project is helpful in early identification of high risk FH individuals. Molecular analysis is an efficient tool in search for FH, particularly among relatives in families with known mutation. More effort is still needed to further increase the number of affected persons detected within Czech FH families.

This project is supported by grant of IGA MZd CR No. NT14186. Thank to all cooperating centres and physicians, nurses and technicians.

Subdivision 5. Not applicable. Abstract matches with track d

Presentation Preference Oral presentation
Additional information
Interesterified fat induces inflammation in a higher level than its native form in LDL Receptor Knockout Mice

Abstract nr. 876
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Co-author(s) - Koike, Marcia
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Co-author(s) - Bombo, Renata de Paula Assis
Co-author(s) - Catanozi, Sergio
Co-author(s) - Gioielli, Luis Antonio
Co-author(s) - Nakandakare, Edna Regina
Co-author(s) - Lottenberg, Ana Maria

Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis, Inflammation, Lipids, Nutrition

Interestereified fat has been used as trans fat substitutes, however, the effects of these fats have not yet been completely elucidated. Thus, the aim of this work was to evaluate the effect of interestereified fats containing palmitic acid on atherosclerosis development and cytokines expression in LDLr knockout mice.

Weaning male LDLr-KO mice were randomly divided into four groups (n=15-20) fed during 16 weeks on a high fat diet (40% of energy as fat) enriched with polyunsaturated (PUFA), TRANS, palmitic (Palm), palmitic interestereified (Palm Inter). Although fats before and after interestereification presented the same fatty acid composition, a large amount of saturated fatty acids migrated to the sn-2 position of the triglycerides after interestereification. Analyses: total plasma cholesterol, lipoprotein profile, arterial lipid accumulation (oil red O), macrophage infiltration (CD68 staining) and arterial MCP-1, IL-1β and TNFα expression and IL-1β and TNFα protein content were determined.

Fats containing palmitic acid increased plasma lipids to a lesser extent than trans fat (499.2 ± 89.9 vs 526.8 ± 90.0, p>0.05), however cholesterol concentration in LDL particle was similar between both groups (265.8 ± 63.5 vs 254.1 ± 29.0, p>0.05, respectively). Regarding cholesterol concentration in HDL particles, TRANS had also the lowest result of all. As expected, TRANS induced the largest lipid accumulation and macrophage infiltration. However, Palm Inter induced the highest lesion area as compared to Palm and PUFA groups. Palm Inter elicit inflammation by IL-1β while TRANS induced by MCP-1 and TNFα mRNA as compared to all (Figure 1). Palm Inter and TRANS elicited the same inflammatory insult as observed with IL-1β (59.2 ± 6.4 vs 52.7 ± 5.7, p>0.05, respectively) and TNFα (69.5 ± 9.7 vs 48.4 ± 7.6, p>0.05, respectively) protein levels. Palmitic acid in the sn-2 position of triglyceride promotes an even higher LDL particle cholesterol
accumulation and inflammation as compared to its native form contributing to atherosclerosis development.

Figure 1. Inflammatory cytokines expression in arterial wall. Different letters indicate statistical differences by ANOVA Newman-Keuls test

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Collapsed Detection of Familial Hypercholesterolemia cases in The Netherlands following discontinuation of Central Screening Program

Abstract nr. 877
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Familial Hypercholesterolemia

Introduction
Familial hypercholesterolemia (FH) is one of the most frequent, autosomal dominant diseases in the Netherlands. After 20 years during which a nationwide screening program for FH was executed, the Ministry of Health decided to discontinue this successful program as of January 2014. Subsequently, cascade screening for FH was referred to the hospitals, coordinated by stichting LEEFH. In the present survey, we evaluated the consequences of this new, decentralized screening method to detect FH.

Methods and results
Using the centralized database for FH, we analyzed retrospectively the yearly number of new ‘index’ patients diagnosed with FH as well as the associated number of relatives screened from 2002 until 2014 in the Netherlands (Table 1). From 2001 onwards, we observed a plateauing increase in ‘index’ FH cases from 131 in 2001 to 1342 in 2013, leading to a cumulative number of FH patients detected of 27,000. Following the discontinuation of the central screening program January 2014, the incidence of DNA-confirmed FH index patients declined by 80% (266 in 2013). In parallel, the number of siblings referred for FH-DNA diagnosis following a positive family index diagnosis declined by more than 95% (from 1000-1500 to < 50 relatives).

Conclusion
The cessation of the central screening program for FH has resulted in a massive decrease in the number of new FH-index cases, with an even higher decrease in the number of relatives detected with FH. These data emphasize the need for the new foundation LEEFH to further develop successful local strategies to promote family cascade screening programs in the Netherlands.
Subdivision 4. Not applicable. Abstract matches with track c

Presentation Preference Oral presentation
Additional information
Exploring the possible relationship between P2X7-ATP and MMP9 pathways on human atherosclerotic carotid plaque

Abstract nr. 878
Author Mantione, Maria Elena, Cardiovascular Research Area, San Raffaele Scientific Institute, Milano, Italy
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Co-author(s) - Ferrara, David
Co-author(s) - Kamami, Mikel
Co-author(s) - Chiesa, Roberto
Co-author(s) - Alfieri, Ottavio
Co-author(s) - Foglieni, Chiara

Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords Atherosclerosis

ATP-activated purinergic receptor channel P2X7 may be expressed on human vessels in relation to phenotype and damage/activation state. The activation/release of MMP9, a major player in atherosclerotic plaque rupture, has been related to P2X7 in circulating cells. We hypothesize a connection between P2X7–ATP and MMPs pathways in atherosclerotic vessels.

To study P2X7 role, we developed human vascular tissue culture models of atherosclerotic carotid artery plaque (PL) and not atherosclerotic internal mammary artery (IMA) incubated in the presence/absence of a P2X7 specific antagonist, A740003 (100 mM, 24 hours). Morphology by histology, expression of P2X7, MMP9 and of aSMA, sm22, FSP1, vimentin, laminin, collagen type I, vWF, CD31, CD68 by western blot and confocal microscopy, ATP content by chemiluminescence were analyzed in PLs (n=10) and IMAs (n=5) samples. In both tissue culture extracts and supernatants, A740003 efficacy in blocking P2X7 was evaluated by IL1β ELISA. MMP9 gelatinolytic activity was semi-quantitated by densitometry on gel zymography.

P2X7 localized on macrophages, endothelial and smooth muscle cells of all PLs, was observed in traces on IMAs endothelium. MMP9 was found in both vessels, localized in the media. Incubation with A740003 did not alter the markers expression. In our settings, where IL1β quantity did not differ in vulnerable vs. unstable PLs, IL1β resulted significantly lower in extracts from PLs A740003-treated as compared to untreated (p=0.0081). No difference was measured in PL supernatants. No IL1β was detected in IMA samples. ATP content was higher in PL than in IMA extracts, was lower in PL extracts from A740003-treated with respect to untreated samples (p=0.0489), in IMA extracts, nor in all supernatants. ProMMP9 and MMP9 active gelatinolytic activity displayed a trend vs. reduction in extracts from PLs but not from IMAs treated with A740003 as compared to untreated. MMP9 release into supernatants was not affected by
incubation with P2X7 antagonist. These preliminary findings suggest that P2X7-ATP pathway, active in atherosclerotic PLs but not in IMAs, might only indirectly modulate MMP9, supporting the idea of a complex intracellular regulation of MMP synthesis/activation. This work is supported by the Italian Ministry of Health, grant number RF2010-2316010

Subdivision 1. Basic Science

Presentation Preference Oral presentation
Additional information
Does Statin Treatment exert Protective action on Polyunsaturated Phospholipids in LDL and on their Susceptibility to Oxidation? Functional effects of Statin-induced HDL3

Abstract nr. 879
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins

AIM: Atherogenic dyslipidemia and insulinoresistance frequently associate with oxidative stress. Polyunsaturated phospholipids in LDL and in HDL constitute a preferential target for oxidation, with formation of oxidized PL possessing potent proinflammatory and pro-oxidative activities. We evaluated the effect of statin treatment on content of polyunsaturated phosphatidylcholine species (PUPC) in LDL and HDL in dyslipidemic subjects, and on their susceptibility to oxidation to the corresponding hydroperoxides (PCOOH).

METHODS: Twelve insulinoresistant, hypertriglyceridemic, obese males with subnormal HDL-C levels were recruited into the CAPITAIN study. Pitavastatin treatment (4mg/day) for 180 days reduced plasma triglycerides (-41%) and LDL-C (-38%), and increased HDL-C and apoAI (+4 and +6% respectively). Density gradient ultracentrifugation allowed isolation of native plasma LDL, HDL2 and HDL3. For each subject, LDL (baseline versus 180 days) was oxidized in vitro with AAPH (2mM) alone or in the presence of the corresponding HDL2 or HDL3 at plasma mass ratios. PUPC and the corresponding PCOOH were analysed by reverse phase HPLC with UV and chemiluminescent detection.

RESULTS: Statin treatment reduced PUPC content in LDL (-23 and -21% for 16:0/22:6 and 16:0/18:2; p<0.01). In contrast, compensatory increases occurred in HDL3 (+11 and + 18% in 16:0/20:4 and 18:0/20:4 respectively; p<0.05), an effect attenuated in HDL2. Statin treatment increased oxidative consumption of 16:0/22:6, 16:0/20:4, and 16:0/18:2 PUPC in LDL by 5-17% (p<0.05); PUPC consumption was attenuated similarly by HDL2 and HDL3. Although statin treatment did not impact reduction in PCOOH formation in LDL by HDL2 or HDL3, nonetheless, the ratio of PCOOH formed to PUPC consumed was preferentially decreased by HDL3 (-15%; 16:0/22.6 + 20:4 species; p<0.05). As conjugated dienes (CD) represent total PCOOH content plus the corresponding hydroxides, (PCOH), and as CD formation was unchanged in the presence of HDL3, these findings indicate that PCOOH formed were more efficiently reduced by HDL3 versus HDL2.

CONCLUSIONS: In dyslipidemia with insulinoresistance, pitavastatin (i) reduced PUPC substrate for oxidation in LDL, thereby attenuating LDL oxidability, and (ii) induced formation of HDL3 with increased capacity to reduce PCOOH to inactive PCOH. Statin treatment therefore attenuated
production of secondary oxidation products, notably aldehydes, potentially reducing deleterious, proatherogenic LDL protein modification.

Subdivision

Presentation Preference Oral presentation
Addtional information
Exploring the role of P2X7 receptor in primary human vascular smooth muscle cells

Abstract nr. 880
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Topic The Pathogenesis of Atherosclerosis; Immunity, Inflammation, and the Endothelium
Keywords

Increasing evidences support the involvement of ATP-activated P2X7 in the cardiovascular regulation, however its expression and role in human vascular smooth muscle cells (VSMC) and in atherosclerosis have been poorly investigated. Our aim was to study P2X7 in primary VSMC from atherosclerotic/not atherosclerotic arteries, and the response to VSMC stimulation with specific P2X7 antagonist and exogenous ATP. VSMC outgrowing from carotid artery plaque (CP) or internal mammary artery (IMA) of patients submitted to carotid thrombo-endo-arterectomy or coronary artery bypass graft respectively, were cultured and characterized. Microscopy, biochemistry and cytofluorimetry methods were applied. After artery harvesting, cells spreaded from CP for up to 2 months, from IMA for up to 5 months. By western blot and confocal microscopy, VSMC from both vessels resulted aSMactin+, SM myosin heavy chain-, vimentin+, collagen type I+, laminin+, CD39low, CD68-. CP-derived cells were sm22+. IMA-spreading cells displayed traces of sm22 but were CD117+. P2X7 was expressed by VSMC from both vessels. Consistently, cryosections from IMA after 3-5 months of culture, but not from freshly collected, displayed aSMactin+, P2X7+, CD117+ outgrowing cells, suggestive of VSMC plasticity. Confocal microscopy and cytofluorimetry of VSMC from CP and IMA demonstrated P2X7 expression on the surface and into the cytoplasm, close to Golgi apparatus, never associated with lysosomes or ovalbumin-loading endosomes. Intra-cytoplasm P2X7 expression in vessel-derived VSMC increased with “aging” of the vascular fragment. VSMC incubation with P2X7 antagonist A740003 (100mM, up to 24 hours) redistributed intracellular P2X7, increased lysosome acidification (by Lysotracker), slightly altered mitochondria oxidative activity (by Mitotracker) and vimentin/aSMactin cytoskeleton, without significant changes in cell viability or in ATP content/release. No additional alteration was observed by single/repeated addition of exogenous ATP (50-200mM), independently from A740003 presence, indicating that ATP alone is not sufficient for VSMC activation.
These findings describe for the first time the expression/localization of P2X7 in primary human VSMC from not atherosclerotic IMA and atherosclerotic CP. The observed differences in VSMC in the presence/absence of P2X7 antagonist hint to a possible additional, novel function for P2X7 in vessels.

This work is supported by the Italian Ministry of Health, grant number RF2010-2316010

Subdivision 1. Basic Science

Presentation Preference Oral presentation

Additional information
Prevalence and management of Familial Hypercholesterolaemia in the EUROASPIRE IV project

Abstract nr. 881
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Co-author(s) - Ray, Kausik
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords

The prevalence of Familial Hypercholesterolaemia (FH) is estimated in the community at 1 / 200-500 persons. In patients with established coronary heart disease (CHD), the prevalence of FH is indeterminate. Purpose: The aim of this substudy of EUROASPIRE IV was to estimate the prevalence of potential FH among patients with CHD and to compare the management of these patients with that of other coronary patients. Methods: In EUROASPIRE IV, data were collected from May 2012 to April 2013 in 24 European countries by means of a standardized interview, bioclinical examination and venous blood sampling. Potential FH was estimated using an adapted version of the Dutch Lipid Clinic Network Criteria; 85.7 % of the patients were on lipid lowering drugs; untreated LDL-cholesterol was estimated using coefficients based on the class and dose of lipid lowering drugs that they currently used. Correction was made for reported non-compliance. Results: Among the 7044 patients eligible for analysis, the prevalence of potential FH was 8.3%; 7.5% in men and 11.1% in women. The prevalence was inversely related to age with a putative prevalence of 1:5 in those with CHD < 50 yrs of age in both sexes. Even among women aged 70, the prevalence was 1:10. Irrespective of age and gender, prevalence differed substantially between European regions; potential FH patients were more likely to smoke, had a lower frequency of low HDL-cholesterol levels but higher triglyceride levels and their blood pressure was less well controlled. The use of cardioprotective drugs and the prevalences of diabetes, obesity and central obesity were similar. Conclusion: The prevalence of potential FH in coronary patients is considerable; the results underscore the need to promote identification of FH in CHD patients and to improve their risk factor profile.

Funding Acknowledgements: The EUROASPIRE IV survey was carried out under the auspices of the European Society of Cardiology, EURObservational Research Programme.
Subdivision

Presentation Preference Oral presentation
Additional information
A multi-tissue eQTL approach to understanding atherosclerosis

Abstract nr. 882
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Topic Epidemiology of CVD; The Risk Factor Concept
Keywords Atherosclerosis, Cardiovascular Disease, Epidemiology, Genetics

Genome-wide association studies have linked multiple loci to cardiovascular traits. However, most such studies have been performed on a single or few tissues, and in most cases the link between the loci and the trait is not known. We have performed transcriptome profiling (RNA-seq) on more than 3600 tissue samples from 9 cardiovascular disease-related tissues, originating from 500 subjects with established atherosclerosis. All individuals were genotyped genome-wide with arrays. We then generated expression quantitative trait loci (eQTLs) using a linear model implemented in the softwares MatrixEQTL and eQtlBma. eQTL analysis using a Bayesian model revealed an unexpected amount of sharing of eQTLs across tissues, and many examples of CAD-relevant GWAS loci linked to expression of suspected and non-expected gene targets. We also observed enrichment of eQTLs for traits not previously linked to CAD. In addition, using the rich information available in RNA-seq data we generated isoform, splice and allele-specific expression QTLs. The latter categories highlighted many known GWAS variants not captured with a regular eQTL analysis.

Subdivision

Presentation Preference Electronic poster presentation
Additional information
Patient and physician perspectives on administration of the PCSK9 monoclonal antibody alirocumab, an injectable medication to lower LDL-C levels

Abstract nr. 1039
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Topic Primary & Secondary prevention of CVD; Lifestyle Measures and Pharmacological Intervention
Keywords Hypolipidemic Drugs,LDL,PCSK9, Therapy

Background: Alirocumab (monotherapy or background statin±other lipid-lowering therapies [LLTs]), has demonstrated therapeutic potential to reduce LDL-C. Many patients requiring LLT do not have injectable medications experience.

Objectives: To assess patients' and physicians' perceptions of usability and acceptance of subcutaneously injected alirocumab 75 or 150 mg (doses per Phase 3 trials; both 1mL injection volumes). Prefilled pen and prefilled syringe delivery devices were evaluated and willingness to self-inject assessed.

Methods: 400 participants (200 physicians, 200 patients) were included. Physicians (99 primary care physicians [PCPs]; 101 specialists) had mean practice experience of 17.8 years since residency and an average of 797 hypercholesterolemic patients. Patients enrolled had LDL-C levels above their goal and at least one of the following characteristics: familial hypercholesterolemia; statin intolerance; high cardiovascular risk; and/or diabetes. Mean patient age was 59.5 years; 52% were female; 25.5% had injectable medication experience.

Participants evaluated pen or syringe administration methods and tested blinded 75 and 150 mg doses*. Following device training, participants tested each dose by prosthetic pad injection (Figure 1). Data were collected via self-administered questionnaire. Safety was not assessed as no human injection occurred.

Results: Participant acceptance of both injection devices was positive with 86-100% agreeing with usability statements (Figure 2). After training with devices, physicians considered 66% (pen) and 58% (syringe) of their patients would be willing to use self-injectable alirocumab (improvement from pre-training of 22% and 16%, respectively; both p<0.05). Specialist estimates were higher than PCP estimates for both devices, before and after training (p<0.05 specialist vs PCP for all assessments). After training, 72% (pen) and 63% (syringe) of patient-participants were very willing to accept self-injectable alirocumab (an improvement from pre-training of 26% [p<0.05] and 11%, respectively).

Initially, patients with previous injectable medication experience were generally more willing to use the pen than injection-naïve patients; however, after training there was no difference. Participant responses were similar irrespective of the dose tested.
Conclusion: Acceptance of alirocumab prefilled pen and syringe devices was generally positive. Devices were considered easy to operate, with most patients willing to use and accept self-injection, particularly after training.

Funding: Sanofi and Regeneron Pharmaceuticals, Inc.
Physician and Patient Usability Statements Tested (Agree Disagree)
Subdivision

Presentation Preference Oral presentation
Additional information
4.5-Year Safety and Efficacy of Mipomersen in Patients With Severe Familial Hypercholesterolemia Uncontrolled by Maximally Tolerated Lipid-Lowering Therapy

Abstract nr. 1040
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Topic Dyslipidemia; Lipids, Lipoproteins and Apolipoproteins
Keywords Apolipoproteins, Familial Hypercholesterolemia, Hypolipidemic Drugs, Lipoproteins

Background: Mipomersen, a second-generation antisense oligonucleotide, blocks translation of apoliprotein B (apo B) mRNA and decreases hepatic synthesis of apo B. In randomized, double-blind, phase 3 studies in patients with familial hypercholesterolemia (FH) taking maximally tolerated lipid-lowering therapy (LLT), mipomersen significantly reduced levels of all atherogenic lipoproteins, including very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL).

Objective: To evaluate long-term safety and efficacy of mipomersen in an open-label extension (OLE) study (NCT00694109).

Methods: Patients with FH taking maximally tolerated LLT who had completed a 6-month phase 3 study participated in this extension study during which they received weekly subcutaneous mipomersen (98% received 200 mg/wk) for up to a total of 4.5 years.

Results: At OLE baseline: mean age was 49 years; 60% of patients were male; 30% had metabolic syndrome; 72% were taking a maximal dose of LLT. Among 141 patients treated in the OLE, 89 completed at least 52 weeks of OLE treatment; 25 completed 208 weeks (4 years) of OLE treatment; 117 returned for follow-up laboratory testing 24 weeks after their final dose of mipomersen. Over 4.5 years of mipomersen treatment, persistent reductions in LDL cholesterol (LDL-C) and apo B levels were observed, as were sustained reductions in lipoprotein(a). Hepatic fat assessed by magnetic resonance imaging increased in some patients, but tended to stabilize or decrease during continued treatment over 4.5 years. Consecutive alanine aminotransferase elevations ≥3x ULN ≥7 days apart did not occur after 2 years of treatment; a small minority of patients had increases during the first 24 months of treatment. Data on flu-like symptoms and injection site reactions are consistent with previously reported information for mipomersen.

Summary/Conclusions: Mipomersen treatment for up to 4.5 years resulted in sustained reductions in atherogenic lipoproteins with no new safety concerns in patients with refractory FH. Efficacy was comparable to findings in phase 3 studies. Hepatic fat stabilized or decreased after the first year. These data support the longer term benefit: risk profile of mipomersen in high-risk FH
patients and the potential viability of sustained treatment with mipomersen in this population. Supported by Genzyme, A Sanofi Company, Cambridge, MA

<table>
<thead>
<tr>
<th>Week</th>
<th>n</th>
<th>LDL C (mg/dL) (SD)</th>
<th>%Change From Baseline (95% CI)</th>
<th>apo B (mg/dL) (SD)</th>
<th>%Change From Baseline (95% CI)</th>
<th>n</th>
<th>Mean Change in % Hepatic Fat (95% CI)</th>
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</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>141</td>
<td>233 (147)</td>
<td>175 (81)</td>
<td>26</td>
<td>130</td>
<td>165 (118)</td>
<td>-28 (-32, -25)</td>
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<tr>
<td>52</td>
<td>111</td>
<td>168 (122)</td>
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<td>126 (71)</td>
<td>-28 (-32, -24)</td>
<td>31</td>
<td>12.50 (8.60, 16.41)</td>
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<td>76</td>
<td>66</td>
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<td>-27 (-33, -22)</td>
<td>110 (58)</td>
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<td>9.34 (6.62, 12.05)</td>
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<td>104</td>
<td>57</td>
<td>117 (55)</td>
<td>-28 (-34, -22)</td>
<td>93 (32)</td>
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<td>7.93 (5.39, 10.48)</td>
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<td>42</td>
<td>138 (85)</td>
<td>-22 (-31, -13)</td>
<td>108 (57)*</td>
<td>-29 (-36, -23)</td>
<td>23</td>
<td>8.33 (4.89, 12.37)</td>
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<td>30</td>
<td>125 (43)</td>
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<td>123 (51)</td>
<td>-24 (-37, -11)</td>
<td>95 (33)</td>
<td>-31 (-40, -22)</td>
<td>21</td>
<td>9.52 (5.71, 14.00)</td>
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<td>234</td>
<td>17</td>
<td>126 (35)</td>
<td>-22 (-34, -11)</td>
<td>96 (24)</td>
<td>-31 (-39, -24)</td>
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<td>24 wks postdose</td>
<td>117</td>
<td>230 (146)</td>
<td>1.6 (-3, 6)</td>
<td>167 (79)</td>
<td>-3.5 (-7, 0)</td>
<td>42</td>
<td>0.76 (0.67, 2.19)</td>
</tr>
</tbody>
</table>

**Subdivision**

**Presentation Preference** Oral presentation

**Additional information**