Molecular Interventions for Cardiovascular Disease: It Isn't That Simple!

A Cautionary Tale
Causes of death, 2001:

1. Infectious and parasitic diseases: 14.9 million
2. Heart diseases: 11.1 million
3. Cancers: 7.3 million
4. Stroke: 5.5 million
5. Respiratory diseases: 3.6 million
6. Accidents, fires, drowning, etc.: 3.5 million
7. Maternal and perinatal: 3.0 million
8. Violence (war, homicide, suicide): 1.6 million

Population: 6,122,210,000
Deaths: 56,554,000

World Health Organization
World Health Report 2002
Atherosclerosis

Risk increases with higher [LDL]
Risk decreases with higher [HDL]
Familial Hypercholesterolemia (FH)

Heterozygotes (1:500)
- 300-500 mg/dl plasma cholesterol
- Xanthomas in third decade
- Coronary heart disease in fourth decade
- Treat w/ statins and bile acid binding resins

Homozygotes (1:10^6)
- 500-1200 mg/dl plasma cholesterol
- Xanthomas at birth
- Death by MI before age 30
- Treat w/ plasma LDL apheresis

Xanthoma
1) Acetyl-CoA → HMG-CoA → Mevalonate

2) Mevalonate (C_6) + 3ATP → Isopentenyl-PP_i (C_5 “isoprene”) + CO_2 + 3ADP + P_i

3) 6 Isoprene units (C_5) → Squalene (C_30)

4) Squalene (C_30) → Cholesterol (C_27)
Isopentylpyrophosphate (MEV Pathway)
Lipid Trafficking Enzymes

Triacylglycerol  \[ \iff \]  3 Fatty Acid + Glycerol

Lipoprotein Lipase, LPL
(located on endothelium of muscle, adipose tissue)

Cholesterol + Phosphatidylcholine \[ \iff \] Cholesterol Ester + Lysolecithin

Lecithin:Cholesterol Acyltransferase (LCAT)
(located on HDL)

Also CETP
## Lipoprotein Particles

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>M.W.</th>
<th>gL(^{-1})</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoA-I</td>
<td>28 kD</td>
<td>1.0-1.2</td>
<td>CMC, HDL; activates LCAT</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>17 kD</td>
<td>0.3-0.5</td>
<td>CMC, HDL</td>
</tr>
<tr>
<td>ApoA-IV</td>
<td>46 kD</td>
<td>0.15-0.16</td>
<td>CMC, HDL</td>
</tr>
<tr>
<td>ApoB-48</td>
<td>264 kD</td>
<td>0.03-0.05</td>
<td>CMC; (intestine only)</td>
</tr>
<tr>
<td>ApoB-100</td>
<td>512 kD</td>
<td>0.7-1.0</td>
<td>VLDL, IDL, LDL; binds receptor</td>
</tr>
<tr>
<td>ApoC-I</td>
<td>7 kD</td>
<td>0.04-0.06</td>
<td>CMC, VLDL, HDL</td>
</tr>
<tr>
<td>ApoC-II</td>
<td>9 kD</td>
<td>0.03-0.05</td>
<td>CMC, VLDL, HDL; activates LPL</td>
</tr>
<tr>
<td>ApoC-III</td>
<td>9 kD</td>
<td>0.12-0.14</td>
<td>CMC, VLDL, HDL</td>
</tr>
<tr>
<td>ApoD</td>
<td>33 kD</td>
<td>0.06-0.07</td>
<td>HDL</td>
</tr>
<tr>
<td>ApoE</td>
<td>38 kD</td>
<td>0.03-0.05</td>
<td>CMC, VLDL, IDL, HDL; binds receptor</td>
</tr>
</tbody>
</table>

### FRAMINGHAM HEART STUDY 1977

For each 1% decrease in [LDL], get an ~1% decrease in RR of CAD mortality. For each 1% increase in [HDL], get a 1.3-3% decrease in RR of CAD mortality. These are independent effects.
Lipoprotein Transport

Dietary fat and cholesterol are absorbed in the intestine and enter the bloodstream. Bile salts and fecal cholesterol are also absorbed. The liver is responsible for the biosynthesis of fats and cholesterol. Lipoproteins such as cholesterol, chylomicrons, and VLDL transport lipids to peripheral tissues. LDL and HDL transport cholesterol back to the liver. The liver also synthesizes remnant chylomicrons and VLDL. Glycerol and fatty acids are transported to peripheral tissues for β-oxidation. Cholesterol and triacylglycerols are transported by lipoprotein lipase in capillaries. RESynthesis and storage of cholesterol mainly in adipose tissue is facilitated by serum albumin.
Trans-Membrane Transport: ABC Transporters

- The A1 and G1 ABC transporters move cholesterol across PM's
- Cholesterol returns to the liver via HDL and LDL

- Largest bacterial protein family (5% of E. coli proteome)
- Two ATP binding cassettes that dimerize in an ATP-gated fashion
- MDR pump is an ABC transporter
- The TAP pore responsible for MHCI presentation is an ABC tp.
- cf C.F.
Intracellular Transport: Niemann-Pick Disease

- Niemann-Pick A..E is a set of five inherited lysosomal storage diseases.
- Neurodegeneration (dramatic loss of Purkinje cells) and liver failure; death in childhood.
- Recessive disease with 1 in 150,000 incidence; in Yarmouth County Nova Scotia 1% with carrier 25%.
- Cholesterol and sphingolipids accumulate in late endosomes and lysosomes of cells; can be observed in vitro by loading fibroblasts with LDL.
- Types A and B from defects in lysosomal sphingomyelinase.
- Type C genes located in 1997/2000; two proteins NPC1 (membrane) and NPC2 (soluble).
- These proteins appear to transport cholesterol out of endosomes and into the cell cytoplasm.
- In NPC cells, cholesterol synthesis is upregulated (via SREBP).
INTERVENTION STRATEGY #1: Lower [LDL]
#1A: The Statins

**Fermentation-Derived Statins**

- Mevastatin
- Lovastatin
- Simvastatin
- Pravastatin

**Synthetic Statins**

- Atorvastatin
- Rosuvastatin
- Fluvastatin
- Cerivastatin*

**SHORT HISTORY**

- 1976 Mevastatin from *Penicillinum citrinum*
- 1980 Mevinolin from *Aspergillus terreus*
- 1987 FDA approves Lovastatin
- 1988 Lovastatin not effective in FH homozygotes
- 1995 Pravastatin decreases heart transplant rejection and mortality independently of lowering cholesterol levels

**STATINS REDUCE [CIRCULATING CHOLESTEROL] BY UP TO 50%**

**FAIRLY SAFE (rhabdomyolysis)**
IDEA:

Statins act by inhibiting cholesterol synthesis in patients.
Clinical Observation

Lovastatin therapy in receptor-negative homozygous familial hypercholesterolemia: Lack of effect on low-density lipoprotein concentrations or turnover

Ricardo Uauy, MD, PhD, Gloria Lena Vega, PhD, Scott M. Grundy, MD, PhD, and David M. Bilheimer, MD

From the Departments of Pediatrics, Internal Medicine, and Clinical Nutrition, and Center for Human Nutrition, University of Texas Southwestern Medical Center at Dallas

To determine whether at least part of the fall in low density lipoprotein (LDL) levels during lovastatin therapy might be the result of a reduced secretion of lipoproteins by the liver, three children 6 to 9 years of age with receptor-negative homozygous familial hypercholesterolemia underwent treatment with lovastatin. These patients have no capacity to synthesize LDL receptors. During lovastatin therapy, at a dose of 2 mg/kg/day, there was no decrease in LDL-cholesterol levels, nor was the turnover rate of LDL affected by the drug. The only significant change was a 74% drop in very low-density lipoprotein during treatment. We conclude that lovastatin is not effective in treatment of receptor-negative homozygous familial hypercholesterolemia. The most likely mechanism of action for this drug is to increase LDL receptor activity. (J Pediatr 1988;113:387-92)

Sterol Regulation of Transcription by SREBP

SREBP Transcription Factor Localization

SCAP/Insig proteins also (HMG CoA R)

ER Retention

Proteases

Goldstein will talk in the Frontiers series on Wednesday Oct. 22 2008, Clark Center Auditorium, 4:00 P.M.
IDEA:

Statins act by activating SREBP, resulting in increased LDL receptor and more efficient recruitment of LDL out of plasma.
Clinical Observations: The Pleiotrophic Effects of Statins

- Statins reduce organ rejection and mortality after cardiac transplant. Randomized, placebo-controlled
  

- Statins reduce inflammation in patients with chronic rheumatoid arthritis. Randomized, placebo-controlled
  

- Statins reduce incidence of MS lesions. Small open-label clinical trial.
  

- Statins reduce proteinuria in systemic lupus erythematosus. Small open-label clinical trial.
  
Multiple Sclerosis

- Effects up to one million worldwide
- 16,000 deaths in 2002 (0.03 %)
- Autoimmune attack of myelin in brain and spinal cord
- Progressive physical disability
- Current therapies include injected Ifn-β and copaxone (basic peptides)
- Statins reverse disease pathology in the EAE mouse model of MS!


Lesions on cerebellum and spinal cord of an MS patient; Jean Cruvelhier circa 1860
Immune System Overview

**Inate**

NK cells

**Adaptive**

APC

B cells

T cells

Ifn-\(\gamma\) induced γδ T cells

Ifn-\(\gamma\) induced CD4 helper

Ifn-\(\beta\)

IL12 from activated macrophages and dendritic cells

CD4 helper

CD8 cytotoxic

IL4, 10

TGF\(\beta\)

Ifn-\(\beta\)

IL2

Ifn-\(\gamma\)

TNF\(\alpha\)

\(\text{T}_{H1}\)

\(\text{T}_{H2}\)
Tc/MHC1/CD8 versus Th/MHC2/CD4
The MHC Molecules

Class I

Class II
MHC Antigen Presentation

Expressed in ALL CELLS

INF-γ can induce expression in non-APC during acute immune response; this is also a hallmark of auto-immune diseases.
Th1 vs. Th2 Helper Cells

- **Th1 Cells**
  - Activated by extracellular pathogens (e.g., helminths) and oral antigens.
  - secretes IL-12.
  - Activates naive helper T cells and B cells.
  - Results in cytokine X production.

- **Th2 Cells**
  - Activated by extracellular pathogens (e.g., helminths) and oral antigens.
  - secretes cytokine X.
  - Activates naive B cells.

LFA-1 is involved in the interaction between Th1 and dendritic cells. TH1 CELLS ACTIVATION

TH2 CELLS ACTIVATION
Th1 vs. Th2 Antagonism

STATINS ALTER THIS BALANCE IN FAVOR OF TH2 HELPER CELLS. BUT HOW?
Isoprenoids in Humans

1. Steroids Hormones
2. Metabolites (Vit. A, E, K; co-Q; 25,000 terpenoids)
3. Isopentyl adenosine (tRNA)
4. Dolichol (N-linked glycosylation)
5. Protein Prenylation
Isoprenoids in G Protein Signaling

Statin influences on prenylation of signaling molecules will likely be "chemocopied" by a new class of drugs, the small-molecule prenylation inhibitors.
Lymphocyte Function Associated Antigen is an integrin on lymphocytes that binds to endothelial cell ICAMS during extravasation of lymphocytes; it is upregulated on activated T cells in a positive feedback loop.
How Do Statins Work?

Reported to:

- Decrease occurrence of AD by 70% in retrospective study
- Suppress MS (clinical trials)
- Block SMC proliferation/migration \textit{in vitro}
- Modulate NF-κB Function \textit{in vitro}
- Block IFN-γ induced MHCII expression \textit{in vitro}
- Suppress expression/secretion of immunoinflammatory molecules \textit{in vitro}
- Direct inhibition of LFA-1

Most \textit{in vitro} effects reversed by mevalonate, \textit{trans}F, \textit{trans}GG, but not \textit{cis}F, \textit{cis}GG, squalene, isopentyl adenosine or ubiquinone; prenylation inhibitors have many of the same immunomodulatory effects that are observed for statins.
#1B,C: Cholestyramine, Apo-B RNAi

B. Cholestyramine and bile-acid chelating resins.

**#1D: Ezetimibe**

- Cholesterol absorption inhibitor derived from ACAT inhibitors.
- Discovered using *in vivo* hypocholesterolemic hamster assay!
- The target was unknown during the discovery process.
- Reduces cholesterol absorption by 75% in mice.
- Reduces plasma cholesterol by 20% in humans.
- Additive effects w.r.t. statins.
INTERVENTION STRATEGY #2: Increase [HDL]
#2A: The CETP Inhibitor Torcetrapib

- Rationally designed inhibitor of CETP.
- Reduces \( \frac{[\text{LDL}]}{[\text{HDL}]} < 1 \! \) in vivo.
- Decreases atherogenesis in the rabbit model.
- Additive effects w.r.t. statins.

- December 2, 2006: Cancellation of all trials due to increased CAD related mortality
B. Niacin

- Can increase [HDL] by 15-30% and reduce triglycerides by 20-40%.
- Side effects are skin flushing (suppressible with a prostaglandin D2 receptor 1 antagonist) and moderate increases in insulin resistance.
- Mechanism unknown, but activates GPCR109A.
- Currently in huge trials with statin combination.

C. Fibrates

- Can increase [HDL] by 5-50%.
- Act as PPAR-α agonists
- Mixed trial results w.r.t. CAD outcomes.

How do Statin Myopathy and Ezetimibe Work?
