Cardiovascular Disease and Inflammation: What Can HDL Do About It?

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Conflicts of Interests: Research grants with MedImmune, Shire, Corvidia, and Liposcience
Overview

- History of Cholesterol Research
- Lipoprotein Metabolism
- Pathogenesis of Atherosclerosis
- HDL and Inflammation
History of Cardiovascular Disease Research

Rudolf Virchow
1821-1902

H. Anitchkov
1885-1964
Evolution of Cholesterol as a CHD Risk Biomarker


LDL (Bad - chol) → LDL (Bad - chol) → LDL subfractions → Non-HDL Lipoproteins

Ox-LDL (Very Bad - chol)

HDL (neutral) → HDL (Good - chol) → Dysfunctional HDL

Bad HDL

Cholesterol (factor in CHD)
Evolution of Cholesterol as a CHD Risk Biomarker

- Cholesterol (factor in CHD)
- LDL (Bad - chol)
- HDL (neutral)
- LDL (Good - chol)
- Ox-LDL (Very Bad - chol)
- HDL
- LDL (Bad - chol)
- LDL subfractions
- Non-HDL Lipoproteins
- Good HDL
- Dysfunctional HDL
- Bad HDL

Timeline:
- 1905
- 1920's-1950's
- 1970's
- 1980's
- 1990's
- 2000's
Evolution of Cholesterol as a CHD Risk Biomarker


LDL (Bad - chol) → LDL (Bad - chol) → LDL subfractions → Non-HDL Lipoproteins

Cholesterol (factor in CHD) → Ox-LDL (Very Bad - chol)

HDL (neutral) → HDL (Good - chol) → Good HDL

Dysfunctional HDL → Bad HDL
Evolution of Cholesterol as a CHD Risk Biomarker


LDL (Bad - chol) → LDL (Bad - chol) → LDL → Non-HDL Lipoproteins

Ox-LDL (Very Bad - chol)

HDL (neutral) → HDL (Good - chol)

Cholesterol (factor in CHD)

Good HDL
Dysfunctional HDL
Bad HDL
Early Lipoprotein Research at the NIH

Circa mid 1960s
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Major Classes of Lipoproteins

- Chylomicron
- VLDL
- IDL
- LDL
- HDL
- Lp(a)

*Cholesterol, Protein, Triglyceride, Phospholipid*
Lipoprotein Metabolism Pathways

Peripheral Cells → Remnants

Endogenous Pathway

- LDL
- VLDL

Liver

Exogenous Pathway

- Chylos
- Dietary Lipids

Intestine

Bile Salts Cholesterol

- Fecal sterols
  - Bile Salts

RBCs

Counter Current Exchange

α₁-3 HDL

Reverse Cholesterol Transport Pathway

α₄ HDL

preβ HDL

Macrophage
Lipoprotein Metabolism Pathways

- Triglyceride delivery for energy metabolism
- Endogenous Pathway
- Exogenous Pathway
- Liver
- RBCs
- Counter Current Exchange
- Macrophage
- α₁-₃ HDL
- Reverse Cholesterol Transport Pathway
- α₄ HDL
- preβ HDL
- Dietary Lipids
- Intestine
- Fecal sterols Bile Salts
Lipoprotein Metabolism Pathways

Peripheral Cells

RBCs

Counter Current Exchange

Macrophage

Remnants

LDL

Endogenous Pathway

Liver

VLDL

α₁-₃ HDL

Reverse Cholesterol Transport Pathway

α₄ HDL

Preß HDL

Cholesterol Removal

Dietary Lipids

Intestine

Bile Salts Cholesterol

Fecal sterols Bile Salts
HDL Structure and Composition

Pre-beta Discoidal Shaped HDL

Initial Cholesterol Acceptor

Delivers Cholesterol to Liver

HDL transports over 80 different proteins and over 200 species of lipids.
Pleiotropic Anti-Atherogenic Effects of HDL

- Antiinfectious Activity
- Antiinflammatory Activity
- Antithrombotic Activity
- Antiapoptotic Activity
- Antioxidative Activity
- Reverse Cholesterol Transport
- Epithelial Repair
- Vasodilatory Activity

Pleiotropic Anti-Atherogenic Effects of HDL

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- Antithrombotic Activity
- Antiinfectious Activity

Reverse Cholesterol Transport Pathway
Reverse Cholesterol Transport Pathway

Preß-HDL

ApoA-I

ABCA1

Small α4 HDL

LCAT

Cholesterol Esterification

Large α1-3 HDL

Macrophage

ABCA1
ABCG1
SR-B1

LDL-R

SR-B1
Reverse Cholesterol Transport Pathway

ApoA-I

Preβ-HDL

LDL-R

SR-B1

ABCA1

Small \(\alpha_4\) HDL

LCAT

Large \(\alpha_1-3\) HDL

ABCA1

ABCG1

SR-B1

Macrophage
Reverse Cholesterol Transport Pathway
Reverse Cholesterol Transport Pathway

ApoA-I

Preβ-HDL

Small α₄ HDL

ABCA1

Large α₁⁻³ HDL

Bile

SR-B1

LDL-R

LCAT

CETP

Macrophage

ABCA1 ABCG1 SR-B1

LDL
Model of Cholesterol Efflux to HDL
Overview

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Early Inflammatory Mediators of Atherosclerosis
Features of a Complex Atherosclerotic Plaque

- Lumen
- Lipid Core
- Fibrous cap
- Shoulder
- Calcification
- Intima
- Media
- Elastic laminae
- Internal
- External
Schematic Timeline for Atherogenesis

Lesion initiation

- No symptoms
- + Symptoms

Time (y)

- Ischemic Heart Disease
- Cerebrovascular Disease
- Peripheral Vascular Disease
Ruptured Atherosclerotic Plaque

- Thrombus
- Thickened Media
- Necrotic Lipid Core
- Ruptured Fibrous Cap
HDL-C is an Independent CVD risk factor

Multiple Anti-atherogenic Effects of HDL
Modulation of LDL-C versus HDL-C for CHD Risk Reduction

![Graph showing RRR (%) for cardiovascular endpoint for different studies: 4S, HPS, ASCOT, PPP, CARDS. Statin monotherapy (mainly LDL-C lowering).]
Modulation of LDL-C versus HDL-C for CHD Risk Reduction

- Statin monotherapy (mainly LDL-C lowering)
  - Combination treatment including nicotinic acid + a statin (lowering LDL-C and raising HDL-C)
First Major Crack in HDL Hypothesis

**Torcetrapib**

**Illuminate Phase 3 trial halted for increased CVD events**

*Forbes, April 2007*

**HDL-C: 72% increase**

**LDL-C: 25% decrease**

**Bartrer P et al. NEJM 2007**
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Potential Anti-inflammatory Mechanisms of HDL

- Cholesterol Removal—Both cellular and extracellular
- Sequestration of oxidized lipids/LPS/cytokines
- Delivery of Anti-inflammatory Proteins and Lipid Cargo
Potential Anti-inflammatory Mechanisms of HDL

• Cholesterol Removal-Both cellular and extracellular

• Sequestration of oxidized lipids/LPS/cytokines

• Delivery of Anti-inflammatory Proteins and Lipid Cargo
5A ApoA-I Mimetic Peptide

5A peptide

Hydrophilic
Alpha-helix cross-section
Hydrophobic

Kidney Int. 89 (2006) 809
JIMD Rep. 2 Dec (2016)
J Lipid Res. 56 (2015) 1727
PLoS One. 8 (2013) e68802
J Biol Chem. 287 (2012) 43730
J Pharm Exp Ther. 344 (2013) 50
Am J Respir Cell Mol Biol. 47 (2012) 186
5A Inhibits Cytokine Release by Macrophages

TNF-alpha release murine macrophages

Cytokine release PHA-treated whole blood

Anna Schwendeman et al. J. Lipid Res. 2015;56:1727-1737
Features of LCAT Deficiency

Partial deficiency: Fish Eye Disease (FED)
- Cloudy cornea

Complete deficiency: Familial LCAT Deficiency (FLD)
- Cloudy cornea
- Normochromic normocytic anemia
- Proteinuria/ESRD
- Low HDL-C
- Presence of LpX

Cloudy Cornea

Renal Lipid Deposits

Bilayer or multi-lamellar complex of phospholipids
LpX Causes Renal Disease in LCAT Deficiency

Synthetic Fluorescent LpX

Plasma Clearance of LpX is Delayed in Lcat−/− Mice

Agarose gel
Plasma PE fluorescence

PLoSOne 26 (2016) e0150083
LpX Causes Renal Disease in LCAT Deficiency

Synthetic Fluorescent LpX

Plasma Clearance of LpX is Delayed in Lcat−/− Mice

Glomerular Deposition of LpX

WT

Lcat−/−

Basement membrane

Mesangium

Plasmas PE fluorescence

Agarose gel

HDL

LpX

PLoS One 26 (2016) e0150083
LpX Causes Podocyte Effacement and Proteinuria

LpX Induces Podocyte Effacement

TEM

SEM

LpX Causes Glomerulosclerosis

WT

WT + LpX

Lcat<sup>-/-</sup>

Lcat<sup>-/-</sup> + LpX

LpX Causes Proteinuria

<table>
<thead>
<tr>
<th>Time</th>
<th>Urine Albumin to Creatinine Ratio (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>10</td>
</tr>
<tr>
<td>Week 2</td>
<td>20</td>
</tr>
<tr>
<td>Week 3</td>
<td>30</td>
</tr>
<tr>
<td>Week 4</td>
<td>40</td>
</tr>
<tr>
<td>Week 5</td>
<td>50</td>
</tr>
</tbody>
</table>

LCAT-KO + LPx

LCAT-KO + saline

WT + LpX

WT + saline

LCAT-KO

PLoSOne 26 (2016) e0150083
LpX Activates the Inflammasome and Fixes Complement

**IL-1β Secretion**

![IL-1β Secretion Graph](image)

**Lysosome Destabilization**

![Lysosome Destabilization Graph](image)

**C3 Fixation**

![C3 Fixation Graph](image)
Complement Fixation of Cholesterol

- **Antigen** binds to **Antibody** forming a complex, which activates the **C1 complex**.
- **C2a & C4b fragments** are produced.
- **Classical pathway** leads to the formation of **C3 convertase**.
- **Alternative pathway** results in **C3 hydrolysis** producing **C3b and C3a fragments**.
- **C3b** cleaves **C5** into **C5a and C5b**.
- **C5b, C6, C7, C8 and C9** together form the membrane attack complex, which causes the cell to swell and burst.

**Cholesterol**

**Cholestane**

**C3 Fixation**

[Graph showing OD values for different compounds: Serum, Cholesterol, Cholestane, Squalene, Squalsterol]
Cholesterol crystals Induce Inflammation

- Crystals can be seen as early as 2 weeks

NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Peter Duewell Eicke Latz

*Immunol 149 (2016) 306*
HDL Suppresses Inflammasome Activation by Cholesterol Crystals

**Image:**
![Diagram showing HDL's role in suppressing inflammasome activation by cholesterol crystals.](image-url)

**Reference:**
Immunol 149 (2016) 306
Potential Anti-inflammatory Mechanisms of HDL

- Cholesterol Removal—Both cellular and extracellular

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- Delivery of Anti-inflammatory Proteins and Lipid Cargo
HDL Dampens LPS Inflammatory Response

Structure of Lipopolysaccharide

[Image showing the structure of lipopolysaccharide with labeled components: O-antigen, core polysaccharide, disaccharide, and fatty acids.]

https://microbeonline.com/lipopolysaccharide

ATVB 27(2007) 1153
Potential Anti-inflammatory Mechanisms of HDL

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HDL-Proteome

Functional Map

Interactome Map


J Clin Invest 2007; 117: 746
Rosuvastatin Increases A1AT on HDL

Molecular Modeling

Cryoelectron Microscopy

Protein Spectral Count Changes

HDL-S

- Vitronectin
- Apolipoprotein C-III
- Complement C2
- Alpha-1-antichymotrypsin

HDL-L

- Alpha-1-antitrypsin
- Ceruloplasmin
- Ig lambda-like polypeptide 5
- Clusterin
- Ig lambda chain V-III (LOI)
- Plasma kallikrein
- Ig lambda chain V-III (SH)
- PGRP-L
- Ig kappa chain V-III

% change from baseline

Mol Cell Proteomics 14 (2015) 3247

A1AT (Red), HDL (Green)
Role of PAR in Inflammation
A1AT-HDL Inhibits Elastase-induced Inflammation

Elastase Induced Inflammation

HDL Delivers A1AT to Lung

Mol Cell Proteomics 14 (2015) 3247
Proposed Role of A1AT-HDL in Atherogenesis
Proposed Role of A1AT-HDL in Atherogenesis
HDL-Lipidome: Major Lipid Classes

- Sterols
- Triglyceride
- Cholesterol
- Glycolipids
- Phospholipids
- Oxy-sterols
- S1P
- Cholesteryl Esters

>200 species of specific lipids
Akt

HDL

S1P1/S1P3

SR-B1

A-I

Gα

β

γ

Bcl-XL

BAD

NF-κB

eNOS

COX-2

Smad2/3

NADPH oxidase

RhoA

Rac1

TGF-β2

MMP9

PLA2

PGI2

VCAM-1

Apoptosis

Adhesion

Vasodilation

Inflammation

Migration
HDL-S1P Inversely Related CHD

Subjects with CHD have decreased S1P on HDL.

*Lipids Health Disease 2011; 10: 70*
HDL-S1P Inversely Related CHD

Subjects with CHD have decreased S1P on HDL. S1P on HDL improves endothelial barrier function.

*S1P Content of HDL*  

*Endothelial Integrity*

*Lipids Health Disease* 2011; 10: 70
Major Questions in HDL Field

• Is it involved in the pathogenesis of CVD?
  Most likely, but maybe not RCT.

• Should we measure it?
  Yes, but need better assays.

• Is it a target of therapy or just a CVD biomarker?
  No for HDL-C, but can be under some conditions.

• What is the future of HDL research?
  Composition studies and how relate to function.

  HDL research is still valuable despite recent clinical trial failures and more research *not less* is needed to harness its potential.
National Institutes of Health

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