New Frontiers of HDL Research

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Institute of Cardiometabolism and Nutrition
Sorbonne Universities
## Disclosures

<table>
<thead>
<tr>
<th>Commercial Interest</th>
<th>Nature of Financial Relationship</th>
<th>(Include all those that apply)</th>
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<tr>
<td>CSL</td>
<td>Research grant</td>
<td>Independent contractor</td>
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2. Altered HDL metabolism and functionally defective HDL
3. Therapeutic normalization of altered HDL metabolism and function
1. Normal HDL metabolism and function

2. Altered HDL metabolism and functionally defective HDL

3. Therapeutic normalization of altered HDL metabolism and function
Why HDL?

Low circulating levels of HDL-cholesterol are a significant, strong and independent predictor of cardiovascular disease

High-Density Lipoprotein Cholesterol and Cardiovascular Disease

Four Prospective American Studies

David J. Gordon, MD, PhD, Jeffrey L. Probstfield, MD,
Robert J. Garrison, MS, James D. Neaton, PhD, William P. Castelli, MD,
James D. Knoke, PhD, David R. Jacobs Jr., PhD,
Shrikant Bangdiwala, PhD, and H. Alfred Tyroler, MD

(Circulation 1989;79:8-15)
Plasma HDL is a small, dense and protein-rich lipoprotein as compared to other lipoprotein classes.

**HDL particles contain apolipoproteins, enzymes, acute-phase proteins, complement proteins, antiproteases and lipids.**

### What is HDL?

<table>
<thead>
<tr>
<th><strong>Major components</strong> (molecules/HDL particle)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface</strong></td>
</tr>
<tr>
<td>ApoA-I (ribbons)</td>
</tr>
<tr>
<td>Phospholipid (light-blue)</td>
</tr>
<tr>
<td>Free cholesterol (orange)</td>
</tr>
<tr>
<td><strong>Core</strong></td>
</tr>
<tr>
<td>Cholesteryl ester (dark-blue)</td>
</tr>
<tr>
<td>Triglyceride (green)</td>
</tr>
</tbody>
</table>
Structures of major HDL apolipoproteins

apoA-I  apoA-II  apoC-I  apoC-II

apoC-III  apoD  apoE  apoM

## Lipoproteinomics of healthy normolipidemic humans: Major lipids

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>VLDL content</th>
<th>LDL content</th>
<th>HDL content</th>
<th>molar % of total lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phospholipids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>19.4-20.3</td>
<td>24.4-36.6</td>
<td>37-54</td>
<td></td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>3.0-3.7</td>
<td>6-12</td>
<td>4.5-6</td>
<td></td>
</tr>
<tr>
<td>Lyso phosphatidylcholine</td>
<td>0.50-0.75</td>
<td>1-1.2</td>
<td>1-8</td>
<td></td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>0.6</td>
<td>0.2</td>
<td>0.5-1.5</td>
<td></td>
</tr>
<tr>
<td>PE plasmalogenes</td>
<td>0.3</td>
<td>0.25</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>ND</td>
<td>ND (18:0/20:3)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td><strong>Sterols</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesteryl ester</td>
<td>11-21</td>
<td>8-17</td>
<td>9-20</td>
<td></td>
</tr>
<tr>
<td>Triacylglycerides</td>
<td>37-52</td>
<td>3-10</td>
<td>5-12</td>
<td></td>
</tr>
<tr>
<td>Diacylglycerides</td>
<td>0.9</td>
<td>0.14</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

*a Main species, b determined by enzymatic assay, c determined by HPLC, ND: not determined

Physicochemical heterogeneity of HDL

**Shape**
- Discoidal HDL
- Spherical HDL

**Density and size**
- HDL2b
- HDL2a
- HDL3a
- HDL3b
- HDL3c

- **HDL2**
  - Density (g/mL): 1.063
  - Diameter (nm): 10.6

- **HDL3**
  - Density (g/mL): 1.21
  - Diameter (nm): 7.6

**Apolipoprotein composition**
- LpA-I
- LpA-I:A-II

**Electrophoretic mobility**
- Pre-β-1
- Pre-β-2
- Pre-β-3
- a1
- a2
- a3
- a4

Models of apoA-I organisation in discoid HDL

**Double belt**
- 5/5 rotamer

**Hairpin**

**_looped belt**
- 5/2 rotamer

Silva, RA et al., Biochemistry 44, 8600, 2005


Structure of spherical plasma HDL: Particles containing apoA-I


Trefoil model of apoA-I organisation in HDL

HDL metabolism:
Major pathways involved in HDL formation

HDL metabolism: Major pathways involved in HDL remodelling

Antiatherogenic and vasculoprotective actions of HDL
Major pathways involved in HDL-mediated cholesterol efflux
Functional heterogeneity of HDL particles

- Large HDL
  - SR-BI
  - ABCG1
  - ABCA1
  - FC, PL

- Small HDL
  - Monocyte
  - Adhesion molecules
  - Endothelial cells

- Monocyte
  - ROS
  - NO•
  - PGI2
  - O2:–

- Endothelial cell

- Small HDL
  - Oxidised PL
  - 1e oxidants
  - Oxidised LDL

- Large HDL
  - Platelets
  - Tissue factor pathway

- Small HDL

- HDL

- Dense HDL
  - LBP
  - LPS
  - Neutrophil

- Trypanosome

- CD14
1. Normal HDL metabolism and function

2. Altered HDL metabolism and functionally defective HDL

3. Therapeutic normalization of altered HDL metabolism and function
HDL metabolism in dyslipidemia, inflammation and insulin resistance: Altered HDL formation

HDL metabolism in dyslipidemia, inflammation and insulin resistance: Altered HDL remodelling

Circulating levels of large HDL particles are typically reduced in CV disease

Framingham Offspring Study

Asztalos et al. ATVB 2004:24;2181
Reduction of plasma HDL concentration may accelerate the development of atherosclerosis, and hence ischaemic heart disease, by impairing the clearance of cholesterol from the arterial wall.
Major pathways involved in altered cholesterol efflux capacity of HDL

- Lipid-free apoA-I
- Lipid-free apoE
- Discoid HDL
- Spherical HDL

- ABCA1
- ABCG1
- SR-BI

- FC, PL

Altered apoA-I
Decreased apoA-I
Deficient LCAT
Subnormal PL
Elevated SM
Altered fatty acids
Oxidised PL
Cholesterol efflux capacity of diluted serum was evaluated in lipid-loaded macrophagic cells, in which ABCA1-mediated efflux to small, dense HDL represents quantitatively the major pathway accounting for up to 80% of effluxed cholesterol.
Clinical relevance of HDL functionality: Cholesterol efflux capacity and risk of incident CVD

1. Normal HDL metabolism and function

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3. Therapeutic normalization of altered HDL metabolism and function
The Concept of HDL-Targeted Therapy

Therapeutic normalization of both HDL quantity (circulating levels) and quality (biological function) can attenuate atherothrombotic disease.
Schematic illustrating plasma HDL-C elevation

Target small HDL

Target large HDL

Increasing plasma HDL-C by increasing the number of lipid-poor nascent HDL particles

Increasing plasma HDL-C by increasing the size and cholesterol content of existing HDL particles

Major pathways involved in enhanced HDL formation
Major pathways involved in the normalization of intravascular HDL remodelling

- CETP inhibitors
- apoC-III ASO
- Niacin
- Fibrates

Hepatocyte

Kidneys
What can CETP inhibition provide?

Potent HDL-C raising

Clark RW et al. *Arterioscler Thromb Vasc Biol* 2004;24:490-497
Torcetrapib Catastrophe of Dec 2, 2006

**A** Death from Any Cause

<table>
<thead>
<tr>
<th>Days after Randomization</th>
<th>Patients without Event (%)</th>
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<tbody>
<tr>
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<td>Atorvastatin only</td>
</tr>
<tr>
<td></td>
<td>Torcetrapib plus atorvastatin</td>
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<tr>
<td>0</td>
<td>100</td>
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<tr>
<td>90</td>
<td>99.5</td>
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<td>180</td>
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<td>270</td>
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<td>360</td>
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<td>720</td>
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<tr>
<td>810</td>
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No. at Risk

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<tr>
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<td>956</td>
<td>943</td>
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<td>109</td>
<td>114</td>
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**B** Major Cardiovascular Events

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<td>1965</td>
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<td>898</td>
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<td>103</td>
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Copyright © 2007 Massachusetts Medical Society.
Torcetrapib Increases Blood Pressure in Patients with Coronary Atherosclerosis

Toxic class-unrelated effect?

Absence of Effects of Dalcetrapib in Patients with a Recent Acute Coronary Syndrome

Gregory G. Schwartz, M.D., Ph.D., Anders G. Olsson, M.D., Ph.D., Markus Abt, Ph.D., Christie M. Ballantyne, M.D., Philip J. Barter, M.D., Ph.D., Jochen Brumm, Ph.D., Bernard R. Chaitman, M.D., Ingar M. Holme, Ph.D., David Kallend, M.B., B.S., Lawrence A. Leiter, M.D., Eran Leitersdorf, M.D., John J.V. McMurray, M.D., Hardi Mundl, M.D., Stephen J. Nicholls, M.B., B.S., Ph.D., Prediman K. Shah, M.D., Jean-Claude Tardif, M.D., and R. Scott Wright, M.D., for the dal-OUTCOMES Investigators*

Absence of Effects of Niacin in Patients with Low HDL Cholesterol Levels Receiving Intensive Statin Therapy

The AIM-HIGH Investigators*
# Recent/In-progress clinical trials of HDL-targeting agents

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<td>A CETP inhibitor which <strong>failed</strong> in a phase 3 ILLUMINATE trial due to excess all-cause mortality in the treatment group (Pfizer)</td>
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<td>A CETP inhibitor which <strong>failed</strong> in a phase 3 dal-OUTCOMES trial due to lack of efficacy (Hoffman - La Roche)</td>
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<tr>
<td>Evacetrapib</td>
<td>A potent CETP inhibitor <strong>abandoned</strong> in a phase 3 ACCELERATE trial due to futility (Eli Lilly)</td>
</tr>
<tr>
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<td>A potent CETP inhibitor currently in a phase 3 REVEAL trial (Merck)</td>
</tr>
<tr>
<td>Niacin</td>
<td>Potent HDL-C-raising agent which <strong>failed</strong> to reduce CV mortality in combination with a statin in AIM-HIGH and HPS2-THRIVE trials</td>
</tr>
<tr>
<td>CSL112</td>
<td>A HDL mimetic manufactured from purified, authentic human plasma apolipoprotein A-I reconstituted with phospholipids (CSL Behring). This formulation supersedes CSL111</td>
</tr>
<tr>
<td>CER-001</td>
<td>A HDL mimetic made from recombinant apolipoprotein A-I produced in mammalian cell expression systems complexed with phospholipids (Cerenis)</td>
</tr>
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<td>MDCO-216</td>
<td>Formerly ETC-216, is a recombinant formulation of apoA-I Milano currently produced by The Medicines Company in a licensing agreement with Pfizer</td>
</tr>
<tr>
<td>RVX-208</td>
<td>An oral apoA-I transcriptional upregulator (Resverlogix Corp)</td>
</tr>
<tr>
<td>ACP-501</td>
<td>Recombinant human LCAT (rhLCAT) is under development for human use (developed by Alphacore Pharma which was recently acquired by AstraZeneca)</td>
</tr>
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</table>
In a ratio of 1:2:2, ACS patients received 5 weekly infusions of placebo or ETC-216 at 15 mg/kg or 45 mg/kg. IVUS was performed within 2 weeks following ACS and repeated after 5 weekly treatments.

Nissen S et al JAMA 2003;290:2292
Why did HDL-C-raising therapies fail?
Possible explanations

1. The potential benefit was abolished by the statin treatment
2. The potential benefit was confined to specific subgroups
3. On-treatment HDL particles were dysfunctional
4. Incorrect HDL particle subpopulation was targeted (large HDL instead of small HDL)
5. HDL-C is not causatively related to CV disease
Failure of HDL-C-raising therapies: Benefit abolished by statin treatment?

Statin therapy interferes with ATP-binding cassette transporter-mediated macrophage cholesterol efflux via miR33 and thus may diminish anti-atherogenic HDL function.
In the dalcetrapib trials, patients homozygous for variant in the ADCY9 gene responded positively to dalcetrapib and experienced a significant reduction in CV risk.

In the torcetrapib trials, patients with highest HDL-C on-treatment levels were less likely to suffer major CV events or death, or to display atherosclerosis progression.

In the AIM-HIGH niacin trial, a significant 33% decrease in primary events in the niacin group beyond that conferred by statins alone was observed in patients with elevated TG (≥200mg/dl) and reduced HDL-C (< 32mg/dl) levels.

**Failure of HDL-C-raising therapies:** Benefit confined to specific subgroups?
Failure of HDL-C-raising therapies: Generation of dysfunctional HDL?

Niacin \hspace{2cm} \text{Anacetrapib}

HDL-C-raising does NOT produce dysfunctional HDL

## Failure of HDL-C-raising therapies: Incorrect HDL subpopulation targeted?

Recent/In-progress clinical trials of HDL-targeting agents

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### Both large and small HDL?

### Number of HDL particles?
Genetically determined low HDL-C is not associated with CV disease in Mendelian randomisation studies

Table 4: Estimate of the association of genetically raised LDL cholesterol or HDL cholesterol and risk of myocardial infarction using multiple genetic variants as instruments

Failure of HDL-C-raising therapies: Low HDL-C is a biomarker for elevated TG?

Although TG and HDL-C levels are inversely correlated, TG concentrations vary more on a daily basis than HDL-C, which may account for why HDL-C is statistically more strongly associated with CV disease than TG.

Summary

Low plasma levels of cholesterol carried by HDL particles (termed HDL-cholesterol) are firmly established as CV risk factor.

HDL particles contain multiple protein and lipid components and are highly heterogeneous in their metabolism, structure and biological function.

Small, dense, protein-rich HDLs display elevated anti-atherogenic activities as compared to large, light, lipid-rich HDLs.

HDL metabolism and function are altered in low HDL-cholesterol states.

Circulating levels of large HDL particles are typically reduced in CV disease.

Reduced capacity of HDL to efflux cholesterol from lipid-loaded macrophages is associated with both the presence of CV disease and the risk of future CV events.

Therapeutic HDL-C raising, which predominantly increases levels of large HDL, have repeatedly failed to reduce CV events in patients treated with statins.

Genetic epidemiology does not support a causal relationship between HDL-cholesterol and CV risk.
HDL remains an exceedingly intriguing therapeutic target to reduce CV risk

There is an urgent need to resolve ongoing controversy between classic epidemiology, genetic epidemiology and clinical trials in order to allow further development of HDL-targeting therapies
HDL remains an exceedingly intriguing therapeutic target to reduce CV risk

There is an urgent need to resolve ongoing controversy between classic epidemiology, genetic epidemiology and clinical trials in order to allow further development of HDL-targeting therapies