The Apolipoprotein C-III Story: Epidemiology, metabolism, and basic science lead to treatment

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ApoB Lipoproteins Have Many Small Apolipoproteins

Small apolipoproteins modulate the metabolism of Chylomicrons, VLDL and LDL
Apo B Lipoprotein Speciation: Based on Presence of Apolipoproteins

ApoC-III and apoE Are Present on some VLDL and LDL particles

- LDL without apoCIII: 85-90% LDL apoB
- LDL with apoCIII: 7-10% LDL apoB
- LDL with apoCIII and apoE: 3-5% LDL apoB
ApoC-III in SDS Micelles:
79 amino acids; 6 amphipathic helices with semi-flexible hinges, negative polar faces

Similarity to LDL receptor binding lysine motifs of apoE, apoB, and RAP.

Causes binding of VLDL and LDL to proteoglycans (Chait; Boren)

Gangabadage..Wijmenga. JBC 2008;283:17456
Apolipoprotein C-III: Associated with CHD

- Apo CIII in apoB lipoproteins (LpB:CIII) associated with progression of coronary atherosclerosis (Blankenhorn, Alaupovic et al. 1990; Hodis 1994)
- “LpB:CIII” strongest lipoprotein association with progression of coronary atherosclerosis in statin-treated patients, LDL=82 mg/dl (Alaupovic 1997)
- Apo CIII in apoB LP associated with CHD in case-control studies (Chivot..Fruchart 1990; Luc..1996).
**Triglycerides, VLDL-Apo B and Apo CIII in VLDL and LDL: Risk Factors for Coronary Events**

N=418 cases of MI or CHD Death: Multivariable RR

LDL particles with apoCIII: Strongest ApoB Lipoprotein Predictor of Recurrent Cardiovascular Events in Diabetes: CARE trial

* without apoCIII, RR=2.2, P=0.07
** with apoCIII, RR=6.6, P < .0001

LDL Particles with apoC-III: Strongest ApoB Lipoprotein Predictor of First Coronary Event in US Men and Women

749 cases of first MI or coronary death

Apolipoprotein B in LDL without apolipoprotein C-III, P for trend=0.81
Apolipoprotein B in LDL with apolipoprotein C-III, P for trend<0.001
Apolipoprotein C-III in LDL, P for trend=0.28
Apolipoprotein B in LDL with apolipoprotein C-III, P for trend=0.029

ApoB Concentrations of VLDL or LDL with apoC-III predict CHD more strongly than apoC-III concentrations

ApoB of LDL with apoC-III: 5th to 1st quint
Men RR = 2.22*; Women RR = 2.43*

ApoC-III in LDL
Men RR = 1.94*; Women RR = 2.26 (ns)

ApoB of VLDL with apoC-III
Men RR = 1.79*; Women RR = 1.85 (ns)

ApoC-III in VLDL
Men RR = 1.49 (ns); Women RR = 1.15 (ns)

Total CIII: Men RR = 1.53*; Women RR = 1.22 (ns)

* P<0.05  Mendivil, Rimm, Furtado, Sacks. Circulation 2011
ApoE on VLDL and LDL Lessens the Risk Associated with ApoC-III

Mendivil C O et al. J Am Heart Assoc 2013;2:e000130
Omega-3 Free Fatty Acids (EPA, DHA; Epanova) increase selectively the apoB concentration of LDL without apoCIII

<table>
<thead>
<tr>
<th>mg/dL</th>
<th>apoB in LDL without apoCIII</th>
<th>apoB in LDL with apoCIII</th>
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<tr>
<td></td>
<td>2g</td>
<td>4g</td>
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<tr>
<td>0,0</td>
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<td>0,2 (p=0.6)</td>
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Morton A…..Sacks F. Presented at ISA 2015
ApoC-III: Mechanistic Studies in Humans

Can we connect the findings on CHD risk to metabolic heterogeneity of the lipoprotein types?
• Secretion rate of apoC-III high in obesity, insulin resistance, hyperTG
• High secretion rate of apoC-III correlated with high secretion rate of VLDL-apoB in these conditions

Watts G, Barrett PH, Ooi E, Chan D: U. Western Australia, Perth
Apolipoprotein C-III and the Metabolic Basis for Hypertriglyceridemia and the Dense LDL Phenotype

Chunyu Zheng
Christina Khoo
Jeremy Furtado
Frank M. Sacks

Circulation 2010;121:1722-34
The Liver Secretes All Sizes of ApoB Lipoproteins, and Subspecies with ApoC-III and ApoE

Sacks FM. Curr Opinion Lipidol 2015
Apolipoprotein C-III and E: A High Carbohydrate Diet Impairs Metabolism Similar to Hypertriglycerideridemia

VLDL E-CIII+
Large TG-rich nascent particle

Dense LDL CIII+
TG-rich remnant

CIII to HDL

VL DL, IDL E+CIII+

Out

Dense LDL CIII-
Major LDL Type

Hypertriglyceridemia or High Carb Diet
Integral Involvement of ApoC-III in Establishing the Dense LDL Phenotype

High secretion of VLDL containing just apoC-III. Most converted to dense LDL. Slow clearance of dense LDL.

Low secretion of VLDL containing apoE and apoC-III

Zheng C…Sacks FM. Circulation 2010; Am J Clin Nutr 2008;88:272
Normal TG

Integral Involvement of ApoE in Normal TG-Rich Lipoprotein Metabolism:

- High secretion of light and medium size LDL E-CIII-; fast clearance of dense LDL.
- Low secretion of VLDL containing just apoC-III.
- High secretion of VLDL containing apoE and apoC-III; Clearance of apoE rich VLDL before it forms LDL.

ApoC-III Principal Mechanism: Inhibition of Clearance of ApoB Lipoproteins

- Protein conformation mimics apoB and apoE
- ApoC-III overexpression in mice: Blocks clearance (lipolysis normal)
- VLDL and LDL with apoC-III: Minimal clearance in humans (fast lipolysis)
- Antagonizes apoE mediated clearance in humans, animal models, cell culture
- Antisense effects: Effective in LpL deficiency

Sacks FM. Curr Opinion Lipidol 2015
ApoC-III: Inhibitor of Lipoprotein Lipase?

- Transgenic human apoC-III mice
  - Low/moderate expressors: normal lipolysis
  - High expressors: inhibited lipolysis
- ApoC-III to C-II ratio
  - In vitro to inhibit LpL = 10 – 100
  - In normal human VLDL with apoC-III = 1
- At high ratio of apoC-III/C-II, C-III displaces LpL from lipid droplet, causing it to be inactivated by angptl2
- Normal lipolytic conversion rates of VLDL with C-III to LDL in humans in vivo
- Human apoA-I/C-III deficiency: Rapid conversion of VLDL to LDL; no inhibition of LpL in vitro

Sacks FM. Curr Opinion Lipidol 2015
Why Hypothesize That ApoC-III Has Direct Effects on Vascular Cells?

ApoC-III containing VLDL and LDL has a strong relation to CVD compared with its low plasma concentration.
Atherogenicity of apoC-III

- VLDL, LDL high apoCIII
- TLR2
- LOX-1
- TNFα, IL1β
- β1integrin
- ligands (VCAM-1)

Monocytes, Macrophages

Endothelial cells

Circulation 2006;113:691; 114:681; ATVB 2007;27:219
ApoCIII induces VCAM-1 accumulation *in vivo*: reduction by pitavastatin

Zheng C…Aikawa M. Eur Heart J 2013
Apolipoprotein C-III in HDL: Recurrent Coronary Events (CARE trial)

- ApoC-III concentration in HDL
  - Relative risk = 1.3 (0.8,2.0) for 5\textsuperscript{th} vs 1\textsuperscript{st} quintile, 8 vs 3 mg/dL
- ApoC-III to apoA-I ratio in HDL
  - Relative risk = 1.6 (1.0,2.5), p=0.05, for 0.4 vs 0.15 mol/mol

HDL with Apo C-III is Associated with Coronary Heart Disease in Turkey

Onat et al. Athero 2003;168:81

**Men**

<table>
<thead>
<tr>
<th>HDL apo C-III mg/dl</th>
<th>OR for CHD</th>
<th>P-value</th>
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<tr>
<td>&lt;4.4</td>
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P<.05

P<.10

Onat et al. Athero 2003;168:81
HDL with apoC-III Predicts *Increased* CHD: US Nurses and Other Health Professionals: 714 with First MI or Coronary Death

Quintiles of HDL-C

| HDL-C with CIII | P = 0.0005 |
| HDL-C without CIII | P = 0.0001 |

HDL in humans with cardiovascular disease exhibits a proteomic signature

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ARTICLE INFO

Article history:
Received 14 January 2010
Received in revised form 9 March 2010
Accepted 15 March 2010
Available online 20 March 2010

Keywords:
Cardiovascular risk score
Inflammation
Mass spectrometry
Oxidized HDL
Partial least squares discriminant analysis

ABSTRACT

Background: Alterations in protein composition and oxidative damage of high density lipoprotein (HDL) have been proposed to impair the cardioprotective properties of HDL. We tested whether relative levels of proteins in HDL2 could be used as biomarkers for coronary artery disease (CAD).

Methods: Twenty control and eighteen CAD subjects matched for HDL-cholesterol, age, and sex were studied. HDL2 isolated from plasma was digested with trypsin and analyzed by high-resolution matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) and pattern recognition analysis.

Results: Partial least squares discriminant analysis (PLS-DA) of mass spectra clearly differentiated CAD from control subjects with area under the receiver operating characteristic curve (ROC_AUC) of 0.94. Targeted tandem mass spectrometric analysis of the model’s significant features revealed that HDL2 of CAD subjects contained oxidized methionine residues of apolipoprotein A-1 and elevated levels of apolipoprotein C-III. A proteomic signature composed of MALDI-MS signals from apoA-1, apoC-III, Lp(a) and apoC-I accurately classified CAD and control subjects (ROC_AUC = 0.82).

Conclusions: HDL2 of CAD subjects carries a distinct protein cargo and that protein oxidation helps generate dysfunctional HDL. Moreover, models based on selected identified peptides in MALDI-TOF mass spectra of the HDL may have diagnostic potential.

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HDL Particles with ApoCIII Do Not Inhibit Monocytic Cell Adhesion to Endothelial Cells

Circulation 2006;113:691
Obese Have Significantly More HDL with apoC-III and apoE; and less HDL without apoC-III or apoE

Normal Weight Group

Obese Group

Talayero B … Sacks F. Presented, AHA Nov. 2012
Treatments That Reduce ApoCIII Containing Lipoproteins

- Fibrates (25-35%) [Ooi, Alaupovic et al. ATVB1997]
- Statins (15-25%) [Dallinga-Thie et al. Diab Care 2004; Lamendola..Reaven et al Am J Cardiol 2005; Sacks et al Am J Cardiol 2002]
- Testosterone in postmenopausal women (62% for apoCIII in VLDL, 35% for apoCIII in LDL) [Chiuve..Sacks. J Clin Endocrinol Metab 2004)]
- Reduced carbohydrate diets, especially diets enriched in protein or unsaturated fat [Furtado..Sacks et al. AJCN2008]
- Weight loss with a range of macronutrient contents
- Mipomersen, an apoB antisense oligonucleotide (Furtado J..Sacks FM. J Lipid Res 2012)
ApoC3Rx Antisense Lowers apoC-III and TG in Normolipidemic People

Graham M, Circ Res 2013
Summary

- ApoC-III concentration in apoB lipoproteins predicts CHD.
- ApoB concentration of LDL with apoC-III is especially strong predictor.
- Causality
  - Adverse effects on apoB metabolism and vascular cells
  - Genetic (Mendelian randomization) studies
  - Antisense drug effects
- Concentration of HDL with apoC-III predicts increased CHD. Possible dysfunctional subspecies.
- Treatment to reduce apoC-III has potential to reduce CHD, especially in hyperTG
ApoC-III and Diabetes
Diabetic Medicine 2009; 26: 981–988

Original Article: Metabolism

Serum apolipoprotein C-III in high-density lipoprotein: a key diabetogenic risk factor in Turks

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Turkish Society of Cardiology, *Department of Cardiology, Cerrahpaşa Medical Faculty, Istanbul University, †Yıldız Technical University, ‡Ersek Cardiovascular Surgery Center, §Kartal Koçüyol Hospital, Istanbul, ¶Department of Cardiology, Centennial University, Van and **Department of Public Health, Cerrahpaşa Medical Faculty, Istanbul University, Turkey

Accepted 17 July 2009

Abstract

Aims We studied determinants of serum apolipoprotein C-III (apoC-III) and whether levels of apoC-III or its fractions predict metabolic syndrome (MetS), Type 2 diabetes and coronary heart disease (CHD).

Methods The predictive value of apoC-III, measured by immunoturbimetric immunoassay in 802 tracked individuals of a Turkish general population in determining cardiometabolic risk was assessed over 4.4 ± 1.2 years’ follow-up. Patients with MetS, Type 2 diabetes and CHD at baseline were excluded.

Results Total apoC-III, as well as both fractions, was significantly, linearly and inversely related to smoking status, positively to alcohol usage and to levels of complement C3. Mid and high tertiles of total or non-high density lipoprotein (HDL) apoC-III predicted significantly and independently incident MetS; they predicted CHD with risk ratios of 1.6 [95% confidence intervals (CI) 1.02–2.5], for 1 sd increment, after adjustments that included HDL cholesterol and body mass index (BMI). The highest tertile of HDL apoC-III was a major independent predictor of new-onset diabetes with a 2.5-fold risk ratio for 1 sd increment (95% CI 1.5–4.0) in combined sexes, after adjustment for waist circumference, HDL cholesterol and other confounders and was a better predictor than waist girth.

Conclusions Serum total apoC-III or its fractions are linearly and inversely associated with smoking, positively with alcohol usage and serum complement C3. The presumably dysfunctional HDL apoC-III is a stronger predictor of Type 2 diabetes than waist girth in Turks. Non-HDL apoC-III predicts strongly the development of MetS as well as incident CHD, independent of HDL cholesterol, BMI and non-lipid factors. The atherogenicity of apoC-III and dysfunctionality of HDL apoC-III carry huge public health implications in Turks.


Keywords apolipoprotein C-III, cigarette smoking, coronary heart disease, diabetes Type 2, high-density lipoprotein

Abbreviations apo, apolipoprotein; BMI, body mass index; CHD, coronary heart disease; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MetS, metabolic syndrome; TRLs, triglyceride-rich lipoproteins; VLDL, very-low-density lipoprotein
ISIS ApoC-III-Rx Antisense Inhibition of apoC-III: Decreases in Glycated Albumin, Fructosamine, and HbA1c

R. Crooke, ISIS Pharmaceuticals, Presented at AHA ATVB Sessions, May 1, 2014
Apolipoprotein CIII links islet insulin resistance to β-cell failure in diabetes

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Edited by Solomon H. Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved April 14, 2015 (received for review December 12, 2014)

Insulin resistance and β-cell failure are the major defects in type 2 diabetes mellitus. However, the molecular mechanisms linking these two defects remain unknown. Elevated levels of apolipoprotein CIII (apoCIII) are associated not only with insulin resistance but also with cardiovascular disorders and inflammation. We now demonstrate that local apoCIII production is connected to pancreatic islet insulin resistance and β-cell failure. An increase in islet apoCIII causes promotion of a local inflammatory milieu, increased mitochondrial metabolism, deranged regulation of β-cell cytoplasmic free Ca2+ concentration ([Ca2+]i) and apoptosis. Decreasing apoCIII in vivo results in improved glucose tolerance, and pancreatic apoCIII knockout islets transplanted into diabetic mice, with high systemic levels of the apolipoprotein, demonstrate a normal [Ca2+]i response pattern and no hallmarks of inflammation. Hence, under conditions of islet insulin resistance, locally produced apoCIII is an important diabetogenic factor involved in impairment of β-cell function and may thus constitute a novel target for the treatment of type 2 diabetes mellitus.

We now show that apoCIII serves as a link between insulin resistance and β-cell failure in T2DM. The mechanistic explanation is that specific insulin resistance within the pancreatic islet leads to local expression of apoCIII, resulting in an autocrine negative feedback loop for β-cell function and survival.

Results

Insulin Signaling in Ob/Ob Islets. The obese/obese (ob/ob) mouse is known to progressively develop insulin resistance and a transient hyperglycemia (14). Using this mouse model at the age of 4–12 wk, we found that blood glucose and body weight increased progressively (Fig. 1 A and B) and that nonfasting insulin levels remained unchanged higher than in control animals (Fig. 1C). We determined the mRNA expression levels of glucokinase (gck), insulin-receptor substrate 1 (irs1), insulin-receptor substrate 2 (irs2), vesicle-associated membrane protein 2 (vamp2), synaptoosomal-associated protein 25 (snap25), and ras-related protein 27a (rab27a), genes that have been demonstrated to be controlled by insulin receptor (IR)-activated phosphatidylinositol-3-kinase (PI3K)