Interactions of Inflammation, Lipoproteins, and Atherosclerosis: How an Innate Immunity Response Can Cause Atherosclerosis

Carl Grunfeld, M.D., Ph.D.
Professor of Medicine, UCSF
Chief, Division of Metabolism and Endocrinology, VAMC, SF
Research Scientist, NCIRE
Inflammation, Lipoproteins, and Atherosclerosis: How an Innate Immunity Response Can Cause Atherosclerosis

Carl Grunfeld, M.D., Ph.D.
Professor of Medicine, UCSF
Chief, Metabolism and Endocrine Sections, VAMC, SF
Research Scientist, NCIRE
Traditional CVD Risk Factors are only the tip of the iceberg.
Both very low (0.5 mg/L) and very high (10 mg/L) levels of hsCRP provide important prognostic information on cardiovascular risk. hsCRP is clinically useful for risk prediction across a full range of values and across a full range of FRS.
Innate Immunity and the Acute Phase Response (APR)

• APR is a host response to infection and inflammation that fights infection and dampens inflammation.

• Changes in production of specific proteins leading to changes in serum and tissue levels.

  (+) APR proteins such as C-Reactive Protein (CRP) and Serum Amyloid A (SAA) increase, while
  (-) APR proteins, such as albumin decrease.

• Expression of 7% of liver genes are altered.

• Induced by cytokines TNF, IL-1 and IL-6.

• Changes are mediated by regulation of gene transcription.

• (+) APR by NF-κB & NF-IL-6; (-) APR ↓NHR
The APR and Lipid Metabolism

- The APR is more than changes in hepatic production of serum proteins.
- Synthesis of specific proteins changes in many other tissues.
- Lipid and carbohydrate metabolism are altered during the APR.
- In humans: $\uparrow$ VLDL (TG), slight $\downarrow$ LDL and significant $\downarrow$ HDL.
- In rodents: $\uparrow$ VLDL (TG), LDL appears, slight $\downarrow$ HDL.
- Protein species of lipoproteins dramatically change.
The APR and Lipid Metabolism

• What changes occur?
• Why do they occur?
• What are the consequences of the changes?
Infection/inflammation Induces the Acute Phase Response (APR)

• The APR is characterized by changes in hepatic production of specific proteins leading to changes in serum levels.
  
  (+) APR proteins such as C reactive protein and serum amyloid A (SAA) increase while (-) APR proteins, such as albumin decrease.

• The specific proteins that change and the magnitude of the changes varies between species.

• The APR is more than changes in hepatic protein production. There are changes in protein synthesis in many tissues in addition to the liver. Moreover, not only protein but also carbohydrate and lipid metabolism are altered during the APR.
How did we get started?
The Cachectin Hypothesis

• Infection and cancers with wasting have hypertriglyceridemia.
• Presume hypertriglyceridemia and wasting linked.
• Product of activated macrophages promotes lipolysis, decreases lipoprotein lipase (LPL) and lipid synthesis (lipogenesis) in cultured fat cells
• Product named “Cachectin”; found to be the cytokine TNF
• We show other cytokines have same effects in cultured fat cells
• TNF & cytokines induce hypertriglyceridemia in animals, but:
  - TNF does not decrease LPL or de novo lipogenesis in fat
  - TNF increased de novo lipogenesis in liver promoting VLDL production
  - TNF did not decrease VLDL triglyceride clearance
  - TNF induced hypertriglyceridemia without causing wasting!
• Hypertriglyceridemia is not linked to wasting in humans.
Animal and *In Vitro* Models of the Acute Phase Response

- Endotoxin (LPS): gram (-) infection
- Lipoteichoic Acid (LTA): gram (+) infection
- Zymosan: fungal infection
- Turpentine: inflammation
- Cytokines:
  - Pro-inflammatory: TNF, IL-1, IL-6
  - Viral: Interferon-α
  - Anti-inflammatory: IL-4, IL-10
**LPS-induced Hypertriglyceridermia**

Low dose LPS:
- mobilizes FFA from adipose tissue,
- increases hepatic *de novo* lipogenesis,
- decreases fatty acid oxidation thereby increasing VLDL production.

Medium dose LPS:
- decreases adipose tissue lipogenesis,
- further stimulating net lipolysis

High dose LPS:
- decreases LPL and triglyceride clearance

The ED\textsubscript{50} for increasing triglycerides is 1/500,000 the LD\textsubscript{50}.

The sensitive nature of the response and the liver as the origin led us to conclude that the changes in lipid metabolism were part of the APR and might be protective.
Should we care about the effects of LPS in humans?

- In humans high fat or high energy diet\(^1\) and type 2 diabetes\(^2\) $\rightarrow$ ↑LPS in plasma
- In mice, diet induced obesity, insulin resistance and dyslipidemia is accompanied by ↑LPS & blocked by antibiotics or germfree breeding\(^3,4\).

LPS is increased in patients with HIV infection and linked to progression\(^5\).

\(^1\)J. Amar AJCN 2008; \(^2\)S. Creeley AJP 2007; \(^3\)F. Backhed PNAS 2007; \(^4\)M. Membrez FASEB J 2008.
The APR Alters Triglyceride Metabolism to Increase VLDL

**Liver**
- Acetate
- FA Oxidation
- CPT-1 & ACO
- FA
- TG
- ApoE
- ApoB/ApoE receptor
- VLDL
- ApoE
- IDL
- LPL
- GLUT 4

**Adipose Tissue**
- FA
- FFA
- FA
- TG
- ApoB/ApoE receptor
- IDL
- LPL
- FAT/P
- GLIT 4

**Extra hepatic tissue**
- P-↑ HSL
- P-↑ Perilipin
- ↓ GPAT
- ↓ MGAT
- ↓ DGAT
- ↓ AGPAT
- ↓ Lipin

- FA Oxidation
- ↓ CPT-1 & ACO
- ↓ FA
- ↓ TG
- ↓ ApoE
- ↓ ApoB/ApoE receptor
- ↓ VLDL
- ↓ ApoE
- ↓ LPL
- ↓ FFA
- ↓ FAT/P
- ↓ GLUT 4
More Cholesterol for VLDL Synthesis

↑ HMG CoA Reductase
→ More cholesterol synthesis

↓ Cholesterol 7α Hydroxylase
↓ Cholesterol 27 Hydroxylase
→ Less bile acid synthesis from cholesterol

↓ ABCG5/G8
→ Less Cholesterol secretion in bile
Reverse Cholesterol Transport

Liver \[\downarrow\text{Apo AI}\] \rightarrow Pre \(\beta\) HDL

Selective uptake

LDL-R LRP

Particle uptake

LDL-R LRP

Endocytotic uptake

Triglyceride-rich lipoproteins

Apo AI

\[\downarrow\text{SR-BI}\]

\[\downarrow\text{PLTP}\]

\[\downarrow\text{HL}\]

\[\downarrow\text{LCAT}\]

Peripheral cell

\[\downarrow\text{ABCA1}\]
<table>
<thead>
<tr>
<th>Nuclear Hormone Receptors</th>
<th>NHR Regulated Metabolic Genes in APR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα &amp; CPT-1, ACO</td>
<td>fatty acid oxidation</td>
</tr>
<tr>
<td>PPARγ LPL, FAT, FATP</td>
<td>fatty acid uptake</td>
</tr>
<tr>
<td>G/M/DG/AGP-AT</td>
<td>triglyceride synthesis</td>
</tr>
<tr>
<td>LXR Cyp7a1</td>
<td>bile acid synthesis</td>
</tr>
<tr>
<td>ABCG5/ABCG8</td>
<td>cholesterol secretion</td>
</tr>
<tr>
<td>CETP</td>
<td>cholesterol ester transfer</td>
</tr>
<tr>
<td>FXR PLTP</td>
<td>phospholipid transfer</td>
</tr>
<tr>
<td>SR-B1</td>
<td>cholesterol ester uptake</td>
</tr>
<tr>
<td>MDR-2</td>
<td>phospholipid secretion</td>
</tr>
<tr>
<td>BSEP</td>
<td>bile salt excretion</td>
</tr>
<tr>
<td>Cyp7a1</td>
<td>bile acid synthesis</td>
</tr>
<tr>
<td>SHP</td>
<td>bile acid synthesis</td>
</tr>
<tr>
<td>LRH-1 Cyp7a1</td>
<td>bile acid synthesis</td>
</tr>
<tr>
<td>PXR &amp; MDR-2</td>
<td>phospholipid secretion</td>
</tr>
<tr>
<td>ERRα MCAD</td>
<td>FA oxidation</td>
</tr>
<tr>
<td></td>
<td>cholic acid synthesis</td>
</tr>
</tbody>
</table>
Role of Acute Phase Response

- Neutralize the invading microorganisms
- Minimize the extent of tissue damage
- Participate in the local immune response and tissue regeneration

- We postulated that lipoproteins play the same roles in the APR plus deliver substrate to immune cells
Endotoxin is in Lipoproteins, But Resists Extraction

*Harris et al. JCI 86:696, 1990*

LPS added to Lipoproteins is hard to detect, even with extraction.

---

**Table 1. Endotoxin Contamination of Ultracentrifugally Isolated Lipoproteins**

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>(A)</th>
<th>(B)</th>
<th>(C)</th>
<th>(D)</th>
<th>(E)</th>
<th>(F)</th>
<th>(G)</th>
<th>(H)</th>
<th>(I)</th>
<th>(J)</th>
<th>Õ</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL #1</td>
<td>42,835</td>
<td>162</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>40</td>
<td>8,314</td>
<td>114</td>
<td>113</td>
<td>125</td>
<td>496</td>
<td>5,755</td>
<td>3,514</td>
</tr>
<tr>
<td>VLDL #2</td>
<td>58,700</td>
<td>584</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>2,000</td>
<td>370</td>
<td>794</td>
<td>132</td>
<td>284</td>
<td>36</td>
<td>5,755</td>
<td>3,514</td>
</tr>
<tr>
<td>LDL #1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>ND</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>LDL #2</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>49</td>
<td>2</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>HDL #1</td>
<td>ND</td>
<td>&lt;1</td>
<td>5,112</td>
<td>ND</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>320</td>
<td>319</td>
</tr>
<tr>
<td>HDL #2</td>
<td>ND</td>
<td>&lt;1</td>
<td>ND</td>
<td>2</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>320</td>
<td>319</td>
</tr>
</tbody>
</table>
Chylomicrons Prevent LPS-Induced Death and Enhance Clearance by Liver.

Harris et al. JCI 91:1028, 1993.

Mortality

Endotoxin Clearance by Organ
Chyomicrons Direct Endotoxin Away from Kupfer Cells to Hepatocytes

_Harris et al. JCI 91:1028, 1993._

LPS + Saline

LPS + Chylomicrons
Chyomicrons Direct Endotoxin Away from Kupffer Cells to Hepatocytes and Into Bile

Lipoproteins Decrease Endotoxemia, Serum TNF and Death in Cecal Ligation & Puncture

*Read et al. J. Exp. Med. 182: 267, 1996*
In Sepsis:
Human VLDL and LDL become enriched in phospholipids & cholesterol and scavenge endotoxin
*Kitchens et al. JLR 44:2339, 2003*

Lipoprotein and apolipoprotein Therapy has been tried in human sepsis, but failed, just as anti-cytokine therapies fail. We diagnose sepsis too late.
Changes in Trace HDL Proteins

Khovidhunkit et al. Am. J. Physiol. 288: R1306, 2005

Saline

Endotoxin
GC-MS Reveals HDL Spot 1 is Parotid Secretory Protein (PSP)  
*Khovidhunkit et al. Am. J. Physiol. 288: R1306, 2005*

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster</td>
<td>MFOGLSLILCGLLIGTSESILGILVSADNLNSVSLAPLNNLNL</td>
</tr>
<tr>
<td>Rat</td>
<td>MFOGLSLILCGLLIGTSESILGILVANAVRNLNLPSLAVSNELNS</td>
</tr>
<tr>
<td>Mouse</td>
<td>MFOGLSLILCGLLIGTSESILGILVAVVNLNSVSLAPLNNLNL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster</td>
<td>YSSLKMKILKLHILNLPLLNLAEVSNLNLKHLKAKLNLNL</td>
</tr>
<tr>
<td>Rat</td>
<td>YSSLKMKILKLHILNLPLLNLAEVSNLNLKHLKAKLNLNL</td>
</tr>
<tr>
<td>Mouse</td>
<td>YSSLKMKILKLHILNLPLLNLAEVSNLNLKHLKAKLNLNL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster</td>
<td>NGLKMTTIGKCSDDKISISLLGRNLIMVDQVSEGALTNTVEV</td>
</tr>
<tr>
<td>Rat</td>
<td>NGLKMTTIGKCSDDKISISLLGRNLIMVDQVSEGALTNTVEV</td>
</tr>
<tr>
<td>Mouse</td>
<td>NGLKMTTIGKCSDDKISISLLGRNLIMVDQVSEGALTNTVEV</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster</td>
<td>NMLCPLLQVLSSLNLTIMGQLSNNLTCGLSLNL</td>
</tr>
<tr>
<td>Rat</td>
<td>NMLCPLLQVLSSLNLTIMGQLSNNLTCGLSLNL</td>
</tr>
<tr>
<td>Mouse</td>
<td>NMLCPLLQVLSSLNLTIMGQLSNNLTCGLSLNL</td>
</tr>
</tbody>
</table>

Has tertiary structure predicted to be similar to:
- CETP  (-) APR protein
- PLTP  (-) APR protein
- LBP   (+) APR protein
- BPI   (+) APR protein
PSP has Anti-Candidal Activity

- PSP has most homology with the *pathogen* binding domain of BPI and LBP
- Saliva has antimicrobial activity
- Thrush is common in Xerostomia
- We expressed and tested recombinant PSP
GC-MS Reveals HDL Spot 1 is Parotid Secretory Protein (PSP)  
*Khovidhunkit et al. Am. J. Physiol. 288: R1306, 2005*

- PSP has most homology with the *pathogen* binding domain of BPI and LBP
- Saliva has anti-microbial activity
- Thrush is common in Xerostomia
- We expressed and tested recombinant PSP

PSP has Anti-Candidal Activity

![Graph showing the effect of PSP concentration on colony-forming units (CFUs)]

PSP is in the CETP family
## Cause of Death in Torcetrapib Trial
*(CETP Inhibitor)*

<table>
<thead>
<tr>
<th>Event</th>
<th>Atorvastatin</th>
<th>Torcetrapib + Atorvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>59</td>
<td>93*</td>
</tr>
<tr>
<td>Any CVD</td>
<td>35</td>
<td>49</td>
</tr>
<tr>
<td>Sudden or MI</td>
<td>31</td>
<td>34</td>
</tr>
<tr>
<td>Stroke</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Other Cardiovascular</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Non CVD</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Cancer</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Infection</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>
What are the consequences of the changes in lipid metabolism?

• While exploring these changes, it became clear to us that many of the changes in lipoproteins were also pro-atherogenic.

• Such changes in lipoproteins may help to explain the epidemiological links of infection and inflammation to cardiovascular disease.
Early ↓HDL, later ↓LDL, then ↑VLDL in HIV & AIDS


↓HDL may promote atherosclerosis more than ↓LDL prevents it
Infectious and Inflammatory Diseases Associated with Atherosclerosis

- Chronic dental infection
- Chronic bronchitis
- HIV infection
- Acute Infection (Gerald Ford Syndrome)
- Chlamydia pneumonia infection?
- Helicobacter pylori infection?
- Systemic Lupus Erythematosus
- Rheumatoid Arthritis
- Psoriasis
Animal and *In Vitro* Models of the Acute Phase Response (APR)

- Endotoxin (LPS): gram (-) infection
- Lipoteichoic Acid (LTA): gram (+) infection
- Zymosan: fungal infection
- Poly I/C: Viral infection
- Turpentine: inflammation
- Cytokines:
  - Pro-inflammatory- TNF, IL-1, IL-6, Interferon-γ
  - Viral- Interferon-α
  - *Anti-inflammatory*- IL-4, IL-10
What promotes or prevents atherosclerosis?

- LDL and VLDL promote Atherosclerosis
- However, LDL must be modified
  - oxidation
  - aggregation (ceramides)
- HDL prevents atherosclerosis
  - anti-oxidation
  - anti-aggregation
  - reverse cholesterol transport
Sources of Oxidized LDL

• From the diet (fried foods)
• In the circulation (reactive oxygen)
  - Reactive oxygen species are used to fight infection
  - Therefore we looked for oxidized lipoproteins in animal models of infection
• In the vessel wall (lipoxygenase)
  - Therefore we determined whether lipoproteins are more easily oxidized in animal models of infection
LPS & Zymosan Increase LDL Lipid Hydroperoxides & Conjugated Dienes
Oxidized LDL is Increased in Systemic Lupus Erythematosus

LPS Increases LDL Susceptibility to \textit{in Vitro} Oxidation (CuSO$_4$)

Most LDL oxidation occurs in the vessel wall.
Paraoxonase (PON)

• Discovered by its ability to metabolize the insecticide paraoxon
• PON destroys oxidized phospholipids
• PON is one of the key antioxidant factors on HDL
• Decreases in PON promote atherosclerosis in mice
• There previously was debate on the role of PON in atherosclerosis, as humans have several PON isoforms with differing catalytic activity towards artificial substrates, but not oxidized lipids
• Decreased PON levels predict atherosclerosis
LPS Decreases Paraoxonase
It is a Negative APR Protein

Activity in Serum

mRNA Levels in Liver

p<0.001

p<0.05

p<0.02

p<0.001

p<0.001

p<0.01

p<0.001
Paraoxonase (PON)

- Discovered by ability to metabolize an insecticide paraoxon
- PON destroys oxidized phospholipids
- PON is a key antioxidant factor on HDL
- Decreases in PON promote atherosclerosis in mice
- There previously was debate on the role of PON in atherosclerosis as humans have several PON isoforms with differing catalytic activity towards artificial substrates, but not towards oxidized lipids
- Decreased PON levels predict atherosclerosis

LPS Decreases PON
It is a Negative APR Protein

Graph: Activity in Serum
- Time (hours): 0, 8, 16, 24, 32, 40, 48
- Percent Control: 125, 100, 75, 50, 25, 0
- Control: Green line, LPS: Yellow line
- p<0.001
An image of a document discussing the topic of Paraoxonase (PON). The document highlights key points about PON:

- Discovered by ability to metabolize an insecticide paraoxon.
- PON destroys oxidized phospholipids.
- PON is a key antioxidant factor on HDL.
- Decreases in PON promote atherosclerosis in mice.
- There previously was debate on the role of PON in atherosclerosis as humans have several PON isoforms with differing catalytic activity towards artificial substrates, but not towards oxidized lipids.
- Decreased PON levels predict atherosclerosis.

The document also notes that LPS decreases PON and that this is a negative APR protein. A graph shows the decrease in PON activity in serum over time with LPS treatment compared to control, indicating a significant decrease with p<0.001.
Paraoxonase (PON) is Reduced in Rheumatoid Arthritis

G. Baskol et al. *Clin Biochem.* 38: 951; 2005

![Graph showing PON1 levels in different groups: Controls (n=25), Active RA (n=31), Inactive RA (n=26). Mean ± SD with p-values: p=0.002 for Controls vs Active RA, p=0.018 for Controls vs Inactive RA.](image-url)
Potential proatherogenic changes affecting LDL during infection and inflammation

<table>
<thead>
<tr>
<th>Changes</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ <strong>LDL oxidation</strong></td>
<td>Increased foam cell formation; activates macrophages &amp; endothelial cells</td>
</tr>
<tr>
<td>↑ <strong>small dense LDL</strong></td>
<td>Increases LDL susceptibility to oxidation, penetration through endothelium, interaction with proteoglycans &amp; retention in arterial wall</td>
</tr>
<tr>
<td>↑ <strong>ceruloplasmin</strong></td>
<td>Increases LDL oxidation</td>
</tr>
</tbody>
</table>
### Other potential proatherogenic changes affecting LDL during the APR → LPC

<table>
<thead>
<tr>
<th>Changes</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Lysophosphatidylcholine (LPC)</td>
<td><em>Mobilizes cells to fight infection</em></td>
</tr>
<tr>
<td></td>
<td>Induces monocyte chemotaxis and endothelial cell adhesion molecules.</td>
</tr>
<tr>
<td></td>
<td>Impairs blood vessel relaxation</td>
</tr>
<tr>
<td>↑ Platelet-Activating Factor acetylhydrolase or Lipoprotein Phospholipase A&lt;sub&gt;2&lt;/sub&gt; (PAF-AH or Lp-PLA&lt;sub&gt;2&lt;/sub&gt;)</td>
<td><em>Decreases inflammation (PAF)</em></td>
</tr>
<tr>
<td></td>
<td>Increases LPC (from oxidized PC); Associated with plaque rupture;</td>
</tr>
<tr>
<td></td>
<td>{Inhibitor decreases atherosclerosis}</td>
</tr>
<tr>
<td>↑ Secretory Phospholipase A&lt;sub&gt;2&lt;/sub&gt; (sPLA&lt;sub&gt;2&lt;/sub&gt;-IIA)</td>
<td>Increases LPC production;</td>
</tr>
<tr>
<td></td>
<td>Releases polyunsaturated FA from phospholipid that become oxidized FA;</td>
</tr>
<tr>
<td></td>
<td>Decreases LDL size</td>
</tr>
</tbody>
</table>
Lysophosphatidyl Choline (LPC)

LPC is another inflammatory mediator that play a positive role in local infection and inflammation, but promotes atherogenesis

• Increases monocyte chemotaxis
• Induces vascular endothelial cell adhesion molecules
• Impairs blood vessel relaxation
LPS Increases PAF-AH and increases LPC in LDL

Memon et al. AJP 46:R94, 1999
PAF-AH Increased in HIV/AIDS: No Effect of HAART

Khovidhunkit et al. Metab. 48:1524, 1999
Increased sPLA$_2$–IIA and Small Dense LDL (LDL-1) in Rheumatoid Arthritis

Hurt-Camejo et al., Arth & Rheum 44:2761, 2001
## Potential proatherogenic changes affecting LDL during infection and inflammation

<table>
<thead>
<tr>
<th>Changes</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ LDL oxidation</td>
<td>Increased foam cell formation; activates macrophages &amp; endothelial cells</td>
</tr>
<tr>
<td>↑ small dense LDL</td>
<td>Increases LDL susceptibility to oxidation, penetration through endothelium, interaction with proteoglycans &amp; retention in arterial wall</td>
</tr>
<tr>
<td>↑ ceruloplasmin</td>
<td>Increases LDL oxidation</td>
</tr>
<tr>
<td>↑ sphingolipid content</td>
<td>Facilitates LDL aggregation and uptake into macrophages</td>
</tr>
</tbody>
</table>
Aggregation, Sphingolipids & LDL

- Aggregation of LDL is another way that LDL becomes more atherogenic
- Aggregation enhances LDL uptake into macrophages and foam cell formation
- Increased sphingolipid content, especially increased ceramide levels, increase LDL aggregation
LPS Increases Serine Palmitoyl-Transferase, Spingolipid Synthesis and Lipoprotein Ceramide Levels

$^3$H-Serine Incorp. (nmol/mg protein)

Ceramides

Sphingomyelin

SPT (pmoles/min/mg protein)

Ceramide content (μg/mg total lipid)

VLDL

IDL

LDL

HDL

Arteriosclerosis, Thrombosis, and Vascular Biology 18:1257, 1998
Aggregation of LDL makes LDL more atherogenic. Aggregation enhances LDL uptake into macrophages. Increased sphingolipid content, especially increased ceramide levels, increase LDL aggregation.

LPS Increases Spingolipid Synthesis and Lipoprotein Ceramide Levels

3H-Serine Incorp. (nmol/mg protein)

Ceramide content (μg/mg total lipid)

Arteriosclerosis, Thrombosis, and Vascular Biology 18:1257, 1998
Potential Proatherogenic changes that affect VLDL during infection and inflammation

<table>
<thead>
<tr>
<th>Changes</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ VLDL levels</td>
<td>Provides lipid substrate for macrophage (mΦ) uptake.</td>
</tr>
<tr>
<td>↓ LPL and HL</td>
<td>Decreases clearance of VLDL,</td>
</tr>
<tr>
<td></td>
<td>↑ LDL production &amp; mΦ uptake</td>
</tr>
<tr>
<td>↑ sphingolipid content</td>
<td>Decreases clearance of VLDL,</td>
</tr>
<tr>
<td></td>
<td>↑ LDL production &amp; mΦ uptake</td>
</tr>
<tr>
<td>↓ tissue apo E expression</td>
<td>Decreases clearance of VLDL,</td>
</tr>
<tr>
<td></td>
<td>↑ LDL production &amp; mΦ uptake</td>
</tr>
</tbody>
</table>

VLDL: very low-density lipoprotein, LPL: lipoprotein lipase, HL: hepatic lipase, apo: apolipoprotein.
Atherosclerotic Plaque Has Foam Cells

LC = lipid core
FC = fibrous cap
F = fissure
* = thrombus

Foam Cells
Reverse Cholesterol Transport

Liver $\downarrow$ Apo Al

LDL-R LRP $\downarrow$ SR-B1 $\downarrow$ PLTP $\downarrow$ HL $\downarrow$ LCAT $\downarrow$ ABCA1

Selective uptake

Particle uptake

Endocytotic uptake

Pre $\beta$ HDL

Peripheric cell

FC $\downarrow$ CE $\downarrow$ TG $\downarrow$ CETP $\downarrow$ α HDL $\downarrow$

Triglyceride-rich lipoproteins

SR-B1: FXR
PLTP: FXR
HL: PGC1, HNF1 & HNF4
CETP: FXR & LXR
Apo Al: ? LXR & PPARα
LCAT Activity Is Reduced in APR HDL: Less Cholesterol Efflux & More Influx

Khovidhunkit et al. JLR 42:907, 2001

HDL - LCAT Activity

**HDL Efflux**

N-ethyl maleimide (NEM) is an LCAT inhibitor
APR HDL: Less Cholesterol Efflux & More Influx
Khovidhunkit et al. JLR 42:907, 2001

HDL Efflux

![HDL Efflux Graph](image1)

HDL Influx

![HDL Influx Graph](image2)

NEM (N-ethyl maleimide) is an LCAT inhibitor
LCAT inactivation of control HDL increases macrophage cholesterol content
Mice were loaded with $^3$H-cholesterol and then given saline or low dose LPS (0.3 ng/kg). Excretion of $^3$H-cholesterol measured.

**Decreased in bile.**

**Decreased in feces.**

The opposite of what lipoproteins do to LPS itself!
Administration of LPS to Humans Reduces HDL’s Ability to Remove Cholesterol
F. McGillicuddy et al. Circ. 119: 1135, 2009

Human volunteers were given low dose LPS (3ng/kg), which induces a flu-like syndrome. An old Endocrine test.

HDL levels don’t change.

Efflux to HDL decreases.
Proinflammatory or Pro-atherogenic HDL in Systemic Lupus Erythematosus and Rheumatoid Arthritis

Cholesterol Efflux

- Apolipoprotein-mediated mechanism
- Diffusion mechanism

- ABCA1
- LCAT
- apo A-I
- Preβ HDL
- FC
- CE
- α HDL
- Hepatic lipase
- Phospholipid transfer protein
Inflammation Initiates and Sustains the Pathogenesis of Atherosclerosis
Macrophages Recognize Inflammatory Stimuli via Toll-like Receptors

TLR4 polymorphisms that attenuate receptor signaling have been associated with reduced atherosclerosis risk
LPS-Activated Macrophages Form Foam Cells From Lipoproteins

_Funk et al, Atherosclerosis 98:67, 1993_

From VLDL

From Native LDL

Unmodified LDL down regulates its receptor and does not form foam cells
Activation of TLR4 or TLR2 Leads to Intracellular Cholesterol Ester Accumulation and Foam Cell Formation

Activation of TLRs Lead to Cholesterol Ester and Triglyceride Storage by:

- ↑ Apo B-48R and LDL-R
- ↑ FA uptake by ↑ FAT/CD36
- ↑ FA movement in cell by AP2 & Mal-1
- ↑ FA → TG by ↑ DGAT
- ↓ FA oxidation by ↓ CPT-1
- ↑ FA and cholesterol synthesis from glucose by ↓ glucose oxidation
Low LPS Concentrations Increase Foam Cell Formation from VLDL via apoB-48R

Kazemi et al (submitted)

LPS + VLDL → CE

LPS ↑ ApoB-48R
Activation of Multiple TLRs Lead to Increased aP2 and mal1 Expression

Kazemi et al, ATVB 2005 & Submitted

Zymosan – TLR2; Poly I:C – TLR3; LPS – TLR4; Imiquimod – TLR7

AP2

Mal1
aP2 Expression is Specifically Induced by LPS, Not Intracellular Lipid Content

Kazemi et al, submitted

**Intracellular TG**

**aP2 Expression**
Glucose is Not Oxidized, but Used to Synthesize Fatty Acids and Cholesterol

Glucose Oxidation

Glucose → Fatty Acid (TG)  Glucose → Cholesterol
Immune Activation of Macrophages Leads to Foam Cell Formation

- Modified LDL
- ↑ CD36/FAT
- LDL
- ↑ LDL-R
- VLDL
- ↑ Apo B48 R
- Fatty Acid
- ↑ CD36/FAT
- Glucose
- Chol
- ↑ AP2
- ↑ Mal1
- ↑ DGAT
- ↑ TG
- CE
- CPT1
- ↓ CPT1
- ▼ Glucose Oxidation

- ABC A1 & G1
- ▼ Apo E
- ▼ Reverse Cholesterol Transport

- LDL-R
- FA
- mΦ
- Glucose
HIV Infection (nef) of mΦ prevents cholesterol efflux.
Mujawar et al., PLoS Biol. 4 e365, 2006

HIV PI increase foam cell formation via CD36.
Dressman et al., JCI 111:389, 2003
HIV Infection (nef) of mΦ prevents cholesterol efflux.
Mujawar et al., PloS Biol. 4 e365, 2006

HIV PI increase foam cell formation via CD36.
Dressman et al., JCI 111:389, 2003

![Graph showing cholesterol efflux](chart1.png)

![Graph showing CD36 expression and cholesterol ester levels](chart2.png)
Metabolic Pathways

- Glucose
- Pyruvate
- Lactate
- Acetate
- CO₂
- Fatty Acid
- Cholesterol
- Cholesteryl Ester
- Triglyceride
Local acidosis is associated with atherosclerotic plaque vulnerability and rupture.

Methods are being developed to detect unstable plaque based on the local acidosis.
LPS Activation Increases Lactate Production and Decreases pH

Kazemi et al, submitted

Lactate in Media $\rightarrow \downarrow 0.5$ pH unit

Local acidosis is associated with atherosclerotic plaque vulnerability and rupture.
Methods are being developed to detect unstable plaque based on the local acidosis.
• **Possible Adaptive Benefits of МΦ Lipid Accumulation in the Short Term...**
  • Lipid loading of macrophages inhibits growth of intracellular pathogens
  • Decreased oxidation with mitochondrial dysfunction facilitates formation of reactive oxygen species (ROS), an essential component of the “respiratory burst”

...**Are Complicated by the Deleterious Consequences of Chronic Activation**
  • Chronic activation leads to accumulation of foam cells and formation of atherosclerotic plaque
  • Local acidosis is associated with plaque vulnerability and rupture
• **Possible Adaptive Benefits of МФ Lipid Accumulation in the Short Term…**
  
  • Lipid loading of macrophages inhibits growth of intracellular pathogens
  
  • Decreased oxidation with mitochondrial dysfunction facilitates formation of reactive oxygen species (ROS), an essential component of the “respiratory burst”

  **…Are Complicated by the Deleterious Consequences of Chronic Activation**

  • Chronic activation leads to accumulation of foam cells and formation of atherosclerotic plaque
Internal/Bulb Carotid IMT is greater in HIV+ vs. Control After Adjusting for CVD Risk Factors

<table>
<thead>
<tr>
<th></th>
<th>HIV+</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Internal cIMT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (SD)</td>
<td>1.17 mm (0.50)</td>
<td>1.06 mm (0.58)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

**Difference of HIV+ vs. Controls**

<table>
<thead>
<tr>
<th></th>
<th>mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted for demographics (age, race, gender)</td>
<td>0.19 mm (0.11, 0.26)</td>
</tr>
<tr>
<td>Adjusted for demographics &amp; traditional CVD risk factors (smoking, diabetes, lipids, BP)</td>
<td>0.15 mm (0.07, 0.22)</td>
</tr>
</tbody>
</table>
# HIV = Male Gender, Smoking and Diabetes

<table>
<thead>
<tr>
<th>Estimated Effect in mm</th>
<th>Internal Carotid</th>
<th>Common Carotid</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV infection</td>
<td>0.15 mm**</td>
<td>0.033 mm *</td>
</tr>
<tr>
<td>Male†</td>
<td>0.13 mm***</td>
<td>0.054 mm***</td>
</tr>
<tr>
<td>Current smoker</td>
<td>0.17 mm***</td>
<td>0.020 mm**</td>
</tr>
<tr>
<td>Past smoker</td>
<td>0.09 mm***</td>
<td>0.020 mm***</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.12 mm***</td>
<td>0.026 mm***</td>
</tr>
<tr>
<td>Age (per 10 years)</td>
<td>0.16 mm***</td>
<td>0.073 mm***</td>
</tr>
<tr>
<td>Systolic BP (per 10 mmHg)</td>
<td>0.05 mm***</td>
<td>0.025 mm***</td>
</tr>
<tr>
<td>Diastolic BP (per 10 mmHg)</td>
<td>-0.07 mm***</td>
<td>-0.026 mm***</td>
</tr>
<tr>
<td>Total Chol (per 10 mg/dl)</td>
<td>0.009 mm***</td>
<td>0.004 mm***</td>
</tr>
<tr>
<td>HDL (per 10 mg/dl)</td>
<td>-0.020 mm***</td>
<td>-0.011 mm***</td>
</tr>
</tbody>
</table>

*p<0.01, **p<.001, ***p<.0001; †There was a significant gender interaction
Both very low (0.5 mg/L) and very high (10 mg/L) levels of hsCRP provide important prognostic information on cardiovascular risk. hsCRP is clinically useful for risk prediction across a full range of values and across a full range of FRS.
Summary and Conclusions

• The host response to infection and inflammation increases VLDL production (in the face of anorexia) and modifies lipoproteins to fight infection and decrease systemic inflammation.

• The very same changes are often pro-atherosclerotic at the level of both lipoproteins and macrophage foam cells, linking infections and inflammatory diseases to atherosclerosis.

→Consider chronic infection and inflammation = a CVD risk factor.

• Is this why so many of us evolved to have pro-atherosclerotic lipoprotein profiles? During evolution few lived long enough to get atherosclerosis and most had reproduced.
Summary and Conclusions

• The host response to infection and inflammation (APR) increases VLDL production (in the face of anorexia) through decreased gene expression driven by decreases in Nuclear Hormone Receptors (NHRs) and Co-Activators.
• The APR modifies lipoproteins to fight infection and decrease systemic inflammation.
• The very same changes are often pro-atherosclerotic at the level of both lipoproteins and macrophage foam cells, linking infections and inflammatory diseases to atherosclerosis.
• Is this why so many of us evolved to have pro-atherosclerotic lipoprotein profiles? During evolution few lived long enough to get atherosclerosis and most had reproduced.
• Changes in NHRs likely lead to decreased drug metabolism, leading to a short-term toxicity.
Colleagues

- Kenneth Feingold
- Riaz Memon
- Weerapan Khovidhunkit
- Ingebjorg Hardardottir
- Hahn Ly
- Anne Beigneux
- Min Sun Kim
- Biao Lu
- Mahmood Kazemi
- Janet Funk
- Arthur Moser

- Joseph Rapp
- Hobart Harris
- Thomas Read
- Maureen Marshall
- Ronald Krauss
- Walter Holleran
- Christopher Fielding
- Judy Shigenaga
- John Kane
- Jennifer Verdier Ratcliffe
- Peter Jensen
Veterans Affairs Medical Center, San Francisco
Sepsis: VLDL and LDL become enriched in phospholipids and scavenge endotoxin

Kitchens et al. JLR 44:2339, 2003
Cholesterol goes down in sepsis, but free cholesterol and phospholipids do not

*Kitchens et al. JLR 44:2339, 2003*
Sepsis: VLDL and LDL become enriched in phospholipids and scavenge endotoxin

Kitchens et al. JLR 44:2339, 2003
In Sepsis: VLDL and LDL become enriched in phospholipids & cholesterol and scavenge endotoxin

*Kitchens et al. JLR 44:2339, 2003*