HDL: Metabolism and Genetic Defects

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Residual Cardiovascular Risk in Statin Treated Patients

- Decrease LDL
- Increase HDL

Reduce Residual Risk in Statin Treated Patients

Percent Decrease in Cardiovascular Risk

4S  WOSCOPS  LIPID STUDIES  AVERRE  ADORIM  JT Meta-Analysis  Jupiter

30%  31%  32%  33%  34%  24%  56%  44%

Percent Residual Risk in Cardiovascular Risk

70%  69%  70%  71%  72%  73%  76%  44%
Preβ-HDL and αHDL Mediated Cellular Cholesterol Efflux

“Dual Pathway”

HDLα (HDL-M,L) are the Ligand for the ABCG1 Transporter

Preβ-HDL is the Ligand for the ABCA1 Transporter

95%

5%
Current Working Model Of HDL Metabolism
Concept of HDL Flux in Reverse Cholesterol Transport
The Percentage of HDL-C Synthesized by the Liver, Intestine, and Peripheral Cells
The Percentage of HDL-C Synthesized by the Liver, Intestine, and Peripheral Cells

Intestine

Liver

HDL-VS Preβ-HDL

~75% Decrease HDL-C

95%

5%

~20% Decrease HDL-C

HDL-S

Plasma

Peripheral Tissues

Macrophages

Wellington CL et al. J Lipid Res. 2003;44: 1470-1480
Current Insights into the Mechanisms by Which HDL Protects Against Cardiovascular Disease
Current Insights into the Mechanisms by Which HDL Protects Against Cardiovascular Disease
Concepts and Definitions of Reverse Cholesterol Transport
Animal Model to Evaluate Reverse Cholesterol Transport

1. **14C-Cholesterol injected IP into Macrophage Donor Mice**
2. 4 Hrs.
3. **14C-Cholesterol Labelled Macrophages isolated and injected IP in Control and Experimental Animals**
4. Control Mice
5. Experimental Mice

**Quantitate 14C-Cholesterol in Plasma Lipoproteins, Liver and Feces**
Methodology for Cholesterol Efflux Capacity of Isolated Lipoproteins and Human Serum

**J774 MACROPHAGE CELLS**

48h w/ $^3$H-Cholesterol labeled 1% FBS RPMI + ACAT Inhibitor (2ug/ml)

15h w/ cAMP + 0.2% BSA

4h w/ 2.8% HDL (apoB-depleted sera)

% FC Efflux

Current Insights into the Mechanisms by Which HDL Protects Against Cardiovascular Disease

- Cholesterol Efflux and RCT
- Anti-Inflammatory Properties
- Stem Cells
- Transport Particle
- Endothelial Function
COLLAR-INDUCED CHANGES IN ENDOTHELIAL EXPRESSION OF VCAM-1 and ICAM-1 FOLLOWING EITHER a SALINE OR rHDL Infusion.

Current Insights into the Mechanisms by Which HDL Protects Against Cardiovascular Disease

- Cholesterol Efflux and RCT
- Anti-Inflammatory Properties
- Protect LDL From Oxidation
- Endothelial Function
MODULATION OF eNOS ACTIVITY IN ENDOTHEIAL CELL BY HDL AND ApoA-I/PC

Adapted from Assanasen J. Clin. Invest. 2005;115:969
Current Insights into the Mechanisms by Which HDL Protects Against Cardiovascular Disease

- Cholesterol Efflux and RCT
- Anti-Inflammatory Properties
- Protect LDL From Oxidation
- Transport Particle
- Endothelial Function
Proteomics of HDL: Proteins Identified in HDL

Current Insights into the Mechanisms by Which HDL Protects Against Cardiovascular Disease

- Cholesterol Efflux and RCT
- Anti-Inflammatory Properties
- Transport Particle
- Protect LDL From Oxidation
- Endothelial Function
- Stem Cells
Clinical Assessment of HDL

Functional vs. Dysfunctional HDL
Potential Mechanisms Involved in the Generation of Dysfunctional HDL

Dysfunctional HDL

Oxidation

Glycation in Diabetes

Myeloperoxidase

HDL


Dysfunctional HDL

Ox. PL

Lyso PC

sPLA2
Myeloperoxidase Catalyzed Nitration and Chlorination Of ApoA-I Decreases ABCA1 Mediated Efflux

The Dysfunctional HDL in Diabetic Patients is Corrected by Niacin Therapy

Effect of HDL Endothelium-dependent relaxations of aortic rings of wild-type mice in response to increasing concentrations of HDL isolated from healthy subjects (n = 5) or diabetic patients (n = 5).

Change in radial artery diameter (FMD) (%) after wrist occlusion during reactive hyperemia in diabetic patients before and after 3 months of niacin therapy (n=15) or control (n=15)

Conversion of Anti-inflammatory HDL to Proinflammatory HDL

Hahn et al. Arthritis Research & Therapy 2008 10:213
Genetic Defects in Lipoprotein Metabolism

- ApoA-I Deficiency
Homozygous Familial ApoA-I Deficiency
Complete ApoA-I Deficiency & Premature CAD in Iraqi Pedigree

Familial Apolipoprotein A-I Deficiency

ApoA-I Gene

Patients who lack ApoA-I, A1 (Gene Mutation) only have marked HDL deficiency, normal TG and LDL-C levels, and premature CHD:

1) Nonsense mutation, codon 84, CHD in Japanese female in her 50s (Matsunaga et al PNAS 1991):
2) four homozygotes in kindred Q[-2]X mutation in Canada, three had CHD in their 30s, one was in her 20s, no CHD, had xanthomas: (Ng et al JCI, 1994)
3) two brothers in Brazil with severe CHD at 38 and 39 requiring CABG, tubo-eruptive and planar xanthomas, normal TG and normal fat absorption, Q[-2]X mutation, (Santos et al, JLR 2008)

ApoA-I/C-III Gene

Patients who lack AI/CIII (DNA rearrangement) have marked HDL deficiency, low TG levels, normal LDL-C levels, premature CHD (two sisters required bypass at 29 and 30 years of age, since then have died of CHD), planar xanthomas, and normal fat absorption (Norum et al NEJM, 1982, Karathanasis PNAS 1987)

ApoA-I/C-III/A-IV Gene

Patients who lack AI/CIII/AIV (deletion of gene complex) have marked HDL deficiency, low TG levels, normal LDL-C levels, premature CHD (female age 43 died of CHD after bypass), and moderate fat malabsorption (function for apoA-IV) (Schaefer et al Arteriosclerosis, 1982, JLR 1985, Ordovas et al JBC 1989)
Genetic Defects in Lipoprotein Metabolism

- ApoA-I Deficiency
- ApoA-I Mutations
Apolipoprotein A-I<sub>Zavalla</sub> (Leu159--&gt;Pro): HDL-C deficiency and Premature CAD

TABLE 3. Mean Age, Lipids (mmol/L), Lipoproteins (mmol/L), and Apolipoproteins (mg/dL) (±SD) Between the 2 Groups

<table>
<thead>
<tr>
<th></th>
<th>With Mutation</th>
<th>Without Mutation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>35 (18.3)</td>
<td>27 (19.0)</td>
<td>NS</td>
</tr>
<tr>
<td>TC</td>
<td>5.13 (1.4)</td>
<td>5.84 (1.6)</td>
<td>NS</td>
</tr>
<tr>
<td>TG</td>
<td>2.44 (1.7)</td>
<td>1.82 (1.2)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.27 (0.19)</td>
<td>1.16 (0.19)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-C</td>
<td>3.71 (1.1)</td>
<td>3.9 (1.6)</td>
<td>NS</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>38.7 (22.3)</td>
<td>124.4 (19.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>14.3 (1.4)</td>
<td>19.0 (3.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoB</td>
<td>133.6 (52.0)</td>
<td>128.0 (37.2)</td>
<td>NS</td>
</tr>
</tbody>
</table>

ApoA-I Milano

They Have The Magic Gene

When a man in Limone visited a doctor, he was found to have a mutation in his ApoA-I gene. This mutation resulted in a change from Arg to Cys.

Clinical Investigation and Reports
Cardiovascular Status of Carriers of the Apolipoprotein A-I Milano Mutant: The Limone sul Garda Study

Table 2. IMT in the CCA, Bifurcation, and ICA Segments in Control Subjects, ApoA-I<sub>W</sub> Carriers, and HA Subjects

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>ApoA-I&lt;sub&gt;W&lt;/sub&gt; Carriers</th>
<th>HA-LC</th>
<th>HA-BD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCA</td>
<td>0.63±0.14</td>
<td>0.61±0.15</td>
<td>0.78±0.36</td>
<td>0.82±0.30†</td>
</tr>
<tr>
<td>Bifurcation</td>
<td>0.70±0.14</td>
<td>0.74±0.18</td>
<td>1.18±0.46</td>
<td>1.11±0.46†</td>
</tr>
<tr>
<td>ICA</td>
<td>0.60±0.13</td>
<td>0.60±0.11</td>
<td>0.76±0.37</td>
<td>0.77±0.34†</td>
</tr>
<tr>
<td>Average</td>
<td>0.64±0.12</td>
<td>0.63±0.10</td>
<td>0.86±0.23</td>
<td>0.88±0.29†</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.83±0.12</td>
<td>0.89±0.36</td>
<td>1.86±0.68</td>
<td>1.73±0.78†</td>
</tr>
</tbody>
</table>

Data are reported as mean±SD.

†Significantly different vs control subjects and ApoA-I<sub>W</sub> carriers.


A

eNOS

GAPDH

B

eNOS

β-actin

<table>
<thead>
<tr>
<th></th>
<th>Medium</th>
<th>Control HDL</th>
<th>A-1&lt;sub&gt;m&lt;/sub&gt; HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS/GAPDH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>eNOS/β-actin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Significant difference versus control subjects.
Infusion of ApoA-I Milano Resulted in Regression of Atherosclerosis in Patients with the Acute Coronary Syndrome

Subjects: 46 Acute Coronary Patients

Infusions: 5 Weekly Infusions
33 ApoA-I Milano Infusion
(15 mg/kg, n=12; 45 mg/kg, n=24; 11 Saline Infusions)

Results: Regression – 14.1% Decrease in Total Atheroma Volume

Genetic Defects in Lipoprotein Metabolism

- ApoA-I Deficiency
- ApoA-I Mutations
- ABCA1 Transporter Deficiency-Tangiers Disease
ABCA1 Transporter Deficiency – Tangier Disease
# Kinetic Analysis of $^{125}$I HDL in Controls and Patients with Tangier Disease

<table>
<thead>
<tr>
<th></th>
<th>Synthesis rate (mg/kg/day)</th>
<th>Residence Time (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HDL</td>
</tr>
<tr>
<td><strong>Control (n=11)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(range)</td>
<td>8.25 (4.40-12.40)</td>
<td>4.89</td>
</tr>
<tr>
<td><strong>Heterozygotes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>6.59</td>
<td>2.59</td>
</tr>
<tr>
<td>Mother</td>
<td>9.28</td>
<td>2.23</td>
</tr>
<tr>
<td><strong>Probands</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brother</td>
<td>3.47</td>
<td>0.47</td>
</tr>
<tr>
<td>Sister</td>
<td>3.11</td>
<td>0.58</td>
</tr>
</tbody>
</table>
KINETIC ANALYSIS OF 125I-ApoA-I CONTROL SUBJECTS, AS WELL AS, TANGIER DISEASE HETEROZYGOTE AND TANGIER HOMOZYGOTE PATIENTS
Tangier Disease

1. Patients who lack ABCA1 function (ABCA1 mutations) have mild hypertriglyceridemia, low LDL cholesterol (usually around 50 mg/dl), and marked HDL deficiency (HDL C < 5 mg/dl) with detectable plasma apolipoprotein A-I (3 mg/dl). The only HDL particle in plasma is very small prebeta1 HDL, which is rapidly cleared from plasma.

2. Clinical features include hepatosplenomegaly and corneal opacification.

3. Tangier patients often develop CHD prior to age 60 years. It has been estimated that homozygotes have a 3 fold increased CHD risk and heterozygotes have 1.5 fold increased CHD risk as compared to Framingham controls.

4. Tangier patient’s LDL is small, TG and beta carotene-enriched – replacing CE, and is rapidly cleared from plasma – which may account for the orange coloration of many tissues in the body.


Genetic Defects in Lipoprotein Metabolism

- ApoA-I Deficiency
- ApoA-I Mutations
- ABCA1 Transporter Deficiency-Tangiers Disease
- LCAT Deficiency
Clinical Features of LCAT Deficiency

LCAT Deficiency


2. These patients have an inability to add a fatty acid derived from phospholipid to cholesterol to form cholesteryl ester, and therefore virtually all of the cholesterol in plasma is in the free form, whereas in normal controls about 70% of cholesterol in plasma is cholesteryl ester.

3. Classification of Mutations in LCAT Gene:
   a. LCAT deficiency – complete LCAT Deficiency
   b. Fish-eye disease – partial LCAT Deficiency-β LCAT on LDL.

4. LCAT deficient patients have marked corneal opacification, and Complete LCAT patients often develop renal insufficiency in the 40s and 50s.

5. These patients have mild hypertriglyceridemia, low LDL cholesterol levels (about 70 mg/dl), very low HDL cholesterol levels (about 10 mg/dl) and apoA-I levels that are about 30 mg/dl.

6. No evidence of CHD prior to age 60 years in homozygous LCAT deficiency.
Genetic Defects in Lipoprotein Metabolism

- ApoA-I Deficiency
- ApoA-I Mutations
- ABCA1 Transporter Deficiency-Tangiers Disease
- LCAT Deficiency
- CETP Deficiency
Current Model of CETP Deficiency

- Cholesterol Pool
- LPL, HL
- B-48
- Triglycerides
- LDL, HDL-VL, HDL-L, HDL-M, HDL-S, HDL-VS
- Arterial Wall Macrophage
- ABCG1, ABCA1
- CETP
- CE
- HL, EL, PLTP
- Modification
- Lipid Transfer Protein (LTP)
- LCAT
- APOA1, APOB, APOE
- Apo AI, Apo B, Apo E
- APOC1, APOC2, APOC3
- HDL, LDL, VLDL
# Cholesteryl Ester Transfer Protein Deficiency

Proband: 42 Year Old Male

<table>
<thead>
<tr>
<th></th>
<th>Proband</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/dl</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>251</td>
<td>162±28</td>
</tr>
<tr>
<td>Plasma Triglycerides</td>
<td>98</td>
<td>72±30</td>
</tr>
<tr>
<td>HDL</td>
<td>117</td>
<td>53±10</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>268</td>
<td>136±15</td>
</tr>
<tr>
<td>LDL</td>
<td>59</td>
<td>139±40</td>
</tr>
<tr>
<td>ApoB</td>
<td>61</td>
<td>120±20</td>
</tr>
</tbody>
</table>

Ikewaki K. J Clin Invest. 1993;92:1650-1658
Plasma Kinetics of $^{125}$I-ApoA-I-HDL in CETP Deficient and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>CETP Deficient</td>
<td>1.00</td>
</tr>
<tr>
<td>Controls</td>
<td>1.00</td>
</tr>
</tbody>
</table>

FCRd$^{-1}$

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CETP Def. (N = 2)</td>
<td>0.135</td>
</tr>
<tr>
<td>Control (N = 4)</td>
<td>0.196±0.04</td>
</tr>
</tbody>
</table>

Ikewaki K. J Clin Invest. 1993;92:1650-1658
Plasma Metabolism of $^3$H-CE-HDL in a CETP Deficient Subject

- **CETP Deficiency**
- **Control**

**Axes:**
- **Y-axis:** Fraction of Dose
- **X-axis:** Time (minutes)
<table>
<thead>
<tr>
<th>CETP Deficiency</th>
<th>LDL (mg/dl)</th>
<th>FCR (d')</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53.2</td>
<td>0.56±0.04</td>
</tr>
<tr>
<td>2</td>
<td>52.3</td>
<td>0.75±0.05</td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>99.0±8.6</td>
<td>0.39±0.11</td>
</tr>
</tbody>
</table>

Take Home Messages

- Epidemiological and clinical trial evidence establishes that low HDL is an independent risk factor for CVD.
- HDL is a complex polyfunctional anti-atherosclerotic lipoprotein.
- HDL-C is a poor biomarker of HDL anti-atherosclerotic properties.
- HDL-C is synthesized primarily by the liver and intestine with <5% from peripheral cells.
Take Home Messages

- The plasma HDL-C level does not indicate the level of "cholesterol flux" from the macrophage to the liver.
- HDL is heterogeneous with different HDL subfractions with separate physiological functions.
- Dysfunctional HDL has now been identified in several different diseases.
- Clinical trials will be required to definitively establish if increasing HDL-C will decrease clinical cardiovascular events.