The Role of Apolipoprotein CIII in Coronary Artery Disease

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Disclosures

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• Consulting:
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  • Merck, Kowa, National Lipid Association, Med Learning Group

Topic Outline:

Triglycerides over 200 & HDL-C <40 mg/dL is a common finding in our patients:
• Major and common risk factor for CVD
• The triglyceride elevation is due to increased VLDL remnants
• Most cases are of polygenic in origin.
• Three specific proteins are important in remnant clearance: ApoE, HTGL and apoCIII
• Plasma triglycerides and apoCIII track very closely.
Topic Outline:

- Risk is reduced when apoCIII concentration or functionality is reduced by genetic variants
- Major genes reducing apoCIII are uncommon but impactful on apoCIII concentration and CAD
- Risk persists after statin treatment
- Fibrates and EPA reduce apoCIII and CAD
- New therapies that suppress apoCIII have profound effects in hypertriglyceridemic states.
- Interventional trials with vascular endpoints are needed in those with TG> 200 and low HDL-C.

Prevalence of Elevated TG in US Ads

**NHANES (1999-2008)**

<table>
<thead>
<tr>
<th>20+ yrs</th>
<th>&gt; 150</th>
<th>&gt; 200</th>
<th>&gt; 500</th>
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<tbody>
<tr>
<td>Overall</td>
<td>31%</td>
<td>16%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Men</td>
<td>35%</td>
<td>20%</td>
<td>1.8%</td>
</tr>
<tr>
<td>Women</td>
<td>27%</td>
<td>13%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Heritage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican</td>
<td>35%</td>
<td>20%</td>
<td>1.4%</td>
</tr>
<tr>
<td>African</td>
<td>16%</td>
<td>8%</td>
<td>0.4%</td>
</tr>
<tr>
<td>European</td>
<td>33%</td>
<td>18%</td>
<td>1.1%</td>
</tr>
</tbody>
</table>


Apolipoprotein CIII

From Teaching Slides by Emilita Breyer. Georgia State University
Does altering the content of apoCIII alter the metabolism of triglyceride rich lipoproteins?

- ApoCIII transgenic mice develop severe hypertriglyceridemia with a gene dose response.
- ApoCIII KO mice have profound reductions in plasma triglycerides with clearance rates 4-5X normal.
- Familial apoCIII deficiency is associated with TG concentrations in the 20 to 50 Mg/dl range and clearance rates of plasma TG 4-5X normal.
Do plasma concentrations of ApoC-III relate to vascular disease?

- **CLAS**: Individuals with the most regression had most apoC-III in HDL. (Circulation 81: 470; 1990)

- **MARS**: apoC-III in VLDL+LDL is strong predictor of progression in spite of lipid-lowering therapy. (Circulation 90: 42; 1994)

- **ECTIM**: In univariate analysis apoCIII-LpB was significantly higher and the ratio of apoCIII-Lp nonB/apoCIII-LpB significantly lower in MI survivors. (JLR 37: 508; 1996)

- **CARE**: Comparing the highest to the lowest quintile for apoC-III in (VLDL+LDL), there was 2.3-fold increase in relative risk. (Circulation 102: 1886; 2000)

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**The TG and HDL Working Group of the Exome Sequencing Project (NHLBI)**

(STUDIES: Atherosclerosis in Communities, Coronary Artery Risk Development in Young Adults, Cardiovascular Health Study, Framingham Heart Study, Jackson Heart Study, Multiethnic Study of Atherosclerosis Women’s Health Initiative and the Myocardial Infarction Genetics Consortium.)

TOTAL COHORT: 110,970

(Sample of 3734 screened for 18,666 genes and related to plasma TG.)

Single gene dysfunctional mutations most commonly associated with:

- **Elevated triglycerides**: ApoA5
- **Reduced triglycerides**: ApoC3

[R19X, IVS2+1G→A, A43T, IVS3+1G→T]

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**GWAS Studies of ApoCIII**

- **Exome Sequencing Project, NHLBI**

  (Working Group: NHLBI 2014; 37:22-31)

  1. Screened 110,970 individuals for apoCIII gene variants associated with low TG
  2. Four found: nonsense mutation, two splice-site mutations and a missense mutation
  3. Prevalence of at least one of these mutations was 1/150 participants.
  4. Mean TG concentrations reduced by 39% and apoCIII concentration by 46%.
  5. CAD disease prevalence was 40% lower in 498 carriers of any of these mutations than the risk among 110,472 noncarriers.

- Genes involved: R19X, IVS2-1G→A, A43T and IVS3+1G→T
GWAS Studies of ApoCIII

- Copenhagen City Heart and the Copenhagen General Population Studies (Jørgensen et al., et al. And Tjønn-Hansen. NEJM 2014; 371:32 – 41)
- Screened 75,725 individuals for apoCIII gene variants associated with low TG.
- Of three mutations identified, at the same sites as the NHLBI findings.
- Prevalence of heterozygosity for any one of these was 1/300 members of the populations.
- Mean TG concentrations in those heterozygous for these variants was 44% less.
- CAD disease prevalence in 260 carrier was reduced by 41% compared to the remainder.

Genes involved: R19X, IVS2=G→A, A43T

A Null Mutation in Human APOC3 Confers a Favorable Plasma Lipid Profile and Apparent Cardioprotection*


The Amish in Pennsylvania have a prevalence of the R19X mutation in the apoC3 gene of approximately 5%.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>183±46</td>
<td>193±35</td>
<td>0.09</td>
<td>183±46</td>
<td>193±35</td>
<td>0.09</td>
<td>183±46</td>
<td>193±35</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>57±42 (91)</td>
<td>19 (25 – 48)</td>
<td>4.1×10−4</td>
<td>57±42 (91)</td>
<td>19 (25 – 48)</td>
<td>4.1×10−4</td>
<td>57±42 (91)</td>
<td>19 (25 – 48)</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>55±13.9</td>
<td>67±17.3</td>
<td>9.0×10−7</td>
<td>55±13.9</td>
<td>67±17.3</td>
<td>9.0×10−7</td>
<td>55±13.9</td>
<td>67±17.3</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>13±10 (18)</td>
<td>18 (14 – 27)</td>
<td>7.0×10−7</td>
<td>13±10 (18)</td>
<td>18 (14 – 27)</td>
<td>7.0×10−7</td>
<td>13±10 (18)</td>
<td>18 (14 – 27)</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>41±7.4</td>
<td>46±9.2</td>
<td>3.0×10−7</td>
<td>41±7.4</td>
<td>46±9.2</td>
<td>3.0×10−7</td>
<td>41±7.4</td>
<td>46±9.2</td>
</tr>
<tr>
<td>Non-HDL Cholesterol (mg/dl)</td>
<td>139±43</td>
<td>132±29</td>
<td>0.0005</td>
<td>139±43</td>
<td>132±29</td>
<td>0.0005</td>
<td>139±43</td>
<td>132±29</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>145±43</td>
<td>116±32</td>
<td>0.001</td>
<td>145±43</td>
<td>116±32</td>
<td>0.001</td>
<td>145±43</td>
<td>116±32</td>
</tr>
</tbody>
</table>

Questions regarding apo CIII.

1. What is the functional value?
2. How does it regulate triglyceride metabolism?
3. How do levels of apoCIII change HDL and LDL?
4. Why do higher levels increase CVD?
5. How can we change apoCIII for potential benefit?
6. Will changing it prevent CVD?

SUMMARY OF APOLIPOPROTEINS AND FUNCTION--PART II

The Plasma Apolipoproteins

<table>
<thead>
<tr>
<th>Name</th>
<th>Lipoprotein</th>
<th>Molecular Weight</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>apo-CI</td>
<td>HDL, chylomicrons, VLDL</td>
<td>8,000</td>
<td>Activator of LCAT</td>
</tr>
<tr>
<td>apo-CII</td>
<td>HDL, chylomicrons, VLDL</td>
<td>9,000</td>
<td>Activator of lipoprotein lipase</td>
</tr>
<tr>
<td>apo-CIII</td>
<td>HDL, chylomicrons, VLDL</td>
<td>9,000</td>
<td>Stabilizes surface and provides negative charge</td>
</tr>
<tr>
<td>apo-D</td>
<td>HDL, chylomicrons*</td>
<td>21,000</td>
<td>Cholesterol ester exchange</td>
</tr>
<tr>
<td>apo-E</td>
<td>VLDL, chylomicrons*</td>
<td>34,000</td>
<td>Binds to receptor on cell membrane of liver (E and BE) and macrophage</td>
</tr>
</tbody>
</table>

*Only in nascent chylomicrons

Fig. 5. Sephadex G-200 chromatography of the Tri-Triton-soluble protein fraction of VLDL lipoproteins. The electrophoretic mobility is indicated by † in Fig. 4. The elution volume of apo-CIII is denoted by ‡ (A). The elution volume is determined by absorption on the right, while with albumin blue is iodinated by †(A). The protein concentration is in the 40-wt unit and is indicated by †(A).

Studies of the Protein in Human Plasma
Very Low Density Lipoproteins
Brown, Levy and Fredrickson JBC 1969; 244:56871
VLDL s are secreted into the space of Disse with a group of apolipoproteins. More are added by transfer from HDL. These protect the VLDL from re-entry into the liver that would cause a feudal cycle of energy transfer.

**Effect of Apolipoprotein CII in Lipase Activity**


**Effect of Apolipoprotein CIII in Lipase Activity**

Triglyceride Kinetics in apoCIII and apoAI Deficiency

Note the rapid clearance of VLDL triglycerides in both patients compared to the normal control. (Endogenous injection of [H3-glycerol])

Lipoprotein Lipase and Hepatic Triglyceride Lipase work in sequence to produce LDL particles from VLDL.

Prolonged residence in the circulation.

Infusion of HTGL, human heptic endothelial lipase, 50% inhibition.

IC50 (µg/ml)

CI - 38
CII - 35
CIII - 18

Fig. 2. Effect of Concentration of human VLDL on the activity of human postheparin plasma lipase. (Left) Effect of apoC (Middle) Effect of apoAI, (Right) Effect of apoCII. This amount of apolipoprotein added to the assay medium is indicated on the abscissa.
Under normal conditions, apoCIII gene expression and synthesis are regulated by several factors, including: PPAR(α), PPAR(γ), Rev-erb (α), farnesoid X receptor, and insulin. Based on the report by Caron et al —glucose (ATVB 2011; vol 31:16)
Reducing synthesis of apoCIII with antisense oligonucleotide
Reduces apoCIII concentration in plasma lipoproteins

ASO treatment of Hypertriglyceridemia.
1. ROBCT - placebo versus weekly doses of ISIS 304801
2. Triglycerides at baseline: 200 - 1400 mg/dL.
3. Fifty seven patients randomized to 4 groups: placebo, 100, 200, 300 mg/wk.

HTGL can lower triglycerides in the absence of LPL if apoCIII is reduced.

Reducing apoCIII with an antisense Oligonucleotide markedly reduces chylomicron triglyceride concentrations.

HTGL is the only known lipase with this capability in the absence of LPL.


Summary:
Triglycerides over 200 & HDL-C <40 mg/dL are:
• Major and common risk factor for vascular disease
• Most are cases are polygenic in origin.
• Risk persists after statin treatment
• Plasma triglycerides and apoCIII track very closely.
• Risk is reduced when apoCIII concentration or functionality is reduced by genetic variants
• Major genes reducing apoCIII are uncommon (1/150) but impactful on apoCIII levels and CAD
Conclusions:

- An imbalance between apoCIII concentration and HTGL activity with VLDL remnant is the most likely mechanism for mild or moderate HiTG.
- This can explain the lower HDL-C and increased concentration of small LDL particles.
- Fibrates and EPA reduce apoCIII and CAD.
- New therapies that suppress apoCIII have profound effects in hypertriglyceridemic states.
- Interventional trials with vascular endpoints are needed in those with TG > 200 and low HDL-C.

Discussion

Question # 1

Apolipoprotein CIII inhibits:

1. Lipoprotein lipase
2. Hepatic triglyceride lipase
3. Is synthesized in the liver and intestine
4. Exchanges between HDL, VLDL and chylomicrons
5. All of the above are correct.
Question #2

Apolipoprotein CIII is suppressed most by:

1. Simvastatin
2. Fenofibrate
3. Glucose
4. Omega 3 fatty acids
5. Bile acid binding resins

Question #3

Genetic deficiency of apoCIII is associated with:

1. Marked reduction of LDL cholesterol and apoB
2. Marked reduction of VLDL and VLDL remnants
3. Increased prevalence of coronary artery disease
4. Suppression of HDL cholesterol
5. Increase in Lp(a)

Question #4

Suppressed synthesis of apoCIII is produced by:

1. Increased glucose in the hepatocyte
2. Increased insulin in the hepatocyte
3. Fatty acid concentrations in the hepatocyte
4. Statins
5. Cholestyramine consumption