Effect of Cholesterol-Lowering Therapy on Coronary Endothelial Vasomotor Function in Patients With Coronary Artery Disease

Joseph A. Vita, MD; Alan C. Yeung, MD; Michael Winniford, MD; John M. Hodgson, MD; Charles B. Treasure, MD; J. Larry Klein, MD; Steven Werns, MD; Morton Kern, MD; Diane Plotkin, PhD; W. Joseph Shih, PhD; Yale Mitchel, MD; Peter Ganz, MD

Background—Improved endothelial function may contribute to the beneficial effects of cholesterol-lowering therapy.

Methods and Results—In this randomized, double-blind study, we compared the effect of 6 months of simvastatin (40 mg/d) treatment with that of placebo on coronary endothelial vasomotor function in 60 patients with coronary artery disease. Simvastatin lowered LDL-cholesterol by 40±12% from 130±28 mg/dL (P<0.001). Peak intracoronary acetylcholine infusion produced epicardial coronary constriction at baseline in both the simvastatin (−17±13%) and placebo (−24±16%) groups. After treatment, acetylcholine produced less constriction in both groups (−12±19% and −15±14%, respectively, P=0.97). The increase in coronary blood flow during infusion of the peak dose of substance P was blunted at baseline in both the simvastatin (42±50%) and placebo (55±71%) groups, reflecting impaired endothelium-dependent dilation of coronary microvessels. After treatment, the flow increase was 82±81% in the simvastatin group and 63±53% in the placebo group (P=0.16).

Conclusions—Six months of cholesterol-lowering therapy has no significant effect on coronary endothelial vasomotor function in the study population of patients with coronary artery disease and mildly elevated cholesterol levels. These findings suggest that the effects of cholesterol lowering on endothelial function are more complex than previously thought. (Circulation. 2000;102:846-851.)

Key Words: endothelium ■ lipids ■ coronary disease

The endothelium controls vascular homeostasis through the elaboration of paracrine factors, including nitric oxide. Endothelium-dependent vasodilation is impaired in epicardial coronary arteries1 and in the coronary microcirculation2 of patients with coronary artery disease (CAD). The loss of endothelium-derived nitric oxide (EDNO) promotes vasospasm, platelet aggregation, and inflammation in atherosclerotic coronary arteries and thus contributes to the clinical expression of CAD.3,4

Several prior studies8–11 examined the effect of cholesterol-lowering therapy on coronary endothelial function in patients with hypercholesterolemia, CAD, or both. In those studies, cholesterol-lowering therapy reduced coronary artery constriction8–11 and increased coronary blood flow responses during intracoronary acetylcholine infusion,9 reflecting improved EDNO action in conduit coronary arteries and coronary microvessels, respectively. Improved endothelium-dependent dilation after cholesterol-lowering therapy has also been shown in the forearm circulation.12,13 Some of those prior coronary studies were not well controlled,8,9 and a relatively small number of subjects were enrolled. The study that examined the coronary microvasculature9 was not randomized. Therefore, the purpose of the present study was to examine the effect of cholesterol-lowering therapy on coro-
nary conduit and microvascular endothelial function in a well-controlled manner.

Methods

Patients

The Coronary Artery Reactivity After Treatment with Simvastatin (CARATS) Study was a randomized, double-blind study that compared the effect of simvastatin with that of placebo on coronary endothelial vasomotor function. Entry criteria were age of 25 to 80 years, total serum cholesterol level of 180 to 300 mg/dL, angiographically documented CAD (diffuse luminal irregularities or >1 vessel with >50% stenosis), and an index vessel for study of >2.0 mm in diameter with no stenosis of >30% that supplied normally contracting myocardium. Exclusion criteria included hypertension (blood pressure of >140/90 mm Hg or antihypertensive treatment), cigarette smoking within 1 month, diabetes mellitus (random glucose level of >200 mg/dL or hypoglycemic treatment), and hypertriglyceridemia (>350 mg/dL). Patients were also excluded if they had left main or 3-vessel disease, CABG within 6 months, coronary angioplasty within 2 weeks, mean pulmonary capillary wedge pressure of >25 mm Hg, or left ventricular ejection fraction of <0.40. Other exclusion criteria included cholesterol-lowering medication within 6 weeks, creatinine kinase level of >1.8 mg/dL, creatine kinase level of >50% above the upper limit of normal, transaminase of >20% above the upper limit of normal, and a history of liver disease within 6 months. All site institutional review boards approved the study, and patients provided informed consent.

Coronary Vasomotor Studies

Coronary vasomotor function was assessed after diagnostic catheterization or before coronary angioplasty. Coronary angioplasty was performed with nonionic contrast medium and a power injector. Serial infusions (0.8 mL/min) were started with low-dose saline at the end of each infusion. Coronary blood flow was estimated as the product of velocity and the cross-sectional area of a 2-mm segment centered 5 mm distal to the flow wire tip. Flow responses were expressed as percent change relative to the baseline blood flow.

Statistical Analysis

The primary end point was the effect of treatment (simvastatin or placebo) on the change in the diameter response of the SMAV to the maximal dose of acetylcholine. The study was planned to have 80% power to detect a difference of 14 percentage points for this end point with a sample size of 80 patients (α=0.05, 2-tailed), with an assumed within-group SD of change in diameter of 22%. The key secondary end point was the effect of treatment on change in the coronary blood flow response to the maximal dose of substance P. The study was planned to have 80% power to detect a difference of 22 percentage points for the key secondary end point with a sample size of 80 patients (α=0.05 2-tailed), with an assumed within-group SD of change in blood flow of 35%.

Other end points included (1) coronary blood flow responses to the maximal doses of acetylcholine and adenosine, (2) diameter responses to the maximal doses of substance P and adenosine, (3) diameter responses to the maximal doses of all 3 agonists with the average of all analyzed segments used for the analysis, rather than the SMAV, and (4) dose response for substance P–mediated changes in coronary blood flow. The dose responses to acetylcholine could not be examined because some patients did not receive all 3 doses due to severe constriction at a submaximum dose.

ANOVA was used to compare the treatment groups for the primary, key secondary, and first 3 other end points. The model included terms for treatment group, investigator, and response to the agonist at the baseline visit. The effect of treatment on dose response to substance P at the follow-up visit was examined with an analysis of repeated measures with use of the mixed model that included dose of substance P, the treatment, and the interaction of dose and treatment. The effect of treatment on lipid profile was compared by ANOVA with treatment group and investigator in the model. The analysis was performed with SAS statistical software. A 2-tailed P value of <0.05 was considered to be statistically significant. All data are expressed as mean±SD unless otherwise indicated.

Results

Clinical Characteristics and Adverse Events

A total of 83 patients were enrolled. Twenty-three patients were withdrawn (simvastatin group n=11, placebo group n=12). Two patients in the simvastatin group had an adverse
drug experience (muscle cramps n=1, urticaria n=1). The remaining patients were withdrawn due to unwillingness to undergo the second catheterization (simvastatin n=6, placebo n=4); protocol violation (placebo n=1); non-medications-related adverse experience, including the development of unstable angina (simvastatin n=2, placebo n=3); or other reasons, including the exacerbation of preexisting non-CAD conditions (simvastatin n=1, placebo n=4). In 1 subject, the follow-up catheterization was complicated by acute myocardial infarction (peak creatine kinase 1300 U/L) due to failure to administer heparin before instrumentation and thrombus formation on the infusion catheter. The subject was treated with heparin and intracoronary urokinase and was discharged with heparin and intracoronary urokinase. Thus, a total of 60 patients were available for analysis. The demographic and clinical characteristics of the evaluable patients are given in Table 1.

**Lipid Effects**

Lipid results are shown in Table 2. The effect of simvastatin treatment was significantly different from that for placebo for total cholesterol ($P<0.001$) and LDL-cholesterol ($P<0.001$) levels. At follow-up, LDL-cholesterol was <100 mg/dL in 30 of 34 patients in the simvastatin group and 4 of 25 patients in the placebo group.

**Epicardial Vasomotor Function**

As shown in Table 3, blood pressure, heart rate, and baseline coronary diameter were similar in both groups and were unaffected by either treatment. Coronary diameter and flow returned to baseline during the vehicle infusion before the next agonist.

The effect of acetylcholine infusion on coronary diameter is shown in Figure 1. The $10^{-9}$ mol/L dose of acetylcholine was omitted in 6 patients in the simvastatin group and 8 patients in the placebo group due to excessive vasoconstriction at $10^{-7}$ mol/L. The maximal dose of acetylcholine produced coronary constriction at the SMAV at baseline in both groups ($-17 \pm 13\%$ and $-24 \pm 16\%$, respectively). After 6 months, acetylcholine produced less severe constriction in both groups ($-12 \pm 19\%$ and $-15 \pm 14\%$, respectively). Because the baseline constrictor responses for the 2 groups were different, the change was adjusted for baseline response with ANCOVA. The adjusted changes in the response to acetylcholine were 6±15 percentage points for the simvastatin group and 6±15 percentage points for the placebo group ($P=0.97$). Adjustment for investigator site, age, extent of atherosclerosis, and baseline LDL level did not alter the findings.

When epicardial function was assessed with the average response of all analyzed segments rather than the 5-mm segment with maximal constriction (SMAV), the extent of coronary constriction was still greater in the placebo group than in the simvastatin group at baseline ($-11\%$ versus $-5\%$, respectively) and at follow-up ($-12\%$ versus $-9\%$, respectively). The adjusted changes in acetylcholine response between baseline and follow-up were equivalent for the simvastatin group ($-3\pm14$ percentage points) and the placebo group ($-3\pm14$ percentage points, $P=0.95$).

The maximal dose of substance P produced modest vaso-dilation at the SMAV at baseline in both the simvastatin (12±10%) and placebo (1±10%) groups. After 6 months, the dilator response to substance P was increased in both groups (11±11% and 13±13%, respectively). The changes in substance P-induced dilation were equivalent in the 2 groups ($P=0.53$). The findings were similar when the average response of all segments was used for the analysis rather than the SMAV (data not shown).

Intracoronary adenosine induced coronary dilation at baseline for the simvastatin (4±12%, n=27) and placebo (5±10%, n=19) groups. After 6 months, the dilator response was greater in both groups (20±19% and 16±23%, respectively). The changes in adenosine-induced dilation were equivalent in the 2 groups ($P=0.45$).

**Microvessel Vasomotor Function**

Coronary blood flow responses to substance P infusion are displayed in Figure 2. The maximal dose of substance P increased coronary blood flow at baseline in both the simvastatin (42±50%) and placebo (55±71%) groups. After 6 months, the response was greater in both groups (82±81% and 63±53%, respectively). The unadjusted improvement was greater in the simvastatin group (39±79 percentage points) than the placebo group (8±79 percentage points). Blood flow responses differed according to participating center, and after adjustment for investigator site and baseline response, the change was 57±70 percentage points for the simvastatin group and 33±67 percentage points for the placebo group ($P=0.16$). Adjustment for age, extent of atherosclerosis, and baseline LDL level did not alter the findings.

When the entire dose response to substance P was considered, the flow response to substance P improved significantly in the simvastatin group ($P=0.03$ by analysis of repeated measures) but not in the placebo group ($P>0.69$) (see Figure 2). However, there was no significant difference

**TABLE 1. Clinical Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Placebo Group (n=26)</th>
<th>Simvastatin Group (n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y (range)</td>
<td>55 (41-71)</td>
<td>55 (39-73)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>23 (88)</td>
<td>28 (82)</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>83±13</td>
<td>84±12</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>128±14</td>
<td>131±11</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79±12</td>
<td>79±9</td>
</tr>
<tr>
<td>Atherosclerosis rating, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade II (luminal irregularities)</td>
<td>14 (54)</td>
<td>16 (47)</td>
</tr>
<tr>
<td>Grade III (stenosis &gt;50%)</td>
<td>12 (46)</td>
<td>18 (53)</td>
</tr>
<tr>
<td>Concurrent treatment with, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>4 (15)</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin E or C</td>
<td>6 (25)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Estrogen</td>
<td>1 (4)</td>
<td>4 (12)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>24 (92)</td>
<td>26 (76)</td>
</tr>
</tbody>
</table>

Values are mean±SD or number (percent of total).
between groups in the degree of improvement for all 3 doses of substance P ($P=0.21$).

The maximal dose of acetylcholine increased coronary blood flow at baseline in both the simvastatin (63±100%) and placebo (33±76%) groups. After 6 months, acetylcholine increased coronary blood flow by 49±87% in the simvastatin group and 35±85% in the placebo group. The change in acetylcholine response was equivalent in the 2 groups (−14±105 and 1±118 percentage points, respectively, $P=0.49$).

The intracoronary infusion of adenosine increased coronary blood flow at baseline in both the simvastatin (327±183%) and placebo (387±296%) groups. After 6 months, the increase was 388±269% and 353±223%, respectively. Thus, the change in adenosine-induced increase in coronary blood flow was 61±246 percentage points for the simvastatin group and 33±334 percentage points for the placebo group ($P=0.36$).

**Discussion**

In these patients with mild CAD and mildly elevated cholesterol levels, acetylcholine infusion produced epicardial coronary vasoconstriction, and there was a blunted blood flow response to substance P. Adenosine, which primarily acts on vascular smooth muscle in the coronary vasculature, produced epicardial dilation and expected increases in coronary blood flow. These findings suggest the presence of endothelial vasomotor dysfunction at baseline. Six-month treatment with simvastatin markedly improved the lipid profile, whereas placebo had no effect. At follow-up, the severity of acetylcholine-induced vasoconstriction was less in both treatment groups, but there was no difference in the extent of improvement. There also was no significant difference between groups in the degree of improvement in the coronary blood flow response to peak substance P dose ($P=0.16$), although the analysis of all 3 doses of substance P raised the possibility of a beneficial effect in the simvastatin group ($P=0.03$). Overall, cholesterol-lowering treatment for 6 months failed to improve coronary endothelial vasomotor function in this patient population.

The present study has a number of strengths. More patients were enrolled than in any prior study of this issue. The investigators were experienced, and the study was rigorously controlled with centralized blinding and core laboratories. In this study, patients with isolated hypercholesterolemia were examined, and thus, the potentially confounding effects of other risk factors were avoided. In addition to acetylcholine, substance P was also used, which permitted an examination of microvascular endothelial function without the simultaneous constriction of epicardial arteries. These features all suggest that the findings are reliable.

**Prior Studies**

Four previous studies examined the effects of cholesterol-lowering therapy on coronary endothelial vasomotor function (Table 4). In an uncontrolled study, Leung et al$^8$ reported that cholestyramine lowered total cholesterol by 29% and converted acetylcholine-induced constriction to vasodilation in hypercholesterolemic men with normal coronary arteries. In an open-label study, Egashira et al$^9$ reported improved epicardial and microvascular responses to acetylcholine after pravastatin treatment in patients with 1-vessel CAD and no change in a nonrandomized control group. In a randomized, double-blind placebo-controlled study, Treasure et al$^{10}$ examined the epicardial coronary responses to acetylcholine in patients with 1-vessel CAD. Lovastatin plus diet reduced total cholesterol by 31% and improved acetylcholine-induced coronary constriction (−16% to 0%), whereas placebo plus diet had no effect (−19 to −18%). Like in the present study, Treasure et al$^{10}$ examined the SMAV and used the same

**TABLE 2. Fasting Serum Lipid Levels**

<table>
<thead>
<tr>
<th></th>
<th>Placebo Plus Diet</th>
<th>Simvastatin Plus Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 mo</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>203±28</td>
<td>200±28</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>41±10</td>
<td>41±9</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>131±24</td>
<td>129±28</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>152±87</td>
<td>147±93</td>
</tr>
</tbody>
</table>

*$P<0.001$, †$P<0.01$ compared with baseline. Values are mean±SD.

**TABLE 3. Hemodynamics and Resting Coronary Diameter**

<table>
<thead>
<tr>
<th></th>
<th>Simvastatin Plus Diet</th>
<th>Placebo Plus Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 mo</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>133±14</td>
<td>129±13</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>78±8</td>
<td>75±9</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>66±12</td>
<td>64±12</td>
</tr>
<tr>
<td>Coronary diameter, mm*</td>
<td>Before ACh</td>
<td>2.11±0.48</td>
</tr>
<tr>
<td></td>
<td>Before substance P</td>
<td>2.05±0.47</td>
</tr>
</tbody>
</table>

*Average of all analyzed segments. Data are mean±SD.
Atherosclerotic coronary disease was relatively mild in the present study population. After 6 months of treatment, the constrictor response improved to a similar extent in both groups. Data are presented as unadjusted mean ± SEM.

Figure 1. Effect of acetylcholine on epicardial coronary diameter before and after treatment. Diameters changes during intracoronary acetylcholine infusion were assessed at baseline and after 6 months of treatment as described in Methods. Acetylcholine produced dose-dependent coronary vasoconstriction at baseline in both groups. After 6 months of treatment, the constrictor response improved to a similar extent in both groups. Data are presented as unadjusted mean ± SEM.

In contrast to those prior studies, the present study demonstrated no effect of cholesterol-lowering therapy on the response to acetylcholine. Several factors might explain these apparently discrepant findings. One possibility is a difference in the study population, and it is notable that baseline total and LDL-cholesterol levels were higher in all 3 studies9–11 that showed a beneficial effect with cholesterol-lowering therapy alone (Table 4). The finding in the present study that there was no relation between baseline LDL-cholesterol and the response to acetylcholine (P = 0.08). Notably, the combination of cholesterol-lowering and antioxidant therapy produced a significant improvement in endothelial vasomotor function.

TABLE 4. Previous Studies of Cholesterol-Lowering and Coronary Artery Endothelial Function

<table>
<thead>
<tr>
<th>Study</th>
<th align="right">n*</th>
<th>Randomized Control Group</th>
<th>Baseline Total Cholesterol, mg/dL</th>
<th>Baseline LDL Cholesterol, mg/dL</th>
<th>Treatment Duration, mo</th>
<th>Improvement Demonstrated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leung et al8</td>
<td align="right">25</td>
<td>No</td>
<td>275 ± 31</td>
<td>220 ± 34</td>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>Egashira et al9</td>
<td align="right">9</td>
<td>No</td>
<td>272 ± 8</td>
<td>195 ± 25</td>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>Treasure et al10</td>
<td align="right">11</td>
<td>Yes</td>
<td>230 ± 33</td>
<td>148 ± 23</td>
<td>5.5</td>
<td>+</td>
</tr>
<tr>
<td>Anderson et al11</td>
<td align="right">21</td>
<td>Yes</td>
<td>209 ± 33</td>
<td>139 ± 38</td>
<td>12</td>
<td>±</td>
</tr>
<tr>
<td>Present study</td>
<td align="right">34</td>
<td>Yes</td>
<td>204 ± 32</td>
<td>130 ± 28</td>
<td>6</td>
<td>–</td>
</tr>
</tbody>
</table>

*No. of subjects in active treatment group. Lipid data are mean ± SD.
therapy on the microvascular response to substance P given that fewer than the planned number of patients were enrolled and the variability was greater than expected. If the planned number of subjects had been studied, the observed changes in microvascular function might have reached statistical significance.

**Clinical Implications and Conclusions**

It has been suggested that improvement of coronary endothelial function contributes to the reduction in CAD events associated with long-term cholesterol-lowering therapy. In the CARE and LIPID studies, which involved patients with CAD and lipid levels comparable to those of the patients in the present study, there were no reductions in cardiovascular disease events during the first 2 to 3 years of treatment. The finding in the present study that endothelial function remained unimproved after only 6 months of treatment is consistent with the results of those clinical trials. It is possible that more prolonged treatment is associated with an improvement in coronary endothelial function that might contribute to the observed reduction in events.

In the present study, there was a suggestion that cholesterol-lowering therapy improved microvascular endothelial function (Figure 2), although the group difference did not reach statistical significance. Recent studies have shown that short-term cholesterol-lowering therapy (3 to 5 months) reduces ambulatory and stress-induced ischemia in patients with CAD and higher total cholesterol levels than those in the present study (238 to 297 mg/dL). Because endothelial vasomotion in coronary microvessels is relevant to metabolic regulation of coronary blood flow, it is possible that improved microvascular endothelial function contributes to the reduction in myocardial ischemia in these patients. However, the present study does not provide conclusive evidence to support this speculation.

In summary, this relatively large, well-controlled study demonstrated that 6 months of cholesterol-lowering therapy has no significant effect on coronary endothelial function in a group of patients with mildly elevated cholesterol levels, no concomitant coronary risk factors, and mild CAD. These findings suggest that the effects of cholesterol lowering on endothelial function are more complex than previously thought.

**Acknowledgments**

The authors thank Francois Charbonneau, MD; J. Jeffery Marshall, MD; Tai-Tien Lim, MD; Richard Bach, MD; Ravi Nar, MD; and John F. Keaney, Jr, MD, who assisted in the completion of study protocols.

**References**


Effect of Cholesterol-Lowering Therapy on Coronary Endothelial Vasomotor Function in Patients With Coronary Artery Disease

Circulation. 2000;102:846-851
doi: 10.1161/01.CIR.102.8.846

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/102/8/846

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/