Metabolism and Protective Properties of HDL

*A riddle wrapped in a mystery inside an enigma*

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Disclosure J. Genest MD 2013

Advisory Board, Speaker’s Bureau, Consultant, Grants, Clinical Trials

- Merck *
- Pfizer
- Novartis
- AMGEN *
- Roche *
- AstraZeneca
- Sanofi/Regeneron *
- Lilly
- Valeant
- Genzyme *
- Aegerion

Stock ownership: none;
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Relevant disclosure: JUPITER, IMPROVE-IT, CANTOS, CAPREE steering Committees; REVEAL, ACCELERATE, AMG145, Lilly Clinical Trials.
The Controversy

- A low level of HDL-C is a major independent risk factor for atherosclerotic cardiovascular disease.
- However, in randomized, controlled trials, high-dose niacin or inhibitors of cholesteryl ester transfer protein did not improve CV outcomes despite significantly increasing the HDL-C level.
- Furthermore, genetic variants associated with HDL-C levels are often not associated with CVD.
- These observations suggest that HDL cholesterol may not be causally associated with CVD

HDL Metabolism

- Complexity of HDL
- HDL Biogenesis
- HDL Metabolism
HDL Metabolism: The Simplified View

Circ Res. 2014;114:171-182
Human Plasma Lipidome
(Quenhenburger N Engl J Med 2011;365:1812-23)

**Figure 1. Relative Distribution of Biologic Molecules in Human Plasma.**
Amino acids and nucleic acids are shown without consideration of the contribution of proteins and DNA or RNA. The relative distribution is based on weight (grams per deciliter). Data were compiled from Lentner,1 Wishart et al.,2 and Quehenberger et al.3

**Figure 2. Relative Distribution of Lipids in Human Plasma.**
Lipidomic analysis has identified, characterized, and quantified almost 600 lipid molecular species in human plasma.3 The relative distribution in each category is given on a molar basis. The nomenclature of the lipid categories conforms to the recently developed LIPID MAPS classification system.6
Human Plasma Lipidome
Apolipoprotein AI
Adenosine Triphosphate Binding Cassette Transporter A1 (ABCA1)

- Full-transporter
- Phospholipid and (cholesterol) efflux to apoA-I
- Early HDL maturation
- LxR/RxR
HDL Biogenesis
Nascent HDL Particles

Figure 6

Sorci-Thomas JLR 2012
Two-D PAGGE Analysis of HDL

Apo AI-containing lipoproteins

Hafiane A. CLC 2013 R17; P18
Lipoprotein Metabolism

**Intestine**
- Chylomicron
  - LPL
  - FFA
  - ApoA-I, A-II
  - ApoC-I, C-II, C-III
  - Phospholipids
  - Free cholesterol

**Liver**
- Nascent HDL
  - LCAT
  - HDL3
  - HDL2
  - CETP
  - PLTP
  - Tg
  - CE
  - LDL
  - IDL
  - CE
  - LPL
  - FFA

**Peripheral Cells**
- Free Cholesterol
- Steroidogenic Cells

**Exogenous Pathway**
- Chylomicron
  - Chylo Remnant
  - HL
  - Liver

**Endogenous Pathway**
- Exogenous Pathway
- Feces

**Liver**
- HL
Genes involved in HDL regulation

- **HDL-C factors:**
  - Environmental
  - Genetic: heritability 50%

- **Known genes:**
  - Mutations in several genes have identified (*ApoAI, ABCA1, LCAT*)

- **Novel genes:** *PCSK5, WWOX*

HDL Functions

- Cellular Cholesterol Efflux
- Anti-oxidant
- Anti-inflammatory
- Vascular Endothelial function
- Anti-thrombotic
**Figure 1** Pleiotropic biological functions of apolipoprotein A-I

ABCA1 –Mediated Cellular Cholesterol Efflux

Severity of cholesterol efflux defect and MI

LxR/RxR

Lipid-free apoA-I → Nascent HDL

TD/ homozygotes (?)

Heterozygotes

Normal

ABCA1 activity
HDL Cholesterol efflux: Biomarker of CVD

The NEW ENGLAND JOURNAL of MEDICINE

HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events


**CVD, HDL-C and Efflux Capacity**

<table>
<thead>
<tr>
<th>Models</th>
<th>No. of Participants with Event/Total No. of Participants</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL cholesterol</td>
<td>132/2416</td>
<td></td>
</tr>
<tr>
<td>Unadjusted analysis</td>
<td></td>
<td>0.64 (0.40–1.03)</td>
</tr>
<tr>
<td>Analysis adjusted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For traditional risk factors</td>
<td></td>
<td>0.80 (0.47–1.37)</td>
</tr>
<tr>
<td>For traditional risk factors and HDL particle concentration</td>
<td></td>
<td>1.08 (0.59–1.99)</td>
</tr>
<tr>
<td>Cholesterol efflux capacity</td>
<td>132/2416</td>
<td></td>
</tr>
<tr>
<td>Unadjusted analysis</td>
<td></td>
<td>0.44 (0.27–0.73)</td>
</tr>
<tr>
<td>Analysis adjusted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For traditional risk factors</td>
<td></td>
<td>0.30 (0.18–0.50)</td>
</tr>
<tr>
<td>For traditional risk factors and HDL cholesterol</td>
<td></td>
<td>0.31 (0.18–0.52)</td>
</tr>
<tr>
<td>For traditional risk factors and HDL particle concentration</td>
<td></td>
<td>0.34 (0.20–0.56)</td>
</tr>
<tr>
<td>For traditional risk factors, HDL cholesterol, and HDL particle concentration</td>
<td></td>
<td>0.33 (0.19–0.55)</td>
</tr>
</tbody>
</table>

Cholesterol Efflux and CVD

**Table of Risk Factors and Odds Ratios**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>1.92 (1.26–2.93)</td>
<td>0.003</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.80 (1.31–2.47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.30 (0.95–1.73)</td>
<td>0.10</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>1.01 (0.86–1.18)</td>
<td>0.93</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.85 (0.70–1.03)</td>
<td>0.09</td>
</tr>
<tr>
<td>Efflux capacity</td>
<td>0.75 (0.63–0.90)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Cholesterol Efflux and CVD

Stable Angiographic Case-Control Cohort (n=1150)

- MI, Stroke (3 years)
  - T1: Unadjusted 1.0, Adjusted 1.2
  - T2: Unadjusted 1.1, Adjusted
  - T3: Unadjusted 2.0, Adjusted 2.2

- MACE (3 years)
  - T1: Unadjusted 1.0, Adjusted
  - T2: Unadjusted 0.8, Adjusted 0.9
  - T3: Unadjusted 1.7, Adjusted 1.9

Hazard Ratio (95% CI)

HDL-Mediated Cellular Cholesterol Efflux in CAS

A

HDL-C (mmol/L)

Control  ACS1  ACS2  CAD

B

hsCRP (mg/L)

Control  ACS1  ACS2  CAD

Hafiane A. CLC 2013  P10
HDL-Mediated Cellular Cholesterol Efflux in CAS

![Graph showing percent cholesterol efflux across different conditions: Control, ACS1, ACS2, CAD. The graph includes error bars and significant differences indicated by ***.](image)
Antioxidant Properties of HDL
Antioxidant Properties of HDL

LDL → PL-ox, POVPC, PGPC, PAPC

HDL

OH•, Apo AI, J, α-Toc

Paraoxonase (PON)
Glutathion peroxidase (GPX)
PAF-acetyl hydrolase (PAF-AH)

Lp-PLA2

12-Lipoxygenase

O₂-
Generation of truncated phospholipids by oxidative fragmentation and chemical synthesis.

Salomon R G Circulation Research. 2012;111:930-946
POVPC activates aortic smooth muscle cell proliferation.
Prothrombotic activities of oxidatively truncated phospholipids.

Salomon R G Circulation Research. 2012;111:930-946
Dehydrative cyclization of isoprostanoïd hydroperoxy endoperoxides generates epoxyisoprostanes.

Salomon R G *Circulation Research*. 2012;111:930-946
No consensus has yet emerged on the proper biomarker of HDL anti-oxidant function.
Endothelial cells and HDL

- HDL exerts beneficial effects on VEC
  - Increase eNOS stability, mRNA and pNOS
  - Decrease in NF-κB-mediated inflammatory gene expression
  - Increases endothelial progenitor cells
HDL- Vascular endothelial Cell Interaction
HDL stimulation of Human Aortic Endothelial eNOS

Loading controls (Ponceau S staining)

Choi, H 2013 Unpublished
HDL_{healthy} prevents NF-KB nuclear translocation/activation

Bessler et al. JCI 2011. Confocal microscopy of GFP-anti RelA/p65
HDL and Endothelial Progenitor Cells

Sorrentino SA et al. Circulation 2010;121;110-122
High-Density Lipoprotein
Vascular Protective Effects, Dysfunction, and Potential as Therapeutic Target

Thomas F. Lüscher, Ulf Landmesser, Arnold von Eckardstein, Alan M. Fogelman

Circ Res. 2014;114:171-182
Cholesterol efflux
ABCA1, ABCG1 and SR-BI

Anti-apoptotic
recruitment of endothelial progenitor cells (EPC)

Anti-inflammatory
inhibit VCAM-1 expression and adhesion molecules

Antioxidative
anti-oxLDL, inactivate LOOH and oxPhospholipase

Anti-infectious
trypanosome lytic factor and LPS inactivation

Vasodilatory
NO release, prostacyclin (PGI2) production and eNOS activation

Anti-thrombotic
inhibition of platelet aggregation and prostacyclin (PGI2) production

Hafiane A. Cholesterol 2013
The HDL Proteome

Over 200 proteins have been identified as part of the HDL proteome

(http://homepages.uc.edu/~davidswm/HDLproteome.html)
The HDL proteome in acute coronary syndromes shifts to an inflammatory profile

Khalid Alwaili¹, Dana Bailey², Zuhier Awan³, Swneke D. Bailey¹, Isabelle Ruel¹, Anouar Hafiane³, Larbi Krimbou³, Sylvie Laboissiere⁴, Jacques Genest¹
Independent confirmation of differentially expressed proteins by Western analysis or ELISA
Independent confirmation of differentially enriched proteins by Western analysis or ELISA - Controls

A. ApoA-I spectral quantification

B. ApoA-I gel quantification

C. α2-antiplasmin spectral quantification

D. α2-antiplasmin gel quantification
HDL-apoA-I Exchange: Rapid Detection and Atherosclerosis

Electron paramagnetic resonance spectroscopy (EPR)

The HDL Lipidome

Specific lipid species may have a physiological role in HDL function

Physicochemical properties and lipid class composition of HDL particle subpopulations.

Specifically, the content of phosphatidylserine revealed positive correlations with all metrics of HDL functionality, reflecting enrichment of phosphatidylserine in small, dense HDL3.
HDL- Vascular endothelial Cell Interaction
The Challenge

Develop *Biomarkers of HDL Function*

- Cellular Cholesterol Efflux
- Anti-oxidant
- Anti-inflammatory
- Vascular Endothelial function
- Anti-thrombotic

That can be tested in outcome studies, be reproducible and brought to high throughput.
The Short Term

Identify Responders, Sub groups

- HDL Biomarkers presently limited in predicting outcomes
- IVUS may be the least bad surrogate currently available
- New imaging modalities (\(^{18}\text{FDG}\)) and PET/CT scanning
- High bar of regulatory agencies
The Long Term

HDL is a Biologically SOUND Target

- Need to identify new pathways of HDL biogenesis and metabolism
- Study the structure of HDL in health and in disease (proteomics, lipidomics)
- Design clinical trials that are highly focused (initially)
Points to Take Home

- HDL particles are complex
- Genes involved in HDL biogenesis may make better therapeutic targets than genes involved in HDL remodeling (Lipases, CETP)
- New Gene, new pathways