Vascular Effects of Estrogen and Vitamin E Therapies in Postmenopausal Women

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Background—Estrogen and vitamin E therapies have been suggested to reduce cardiovascular risk, but comparison of the vascular effects of these therapies to determine mechanisms of potential benefit has not been performed in postmenopausal women.

Methods and Results—In a double-blind, 3-period crossover study, we randomly assigned 28 healthy postmenopausal women to conjugated equine estrogens (CE) 0.625 mg/d, vitamin E 800 IU/d, and their combination, with measurements made before and after each 6-week treatment period. The ratio of LDL to HDL cholesterol and lipoprotein(a) decreased on therapies including CE but increased on vitamin E alone (P<0.001 and P=0.002, respectively, by ANOVA). Brachial artery flow–mediated dilation improved on all therapies (all P<0.001 versus pretreatment values) and to a similar degree (P=0.267 by ANOVA). No therapy improved the dilator response to nitroglycerin. CE lowered serum levels of cell adhesion molecules E-selectin, ICAM-1, and VCAM-1 (all P<0.05 versus pretreatment values). Vitamin E had no significant effect on levels of these markers of inflammation (P>0.001 by ANOVA for E-selectin). CE alone or combined with vitamin E but not vitamin E alone lowered or showed a trend for lowering plasma levels of plasminogen activator inhibitor type-1 (P=0.069 by ANOVA).

Conclusions—Estrogen and vitamin E therapies similarly improved arterial endothelium-dependent vasodilator responsiveness consistent with increased nitric oxide in healthy postmenopausal women, despite divergent effects on atherogenic lipoproteins. However, only estrogen reduced markers of vascular disease. (Circulation. 1999;100:1851-1857.)

Key Words: atherosclerosis ■ endothelium ■ antioxidants ■ cell adhesion molecules ■ fibrinolysis

Prospective cohort surveys such as the Nurses’ Health Study suggest that estrogen therapy decreases the risk of coronary artery disease in postmenopausal women initially healthy at the time of enrollment.1 The mechanisms of this apparent benefit of hormone therapy likely include lipoprotein effects: Orally administered estrogen raises levels of HDL cholesterol and lowers levels of LDL cholesterol and lipoprotein(a) in serum.2–4 Other mechanisms of potential benefit include protection of LDL from oxidation,5 potentiation of fibrinolysis,6 and improvement in endothelium-dependent vasodilator function because of increased nitric oxide bioavailability.7–9

Use of vitamin E supplements was also associated with decreased risk of cardiovascular events in the Nurses’ Health Study10 and reduced risk of nonfatal myocardial infarction (but not cardiovascular death) in the Cambridge Heart Antioxidant Study.11 Vitamin E is the most abundant lipid-soluble antioxidant in biological membranes and has been shown to protect LDL from oxidation and improve endothelium-dependent relaxation in animal models.12–16 We previously administered vitamin E 800 IU to 10 healthy postmenopausal women for 6 weeks, raising vitamin E levels in plasma from 1.8±0.9 to 3.0±1.6 mg/dL.13 Vitamin E prolonged the time to onset of copper-induced oxidation of LDL isolated from these women by 29% (173±55 versus 135±18 minutes at baseline). Although the specific vascular effect of vitamin E in postmenopausal women is unknown, the animal data are consistent with data from studies showing that antioxidant therapies improve endothelial function in patients with coronary artery disease or its risk factors.17–21

Because the vasculoprotective mechanisms of these therapies may differ, their combination may be additive, an effect of potential atheroprotective importance to postmenopausal women. This study was designed to assess the effects of these therapies, independently and in combination, on vascular dilator and other homeostatic functions potentially affected by their antioxidant and nitric oxide–potentiating properties in healthy postmenopausal women.

Methods

Study Population and Design

Thirty postmenopausal women (age, 57±7 years [mean±SD]) participated in this study, all with plasma 17β-estradiol levels <50 ng/mL and <200 mg/dL (normal range). Women were not taking estrogen, progestogens, oral contraceptives, or other lipid-lowering therapies. Women visited the Clinical Investigation and Reports...
pg/mL and cessation of menses for at least 1 year. Eight hypercholesterolemic women had participated previously in a study assessing the effect of estrogen and simvastatin on vascular function. None was diabetic, hypertensive, or a current cigarette smoker. Sixteen women had LDL cholesterol levels ≥130 mg/dL; 9 had levels ≥160 mg/dL. 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 beginning 10 days before study. Two women requested withdrawal from the study but denied side effects of therapy. Thus, 28 women completed all phases of the study. This study was a randomized, double-blind, 3-period crossover treatment trial. Study participants received conjugated equine estrogens (CE) 0.625 mg and placebo, vitamin E 800 IU and placebo, or a combination of the 2 therapies per day for each of three 6-week treatment periods, with 6 weeks off all therapies between treatment periods. The study was approved by the National Heart, Lung, and Blood Institute Review Board, and all participants gave written, informed consent.

**Laboratory Assays**

Subjects were placed on a nitrate-restricted diet (≤15 mg/d) for 3 days before each pretreatment and treatment study to reduce the contribution of dietary nitrates (usual daily intake 75 to 100 mg/d) to serum nitrogen oxide levels. We had previously found this diet to reduce serum nitrogen oxide levels from 66.2 ± 46.1 to 46.4 ± 26.1 μmol/L (P≤0.001) in 30 healthy subjects. Blood samples for laboratory assays were obtained at ~8 AM after an overnight fast and before and at the end of each treatment period; samples were immediately coded so that investigators performing laboratory assays were blinded to subject identity or study sequence. Assays for lipids, apolipoproteins, nitrogen oxides, plasmogen activator inhibitor type 1 (PAI-1), and cell adhesion molecules were performed as previously described.

**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 before and at the end of each of the 3 treatment periods on the basis of a previously published technique and as reported by us previously. Endothelium-dependent vasodilation was assessed by measurement of 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. Arterial flow velocity was measured for the first 15 seconds after cuff deflation. After baseline conditions were reestablished 15 minutes later, measurements of arterial diameter and flow velocity were repeated, followed by administration of nitroglycerin 0.4 mg by spray under the tongue to assess endothelium-independent vasodilation. Repeated measurements of arterial diameter and flow velocity were made 3 minutes later. All images were coded and recorded on VHS videotape for subsequent blinded analysis. Measurements of flow-mediated dilation were made on 2 occasions from the videotapes of 10 studies selected at random. The mean±SD of intraobserver differences in measurements was 0.4±0.3% (range, 0.1% to 1.1%), yielding a coefficient of variation of 1.28 and a coefficient of repeatability of 0.6%. Statistical Analysis

Data are expressed as mean±SD. After testing data for normality, we used Student’s paired t test or the Wilcoxon signed-rank test to compare values before and after each therapy and the relative changes in values in response to each therapy, as reported in Tables 1 and 2. The effects of the 3 therapies on vascular function and markers of inflammation and fibrinolysis inhibition relative to respective pretreatment values were analyzed by 1-way repeated-measures ANOVA or Friedman’s repeated ANOVA on ranks. After demonstration of significant differences among therapies by ANOVA, post hoc comparisons between treatment pairs were made by use of the Student-Newman-Keuls multiple comparison procedures. Pearson’s correlation coefficient analysis was used to assess associations between measured parameters. The comparison of endothelium-dependent dilation among the 3 treatment schemes was prospectively designated as the primary end point of the study. All other comparisons were considered secondary. Therefore, probability values less than the Bonferroni-adjusted α of 0.05/3=0.017 were deemed statistically significant for the 3 primary hypothesis pairwise comparisons. No adjustments were made for the number of secondary hypothesis.

**Results**

Baseline values before each treatment period were compared, and no significant differences were noted (Tables 1 and 2). To assess the possibility of a carryover effect from the initial treatment periods to the next treatment period, we compared the baseline values before the first treatment period to those before the second and third treatment periods (data not
were noted with vitamin E alone.

No changes in hormone levels compared with respective pretreatment values, CE alone or combined with vitamin E, plasma levels of IL-6, TNF-α, and fibrinogen were significantly higher than baseline (Figure 1). No significant differences were found. After 6 weeks of CE alone or combined with vitamin E, plasma levels of lipids decreased more from respective pretreatment values on CE alone compared with the other treatment periods (Figure 1). CE alone or combined with vitamin E decreased lipoprotein(a) levels from pretreatment values, whereas vitamin E alone significantly increased these levels (P=0.002 by ANOVA; Figure 2).

**Effects of Therapies on Nitric Oxide Bioactivity**

Basal brachial artery diameter and forearm blood flows were similar during the 3 treatment periods (P=0.330 and P=0.964 by ANOVA, respectively), as were the peak brachial artery diameters and forearm blood flows during reactive hyperemia (P=0.472 and P=0.761 by ANOVA, respectively) and the percent increase in flow during hyperemia (P=0.558 and P=0.350 by ANOVA, respectively; Table 2). CE and vitamin E therapies improved the flow-mediated dilator response to hyperemia relative to respective pretreatment measurements (Figure 3) without additive effects when these therapies were combined (P=0.267 by ANOVA). The 95% CI for the absolute differences in flow-mediated dilation between CE alone and the therapies combined was −1.4% to 2.4%; between vitamin E alone and the therapies combined, −1.2% to 2.0%. Thus, with our study of 28 subjects, we identified by bars.

**Effects of Therapies on Lipids**

The effects of therapies on lipids are shown in Table 1. Compared with respective pretreatment values, CE alone or combined with vitamin E lowered total cholesterol levels by 4±10% and 4±11%, respectively (both P=0.03); lowered LDL cholesterol levels by 9±12% (P<0.001) and 5±16% (P=0.04); and increased HDL cholesterol levels by 16±20% (P=0.001) and 12±20% (P=0.008). In contrast, vitamin E alone increased total cholesterol levels by 4±8% (P<0.05), with LDL and HDL cholesterol levels unchanged from pretreatment values. The ratio of LDL to HDL cholesterol levels decreased more from respective pretreatment values on CE alone compared with the other treatment periods (Figure 1). CE alone or combined with vitamin E decreased lipoprotein(a) levels from pretreatment values, whereas vitamin E alone significantly increased these levels (P=0.002 by ANOVA; Figure 2).

**TABLE 2. Effect of CE, Vitamin E, or Combined Therapy on Endothelial Function**

<table>
<thead>
<tr>
<th>Vasomotor function</th>
<th>CE</th>
<th>After Therapy</th>
<th>CE</th>
<th>After Therapy</th>
<th>Combined Therapy</th>
<th>Baseline</th>
<th>After Therapy</th>
<th>Baseline</th>
<th>After Therapy</th>
<th>Baseline</th>
<th>After Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum nitrogen oxides, μmol/L</td>
<td>58.2±28.8</td>
<td>56.7±29.8</td>
<td>55.2±27.5</td>
<td>50.9±28.6</td>
<td>50.9±18.9</td>
<td>52.8±23.5</td>
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<tr>
<td>Basal brachial artery diameter, mm</td>
<td>3.77±0.63</td>
<td>3.71±0.57</td>
<td>3.75±0.51</td>
<td>3.72±0.49</td>
<td>3.87±0.50</td>
<td>3.73±0.49*</td>
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</tr>
<tr>
<td>Hyperemia</td>
<td>3.92±0.62</td>
<td>4.04±0.66</td>
<td>3.92±0.53</td>
<td>4.04±0.53*</td>
<td>4.03±0.52</td>
<td>4.08±0.54</td>
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<tr>
<td>Basal-2</td>
<td>3.74±0.58</td>
<td>3.72±0.55</td>
<td>3.78±0.49</td>
<td>3.71±0.49</td>
<td>3.86±0.50</td>
<td>3.77±0.48</td>
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<tr>
<td>Nitroglycerin</td>
<td>4.30±0.62</td>
<td>4.25±0.59</td>
<td>4.34±0.54</td>
<td>4.25±0.54</td>
<td>4.40±0.56</td>
<td>4.34±0.54</td>
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<tr>
<td>Basal-1, mL/min</td>
<td>124±78</td>
<td>111±58</td>
<td>124±58</td>
<td>126±64</td>
<td>136±61</td>
<td>135±72</td>
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<tr>
<td>Hyperemia, mL/min</td>
<td>635±247</td>
<td>677±343</td>
<td>611±176</td>
<td>714±272†</td>
<td>678±241</td>
<td>675±279</td>
<td></td>
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<tr>
<td>Increase in flow, %</td>
<td>510±250</td>
<td>586±355</td>
<td>458±211</td>
<td>524±195</td>
<td>451±255</td>
<td>477±219</td>
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<tr>
<td>Basal-2, mL/min</td>
<td>110±55</td>
<td>103±51</td>
<td>119±51</td>
<td>104±45</td>
<td>136±99</td>
<td>125±77</td>
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<tr>
<td>Nitroglycerin, mL/min</td>
<td>144±89</td>
<td>147±83</td>
<td>145±53</td>
<td>137±60</td>
<td>156±66</td>
<td>155±71</td>
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</table>

Data are expressed as mean±SD.
*P<0.05, †P<0.01, and ‡P<0.001 vs respective baseline value.
would have excluded unknown values outside the 95% CIs as plausible values for differences in the flow-mediated dilator response on each therapy alone and the therapies combined at \( \alpha = 0.05 \). The brachial artery dilator response to nitroglycerin was similar for all therapies (\( P = 0.362 \) by ANOVA) and was not significantly changed from respective pretreatment measurements (Table 2 and Figure 3). The greatest treatment effect on serum nitrogen oxides was noted during treatment with vitamin E alone, during which a 10\% (\( P = 0.152 \)) reduction in levels relative to the pretreatment value was noted (Table 2). By ANOVA, there was a trend toward significance (\( P = 0.108 \)) regarding vitamin E effects on serum nitrogen oxide levels relative to the other treatment periods.

**Effects of Therapies on Markers of Inflammation and Fibrinolysis Inhibition**

CE alone or combined with vitamin E significantly decreased E-selectin levels from respective pretreatment values (Table 2), an effect not seen with vitamin E alone (\( P < 0.001 \) by ANOVA; Figure 4). CE alone significantly decreased VCAM-1 levels by 6\% (\( P = 0.008 \)) from pretreatment values, although this effect was not significantly greater than the nonsignificant 2\% reduction in levels on CE combined with vitamin E or the 3\% reduction in levels on vitamin E alone (\( P = 0.651 \) by ANOVA). CE alone significantly decreased ICAM-1 levels by 7\% (\( P = 0.015 \)) from pretreatment values, although this effect was not significantly greater than the marginally significant 7\% (\( P = 0.066 \)) reduction in levels on CE combined with vitamin E or the nonsignificant 3\% reduction in these levels on vitamin E alone (\( P = 0.630 \) by ANOVA).

Because the effects of therapies including CE were most robust for reduction in E-selectin levels, associations were determined between changes in these levels and changes in lipoprotein levels and in flow-mediated dilatation of the brachial artery. There was a weak but statistically significant correlation between the reduction in E-selectin levels and the reduction in LDL cholesterol levels on CE combined with vitamin E (\( r = 0.446, P = 0.017 \)) but not with CE alone (\( r = 0.009 \)). There were no associations between the reduction in lipoprotein(a) levels and the reduction in E-selectin levels on CE alone (\( r = -0.081 \)) or CE combined with vitamin E (\( r = -0.100 \)). There were no associations between the increase in HDL cholesterol levels and the reduction in E-selectin levels during CE alone (\( r = -0.219 \)) or CE combined with vitamin E (\( r = 0.161 \)). There were no associations between the improvement in flow-mediated dilatation or nitrogen oxide levels and changes in E-selectin levels on CE alone (\( r = -0.073 \) and \( r = -0.171 \), respectively) or CE combined with vitamin E (\( r = -0.018 \) and \( r = 0.219 \)).

CE alone or combined with vitamin E lowered plasma PAI-1 levels from pretreatment values (Table 2), in contrast to the minimal change in these levels during treatment with vitamin E alone (\( P = 0.069 \) by ANOVA).

**Discussion**

We reasoned that differing biological effects of estrogen and vitamin E therapies might have dissimilar vascular effects when administered to postmenopausal women. However, despite differences in lipoprotein effects of these therapies, we found that conventional-dose CE therapy and high-dose vitamin E therapy had similar effects on brachial artery
flow–mediated dilation, a bioassay for nitric oxide bioavailability, but with no additive effects when the therapies were combined. Slight but statistically nonsignificant increases in brachial artery blood flow were noted during hyperemia with all therapies relative to respective pretreatment values and may represent improvement in forearm microvascular endothelial responsiveness. The absence of an additive effect of therapies may be due to the robust improvement in flow-mediated dilation on each therapy alone. Thus, demonstration of an additive effect of estrogen and vitamin E may not be possible in healthy postmenopausal women.

The mechanism of enhanced nitric oxide bioavailability may differ between CE and vitamin E. Estrogen has been shown in endothelial cell culture studies to increase transcription and activity of nitric oxide synthase. Vitamin E, by protection of LDL from oxidation and scavenging of free radical molecules, may reduce the oxidative degradation of nitric oxide. We found a reduction in nitrogen oxide levels in serum during the vitamin E treatment period, with a trend toward significance in this effect relative to the other treatment schemes. With reduced degradation of nitric oxide, nitric oxide synthesis may be decreased because of feedback effects of increased cytosolic levels of nitric oxide on nitric oxide synthase. Although we failed to detect an additive effect of vitamin E and estrogen on flow-mediated dilation in our postmenopausal subjects, vitamin E combined with simvastatin was found to improve both flow-mediated and nitroglycerin-induced brachial artery dilation in 7 hypercholesterolemic men. Thus, vitamin E may be useful as an adjunctive therapy with lipid-lowering therapy or in patients with coronary artery disease in whom endothelial function may be more impaired than in healthy postmenopausal women.

To gain additional insight into the mechanisms of potential vasculoprotective effects of CE and vitamin E therapies, we measured markers of fibrinolysis inhibition and inflammation that, on the basis of clinical and experimental studies, are potentially affected by these therapies. We previously demonstrated that in postmenopausal women CE reduced PAI-1 levels with concomitant increases in levels of d-dimer, a product of cross-linked fibrin degradation by plasmin, thus providing evidence of enhanced fibrinolysis. In the present study, therapies including CE likewise reduced PAI-1 levels. However, vitamin E alone did not change PAI-1 levels, despite experimental evidence that oxidized LDL promotes the transcription and release of PAI-1 from endothelial cells in culture and our prior demonstration that this dose of vitamin E protects LDL from oxidation when administered to postmenopausal women.

Serum concentrations of E-selectin, ICAM-1, and VCAM-1 have been reported to be higher in postmenopausal women with coronary artery disease who are not on hormone therapy than postmenopausal women with coronary artery disease who are on hormone therapy at the time of cardiac catheterization. However, conflicting findings have been reported from cell culture studies regarding the effect of estrogen on cell adhesion molecule expression. Caulin-Glaser et al found 17βestradiol pretreatment for 48 hours to inhibit interleukin-1–induced expression of cell adhesion molecules in endothelial cell cultures, but Cid et al found estradiol to increase the expression of cell adhesion molecules on endothelial cells in culture during simultaneous stimulation by tumor necrosis factor-α, with increased adherence to mononuclear cells. We found that CE significantly reduced levels of the 3 cell adhesion molecules—E-selectin, ICAM-1, and VCAM-1—measured in our study relative to respective pretreatment values, with the greatest effect noted on E-selectin, the cell adhesion molecule specific to the activated endothelium. The pathophysiological relevance of E-selectin in humans has been suggested by their localization in atherosclerotic plaques, higher levels of E-selectin in patients with coronary artery disease or carotid artery atherosclerosis relative to control subjects, correlation of E-selectin levels with carotid artery wall thickness by ultrasound, and high levels of E-selectin in patients undergoing peripheral balloon angioplasty who developed restenosis.

To identify a mechanism for the CE treatment effects on cell adhesion molecules, we assessed correlations between changes in levels of E-selectin—the cell adhesion molecule in which reduction from pretreatment levels was most robust—and changes in LDL cholesterol, lipoprotein(a), and HDL cholesterol levels on the basis of experimental studies showing stimulatory [(LDL40 or lipoprotein(a)41] or inhibitory (HDL)42 effects of these lipoproteins on cell adhesion molecule expression. However, no significant or consistent (between the 2 treatment schemes that included CE) correlations were determined. Furthermore, vitamin E alone did not significantly change levels of cell adhesion molecules in our study participants, despite similar improvement in flow-mediated vasodilator responsiveness and thus nitric oxide bioavailability to therapies including CE. The absence of an effect of vitamin E on cell adhesion molecule levels in our study participants stands in contrast to experimental studies showing that nitric oxide donors, antioxidants, and vitamin E reduce the expression of cell adhesion molecules on cytokine–activated cells. However, our results are consistent with a recent study of male smokers with hypercholesterolemia that found no difference in levels of the soluble cell adhesion molecules E-selectin and VCAM-1 in subjects randomized to vitamin E 75 mg, vitamin C 150 mg, and β-carotene 15 mg for 6 weeks.

Thus, estrogen and vitamin E therapies provide similar (albeit not additive) improvement in endothelium-dependent vasodilator responsiveness consistent with enhanced nitric oxide bioavailability in healthy postmenopausal women, despite divergent effects of estrogen and vitamin E on levels of lipoproteins that potentially influence vascular function. Only therapies including estrogen, however, reduce the levels of atherogenic lipoproteins and markers of inflammation and fibrinolysis inhibition potentially important in the pathogenesis of atherosclerosis. Randomized clinical trials currently in progress may determine the appropriate role of estrogen and vitamin E therapies as primary prevention strategies against atherosclerotic cardiovascular disease in healthy postmenopausal women.

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