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

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Disclosures

- Inventor on patent to Harvard U. on methods to measure and use apoC3.
- Consultant: Lilly, Amgen, ISIS, Pfizer
ApoB Lipoproteins Have Many Small Apolipoproteins

Small apolipoproteins modulate the metabolism of Chylomicrons, VLDL and LDL
Apolipoprotein CIII and E

Present on some VLDL, LDL, and HDL particles

LDL without apoCIII

LDL with apoCIII

LDL with apoCIII and apoE
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).
• Do prospective studies confirm high risk of apoCIII?
Triglycerides, VLDL-Apo B and Apo CIII in VLDL and LDL: Risk Factors for Coronary Events

N=418 cases of MI or CHD Death

**LDL particles with apoCIII: Risk of Recurrent Cardiovascular Events in Diabetes: CARE trial**

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

LDL with apoC-III and LDL without apoC-III: First coronary event in US Men and Women

A

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

749 cases of first MI or coronary death

LDL subpopulations according to contents of apoE and apoC-III

Mendivil CO et al. J Am Heart Assoc 2013;2:e000130
ApoE on VLDL and LDL Lessens the Risk Associated with ApoC-III

Mendivil C O et al. J Am Heart Assoc 2013;2:e000130
ApoC-III: Mechanistic Studies in Humans

Can we connect the findings on CHD risk to metabolic diversity of the lipoprotein types?
The Liver Secretes All Sizes of ApoB Lipoproteins, and Subspecies with ApoC-III and ApoE

Sacks FM. Curr Opinion Lipidol 2015
Complexity of VLDL and LDL Metabolism in Plasma

ApoB Secretion (11 mg·kg⁻¹·day⁻¹)

Zheng C., Sacks F. JLR 2007;48:1190
Apolipoprotein C-III and E: A High Carbohydrate Diet and Hypertriglyceridemia Impair VLDL Metabolism

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.

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

Why Hypothesize That ApoC-III Has Direct Effects on Vascular Cells?

ApoC-III containing VLDL and LDL have an outsized relation to CVD compared with its low plasma concentration
Atherogenicity of apoC-III

VLDL, LDL high apoCIII

Monocytes, Macrophages

Endothelial cells

ApoCIII-induces VCAM-1 accumulation in vivo: reduction by pitavastatin

Zheng C…Aikawa M. Eur Heart J 2013
HDL with ApoC-III: A Subspecies With an Adverse Relation to CHD and Metabolism
HDL Particles with ApoCIII Do Not Inhibit Monocytic Cell Adhesion to Endothelial Cells

*P<0.05

Circulation 2006;113:691
Apolipoprotein C-III in HDL: Recurrent Coronary Events (CARE trial)

- ApoC-III concentration in HDL
  - Relative risk = 1.3 (0.8, 2.0) for 5th vs 1st 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

Men

Women

HDL apo C-III mg/dl

Onat …Assmann et al. Athero 2003;168:81
Nurses and Other Health Professionals: Risk of First MI for HDL-C with and without apoCIII: 714 with First MI or Coronary Death

HDL-C with CIII
P trend = 0.0005

HDL-C without CIII
P trend = 0.0001

ApoC-III in HDL Is a Component of a Proteomic Signature for CVD

HDL in humans with cardiovascular disease exhibits a proteomic signature

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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 (ROCAUC) 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-I and elevated levels of apolipoprotein C-III. A proteomic signature composed of MALDI-MS signals from apoA-I, apoC-III, Lp(a) and apoC-I accurately classified CAD and control subjects (ROCAUC = 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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Obese Have Significantly More HDL with apoC-III and apoE; and less HDL without apoC-III or apoE

Talayero B, Sacks F. J Lipid Res 2014
ApoC3 Null Mutation Protects Against Coronary Atherosclerosis: Carriers vs noncarriers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Control</th>
<th>Carriers</th>
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<tr>
<td>ApoC-III</td>
<td>50% of control</td>
<td></td>
<td></td>
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<tr>
<td>TG</td>
<td>31 vs 57 mg/dl*</td>
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<tr>
<td>LDL-C</td>
<td>116 vs 140 mg/dl*</td>
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<tr>
<td>HDL-C</td>
<td>67 vs 55* mg/dl*</td>
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<tr>
<td>Coronary Ca+</td>
<td>34% vs 54%*</td>
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Pollin et al. Science 2008;322:1702
ApoC3 Variants Associated with Reduced TG Levels

Figure 2. Mean Plasma Levels of Nonfasting Triglycerides as a Function of APOC3 Genotype.

Jorgensen et al. NEJM 2014
ApoC3 Variants Associated with Reduced Incidence of Vascular Disease

Jorgensen et al. NEJM 2014
ApoC3 Variants Associated with Reduced Incidence of Vascular Disease

Figure 2. Association of APOC3 Loss-of-Function Mutations with Risk of Coronary Heart Disease among 110,970 Participants in 15 Studies.

Kathiresan et al. NEJM 2014
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)
- Anti-apoC-III antisense oligonucleotide (ISIS)
ISIS-ApoC3Rx Lowers apoC3 and TG in Healthy Participants

Graham M, Circ Res 2013
ISIS-ApoC3Rx Lowers apoC3 and TG in Familial Chylomicronemia: LpL Deficiency

ApoC-III: Putting It Together

• Total and apoB lipoprotein apoC-III predicts CHD.
• Even more, apoB concentration of LDL with apoC-III is especially strong, independent predictor.
• Adverse effects of apoC-III on apoB metabolism and vascular cells, and Mendelian randomization studies provide a strong case for causality.
• ApoA-I concentration of HDL with apoC-III predicts increased CHD. Possible dysfunctional subspecies.
• Treatment to reduce apoC3 has potential to reduce CHD, especially in hyperTG
ISIS-APOCIII$_{Rx}$ Treatment Significantly Reduced ApoC-III

Mean % Change in Fasting ApoC-III

R. Crooke, ISIS Pharmaceuticals, Presented at AHA ATVB Sessions, May 1, 2014
ISIS-APOCIII\(_{Rx}\) Treatment Significantly Reduced Triglycerides

Mean % Change in Fasting Triglycerides

R. Crooke, ISIS Pharmaceuticals, Presented at AHA ATVB Sessions, May 1, 2014
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