Effects of HMG-CoA Reductase Inhibitors on Inflammation and Endothelial Function

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Integrated Perspective on CV Risk Factors and Vascular Disease


Oxidative Stress & Inflammation

Endothelial Dysfunction

Diabetes

An Established Fibrofatty Plaque with Excessive Lipid Core
Statins Reduce Morbidity and Mortality with Atherosclerosis
### Reduction in Major Coronary Events: Statin Trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>AF/TexCAPS</th>
<th>WOS</th>
<th>HPS</th>
<th>4S</th>
<th>LIPID</th>
<th>CARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>6605</td>
<td>6595</td>
<td>20,536</td>
<td>4444</td>
<td>9014</td>
<td>4159</td>
</tr>
<tr>
<td>ΔLDL</td>
<td>-27%</td>
<td>-26%</td>
<td>-29%</td>
<td>-36%</td>
<td>-25%</td>
<td>-28%</td>
</tr>
</tbody>
</table>

**Secondary**

<table>
<thead>
<tr>
<th>Trial</th>
<th>HPS</th>
<th>4S</th>
<th>LIPID</th>
<th>CARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20,536</td>
<td>4444</td>
<td>9014</td>
<td>4159</td>
</tr>
<tr>
<td>ΔLDL</td>
<td>-25*</td>
<td>-25*</td>
<td>-25‡</td>
<td></td>
</tr>
<tr>
<td>% Reduction</td>
<td>-38*</td>
<td>-31*</td>
<td>-27†</td>
<td>-38*</td>
</tr>
</tbody>
</table>

*P<0.001; †P<0.0001; ‡P=0.002.

<table>
<thead>
<tr>
<th>Study</th>
<th>Deaths Not Prevented</th>
</tr>
</thead>
<tbody>
<tr>
<td>4S</td>
<td>70%</td>
</tr>
<tr>
<td>WOSCOPS</td>
<td>78%</td>
</tr>
<tr>
<td>CARE</td>
<td>80%</td>
</tr>
</tbody>
</table>
The Challenge of Residual Morbidity and Mortality due to Atherosclerotic Disease Beyond LDL
Statins and Inflammation: CRP Additive With Lipids

Data From NHANES

Men

Women

Inflammatory Markers of Coronary Risk

Proinflammatory Risk Factors
(oxidized LDL, infectious agents, etc.)
Vascular and Extravascular Sources

Primary Proinflammatory Cytokines
(e.g., IL-1, TNF-α)

ICAM-1
Selectins, HSPs, etc.
Endothelium and Other Cells

IL-6
“Messenger” Cytokine

CRP
SAA
Liver

Circulation

Statins have Anti-inflammatory Actions: A Novel Mechanism of Action
Statin Therapy and hsCRP

- **PRINCE**
  - Pravastatin
  - 24 weeks
  - n = 2884
  - % change in median CRP: -16.9

- **Cerivastatin**
  - 8 weeks
  - n = 785
  - % change in median CRP: -13.3

- **4S**
  - Simvastatin
  - 4 months
  - n = 249
  - % change in median CRP: -20.9

- **REVERSAL**
  - Atorvastatin
  - 18 months
  - n = 253
  - % change in median CRP: -36.4

*P = 0.009
**P < 0.001

Lipophilic Statins have Anti-inflammatory Actions that Accelerate Benefit
SEPTEMBER 5, 2005

Time to Benefit in Lipid-Lowering Trials

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Elsevier Inc.

Supplement available at www.AJConline.org
PROVE IT-TIMI 22
Study Design

- N = 4162
- Randomized to standard (40 mg/day pravastatin) or intensive (80 mg/day atorvastatin) therapy for 2 years
- Primary outcome: composite of time to all-cause mortality, MI, unstable angina requiring hospitalization, revascularization ≥30 days after randomization, and stroke

PROVE IT-TIMI 22: A Major Cardiovascular Event Or Death From Any Cause

Primary End Point

Death Or Major Cardiovascular Event (%)

Standard Care

Intensive Statin Use

$P = .005$ Overall

$P = .03$

Months Of Follow-Up


Ray and Cannon. *Am J Cardiol*. 2005;96(suppl):54F.
PROVE IT-TIMI 22: A Major Cardiovascular Event Or Death From Any Cause At Different Censoring Times

<table>
<thead>
<tr>
<th>Censoring Time</th>
<th>Hazard Ratio (95% CI)</th>
<th>Risk Reduction (%)</th>
<th>Event Rate (%)</th>
<th>Intensive</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 days</td>
<td>1.25</td>
<td>17</td>
<td>1.9</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>90 days</td>
<td>1.50</td>
<td>18</td>
<td>6.3</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>180 days</td>
<td>0.50</td>
<td>14</td>
<td>12.2</td>
<td>14.1</td>
<td></td>
</tr>
<tr>
<td>End of follow-up</td>
<td>0.75</td>
<td>16</td>
<td>22.4</td>
<td>26.3</td>
<td></td>
</tr>
</tbody>
</table>

Ray and Cannon. *Am J Cardiol.* 2005;96(suppl):54F.

### PROVE IT-TIMI 22: Effect Of Different Statin Regimens On LDL Cholesterol And CRP

<table>
<thead>
<tr>
<th>Biological Response</th>
<th>Statin Regimen</th>
<th>Baseline</th>
<th>30 Days</th>
<th>4 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LDL mg/dL (mean)</strong></td>
<td>Standard</td>
<td>106</td>
<td>88</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Intensive</td>
<td>106</td>
<td>60</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td><em>P</em> value</td>
<td>NS</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>CRP mg/L (median)</strong></td>
<td>Standard</td>
<td>11.9</td>
<td>2.3</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Intensive</td>
<td>12.2</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td><em>P</em> value</td>
<td>NS</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>


PROVE IT-TIMI 22: CRP Levels At Enrollment And During Follow-Up

Atorvastatin 80 mg

Baseline 12.2
30 Days 1.6
4 Months 1.3
End of Study 1.3

Pravastatin 40 mg

Baseline 11.9
30 Days 2.3
4 Months 2.1
End of Study 2.1

* P<.001 vs baseline.
** P<.001 vs pravastatin.

PROVE IT-TIMI 22: Relationship Between CRP And Clinical Outcomes

- CRP is a clinical marker of inflammation
- CRP as an independent predictor of cardiovascular risk may be more important than LDL
- Early and large decreases in CRP following intensive statin therapy (80 mg/day atorvastatin) were closely related to reduced morbidity and slower progression of atherosclerosis

Ray and Cannon. Am J Cardiol. 2005;96(suppl):54F.
PROVE IT-TIMI 22: Prognostic Value Of 30-Day Achieved LDL And CRP On Recurrent MI Or Death From Cardiovascular Causes

Cumulative Rate Of Recurrent Myocardial Infarction Or Death From Coronary Causes

LDL Cholesterol ≥70 mg/dL
CRP ≥2 mg/L

LDL Cholesterol <70 mg/dL
CRP ≥2 mg/L

LDL Cholesterol ≥70 mg/dL
CRP <2 mg/L

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

Ray and Cannon. *Am J Cardiol.* 2005;96(suppl):54F.
PROVE IT-TIMI 22
Conclusions

• Intensive statin therapy with resulted in apparent clinical benefit observed as early as 30 days

• Significant reduction in all-cause mortality, MI, unstable angina, revascularization ≥30 days, and stroke apparent at 4 months ($P=.03$)

• Additional benefits gained in patients with concurrent reductions in CRP beyond LDL reduction
ASCOT-LLA
Study Design

- N = 10,305
- Randomized to atorvastatin 10 mg/d or placebo for 5 years (stopped after 3.3 years)
- Primary outcome: time to first nonfatal MI and fatal CAD

ASCOT-LLA: Nonfatal MI And Fatal CAD
Primary End Point

Atorvastatin 10 mg
Number of Events 100
HR = 0.64 (0.50-0.83)
P = .0005

Placebo
Number of Events 154
Cumulative Incidence (%)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
Years

36% Reduction

Sever et al. *Am J Cardiol*. 2005;96(suppl):39F.
ASCOT-LLA: Fatal And Nonfatal Stroke
Secondary End Point

Number of Events 89
HR = 0.73 (0.56-0.96)

Number of Events 121

27% Reduction

HR = hazard ratio.
Adapted from Sever et al. Lancet. 2003;361:1149, with permission.
ASCOT-LLA

Post Hoc Analysis Of Time To Benefit

• To assess benefit at specific time points (30 days, 90 days, 180 days, 1 y, 2 y, end of study), cumulative hazard ratio was calculated at different censoring times

• Time to 1st primary end point event in the atorvastatin and placebo groups was compared in an intention-to-treat basis

• Log-rank procedure and Cox proportional hazards model was used to calculate confidence interval

ASCOT-LLA Time-To-Benefit Analysis: Cardiac Events

<table>
<thead>
<tr>
<th>Censoring Time</th>
<th>Hazard Ratio (95% CI)</th>
<th>Risk Reduction (%)</th>
<th>Statin</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 days</td>
<td></td>
<td></td>
<td>83</td>
<td>2.4</td>
</tr>
<tr>
<td>90 days</td>
<td></td>
<td></td>
<td>67</td>
<td>5.5</td>
</tr>
<tr>
<td>180 days</td>
<td></td>
<td></td>
<td>48</td>
<td>7.5</td>
</tr>
<tr>
<td>1 year</td>
<td></td>
<td></td>
<td>45</td>
<td>6.6</td>
</tr>
<tr>
<td>2 years</td>
<td></td>
<td></td>
<td>38</td>
<td>5.9</td>
</tr>
<tr>
<td>End of study</td>
<td></td>
<td></td>
<td>36</td>
<td>6.0</td>
</tr>
</tbody>
</table>

* Per 1000 patient-years.

CI = confidence interval.

## ASCOT-LLA Time-To-Benefit Analysis: Stroke Events

<table>
<thead>
<tr>
<th>Censoring Time</th>
<th>Hazard Ratio (95% CI)</th>
<th>Risk Reduction (%)</th>
<th>Event Rate*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Statin</td>
</tr>
<tr>
<td>30 days</td>
<td></td>
<td>34</td>
<td>9.4</td>
</tr>
<tr>
<td>90 days</td>
<td></td>
<td>19</td>
<td>10.2</td>
</tr>
<tr>
<td>180 days</td>
<td></td>
<td>31</td>
<td>7.9</td>
</tr>
<tr>
<td>1 year</td>
<td></td>
<td>11</td>
<td>6.6</td>
</tr>
<tr>
<td>2 years</td>
<td></td>
<td>29</td>
<td>5.5</td>
</tr>
<tr>
<td>End of study</td>
<td></td>
<td>27</td>
<td>5.4</td>
</tr>
</tbody>
</table>

* Per 1000 patient-years.

ASCOT-LLA Time-To-Benefit Analysis

Conclusions

• CAD risk reduction with atorvastatin occurred much earlier than expected based on lipid-lowering effects only
  – CAD relative risk reduction with statin was noticeable at 30 days and was significant at 3 months \((P=.008)\) and through termination of trial (3.3 years)
  – stroke relative risk reduction with statin was noticeable at 30 days, and was significant at 2 years \((P=.05)\) and termination \((P=.02)\)
Intermolecular Differences of Statins Contribute to Distinct Actions
Intermolecular Similarities And Differences Of Statins

• Intermolecular similarities
  – all statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase
  – all statins share a common dihydroxy group necessary for HMG-CoA reductase enzyme inhibition

• Intermolecular difference
  – substituents on pharmacophore moiety are responsible for pharmacokinetic and pharmacodynamic differences, which in turn affect efficacy, safety, and pleiotropic effects

Mason et al. *Am J Cardiol.* 2005;96(suppl):11F.
Equilibrium Molecular Locations Of Statins Based On X-Ray Diffraction Analysis

Oxidative Stress

Endothelial Dysfunction

Inflammation
Effect of Native and Oxidized LDL on Endothelial NO Production

Vergnani et al. 

![Graph showing the effect of LDL on NO concentration. The x-axis represents LDL concentration (mg chol/dL), and the y-axis represents NO concentration (nM). The graph compares native LDL (n-LDL) and oxidized LDL (ox-LDL).](image-url)
Early (LOOH) Versus Late (MDA) Markers of LDL Oxidation

- Oxidative Stress
- FOAM-cell formation
- Monocyte motility
- Endothelial adhesion
- Chemoattraction
- Free-radical production

RP Mason
Analysis of CV Risk Prediction With MDA-LDL in 634 Pts with Stable CAD

- Major Vascular Events (stroke/MI)
  - Quartile 1: $P = .0038$
  - Quartile 4: $P < .0001$

- Nonfatal Vascular Events (angina/CHF)
  - Quartile 1: $P = .0038$
  - Quartile 4: $P < .0001$

- Major Vascular Procedures (CABG/PTCA)
  - Quartile 1: $P = .0038$
  - Quartile 4: $P < .0001$

- All Vascular Events and Procedures
  - Quartile 1: $P = .0038$
  - Quartile 4: $P < .0001$

Walter MF, Mason RP. JACC 44:1996;2004
Plasma Lipid Oxidation Markers: An Independent Biomarker of CV Risk?

oxLDL Predicted Events Independently of other Risk Factors:

1) Lipids (LDL, HDL, triglycerides)
2) Blood Pressure (SBP, DBP)
3) Age
4) Body Mass Index (BMI)
5) Inflammatory Marker: hsCRP

Walter MF, Mason RP. JACC 44:1996;2004
What’s Wrong with Natural Antioxidants?
## Antioxidant Vitamins and CVD

<table>
<thead>
<tr>
<th></th>
<th>Trial</th>
<th>Odds ratio (95% CI)</th>
<th>Absolute event rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \beta ) carotene</td>
</tr>
<tr>
<td>( \beta ) Carotene</td>
<td></td>
<td></td>
<td>( p )</td>
</tr>
<tr>
<td></td>
<td>Mortality ((n=138,113))</td>
<td></td>
<td>( p=0.003)</td>
</tr>
<tr>
<td></td>
<td>CV Death ((n=131,551))</td>
<td></td>
<td>( p=0.92)</td>
</tr>
<tr>
<td></td>
<td>Stroke ((n=82,483))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( p=0.42)</td>
</tr>
<tr>
<td></td>
<td>Mortality ((n=81,788))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV Death ((n=77,031))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stroke ((n=45,896))</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Antioxidant Effects of Vitamin E Diminished Under Atherosclerotic Conditions

Mean ± S.D. (n=5-6)
Walter WF, Mason RP. American College of Cardiology (2004)
Statins Interfere with Oxidation Pathways
Mechanisms by which Statins Interfere with Oxidative Stress

Reduce Expression of NADPH Oxidase Subunits

Block isoprenylation of rac-1

Reduce LDL levels

Enhance catalase levels

Effect of Atorvastatin o-Hydroxy Metabolite on Human LDL Oxidation

TBARS (% of Control)

* $p < 0.05$ versus untreated.

Effects of Atorvastatin Active Metabolite vs Antioxidants on Cu²⁺-Induced LDL Oxidation

Atorvastatin Trolox Probucol

0 5 10 15 20 25 30 35

% Inhibition
Human LDL Oxidation (TBARS)

*p < 0.001, **p < 0.001 and † p < 0.0001 versus Control
Effects of Statins on Human LDL Conjugated Diene Formation

*\( p < 0.005 \) vs untreated.

Walter MF, Mason RP. American College of Cardiology (2004)
Statins Improve Endothelial Function
Prenylation of the G-Protein Rho is Central for Mediating Many Pleiotropic Effects of Statins

Acetyl CoA → HMG CoA

↓

Mevalonate

↓

Farnesyl-PP

Cholesterol

Rho

GDP

GG

Inactive

P+

Rho

GTP

GG

Active

Geranyl Geranyl-PP

Statins

PP

↓ NO inflammation vasoconstriction

↑ preproET-1 oxidation

Role of Microdomains in Atherosclerosis

Cholesterol Crystals Associated with Apoptotic Cell Death

Caveolin and HSP/Akt and eNOS Activation

1. Statins decrease caveolin expression
2. Statins increase eNOS activation by HSP/Akt phosphorylation
3. Activated eNOS increases NO production

Statin Promotes eNOS Activation: Decrease in Caveolin Abundance in EC

Effects of Statin Treatment on Endothelial Cell Membranes and Vasculature in Atherosclerosis

Atheroprotection with Lipophilic Statin: HMG-CoA Inhibition and Beyond

**LDL-Dependent**
- ↓ Plasma LDL Levels
- ↑ HDL
- ↓ hsCRP
- ↓ Atheroma Progress
- ↓ Small Dense LDL

**LDL-Independent**
- ↑ Endothelial Nitric Oxide
- ↓ LDL Oxidation
- ↑ Endothelial Function
- ↑ Plaque Stabilization
- ↓ Endothelial Caveolae
- ↓ Inflammation
- ↓ NADPH Oxidase
- ↓ hsCRP
- ↓ Atheroma Progress
Cardiovascular Division
Brigham & Women’s Hospital
Harvard Medical School