Clinical Benefits of Statin Therapy: LDL-lowering and/or Anti-Inflammatory?

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Cardiology Update
Davos, CH   February 2013
Dr. Libby does not accept payments from Pharma. He serves as an unpaid consultant and contributes to clinical trials sponsored by Pharma. BWH has patents concerning the use of CRP in CVD.
Clinical Benefits of Statin Therapy: LDL-lowering and/or Anti-Inflammatory? YES
Lifestyle a key to CV prevention

Libby & Crea

European Heart Journal 2010
Mechanisms of Reduction of Cardiovascular Events by Statin Therapy?

❤️ LDL-Lowering effect

❤️ “Pleiotropic” effects

Putative Pleiotropic Effects of Statins

- Platelet activation
- Thrombotic effect
- Plaque stability
- Vascular inflammation
- SMC hypertrophy
- Endothelial dysfunction
- SMC proliferation
- Vasoconstriction
- TXA₂
- t-PA
- PAI-1
- Macrophage growth
- MMPs
- TF
- hs-CRP
- Adhesion molecule
- Rac1
- RhoA
- ET-1
- AT1 receptor

Cardiovascular Diseases

- Atherosclerosis
- Hypertension

Possible Non-LDL Lowering Effects of Statins Relevant to Atherosclerosis

- Reducing thrombogenicity
- Opposing vasospasm
- Decreasing inflammation
- Stabilizing the fibrous cap
Possible Non-LDL Lowering Effects of Statins Relevant to Atherosclerosis

♥ Decreased inflammation
Statins Decrease Inflammation in Mouse Atheromata Despite Modest LDL Lowering

Nahrendorf et al.

Noninvasive MR Imaging of VCAM-1 Expression: 8 weeks of statin treatment reduced serum cholesterol moderately, as expected, in mice (HCD, 601 ± 34 mg/dL; HCD plus atorvastatin, 506 ± 79 mg/dL; P<0.05)
Can we use inflammatory markers in the clinic?

C-reactive protein: CRP
Statin Therapy Reduces C-Reactive Protein

4,162 patients with an Acute Coronary Syndrome < 10 days

ASA + Standard Medical Therapy

Standard Therapy (Pravastatin 40 mg)

Intensive Therapy (Atorvastatin 80 mg)

Duration: Mean 2 year follow-up (>925 events)

Primary Endpoint: Death, MI, Documented UA requiring hospitalization, revascularization (> 30 days after randomization), or Stroke
All-Cause Death or Major CV Events in All Randomized Subjects

% with Event

Pravastatin 40mg (26.3%)

Atorvastatin 80mg (22.4%)

16% RR (P = 0.005)

Clinical Relevance of Achieved LDL and Achieved CRP After Treatment with Statin Therapy

Ridker et al. NEJM 2005
• Statins reduce C-reactive protein (CRP)- *but* the individual magnitude of CRP reduction does not correlate well with the drop in LDL-C levels

Blake GJ et al. *Am J Cardiol* 2003;41:37S-42S.
Minimal Relationship Between Achieved LDL and Achieved CRP After Initiation of Statin Therapy

\[ r = 0.18 \]

Variance = 3 percent

Ridker et al. NEJM 2005

PROVE IT – TIMI 22
Clinical Relevance of Achieved LDL and Achieved CRP After Treatment with Statin Therapy

Ridker et al. NEJM 2005

Recurrent MI or Coronary Death

(percent)

Follow-Up (Years)

LDL ≥ 70 mg/dL, CRP ≥ 2 mg/L

LDL ≥ 70 mg/dL, CRP < 2 mg/L

LDL < 70 mg/dL, CRP ≥ 2 mg/L

LDL < 70 mg/dL, CRP < 2 mg/L

LDL < 70 mg/dL, CRP < 1 mg/L
Clinical Importance of Achieving LDL-C < 70 mg/dL and hsCRP < 2 mg/L Following Initiation of Statin Therapy

PROVE IT – TIMI 22

A to Z
Circulation 2006;114:281-8
JUPITER
Does clinical benefit associate with achieved LDLC, achieved hsCRP, or both?

Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosvastatin: a prospective study of the JUPITER trial

Paul M Ridker, Eleanor Danielson, Francisco A H Fonseca, Jacques Genest, Antonio M Gotto Jr, John J P Kastelein, Wolfgang Koenig, Peter Libby, Alberto J Lorenzatti, Jean G MacFadyen, Børge G Nordestgaard, James Shepherd, James T Willerson, Robert J Glynn, on behalf of the JUPITER Trial Study Group

Summary
Background Statins lower high-sensitivity C-reactive protein (hsCRP) and cholesterol concentrations, and hypothesis generating analyses suggest that clinical outcomes improve in patients given statins who achieve hsCRP concentrations less than 2 mg/L in addition to LDL cholesterol less than 1.8 mmol/L (<70 mg/dL). However, the benefit of lowering both LDL cholesterol and hsCRP after the start of statin therapy is controversial. We prospectively tested this hypothesis.

www.thelancet.com Published online March 29, 2009
An individual’s drop in LDL and drop in hsCRP on statin treatment vary independently.

<table>
<thead>
<tr>
<th>Achieved</th>
<th>Achieved hsCRP</th>
<th>Percent change in LDL</th>
<th>Percent change in hsCRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLC</td>
<td>0.10</td>
<td>0.10</td>
<td>0.15</td>
</tr>
</tbody>
</table>

The variance in achieved LDLC explains less than 2 percent of the variance in achieved hsCRP.
**JUPITER**

**Dual Cutpoint Analysis: LDLC<70 mg/dL, hsCRP<2 mg/L**

- **LDL > 70 mg/dL and/or hsCRP > 2 mg/L**
  - HR 0.64 (0.49-0.84)

- **LDL < 70 mg/dL and hsCRP < 2 mg/L**
  - HR 0.35 (0.23-0.54)

- **Placebo**
  - HR 1.0 (referent)

**Cumulative Incidence**

**Follow-up (years)**

<table>
<thead>
<tr>
<th>Number at Risk</th>
<th>Rosuvastatin</th>
<th>Placebo</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>7,716</td>
<td>7,832</td>
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<tr>
<td>1</td>
<td>7,678</td>
<td>7,806</td>
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<tr>
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<td>7,678</td>
<td>7,777</td>
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<td>3</td>
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<td>6,114</td>
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<td>4</td>
<td>3,608</td>
<td>3,656</td>
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<tr>
<td>5</td>
<td>1,812</td>
<td>1,863</td>
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<tr>
<td>6</td>
<td>1,254</td>
<td>1,263</td>
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<tr>
<td>7</td>
<td>913</td>
<td>905</td>
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<tr>
<td>8</td>
<td>508</td>
<td>507</td>
</tr>
<tr>
<td>9</td>
<td>145</td>
<td>168</td>
</tr>
</tbody>
</table>

**P < 0.0001**
How might lipid lowering prevent cardiovascular events?

❤️ Stabilizing the plaque’s fibrous cap
Plaque “Stabilization”: Plaques with a thick fibrous cap may have less tendency to rupture and cause thrombosis.

“Unstable” plaque:
- Fibrous cap
- Lipid core
- Inflammatory cells

“Stable” plaque:
- Fibrous cap
- Lipid core
- Fewer inflammatory cells

Matrix metabolism and integrity of the plaque’s fibrous cap

After Libby P. Circulation 1995
Lipid lowering reduces collagenase expression and increases collagen accumulation in rabbit atheroma

Masanori Aikawa,
Elena Rabkin,
Yoshikatsu Okada,
Sami J. Voglic,
Steven K. Clinton,
Constance E. Brinckerhoff,
Galina K. Sukhova,
Peter Libby
Circulation 1998;97:2433
Lipid Lowering Reduces Plaque Tissue Factor Expression


Plaque “stabilization” by lipid lowering: an anti-inflammatory therapy

<table>
<thead>
<tr>
<th>Cholesterol by diet</th>
<th>Macrophage accumulation ↓</th>
<th>MMP-1, -2, -3, -9 expression ↓</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Collagen accumulation ↑</td>
<td>Tissue factor expression/activity ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD40L / CD40 expression ↓</td>
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<tr>
<td></td>
<td></td>
<td>PDGF-B expression ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SMC maturation/differentiation ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LDL accumulation ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>oxLDL/oxPL accumulation ↓</td>
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<tr>
<td></td>
<td></td>
<td>Anti-oxLDL autoantibody ↓</td>
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<tr>
<td></td>
<td></td>
<td>ROS production ↓</td>
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<tr>
<td></td>
<td></td>
<td>VCAM-1 expression ↓</td>
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<tr>
<td></td>
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<td>MCP-1 expression ↓</td>
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<tr>
<td></td>
<td></td>
<td>eNOS expression ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microvessels ↓</td>
</tr>
</tbody>
</table>

Aikawa et al. Circulation ‘98
Aikawa et al. Circulation ‘99
Aikawa et al. Circulation ‘02
Aikawa et al. CV Pathol ‘04
Tsimikas et al. ATVB ‘06

<table>
<thead>
<tr>
<th>Cholesterol by statins</th>
<th>Macrophage accumulation/growth ↓</th>
<th>MMP-1, -3, -9 expression ↓</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Collagen accumulation ↑</td>
<td>Tissue factor expression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD40L / CD40 expression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAI-I expression ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>oxLDL accumulation ↓</td>
</tr>
</tbody>
</table>

Aikawa et al. Circulation ‘01
Fukumoto et al. Circulation ‘01
How can we translate inflammation biology to the clinic?
Clinical Evidence for Statin Treatment
Increasing Fibrous vs. Lipid Character of Atherosclerotic Plaques

Stabilization of Carotid Atheroma Assessed by Quantitative Ultrasound Analysis in Nonhypercholesterolemic Patients With Coronary Artery Disease

Keisuke Watanabe, MD,* Seigo Sugiyama, MD, PhD,* Kiyotaka Kugiyama, MD, PhD;† Osamu Honda, MD,* Hironobu Fukushima, MD,* Hidenobu Koga, MD,* Yoko Horiibata, MD,* Toshinori Hirai, MD, PhD;† Tomohiro Sakamoto, MD, PhD,* Michihiro Yoshimura, MD, PhD,* Yasuyuki Yamashita, MD, PhD;† Hisao Ogawa, MD, PhD*

Kumamoto and Yamanashi, Japan
Stabilization of carotid atheroma assessed by quantitative ultrasound analysis in nonhypercholesterolemic patients with coronary artery disease.

Watanabe et al. JACC 2005;46:2022-30

Figure 4. Representative IBS images of carotid atheroma from baseline to follow-up. (A) Carotid atheroma at pretreatment. Values of cIBS and plaque-IMT_{max} of this plaque are −17.8 dB and 2.05 mm, respectively. (B) The same carotid atheroma post-pravastatin therapy (6 months). Values of cIBS and plaque-IMT_{max} of this plaque are −14.2 dB and 2.10 mm, respectively.

IBS=Integrated Back Scatter

c-IBS:  -17.8 dB
IMT_{max}: 2.05 mm

c-IBS:  -14.2 dB
IMT_{max}: 2.10 mm
Statin Treatment Increases Fibrous Character of Atherosclerotic Plaques but not Intima-Media Thickness

Abstract: We determined time course of stabilization of echolucent carotid plaques by statin therapy in patients with acute coronary syndrome (ACS). Treatment with 4 mg/d pitavastatin (n = 33) or placebo (n = 32) was initiated within 3 days after onset of ACS in 65 patients with echolucent carotid plaque. Vulnerable carotid plaques were assessed by measuring plaque echolucency using carotid ultrasound with integrated backscatter (IBS) analysis before and 1 month after treatment in all patients. The calibrated IBS value (intima-media IBS value minus adventia IBS) of vulnerable carotid plaques favorably changed at 1 month after treatment in both groups, but the echo-lucency at 1 month improved more in the pitavastatin than in the placebo group (pitavastatin group: –18.7 6 3.3 dB at pretreatment versus –12.7 6 2.3 dB at 1 month *P, 0.001; placebo: –19.0 6 3.5 dB versus –16.9 6 3.2 dB, P, 0.05, *P, 0.01 versus the value at 1 month in placebo group). Levels of CRP, VEGF, and TNFa at 1 month were significantly lower in pitavastatin than placebo group. In conclusion, pitavastatin improved carotid plaque echolucency within 1 month of therapy in patients with ACS, in association with decrease in the inflammatory biomarkers related to vulnerable plaques.
Rapid Stabilization of Vulnerable Carotid Plaque Within 1 Month of Pitavastatin Treatment in Patients With Acute Coronary Syndrome

Representative IBS images of carotid atheroma from pretreatment to 1-month treatment with pitavastatin. A, Carotid atheroma at pretreatment. B, The same carotid atheroma after 1-month treatment with pitavastatin. The white line indicates the region of interest (ROI) in the plaque (intima-media complex), and the white dotted line indicates the ROI in the adventitia using the manual outline definition mode. CCA, common carotid artery; IBS, integrated backscatter; ICA, internal carotid artery; IMT, intima-media thickness.

Comparison between pitavastatin (n = 33) and placebo (n = 32) treatment on calibrated integrated backscatter (IBS) levels A, and CRP levels B. Mean +/- SD.

*P < 0.01 between pitavastatin and placebo groups using 2-way ANOVA.

Relationship of the percent change in calibrated IBS values with the percent change in CRP levels from baseline to 1 month of follow-up.

Possible Non-LDL Lowering Effects of Statins Relevant to Atherosclerosis

❤ Reducing thrombogenicity
❤ Opposing vasospasm
❤ Decreasing inflammation
❤ Stabilizing fibrous cap
What is the molecular basis of statins’ LDL-independent effects?
A Molecular Mediator of Statins’ “Pleiotropic” Effects: the Transcriptional Regulator Kruppel-like Factor-2 (KLF-2)
Krupple-like factor 2 (KLF-2) Antagonizes Cytokine-Induced Endothelial Activation and Promotes Vasculoprotective Gene Expression

Cytokine
- E-selectin
- VCAM-1
- ICAM-1
- tissue factor
- PAI-1

Laminar Flow
- eNOS
- thrombomodulin
- PGI₂
- tPA
- Superoxide dismutase

pro-adhesive
pro-coagulant
anti-fibrinolytic
KLF2 Inhibits

anti-adhesive
anti-coagulant
anti-oxidant
KLF2 Stimulates
Statins Exert Endothelial Atheroprotective Effects via the KLF2 Transcription Factor*

Kush M. Parmar, Vinod Nambudiri, Guohao Dai, H. Benjamin Larman, Michael A. Gimbrone, Jr., and Guillermo García-Cardeña‡

From the Center for Excellence in Vascular Biology, Departments of Pathology, Harvard Medical School and Brigham and Women’s Hospital, Boston, Massachusetts 02115
Statins raise KLF2 levels

(SenBanerjee, Circulation, 2005)

(Slide courtesy of M. Jain)
Do statins affect adaptive as well as innate immunity?
Statins as a newly recognized type of immunomodulator

BRENDA KWAK, FLORE MULHAUPT, SAMIR MYTT & FRANÇOIS MACH

Cardiology Division, Department of Medicine, University Hospital, Geneva Medical School, Foundation for Medical Research, Geneva, Switzerland.
Correspondence should be addressed to F.M.; email: machf@cmu.unige.ch
Inhibition of MHC-II antigens by statins reduces T-lymphocyte proliferation and interleukin-2 production

**Diagram:**

- **Stimulator cells treated with interferon-γ**
  - EC: 
    - [3H]Thymidine incorporation
    - + statin
  - MΦ: 
    - [3H]Thymidine incorporation
    - + statin

**Legend:**
- **a:** [3H]Thymidine incorporation measured in allogeneic T lymphocytes exposed (five days) to human ECs or MΦ pretreated for 48 h with IFN-γ (500 U/ml) alone, or IFN-γ (500 U/ml) with Atorvastatin (10 μM). Similar results were obtained in independent experiments with ECs or MΦ from three different donors. *P < 0.02 compared to IFN-γ treated cells.
- **b:** IL-2 release measured by ELISA in supernatants of allogeneic T lymphocytes exposed (48 h) to human ECs (+) or MΦ (+) pretreated during 48 h with IFN-γ (500 U/ml) alone, or IFN-γ (500 U/ml) with Atorvastatin (10 μM). Similar results were obtained in independent experiments with ECs or MΦ from four different donors. **P < 0.01 compared to IFN-γ treated cells.

Direct Anti-Inflammatory Mechanisms Contribute to Attenuation of Experimental Allograft Arteriosclerosis by Statins

Koichi Shimizu, MD, PhD; Masanori Aikawa, MD, PhD; Kiyoshi Takayama, PhD; Peter Libby, MD; Richard N. Mitchell, MD, PhD

Background — Despite the development of effective immunosuppressive therapy, transplant graft arterial disease (GAD) remains the major limitation to long-term graft survival. The interplay between host inflammatory cells and donor vascular wall cells results in an intimal hyperplastic lesion, which leads to ischemia and graft failure. HMG-CoA reductase inhibitors (statins) reduce GAD in human cardiac allografts, although it is unclear whether this is secondary to cholesterol lowering or other mechanisms. This study tested the hypothesis that statins can suppress GAD by cholesterol-independent pathways.

Methods and Results — We performed heterotopic murine cardiac transplants in total allogeneic or major histocompatibility complex class II–mismatched combinations. Transplanted animals received either control chow, chow containing 25 ppm cerivastatin (low dose), or chow containing 125 ppm cerivastatin (high dose). Mean plasma cerivastatin concentrations were 0.0 (control), 10.1 (low dose), and 21.9 (high dose) nmol/L, respectively. Plasma cholesterol levels were the same in all groups. GAD scores decreased in low-dose ($P<0.05$) and high-dose ($P<0.0001$) cerivastatin groups compared with controls, with concomitant reduction in graft-infiltrating cells and significantly decreased intragraft RANTES and monocyte chemotactic protein-1 mRNA expression. Cerivastatin, as well as other statins, also reduced RANTES and monocyte chemotactic protein-1 production in mouse endothelial cells stimulated with interferon-γ and tumor necrosis factor-α in vitro.

Conclusions — Clinically achievable levels of an HMG-CoA reductase inhibitor attenuate GAD in murine heart transplants, diminish host inflammatory cell recruitment, and do not alter cholesterol levels. These results indicate that statins can affect arterial biology and inflammation independently of their effects on cholesterol metabolism. (Circulation. 2003; 108:2113-2120.)
Statin-induced Kruppel-like factor 2 expression in human and mouse T cells reduces inflammatory and pathogenic responses

De-xiu Bu,1 Margarite Tarrio,1 Nir Grabie,1 Yuzhi Zhang,1 Hiroyuki Yamazaki,2 George Stavrakis,1 Elena Maganto-Garcia,1 Zachary Pepper-Cunningham,1 Petr Jarolim,1 Masanori Aikawa,2 Guillermo García-Cardeña,1 and Andrew H. Lichtman1

1Department of Pathology and 2Cardiovascular Division, Department of Medicine, Brigham and Women’s Hospital, Boston, Massachusetts, USA.

The transcription factor kruppel-like factor 2 (KLF2) is required for the quiescent and migratory properties of naive T cells. Statins, a class of HMG-CoA reductase inhibitors, display pleiotropic immunomodulatory effects that are independent of their lipid-lowering capacity and may be beneficial as therapeutic agents for T cell–mediated inflammatory diseases. Statins upregulate KLF2 expression in endothelial cells, and this activity is associated with an antiinflammatory phenotype. We therefore hypothesized that the immunomodulatory effects of statins are due, in part, to their direct effects on T cell KLF2 gene expression. Here we report that lipophilic statin treatment of mouse and human T cells increased expression of KLF2 through a HMG-CoA/prenylation–dependent pathway. Statins also diminished T cell proliferation and IFN-γ expression. shRNA blockade of KLF2 expression in human T cells increased IFN-γ expression and prevented statin-induced IFN-γ reduction. In a mouse model of myocarditis induced by heart antigen–specific CD8+ T cells, both statin treatment of the T cells and retroviralmediated overexpression of KLF2 in the T cells had similar ameliorating effects on disease induction. We conclude that statins reduce inflammatory functions and pathogenic activity of T cells through KLF2-dependent mechanisms, and this pathway may be a potential therapeutic target for cardiovascular diseases.
Statins reduce T cell proliferation and cytokine expression in vitro

Do Statins’ Benefits Reach Beyond Lowering LDL?

❤️ LDL-Lowering effect

❤️ “Pleiotropic” effects

Both mechanisms contribute to statins’ clinical benefits