Vascular Effects of Estrogen and Cholesterol-Lowering Therapies in Hypercholesterolemic Postmenopausal Women

Kwang Kon Koh, MD; Carmine Cardillo, MD; Minh N. Bui, MD; Londa Hathaway, RN; Gyorgy Csako, MD; Myron A. Waclawiw, PhD; Julio A. Panza, MD; Richard O. Cannon III, MD

Background—Lipoproteins affect endothelium-dependent vasomotor responsiveness. Because lipoprotein effects of estrogen and cholesterol-lowering therapies differ, we studied the vascular responses to these therapies in hypercholesterolemic postmenopausal women.

Methods and Results—We randomly assigned 28 women to conjugated equine estrogen (CE) 0.625 mg, simvastatin 10 mg, and their combination daily for 6 weeks. Compared with respective baseline values, simvastatin alone and combined with CE reduced LDL cholesterol to a greater extent than CE alone (both P<0.05). CE alone and combined with simvastatin raised HDL cholesterol and lowered lipoprotein(a) to a greater extent than simvastatin alone (all P<0.05). Flow-mediated dilation of the brachial artery (by ultrasonography) improved (all P<0.001 versus baseline values) on CE (4.0±2.6% to 10.2±3.9%), simvastatin (4.3±2.4% to 10.0±3.9%), and CE combined with simvastatin (4.6±2.0% to 9.8±2.6%), but similarly among therapies (P=0.507 by ANOVA). None of the therapies improved the dilator response to nitroglycerin (all P=0.184). Only therapies including CE lowered levels of plasminogen activator inhibitor type 1 and the cell adhesion molecule E-selectin (all P<0.05 versus simvastatin).

Conclusions—Although estrogen and statin therapies have differing effects on lipoprotein levels, specific improvement in endothelium-dependent vasodilator responsiveness is similar. However, only therapies including estrogen improved markers of fibrinolysis and vascular inflammation. Thus, estrogen therapy appears to have unique properties that may benefit the vasculature of hypercholesterolemic postmenopausal women, even if they are already on cholesterol-lowering therapy. (Circulation. 1999;99:354-360.)

Key Words: lipoproteins ■ endothelium ■ hormones ■ cell adhesion molecules ■ fibrinolysis

Observational studies suggest that estrogen therapy decreases the risk of coronary artery disease in postmenopausal women.1 The mechanisms of this apparent benefit of hormone therapy most likely include lipoprotein effects: Orally administered estrogen raises plasma levels of HDL cholesterol and lowers plasma levels of LDL cholesterol2 and lipoprotein(a)3 and protects LDL from oxidation.4 These lipoprotein effects may account for improvement in coronary and systemic vasomotor responsiveness due to reduction in inhibitory effects of LDL and lipoprotein(a) in the vessel wall5-7 and facilitatory effects of HDL,8 in addition to enhanced nitric oxide bioactivity.9,10 Statin (β-hydroxy-β-methylglutaryl-coenzyme A [HMG-CoA] reductase inhibitor) therapy has also been shown to improve vasomotor responsiveness,11-14 possibly through enhanced nitric oxide bioactivity. However, important differences in the lipoprotein effects of estrogen versus statin therapy may result in differing effects on vasomotor responsiveness. For example, at conventional dosages, statins lower LDL cholesterol to a greater degree than estrogen therapy and have a smaller effect on HDL cholesterol and lipoprotein(a) levels than does estrogen administered to postmenopausal women.15,16

Thus, because the lipoprotein effects that could influence nitric oxide bioactivity differ between estrogen and statin therapies and because estrogen may directly stimulate the release of nitric oxide, as shown in endothelial cells in culture,17-20 it is possible that the impact of these therapies on nitric oxide bioactivity and its subsequent effects on endothelial homeostasis may differ. Furthermore, because the mechanisms of the biological effects of these therapies differ, the combination of the therapies may be additive, an effect of potential importance to women at high risk for atherosclerosis or with established atherosclerotic disease. Thus, this study was designed to assess the effect of these therapies, independently and in combination, on vascular function in hypercholesterolemic postmenopausal women.

Methods

Study Population and Design

Thirty-one hypercholesterolemic postmenopausal women (LDL cholesterol levels >130 mg/dL) participated in this study, all with...
plasma 17β-estradiol levels <50 pg/mL and cessation of menses for at least 1 year. None were diabetic or current cigarette smokers. No subject had taken any cholesterol-lowering agent, estrogen therapy, or antioxidant vitamin supplements during the preceding 2 months. Aspirin and nonsteroidal anti-inflammatory agents were stopped for 10 days before study; study participants remained off such drugs throughout the study. Two women withdrew from the study but denied side effects of therapy. A third woman dropped out after the second treatment period because of changes in visual acuity, believed by her ophthalmologist to be unrelated to therapy. Thus, a total of 28 women (age, 57±2 years; LDL cholesterol, 163±36 mg/dL) completed all phases of the study. This study was a randomized, double-blind, double-crossover trial. The study participants received conjugated equine estrogen (CE) 0.625 mg each morning and placebo each night, placebo each morning and simvastatin 10 mg each night, or a combination of the 2 therapies per day for each of three 6-week treatment periods, with 6 weeks between treatment periods. Subjects were placed on a low-nitrate diet for 72 hours before each study to reduce the contribution of dietary nitrates to serum nitrogen oxide levels. The study was approved by the National Heart, Lung, and Blood Institute Review Board, and all participants gave written informed consent.

**Laboratory Assays**

Blood samples for laboratory assays were obtained between 8:00 and 9:00 AM after overnight fasting (including caffeine) and with the patient recumbent for at least 15 minutes before and at the end of each treatment period and were immediately coded so that investigators performing laboratory assays were blinded to subject identity and study sequence. Plasma estrone and 17β-estradiol levels were measured by radioimmunoassay. All samples were stored at −70°C until analysis. Total cholesterol and glycerol-glyceryl triglycerides in the serum were quantified by automated enzymatic techniques. Serum HDL cholesterol was quantified after dextran sulfate precipitation of other lipoproteins. Serum LDL cholesterol levels were directly quantified by an immunoabsorption method. Serum lipoprotein(a) levels were measured by an immunoturbidimetric assay (Incstar) with a lower limit of detectability of 4.9 mg/dL. Serum nitrate/nitrite levels were measured in triplicate by conversion of nitrate (NO3−) to nitrite (NO2−) by nitrate reductase, followed by addition of Griess reagents and photometric measurement of absorbance (Oxford Biomedical Research). Intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1), and E-selectin levels were measured in triplicate by ELISA (R and D systems). Plasminogen activator inhibitor type 1 (PAI-1) antigen levels were determined in duplicate by a sandwich ELISA (Biopool).

**Vascular Studies**

Imaging studies of the left brachial artery were performed with a Hewlett-Packard SONOS 2500 ultrasound machine equipped with a 7.5-MHz linear-array transducer after 10 minutes of rest before and at the end of each of the 3 treatment periods. Imaging of the artery proximal to the antecubital fossa was done longitudinally, with the center of the artery identified by the clearest visualization of the anterior and posterior intimal layers. The transmit (focus) zone was set to the depth of the near wall. Depth and gain settings were set to optimize images of the interface between the lumen and the arterial wall; images were magnified by use of a resolution box function. After a satisfactory transducer position was found, the skin was marked, and the arm remained in that position throughout the study. A baseline measurement of brachial arterial diameter was made, as well as a baseline measurement of the velocity of arterial flow by pulsed Doppler with the range gate (1.5 mm) in the center of the artery. The system permitted a direct assessment of the angle between the blood stream and the intersecting ultrasound beam, which was then used to calculate blood flow velocity. Endothelium-dependent vasodilation was assessed by measuring the change in the diameter of the brachial artery after 60 seconds of reactive hyperemia relative to baseline measurements after deflation of a cuff on the forearm inflated to 250 mm Hg for 5 minutes, a response previously shown to be mediated primarily by nitric oxide. Arterial flow velocity was measured for the first 15 seconds after cuff deflation. After baseline conditions had been reestablished 10 to 15 minutes later, measurements of arterial diameter and flow velocity were repeated, followed by nitroglycerin at a dose of 0.4 mg administered by spray under the tongue to assess endothelium-independent vasodilation. Three minutes later, repeat measurements of arterial diameter and flow velocity were made. All images were coded and recorded on VHS videotape for subsequent blinded analysis. Arterial diameter was measured in millimeters as the distance between the anterior wall media-adventitial interface (“m” line) and the posterior wall intima-lumen interface at end diastole, coincident with the R wave on the ECG at 2 sites along the artery and for 3 cardiac cycles, with these 6 measurements averaged. Blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal by the heart rate and the cross-sectional area of the vessel. Sixteen studies were independently analyzed on 2 occasions; intraobserver correlation for maximum diameter was 0.97 and for percent dilation 0.78.

**Statistical Analysis**

Data are expressed as mean±SD. Student’s paired t test was used to compare values before and after each treatment and the relative changes in values in response to treatment. The effects of CE, simvastatin, and the two combined were analyzed by 1-way repeated-measures ANOVA. Post hoc comparisons between different treatment pairs were made by the Student-Newman-Keuls multiple-comparison procedures. Pearson correlation coefficient analysis was used to assess associations between values. Friedman repeated-measures ANOVA on ranks was used to compare the effects of CE, simvastatin, and the two combined on lipoprotein(a) levels. The comparison of endothelium-dependent dilation among the 3 treatment schemes was prospectively designated as the primary endpoint of the study. All other comparisons were considered secondary end points. Therefore, probability values less than the Bonferroni-adjusted α of 0.05/3=0.017 are deemed as statistically significant for the primary hypothesis. No adjustments were made for the number of secondary hypotheses.

**Results**

Baseline values before each treatment period were compared: no significant differences were noted (Tables 1 and 2). To assess the possibility of a carryover effect from the initial treatment phase to the next treatment phase, we compared the baseline values before the first treatment phase with those before the second and third treatment phases. No significant differences were found. After 6 weeks of oral CE alone or combined with simvastatin, plasma levels of estrone and 17β-estradiol increased to a similar degree (Table 1). No changes in hormone levels were noted with simvastatin alone.

**Effects of Treatments on Lipoproteins**

All therapies lowered total and LDL cholesterol levels from baseline values (all P<0.001, Table 1), with greater effect on LDL cholesterol for simvastatin (−25±14%) and CE combined with simvastatin (−32±14% than for CE alone (−11±11%; both P<0.05 versus CE alone). The differences in effects of therapies on apolipoprotein B levels were similar. In contrast, only therapies with CE alone or combined with simvastatin increased HDL cholesterol levels (both 17±15% from baseline values; both P<0.05 versus simvastatin alone). All therapies increased apolipoprotein A-I levels from baseline values (all P<0.02) and to a similar degree (P=0.367 by ANOVA). The ratio of LDL to HDL cholesterol levels and the ratio of the apolipoproteins to these lipoproteins decreased to a greater degree on simvastatin
combined with CE than with CE or simvastatin alone (Figure 1). Only CE alone or combined with simvastatin lowered lipoprotein(a) levels from baseline values (both \( P < 0.05 \) versus simvastatin alone, Table 1). Only simvastatin alone significantly reduced triglyceride levels (\(-7 \pm 43\% ; \ P = 0.023\)); therapies including CE did not significantly change these levels from baseline values (both \( P < 0.05 \) versus simvastatin alone).

### TABLE 1. Effects of Oral Conjugated Estrogen, Simvastatin, or Combined Therapy on Hormones and Lipids

<table>
<thead>
<tr>
<th>Variable</th>
<th>Conjugated Estrogen Alone</th>
<th>Simvastatin Alone</th>
<th>Combination Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormones, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrone</td>
<td>30 \pm 17</td>
<td>30 \pm 18</td>
<td>32 \pm 17</td>
</tr>
<tr>
<td>17\beta-Estradiol</td>
<td>21 \pm 12</td>
<td>21 \pm 25</td>
<td>21 \pm 11</td>
</tr>
<tr>
<td>Lipids, mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>121 \pm 69</td>
<td>134 \pm 96</td>
<td>113 \pm 81</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>239 \pm 40</td>
<td>240 \pm 45</td>
<td>242 \pm 41</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>58 \pm 13</td>
<td>58 \pm 16</td>
<td>59 \pm 16</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>151 \pm 21</td>
<td>149 \pm 29</td>
<td>152 \pm 23</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>163 \pm 36</td>
<td>162 \pm 35</td>
<td>167 \pm 32</td>
</tr>
<tr>
<td>Apo B</td>
<td>121 \pm 28</td>
<td>119 \pm 31</td>
<td>119 \pm 31</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>3.00 \pm 1.20</td>
<td>3.00 \pm 1.20</td>
<td>3.04 \pm 1.23</td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>29.7 \pm 27.1</td>
<td>28.8 \pm 25.9</td>
<td>31.8 \pm 26.4</td>
</tr>
</tbody>
</table>

Apo indicates apolipoprotein. Data are expressed as mean \pm SD.

\( \ast P < 0.05 \)

\( \dagger P < 0.01 \)

\( \ddagger P < 0.001 \) for comparison with the baseline value.

### Effects of Treatments on Vasomotor Function

Brachial artery diameter and basal forearm blood flow were similar during each treatment period (\( P = 0.490 \) and \( P = 0.105 \) by ANOVA, respectively), as were the peak forearm blood flow and brachial artery diameter during reactive hyperemia (\( P = 0.941 \) and \( P = 0.248 \) by ANOVA, respectively; Table 2). All therapies increased flow-mediated dilation relative to baseline measurements (Figure 2) and to a similar degree

### TABLE 2. Effects of Oral Conjugated Estrogen, Simvastatin, or Combined Therapy on Endothelial Function

<table>
<thead>
<tr>
<th>Variable</th>
<th>Conjugated Estrogen Alone</th>
<th>Simvastatin Alone</th>
<th>Combination Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasomotor function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum nitrate/nitrite, ( \mu )mol/L</td>
<td>43.1 \pm 15.8</td>
<td>53.0 \pm 45.2</td>
<td>50.6 \pm 30.4</td>
</tr>
<tr>
<td>Brachial artery diameter, mm</td>
<td>3.92 \pm 0.39</td>
<td>3.93 \pm 0.46</td>
<td>3.88 \pm 0.38</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>4.08 \pm 0.40</td>
<td>4.09 \pm 0.44</td>
<td>4.07 \pm 0.41</td>
</tr>
<tr>
<td>Brachial artery flow, mL/min</td>
<td>108 \pm 42</td>
<td>128 \pm 65</td>
<td>117 \pm 44</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>666 \pm 173</td>
<td>712 \pm 252</td>
<td>677 \pm 225</td>
</tr>
<tr>
<td>Flow-mediated dilation, %</td>
<td>4.0 \pm 2.6</td>
<td>4.3 \pm 2.4</td>
<td>4.6 \pm 2.0</td>
</tr>
<tr>
<td>Nitroglycerin, %</td>
<td>14.8 \pm 4.8</td>
<td>15.3 \pm 4.9</td>
<td>15.5 \pm 4.5</td>
</tr>
<tr>
<td>Fibrinolysis</td>
<td>20.2 \pm 11.9</td>
<td>19.2 \pm 11.9</td>
<td>19.8 \pm 13.6</td>
</tr>
<tr>
<td>Cell adhesion molecules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-selectin, ng/mL</td>
<td>52.5 \pm 19.0</td>
<td>49.1 \pm 17.6</td>
<td>52.7 \pm 20.4</td>
</tr>
<tr>
<td>ICAM-1, ng/mL</td>
<td>281 \pm 69</td>
<td>268 \pm 59</td>
<td>284 \pm 65</td>
</tr>
<tr>
<td>VCAM-1, ng/mL</td>
<td>732 \pm 254</td>
<td>747 \pm 321</td>
<td>763 \pm 266</td>
</tr>
</tbody>
</table>

Data are expressed as means \pm SD.

\( \ast P < 0.05 \)

\( \dagger P < 0.01 \)

\( \ddagger P < 0.001 \) for comparison with the baseline value.
The brachial artery dilator responses to nitroglycerin were similar for all therapies ($P=0.878$ by ANOVA) and were not significantly increased compared with respective baseline values ($P=0.851$ for CE alone, $P=0.534$ for simvastatin alone, $P=0.184$ for the two combined). Improvement in flow-mediated dilation did not correlate strongly with changes in lipoprotein levels during any of the treatment periods (all $r#0.366$). CE increased serum nitrate/nitrite levels by $5\pm38\%$, but the degree of change was not significant compared with pretreatment values (Table 2). Simvastatin alone and simvastatin combined with CE lowered serum nitrate/nitrite levels with marginal significance, by $5\pm37\%$ and $6\pm30\%$, respectively ($P=0.104$ and $P=0.109$ versus baseline values), albeit without significant differences among therapies ($P=0.441$ by ANOVA). Improvement in flow-mediated dilation did not correlate with changes in serum nitrate/nitrite levels during any of the treatment periods (all $r<0.053$).

**Discussion**

In this study, CE 0.625 mg and simvastatin 10 mg, administered alone and in combination, each for 6 weeks, had effects on LDL and HDL cholesterol levels comparable to the report by Davidson et al.,$^{15}$ in which CE 0.625 mg, pravastatin 20 mg, and a combination of these therapies were administered daily to hypercholesterolemic postmenopausal women, each for 16 weeks. We found that the combination of estrogen and statin therapies lowered LDL cholesterol to a greater degree than either therapy alone, with elevation of HDL cholesterol similar to that with estrogen alone. Thus, the combination of these therapies decreased the ratio of LDL to HDL cholesterol and apolipoprotein levels to a greater degree than either therapy administered alone. Consistent with the findings of Darling et al.,$^{16}$ therapies including CE, but not simvastatin alone, reduced lipoprotein(a) levels.

We reasoned that differing effects of estrogen and statin therapies on lipoproteins that have been shown to affect...
vascular function might result in differential effects on endothelium-dependent vasodilator responsiveness, with possible additive effects when the therapies are combined. Indeed, CE significantly improved flow-mediated dilation of the brachial artery, an effect consistent with enhanced release of nitric oxide. Nitrate/nitrite levels, which reflect in part the luminal release of nitric oxide, were marginally reduced with simvastatin alone or combined with CE, but not with CE alone. Reduction in luminal release of nitric oxide after statin therapy may indicate reduced synthesis of nitric oxide required for endothelial homeostasis as a consequence of reduced degradation of nitric oxide by oxidized lipoproteins and free radical molecules from the endothelium and from inflammatory cells. In contrast, CE did not lower nitrate/nitrite levels in our study participants, despite significant reductions in LDL cholesterol and lipoprotein(a) levels and may reflect competing stimuli to reduce and increase nitric oxide synthesis.

To determine the extent of vascular effects of CE and statin therapies, we measured markers of fibrinolysis and inflammation considered important in the pathogenesis of atherosclerosis. CE alone or combined with simvastatin reduced PAI-1 levels. In a previous study, we demonstrated that the relative reduction in PAI-1 levels on CE was significantly associated with comparable increases in levels of α1-microglobulin, a product of cross-linked fibrin degradation by plasmin, providing evidence of enhanced fibrinolysis. In contrast to the effects of CE, simvastatin did not change PAI-1 levels, consistent with the observation of Zambrana et al. in 21 hyperlipemic heart transplant patients. However, Isaacsohn et al. observed a decrease in plasma PAI-1 levels in hypercholesterolemic patients (menopausal status of women not reported) treated with high doses of lovastatin (up to 80 mg/d).

Cell adhesion molecules are expressed after transcriptional activation by a variety of proinflammatory stimuli, including cytokines and oxidized LDL. These molecules are then positioned across the endothelial cell membrane and bind to ligands on inflammatory cells and facilitate their subsequent entry into the vessel wall. 17β-Estradiol has been shown in endothelial cell cultures to inhibit the expression of cell adhesion molecules in one study but to promote their expression in another study. Experimental evidence suggests that cell adhesion molecules, once expressed on the endothelial cell surface, may be shed from the surface. Several groups have reported the presence of E-selectin, ICAM-1, and VCAM-1 in the culture supernatant within 4 to 6 hours of endothelial or leukocyte cell activation and in sera of humans as shown by the same monoclonal antibody assay as used to demonstrate adhesion molecules in the supernatant of activated endothelial cells in culture. Serum concentrations of E-selectin, ICAM-1, and VCAM-1 have been reported to be higher in patients with coronary artery disease and dyslipidemia than in healthy control subjects. Although the biological function in sera remains unclear, the clinical relevance of cell adhesion molecules has been suggested by several observational studies. Thus, E-selectin, ICAM-1, and VCAM-1 have been demonstrated in human coronary atherosclerotic arteries by immunohistochemistry. In the Atherosclerosis Risk in Communities (ARIC) study, higher serum levels of E-selectin and ICAM-1 were found in patients with coronary heart disease and carotid artery atherosclerosis than in healthy control subjects: E-selectin levels correlated positively with the carotid artery thickness measured by ultrasound in this study. Belch et al. reported that patients who underwent peripheral artery balloon angioplasty and developed restenosis at higher serum levels of E-selectin than patients without restenosis. Recently,
men in the Physician’s Health Study with the highest quartile of ICAM-1 levels were found to be of greater cardiovascular risk than men in the lowest quartile; E-selectin levels were not reported in this study.44 We found that only therapies including CE alone or combined with statin therapy significantly reduced E-selectin, ICAM-1, and VCAM-1 levels from respective pretreatment values. These findings are consistent with the recent report of Caulin-Glaser et al,45 who found that men and postmenopausal women not on hormone therapy who had coronary artery disease shown by coronary angiography had higher levels of soluble cell adhesion molecules than men and women without coronary artery disease or postmenopausal women with coronary artery disease who were current users of hormone therapy.

The mechanisms by which CE therapies, but not simvastatin alone, reduced levels of cell adhesion molecules cannot be determined from our study, but they are probably independent of nitric oxide, because nitric oxide bioactivity as manifest by improvement in brachial artery flow-mediated dilation was increased equally by simvastatin and CE therapies from respective pretreatment values. Of interest, a recent study showed that HDL, levels of which were significantly increased in our study with therapies including CE but not simvastatin alone, inhibits cytokine-induced expression of cell adhesion molecules in cultured endothelial cells.46 Furthermore, CE therapies, but not simvastatin alone, reduced levels of lipoprotein(a), recently shown to stimulate the expression of ICAM-1 in cultured endothelial cells.47 We conclude from our study that although estrogen and statin therapies at the dosages used have differing effects on cell adhesion molecules in cultured endothelial cells,48 furthermore, CE therapies, but not simvastatin alone, reduce levels of lipoprotein(a), recently shown to stimulate the expression of ICAM-1 in cultured endothelial cells.47

We conclude from our study that although estrogen and statin therapies at the dosages used have differing effects on lipoprotein levels, improvement in endothelium-dependent responsiveness is similar. However, only therapies including estrogen improved other vascular homeostatic factors potentially important in atherogenesis. Thus, estrogen therapy may provide vasculoprotective benefit to hypercholesterolemic postmenopausal women, even if they are already on statin therapy.

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References


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