Majken K. Jensen, PhD.
HDL that contains apoCIII is not inversely associated with risk of coronary events: The MESA Study

FINANCIAL DISCLOSURE:
Funding from Roche for HDL-apoCIII measurements

Harvard University holds a patent for apoCIII defined HDL subfractions and risk of CHD. Drs. Jensen, Furtado, Rimm and Sacks are named inventors
HDL that contains apolipoprotein CIII is not inversely associated with risk of coronary events

Majken K. Jensen, Sarah Aroner, Jeremy Furtado, Kenneth J Mukamal, Wendy Post, Michael Tsai, Joseph F. Polak, Frank Sacks, Robyn McClelland
Current debate about high-density lipoprotein (HDL)

- Potential cardioprotective role of HDL called into question

- Emerging measures of HDL function
  - HDL properties that are not captured by plasma HDL-C/apoA1 levels
Emerging measures of HDL function

- Cholesterol efflux from macrophages (Rader et al)
Emerging measures of HDL function

- Cholesterol efflux from macrophages (Rader et al)
  - First step in reverse cholesterol transport
Emerging measures of HDL *function*

- Cholesterol efflux from macrophages (Rader *et al*)
  - First step in reverse cholesterol transport

- Other HDL functions:
  - Cholesterol delivery to liver
  - Anti-oxidation
  - Endothelial repair
  - Vasodilation
  - Anti-inflammatory
  - Immunofunction
  - Anti-apoptotic
  - Anti-thrombotic

Mediated by HDL protein cargo
Emerging measures of HDL function

• Cholesterol efflux from macrophages (Rader et al)
  - First step in reverse cholesterol transport

• Other HDL functions:
  - Cholesterol delivery to liver
  - Vasodilation
  - Anti-oxidation
  - Anti-inflammatory
  - Immunofunction
  - Endothelial repair
  - Anti-apoptotic
  - Anti-thrombotic
  - Mediated by HDL protein cargo
  - Apolipoprotein CIII
Apolipoprotein CIII (apoCIII)

- Present on 5-20% of LDL, VLDL and HDL

- Impairs clearance of cholesterol (via liver)
  - Raises triglycerides and LDL-C

- LDL with apoCIII much stronger associated with CHD-risk than LDL without apoCIII
  (Mendivil, Circ 2012)
Does ApoC-III affect HDL function?

- HDL with apoCIII:
  - Impaired anti-inflammatory properties  
    (Kawakami, Circ 2006)
  - Impaired endothelial anti-apoptotic function  
    (Riwanto, Circ 2013)
  - Not inversely associated with risk of CHD  
    (Jensen, JAHA 2012)
Quintiles of HDL-C, according to apoCIII

HDL-C without apoCIII
HDL-C with apoCIII

NHS/HPFS case control studies

Jensen et al. JAHA 2012

P interaction = 0.02
HDL with apoCIII
P trend = 0.02
HDL without apoCIII
P trend = 0.01

Quintiles of HDL-C, according to apoCIII
Objective

- Investigate associations of HDL subtypes defined by apoCIII with
  - subclinical atherosclerosis
  - risk of coronary events

in the Multi-Ethnic Study of Atherosclerosis
Multi-Ethnic Study of Atherosclerosis (MESA)

- Multi-center cohort study
- Baseline visit in 2000 – 2002
- 6,814 men and women
  - 45-84 years at baseline
    - Caucasian (38%)
    - African American (28%),
    - Hispanic (22%)
    - Chinese American (12%)
- All participants free of clinical CVD at baseline

http://depts.washington.edu/mesair/study.html
Multi-Ethnic Study of Atherosclerosis (MESA)

- Multi-center cohort study
- Baseline visit in 2000 – 2002
- 6,814 men and women
  - 45-84 years at baseline
    - Caucasian (38%)
    - African American (28%),
    - Hispanic (22%)
    - Chinese American (12%)
- All participants free of clinical CVD at baseline
- N=5,800 plasma samples available

http://depts.washington.edu/mesaaair/study.html
ApoAI with/without apoCIII: Modified sandwich ELISA

1. Capture apoCIII

2. Measure apoCIII

3. Dissociate lipoproteins & measure apoAI

4. Transfer unbound proteins & measure apoAI

Concentration of ApoAI with apoCIII apoAI

Concentration of ApoAI without apoCIII apoAI
• cIMT: maximal thickness measured at 4 sites for both common (CCA) and internal (ICA) carotid arteries
  - Composite measurement = average ICA IMT and CCA IMT

• Incident CHD events, n=386
  - MI, CHD death, resuscitated cardiac arrest, definite and probable angina (followed by revascularization) by medical records until 2012
Statistical Analysis

- cIMT: Generalized linear models
  - LSMEANS and per SD

- CHD: Cox proportional hazard regressions
  - Penalized splines
  - Per 1 standard deviation (sd)
  - Quintiles

- Multivariable model: Age, race/ethnicity, sex, field site, smoking, alcohol, body mass index, income
  - + LDL-C, triglycerides, diabetes, family history of CHD, blood pressure
Results
<table>
<thead>
<tr>
<th>Variable</th>
<th>N=5658</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years (SD)</td>
<td>62.7 (10.3)</td>
</tr>
<tr>
<td>Female, N (%)</td>
<td>2940 (52.0)</td>
</tr>
<tr>
<td>Postmenopausal N (%)</td>
<td>2443 (83.2)</td>
</tr>
<tr>
<td>Race/ethnicity, N (%)</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>2103 (37.2)</td>
</tr>
<tr>
<td>Chinese-American</td>
<td>694 (12.3)</td>
</tr>
<tr>
<td>African-American</td>
<td>1618 (28.6)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1243 (22.0)</td>
</tr>
<tr>
<td>Mean carotid intima-media thickness, um* (SD)</td>
<td>978 (361)</td>
</tr>
<tr>
<td>Diabetes, N (%)</td>
<td>722 (12.8)</td>
</tr>
<tr>
<td>Hypertension, N (%)</td>
<td>2580 (45.6)</td>
</tr>
<tr>
<td>Hypercholesterolemia, N (%)</td>
<td>543 (9.6)</td>
</tr>
<tr>
<td>Use of lipid lowering drugs, N (%)</td>
<td>953 (16.8)</td>
</tr>
<tr>
<td>Mean body mass index, kg/m² (SD)</td>
<td>28.3 (5.4)</td>
</tr>
<tr>
<td>Current smoker, N (%)</td>
<td>712 (12.6)</td>
</tr>
</tbody>
</table>
**Majority of apoAI is without apoCIII**

<table>
<thead>
<tr>
<th>Variable</th>
<th>N=5658</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median apoAI, mg/dL (IQR)</td>
<td>125 (107, 149)</td>
</tr>
<tr>
<td>Median total apoC-III, mg/dL (IQR)</td>
<td>8.7 (6.9, 11.0)</td>
</tr>
<tr>
<td>Median apoAI without apoCIII, mg/dL (IQR)</td>
<td>117 (101, 138)</td>
</tr>
<tr>
<td>Median apoAI with apoCIII, mg/dL (IQR)</td>
<td>7.9 (6.3, 9.9)</td>
</tr>
<tr>
<td>% of apoAI that has apoCIII, mg/dL (IQR)</td>
<td>6.3 (5.4, 7.3)</td>
</tr>
</tbody>
</table>
cIMT: ApoAI and apoCIII defined subtypes
Quintiles and per SD

<table>
<thead>
<tr>
<th>IMT (μm)</th>
<th>ApoAI</th>
<th>ApoAI without apoCIII</th>
<th>ApoAI with apoCIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>850</td>
<td>900</td>
<td>950</td>
</tr>
<tr>
<td>Q2</td>
<td>900</td>
<td>950</td>
<td>1000</td>
</tr>
<tr>
<td>Q3</td>
<td>950</td>
<td>1000</td>
<td>1050</td>
</tr>
<tr>
<td>Q4</td>
<td>1000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q5</td>
<td>1050</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ApoAI p trend = 0.0001
ApoAI without apoCIII p trend = 0.003
ApoAI with apoCIII p trend = 0.4

-13μm (4.7)  -16μm (5.9)  3.8μm (5.9)
ApoA1 without apoCIII and CHD

P trend < 0.001
Per SD:
0.80 (0.70-0.93)
ApoA1 without apoCIII and CHD

Hazard Ratio for CHD

P trend < 0.001
Per SD:
0.80 (0.70-0.93)

+LDL, TG:
P trend = 0.001
0.83 (0.72-0.97)

+LDL, TG, Statin
users excluded:
P trend = 0.001
0.79 (0.67-0.94)
ApoA1 with apoCIII and CHD

Hazard Ratio for CHD

P trend=0.02
Per SD:
1.12 (0.98-1.27)
ApoAl with apoCIII and CHD

P trend=0.02
Per SD:
1.12 (0.98-1.27)

+LDL, TG:
P trend=0.07
1.09 (0.95-1.24)

+LDL, TG, Statin users excluded:
P trend=0.09
1.09 (0.93-1.27)
ApoA1 without apoCIII significantly different from ApoA1 with apoCIII

• P for heterogeneity in regression slopes:
  - Multivariable model: p=0.007
  - With triglycerides and LDL-C: p=0.04
  - With triglycerides and LDL-C without statin users: p=0.03
• ApoCIII separates HDL into a component that is and one that isn’t associated with cardioprotection

• Findings consistent across four prospective studies
  - MESA: CHD cases n=386
  - NHS: CHD cases n=286
  - HPFS: CHD cases=348
  - Danish EPIC: CHD=1949
Meta-analysis

2,969 incident CHD events across four studies

HDL with apoCIII
Per SD=1.12 (1.01-1.25)

HDL without apoCIII
Per SD=0.76 (0.68-0.84)

P difference=0.001
Conclusion

- Two HDL subfractions defined on the basis of apoCIII
- HDL with apoCIII not associated with benefit
  - Consistent with functional role of apoCIII
- HDL without apoCIII strongly inverse
- Validates novel modified sandwich ELISA with potential for widespread use
- Assay can be adapted to other protein cargo of HDL
Thank you

Questions?
• Prelim analyses (HPFS/NHS):
  - CETP TaqIB variants have higher HDL, but not lower CHD-risk

<table>
<thead>
<tr>
<th>CETP TaqIB</th>
<th>Wildtype</th>
<th>BB homozygous</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C without apoC-III</td>
<td>48.2 mg/dL</td>
<td>52.8 mg/dL</td>
</tr>
<tr>
<td>HDL-C with apoC-III</td>
<td>7.7 mg/dL</td>
<td>8.7 mg/dL</td>
</tr>
<tr>
<td>Proportion of HDL-C with apoC-III</td>
<td>14.0%</td>
<td>14.9%</td>
</tr>
</tbody>
</table>
### LSMEANS by race/ethnicity and sex

<table>
<thead>
<tr>
<th>Race/Ethnicity</th>
<th>Total apoCIII</th>
<th>ApoAI without apoCIII</th>
<th>ApoAI with apoCIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>9.9</td>
<td>120.2</td>
<td>8.4</td>
</tr>
<tr>
<td>Chinese-American</td>
<td>9.5</td>
<td>118.7</td>
<td>8.1</td>
</tr>
<tr>
<td>African-American</td>
<td>8.2</td>
<td>121.9</td>
<td>8.3</td>
</tr>
<tr>
<td>Hispanic</td>
<td>9.6</td>
<td>118.8</td>
<td>8.2</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td><strong>P=0.001</strong></td>
<td><strong>P=0.001</strong></td>
</tr>
<tr>
<td>Women</td>
<td>9.8</td>
<td>125.6</td>
<td>8.7</td>
</tr>
<tr>
<td>Men</td>
<td>8.7</td>
<td>114.2</td>
<td>7.8</td>
</tr>
</tbody>
</table>
Potential effects of apoCIII on functional aspects of HDL

- HDL with apoCIII less capable of reducing inflammation (Kawakami 2006)

- Elevated apoCIII in “dysfunctional” HDL proteome from CHD patients, compared to healthy controls (Riwanto 2012)

- Preliminary lab data: HDL with apoCIII delivers less cholesterol to the liver
Proteins Associated with Human HDL

Vaisar T, et al. JCI 2007
Estimates by race/ethnicity (per SD)

Caucasians (N=2106, 168 cases)
apoA1 w/o C3: HR = 0.81 (0.68, 0.95)
apoA1 w/ C3: HR = 1.07 (0.88, 1.30)
p-het = 0.07

African Americans (N=1623, 107 cases)
apoA1 w/o C3: HR = 0.87 (0.66, 1.16)
apoA1 w/ C3: HR = 1.09 (0.82, 1.47)
p-het = 0.39

Chinese Americans (N=694, 31 cases)
apoA1 w/o C3: HR = 0.65 (0.34, 1.26)
apoA1 w/ C3: HR = 0.96 (0.56, 1.65)
p-het = 0.45

Hispanics (N=1245, 80 cases)
apoA1 w/o C3: HR = 0.92 (0.66, 1.28)
apoA1 w/ C3: HR = 0.97 (0.71, 1.34)
p-het = 0.85
Estimates by gender

**Women**
apoA1 w/o C3: HR = 0.81
apoA1 w/ C3: HR = 1.16
p-het = 0.08

**Men**
apoA1 w/o C3: HR = 0.80
apoA1 w/ C3: HR = 1.07
p-het = 0.08
Estimates by smoking status

Non-smokers (never and past, n=4902)
apoA1 w/o C3: HR = 0.83
apoA1 w/ C3: HR = 1.09
p-het = 0.04

Current (n=694)
apoA1 w/o C3: HR = 0.52
apoA1 w/ C3: HR = 1.61
p-het = 0.03
Estimates by smoking status

**Never (n=2845)**
apoA1 w/o C3: HR = 0.83
apoA1 w/ C3: HR = 1.02
p-het = 0.2

**Past (n=2051)**
apoA1 w/o C3: HR = 0.84
apoA1 w/ C3: HR = 1.20
p-het = 0.06

**Current (n=694)**
apoA1 w/o C3: HR = 0.52
apoA1 w/ C3: HR = 1.61
p-het = 0.03
## Correlations

Unadjusted Pearson Correlation Coefficients. All p<0.001

<table>
<thead>
<tr>
<th>Variable</th>
<th>ApoAI without apoCIII</th>
<th>Total apoAI</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoAI with apoCIII</td>
<td>0.6</td>
<td>0.66</td>
<td>0.04</td>
</tr>
<tr>
<td>ApoAI without apoCIII</td>
<td></td>
<td>0.99</td>
<td>0.12</td>
</tr>
<tr>
<td>Total apoCIII</td>
<td>0.25</td>
<td>0.38</td>
<td>0.64</td>
</tr>
<tr>
<td>% apoAI with apoCIII</td>
<td>-0.22</td>
<td>0.44</td>
<td>0.13</td>
</tr>
</tbody>
</table>
## Correlations: spearman sex and age adjusted

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total # HDL particles</th>
<th>Large HDL</th>
<th>Medium HDL</th>
<th>Small HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoAI with apoCIII</td>
<td>0.50</td>
<td>0.50</td>
<td>0.27</td>
<td>-0.07</td>
</tr>
<tr>
<td>ApoAI without apoCIII</td>
<td>0.60</td>
<td>0.55</td>
<td>0.32</td>
<td>-0.04</td>
</tr>
<tr>
<td>% apoAI with apoCIII</td>
<td>0.09</td>
<td>0.14</td>
<td>0.06</td>
<td>-0.006</td>
</tr>
<tr>
<td>Ratio apoAI with/apoAI without</td>
<td>0.09</td>
<td>0.14</td>
<td>0.06</td>
<td>-0.06</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.08</td>
<td>-0.46</td>
<td>-0.15</td>
<td>0.32</td>
</tr>
<tr>
<td>LDL-C</td>
<td>-0.12</td>
<td>-0.13</td>
<td>-0.05</td>
<td>0.02</td>
</tr>
</tbody>
</table>
1. Capture apoCIII

2. Measure apoCIII

3. Dissociate lipoproteins & measure apoAI

4. Transfer unbound proteins & measure apoAI

Concentration of ApoAI with apoCIII apoAI

Concentration of ApoAI without apoCIII apoAI
## LSMEANS by Smoking, Diabetes, and Statin use

<table>
<thead>
<tr>
<th></th>
<th>Total apoCIII</th>
<th>ApoAI without apoCIII</th>
<th>ApoAI with apoCIII</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non smoker</td>
<td>P=0.6</td>
<td>P=0.04</td>
<td>P=0.01</td>
</tr>
<tr>
<td>Past smoker</td>
<td>9.2</td>
<td>121.3</td>
<td>8.4</td>
</tr>
<tr>
<td>Current smoker</td>
<td>9.3</td>
<td>122.0</td>
<td>8.5</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>P&lt;0.0001</td>
<td>P=0.0001</td>
<td>P=0.01</td>
</tr>
<tr>
<td>Yes</td>
<td>10.2</td>
<td>116.1</td>
<td>8.6</td>
</tr>
<tr>
<td><strong>Use of lipid-lowering drugs</strong></td>
<td>p=0.001</td>
<td>P=0.2</td>
<td>P=0.003</td>
</tr>
<tr>
<td>No</td>
<td>9.1</td>
<td>120.1</td>
<td>8.2</td>
</tr>
<tr>
<td>Yes</td>
<td>10.2</td>
<td>118.9</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Apolipoprotein concentration in mg/dl. LSMEANS and 95% CI, adjusted for age, sex, race/ethnicity, study site, smoking, alcohol, income, BMI.
### LSMEANS by adiposity

<table>
<thead>
<tr>
<th></th>
<th>Total apoCIII</th>
<th>ApoAI without apoCIII</th>
<th>ApoAI with apoCIII</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal-weight</td>
<td>p&lt;0.0001</td>
<td>P=0.001</td>
<td>P=0.001</td>
</tr>
<tr>
<td>Overweight</td>
<td>8.8</td>
<td>124.0</td>
<td>8.7</td>
</tr>
<tr>
<td>Obese</td>
<td>9.5</td>
<td>120.1</td>
<td>8.3</td>
</tr>
<tr>
<td><strong>Waist</strong></td>
<td>p=0.001</td>
<td>P&lt;0.001</td>
<td>P=0.003</td>
</tr>
<tr>
<td>Normal</td>
<td>8.9</td>
<td>123.0</td>
<td>8.5</td>
</tr>
<tr>
<td>Overweight (88/102cm)</td>
<td>9.6</td>
<td>118.6</td>
<td>8.1</td>
</tr>
</tbody>
</table>

Apolipoprotein concentration in mg/dl. LSMEANS and 95% CI, adjusted for age, sex, race/ethnicity, study site, smoking, alcohol, income, BMI.
Meta-analysis: Adjusted TG and LDL/apoB

HDL with apoCIII
Per SD=1.06 (0.98-1.15)

HDL without apoCIII
Per SD=0.82 (0.75-0.90)

Quintiles of ApoA-I with and without apoC-III

HR for CHD

Q1 Q2 Q3 Q4 Q5
### ApoCIII, apoA1 subtypes and cIMT

<table>
<thead>
<tr>
<th></th>
<th>ApoCIII</th>
<th>ApoA1</th>
<th>ApoA1 without apoCIII</th>
<th>ApoA1 with apoCIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted for</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age, sex, race,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>study site,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>smoking, alcohol,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>income, BMI.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ApoCIII** 16um (4.5)  
**ApoA1** -13um (4.7)  
**ApoA1 without apoCIII** -16um (5.9)  
**ApoA1 with apoCIII** 3.8um (5.9)
Total apoCIII and risk of CHD

P trend=0.05
Per SD:
1.11 (1.01-1.23)
Total apoCIII and risk of CHD

Adjusted for age, sex, site, race/ethnicity, alcohol, income, BMI

HR for CHD

P trend = 0.05
Per SD = 1.11 (1.01; 1.23)
Adjustment for LDL-C and triglycerides

Quintiles of total ApoCIII:

- Q1
- Q2
- Q3
- Q4
- Q5

HR for CHD:

- Q1: 0.6
- Q2: 0.8
- Q3: 1.0
- Q4: 1.2
- Q5: 1.4

Per SD:

- Q1: 1.11 (1.01; 1.23)
- Q2: 1.01 (0.88; 1.16)

P trend:

- Q1: 0.05
- Q2: 0.9