Oral Conjugated Equine Estrogen Increases Plasma von Willebrand Factor in Postmenopausal Women

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OBJECTIVES

We sought to test whether one month of daily oral conjugated equine estrogen (CEE) or transdermal estradiol alters hemostatic factors in postmenopausal subjects.

BACKGROUND

Estrogen replacement therapy and hormonal replacement therapy (HRT) effect an early increase in cardiovascular events in postmenopausal women. Circulating plasma von Willebrand factor (vWF) antigen is a marker of generalized endothelial dysfunction and atherothrombosis.

METHODS

Thirty-eight healthy postmenopausal women (average 59 ± 7 years) were randomized to receive daily oral CEE, 0.625 mg (n = 21); transdermal estradiol, 0.1 mg/day (n = 7); or oral placebo (n = 10) for one month. Blood samples were collected at baseline and after two weeks and four weeks of therapy for measurement of circulating plasma hormones, lipid concentrations, and hemostatic factors.

RESULTS

Oral CEE decreased total cholesterol (p < 0.01) and low-density lipoprotein cholesterol (p < 0.01), although it increased both triglycerides (p < 0.05) and high-density lipoprotein cholesterol (p < 0.01). Transdermal estradiol had no significant effect on lipids. Plasminogen activator inhibitor-1 antigen declined in both oral CEE and transdermal estradiol users, but did not achieve statistical significance. Fibrin D-dimer antigen did not vary significantly in any group. However, oral CEE users had a significant increase in vWF from baseline to four weeks (p < 0.03) and a decrease in tissue-type plasminogen activator antigen from baseline to four weeks (p < 0.004), which was significantly different from the change observed in the transdermal estradiol group (p < 0.05).

CONCLUSIONS

These data suggest that the oral CEE-mediated increase in plasma vWF may have clinical relevance given the early atherothrombotic effects of HRT in postmenopausal women. (J Am Coll Cardiol 2002;40:1991–9) © 2002 by the American College of Cardiology Foundation

Cardiovascular disease is the leading cause of mortality for women in the U.S. (1,2). The role of hormone replacement therapy (HRT) with estrogen and progesterone for primary and secondary prevention of coronary artery disease in women remains controversial. Observational studies have demonstrated that women who had used estrogen-replacement therapy (ERT) with or without progestins (i.e., HRT) during the menopausal years had a lower incidence of cardiovascular events, as compared with untreated women (3,4). Indeed, several potential mechanisms attributed to ERT or HRT had been considered to account for a beneficial effect. For example, oral exogenous estrogen increases the level of high-density lipoprotein (HDL) cholesterol and decreases the concentration of low-density lipoprotein (LDL). Moreover, several studies have suggested that ERT or HRT exerts a profibrinolytic effect by reducing plasminogen activator inhibitor-1 (PAI-1), the endogenous inhibitor of tissue-type plasminogen activator (t-PA) and urokinase plasminogen activator (5–15).

However, recent studies have cast doubt on the cardio-protective effects of HRT. The secondary prevention trial—the Heart and Estrogen/progestin Replacement Study (HERS)—examined 2,763 subjects with proven coronary artery disease and randomized them to conjugated equine-estrogen (CEE) with medroxyprogesterone acetate (MPA) or placebo (16). Over a four-year period, there was no difference in the number of secondary cardiovascular events and adverse events from peripheral vascular disease between the HRT-treated subjects and the placebo-treated subjects (16). The group receiving HRT experienced a 50% higher cardiac event rate during the first year (absolute excess of 1.4%) and a lower rate from the third year onward. The investigators considered that the results might be attributable to dual effects, such as an immediate prothrombotic, proarrhythmic, or proischemic effect that was later outweighed by slower progression of atherosclerosis. In further support of the lack of a benefit from HERS, the Estrogen Replacement and Atherosclerosis (ERA) trial demonstrated that three years of treatment with either CEE or CEE with MPA did not slow the rate of angiographic coronary artery disease progression, compared with placebo, in 309 postmenopausal women with established coronary artery disease (17).

The von Willebrand factor (vWF) has recently been proposed as a marker of generalized endothelial dysfunction, and as such, may contribute to the development of

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versus transdermal estradiol or placebo for one month. In
the concentrations of vWF, lipids, and other hemostatic
factors associated with endothelial dysfunction, such as vWF.
oral CEE in postmenopausal women might increase mark-
er all-cause mortality in both diabetic and nondiabetic patients
(26).
Hoorn study of 631 patients demonstrated that increased
vWF is independently associated with cardiovascular and all-cause mortality in both diabetic and nondiabetic patients
(26).
We hypothesized that the early effects of treatment with
oral CEE in postmenopausal women might increase mark-
ers associated with endothelial dysfunction, such as vWF.
Therefore, we conducted a short-term study and measured
the concentrations of vWF, lipids, and other hemostatic
factors in postmenopausal subjects receiving daily oral CEE
versus transdermal estradiol or placebo for one month. In
this study, we demonstrate that one-month treatment with
oral CEE, but not transdermal estradiol, significantly in-
creases vWF, thereby potentially increasing the likelihood of
cardiovascular events.

METHODS
The Institutional Review Board of the Columbia–
Presbyterian Medical Center approved the protocol, and
written, informed consent was obtained from each subject,
in accordance with institutional guidelines. Subjects who
were postmenopausal for at least one year participated.
Menopausal status was confirmed by measures of the
estradiol concentration (<50 pg/ml) and follicle-
stimulating hormone (>20 mU/ml), consistent with men-
opause. No subject was currently taking or had previously
taken ERT within the past year. Exclusion criteria included
a personal or immediate family history of breast or endo-
metrial cancer, previous myocardial infarction, coronary
artery disease, thrombophlebitis or a history of thromboem-
bolic disease, and a previous adverse reaction to estrogen.
Only subjects with a normal mammogram within the year
were enrolled. Subjects with an abnormal metabolic-renal
profile, including hyperglycemia and hypercalcemia, were
not enrolled.
Subjects underwent testing at baseline and after two and
four weeks of therapy. At enrollment, each had a history and
physical examination. Venous blood samples were collected
during each visit. At baseline and after four weeks, blood
was collected for a complete blood count, chemi-

meteor profile—including electrolytes, hepatic and renal
function, total cholesterol, LDL cholesterol, HDL chole-
sterol, triglycerides, estradiol, follicle-stimulating hormone,
progesterone, luteinizing hormone—and urinalysis, as well as
t-PA antigen, PAI-1 antigen, fibrin D-dimer, vWF
antigen, and fibrinogen. After two weeks, blood was also
collected for hormonal and hemostatic factors to assess
patient compliance.

Subjects were entered into a randomized, double-blind,
placebo-controlled trial using one of two forms of hormonal
replacement: oral CEE (Premarin), 0.625 mg/day, or trans-
dermal estrogen (Climara), 0.1 mg/day. Because concurrent
progestins given in conjunction with CEE may offset
estrogenic effects, we elected to treat subjects with estrogen
monotherapy. Subjects were randomized by means of a
random table to three groups in a ratio of 2:1:1, as to
whether they received oral CEE, transdermal estrogen, or
oral placebo, respectively. The rationale for the randomiza-
tion scheme, which favored oral administration, was the
consideration that the majority of women prescribed post-
menopausal ERT use CEE.

There were 55 subjects enrolled: 48 were randomized,
and 38 completed the trial. Three subjects randomized to
oral CEE did not complete the study because of stomach
upset (n = 1) or noncompliance (n = 2). Three subjects
randomized to transdermal estrogen did not complete the
study because of vaginal bleeding (n = 1), noncompliance
(n = 1), and elective withdrawal (n = 1). Four randomized
to placebo did not complete the study because of migraine
headache (n = 1), noncompliance (n = 1), and generalized
discomfort following medication (n = 1). Thirty-eight postmenopausal subjects completed
the study. The subjects (average age 59 ± 7 years) received
daily oral CEE, 0.625 mg (n = 21); transdermal estradiol,
0.1 mg/day (n = 7); or oral placebo (n = 10) for one month.

Plasma samples were collected before and after treatment
in the morning, at the same time of the day for each visit.
Blood samples were drawn at rest from a large antecubital
vein into 0.1 mol/liter trisodium citrate tubes for analysis of
t-PA antigen, PAI-1 antigen, fibrin D-dimer antigen, and
vWF. The tubes were immediately centrifuged and spun at
3,000 g for 20 min at 4°C. This centrifugation was per-
formed within 1 h. The plasma was removed into aliquots
and stored at -80°C for up to four months before analysis.
The assays for PAI-1, t-PA, and vWF were determined
using the respective enzyme-linked immunosorbent assay
(ELISA) kits purchased from American Diagnostics, Inc.
(Greenwich, Connecticut). The t-PA antigen measured by
ELISA was the total amount of t-PA present (free and
complexed t-PA, single-chain and double-chain t-PA) (27).
The PAI-1 antigen measured by ELISA was the total

}\n\textbf{Abbreviations and Acronyms}\n\begin{itemize}\n\item CEE = conjugated equine estrogen\n\item CRP = C-reactive protein\n\item ERT = estrogen replacement therapy\n\item HDL = high-density lipoprotein cholesterol\n\item HRT = hormone replacement therapy\n\item LDL = low-density lipoprotein cholesterol\n\item MPA = medroxyprogesterone acetate\n\item PAI-1 = plasminogen activator inhibitor-1\n\item t-PA = tissue-type plasminogen activator\n\item vWF = von Willebrand factor\n\end{itemize}
quantity of PAI-1 present (free or complexed with t-PA; active or inactive form) (27). Fibrin D-dimer was analyzed using the Asserachrom D-Di ELISA from Diagnostica Stago (Asnieres-Sur-Seine, France). Estradiol, progesterone, follicle-stimulating hormone, and luteinizing hormone concentrations were determined by their specific immunoassays from Diagnostic Products Corp. (Los Angeles, California), according to the manufacturer’s instructions. Interassay coefficient of variation for estradiol at high, middle, and low control concentrations are 3.5%, 4.6%, and 9.6%, respectively. Total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, and fibrinogen concentrations were determined by conventional enzymatic methods.

**Statistical analysis.** Data on baseline characteristics were analyzed by ANOVA, with post-hoc differences in mean values among groups assessed using Tukey’s procedure. Statistical significance was defined as a two-sided p value <0.05. The effect of estrogen within each group on hormones and lipid concentrations was determined using a two-tailed, paired Student t test. In addition, to correct for individual differences in baseline values among subjects, relative changes in the effect of estrogen treatment within each group on hormones, lipids, and hemostatic factors were assessed using a log-transform of the various factors.

**RESULTS**

Table 1 shows the baseline characteristics of the assigned subjects. There were no significant differences among the placebo, oral CEE, and transdermal estradiol groups at baseline.

Table 2 shows the effect of oral CEE and transdermal estradiol therapy on hormones and lipids after one month. Subjects assigned to oral CEE experienced a significant increase in estradiol (p < 0.01) and a decrease in follicle-stimulating hormone from baseline to four weeks (p < 0.01). Transdermal estradiol users experienced a nonsignificant increase in estradiol (p = NS) and a significant decrease in follicle-stimulating hormone at four weeks (p < 0.01). Neither transdermal estradiol nor placebo had a significant effect on lipids.

Table 3 and Figure 1 show the effect of oral CEE and transdermal estradiol on hemostatic factors; there was no effect of placebo on hemostatic factors. For the subjects randomized to oral CEE and transdermal estradiol, PAI-1 (ng/ml) declined, but it achieved only borderline significance (p < 0.08), and only in the oral CEE subjects. There was a significant decrease in t-PA antigen (p < 0.004) after oral CEE. Fibrin D-dimer did not vary significantly in any group. However, oral CEE users experienced a significant increase (p < 0.03) in vWF from baseline to four weeks, which was significantly different from the change observed in the transdermal estradiol group. Neither oral CEE nor transdermal estradiol had significant effects on fibrinogen.

The change observed for t-PA in the oral CEE group was significantly different from that observed in the transdermal group (p < 0.05) and that in the placebo group (p < 0.05). Subjects using placebo experienced no significant change in any hormone, lipid, or hemostatic factor values from baseline to two and four weeks.

**Table 1. Baseline Characteristics of Subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Oral CEE</th>
<th>Transdermal Estriol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>60 ± 4</td>
<td>58 ± 7</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>Time since menopause (yrs)</td>
<td>11 ± 6</td>
<td>8 ± 9</td>
<td>9 ± 8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70 ± 14</td>
<td>68 ± 11</td>
<td>70 ± 10</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28 ± 6</td>
<td>27 ± 5</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>15 ± 9</td>
<td>18 ± 5</td>
<td>16 ± 10</td>
</tr>
<tr>
<td>Follicle-stimulating hormone</td>
<td>63 ± 18</td>
<td>69 ± 20</td>
<td>63 ± 28</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Luteinizing hormone (mU/ml)</td>
<td>24 ± 12</td>
<td>24 ± 9</td>
<td>27 ± 17</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>221 ± 39</td>
<td>229 ± 32</td>
<td>218 ± 34</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>139 ± 54</td>
<td>121 ± 75</td>
<td>134 ± 46</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>53 ± 16</td>
<td>61 ± 15</td>
<td>43 ± 8</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>140 ± 31</td>
<td>144 ± 29</td>
<td>148 ± 32</td>
</tr>
</tbody>
</table>

Data are presented as the mean value ± SD.

CEE = conjugated equine estrogen; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

**Table 2. Effect of Oral CEE or Transdermal Estradiol on Hormones and Lipids After One Month**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Oral CEE</th>
<th>Transdermal Estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (pg/ml)</td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td></td>
<td>18 ± 5</td>
<td>76 ± 28</td>
</tr>
<tr>
<td>Follicle-stimulating hormone</td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td>(ng/ml)</td>
<td>69 ± 20</td>
<td>39 ± 13</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td></td>
<td>0.3 ± 0.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Luteinizing hormone (mU/ml)</td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td></td>
<td>24 ± 9</td>
<td>21 ± 11</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td></td>
<td>229 ± 32</td>
<td>209 ± 24</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td></td>
<td>121 ± 75</td>
<td>144 ± 75</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td></td>
<td>61 ± 15</td>
<td>65 ± 14</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td></td>
<td>144 ± 29</td>
<td>115 ± 27</td>
</tr>
</tbody>
</table>

Data are presented as the mean value ± SD.

NS = not significant; other abbreviations as in Table 1.
DISCUSSION

In this study, we report that oral CEE but not transdermal estradiol therapy for one month significantly increases the plasma concentration of vWF antigen in postmenopausal women. Our findings with CEE and vWF in postmenopausal women are intriguing when viewed in the context of several recent trials such as HERS and ERA, which have cast doubt on the cardioprotective effect of HRT in postmenopausal women (16,17). Although we found that treatment for one month with oral CEE decreased PAI-1, t-PA antigen, total cholesterol, and LDL cholesterol, it significantly increased vWF antigen. Oral CEE also increased HDL cholesterol and triglyceride concentrations. Oral CEE had no significant effect on fibrin d-dimer or fibrinogen. In contrast, one month of transdermal estradiol therapy or placebo had no effect on PAI-1, t-PA, fibrin d-dimer, vWF antigen, fibrinogen, or lipids. The observation that oral, but not transdermal, administration of estrogen alters several markers, including lipoproteins and PAI-1 levels, suggests that the hepatic effects of estrogen regulate their synthesis and/or clearance (5,10,15), accounting for the difference in response.

Elevated serum t-PA levels are present in cardiovascular conditions involving endothelial cell damage (28). Most of the t-PA in the plasma travels while bound to PAI-1 molecules to form circulating, inactive t-PA/PAI-1 complexes. The percent change after oral conjugated equine estrogen (CEE) or transdermal estrogen on plasminogen activator inhibitor-1 (PAI-1), tissue-type plasminogen activator (t-PA), and von Willebrand factor (vWF), compared with baseline. For oral CEE, the test result of the percent change (p value by analysis of variance [ANOVA]) was p = 0.08 for PAI-1, p < 0.004 for t-PA, and p = 0.03 for vWF. For transdermal estrogen, the test result of the percent change (p value by ANOVA) was not significant: p = 0.34 for PAI-1; p = 0.23 for t-PA; and p = 0.24 for vWF. Solid bars = PAI-1; hatched bars = t-PA; and open bars = vWF.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Treatment</th>
<th>After Treatment</th>
<th>% Change</th>
<th>p Value*</th>
<th>Before Treatment</th>
<th>After Treatment</th>
<th>% Change</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-1 (mg/ml)</td>
<td>42 ± 21</td>
<td>36 ± 19</td>
<td>−8.2</td>
<td>0.08</td>
<td>34 ± 10</td>
<td>30 ± 12</td>
<td>−9.2</td>
<td>0.34</td>
</tr>
<tr>
<td>t-PA (mg/ml)</td>
<td>11.2 ± 8.0</td>
<td>9.7 ± 8.1</td>
<td>−17.8</td>
<td>0.004</td>
<td>7.8 ± 4.0</td>
<td>9.0 ± 4.0</td>
<td>19.5</td>
<td>0.23</td>
</tr>
<tr>
<td>vWF (mU/ml)</td>
<td>835 ± 365</td>
<td>963 ± 372</td>
<td>22.7</td>
<td>0.03</td>
<td>1,131 ± 159</td>
<td>1,025 ± 238</td>
<td>−10.5</td>
<td>0.24</td>
</tr>
<tr>
<td>Fibrin d-dimer (mU/ml)</td>
<td>339 ± 141</td>
<td>467 ± 354</td>
<td>67.7</td>
<td>0.06</td>
<td>452 ± 116</td>
<td>446 ± 222</td>
<td>−4.7</td>
<td>0.72</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>354 ± 67</td>
<td>368 ± 65</td>
<td>6.9</td>
<td>0.33</td>
<td>363 ± 61</td>
<td>348 ± 45</td>
<td>−2.7</td>
<td>0.54</td>
</tr>
</tbody>
</table>

*Relates to the log difference reflecting the percent change. Data are presented as the mean value ± SD.

CEE = conjugated equine estrogen; PAI-1 = plasminogen activator inhibitor-1; t-PA = tissue-type plasminogen activator; vWF = von Willebrand factor.
plexes (29,30). Therefore, oral CEE-induced decreased t-PA and PAI-1 antigen levels may reflect diminished t-PA/PAI-1 complexes and, hence, augmented fibrinolytic activity.

Recent studies have suggested that increased circulating vWF antigen denotes a poor prognosis in unstable angina and non–ST-segment elevation myocardial infarction (31). Moreover, patients undergoing percutaneous coronary interventions who have unstable angina have higher vWF antigen levels than do patients with stable angina, and the decrease in vWF antigen after a successful percutaneous intervention may reflect plaque re-endothelialization and stabilization (32). Indeed, vWF facilitates platelet aggregation at sites of vascular injury under high shear stress, and elevated plasma vWF antigen concentrations correlate with enhanced platelet adhesion to collagen types I, III, and IV—all extremely potent physiologic inducers of platelet aggregation at the site of plaque rupture and vascular injury (33). Studies with vWF-deficient mice indicate that the absence of vWF predominantly affects aortic regions with disturbed flow that are prone to atherosclerosis (34). vWF may recruit platelets and leukocytes to the lesion in a flow-dependent fashion or may be part of the mechano-transduction pathway that regulates the endothelium’s response to shear stress (34).

Therefore, the oral CEE-mediated increase of vWF antigen at one month in our study may partially explain the results of HERS, which found an early increase in cardiovascular events after oral CEE and MPA. It should be noted that van Baal et al. (35) found a 14% decrease in vWF in 14 healthy postmenopausal subjects after three months of oral CEE. Cushman et al. (36) found no effect of four different estrogen/progestin hormone preparations on vWF antigen levels in 365 patients after 12 months. One study measured the effect of oral CEE and transdermal estrogen after one month; however, in that study, vWF was not measured (5). In a study by Lip et al. (37), 27 premenopausal women (average age 43.6 years) received CEE, 0.625 mg, following a hysterectomy and bilateral salpingo-oophorectomy. They found a decrease in plasma levels of both vWF and t-PA after six weeks of CEE (37). In contrast to the subjects described here, no subjects were naturally postmenopausal for at least one year; all had surgically induced menopause; all were considerably younger (as expected of a premenopausal population); and all had vasomotor symptoms. Furthermore, the investigators acknowledge that stress and blood loss, a consequence of surgery versus naturally occurring menopause, might have accounted for the findings.

The opportunity to evaluate the effects of CEE and transdermal estradiol after one month may provide insight into the time course of variables affected by estrogen. Although there are a considerable number of reports documenting estrogen’s effect on markers of fibrinolysis and hemostasis, the results are not uniform. Tables 4 and 5 summarize the existing, sometimes contradictory, findings of 20 studies (5,10–13,15,35,36,38–49). From this sum-
mary, we conclude that variability in the findings could be related to study design: estrogen formulations (e.g., CEE, estradiol valerate, micronized estradiol, 17-beta-estradiol), the size of dose, the method of estrogen replacement (e.g., cyclical, continuous), monotherapy or combination with progestin, the age of subjects, the time since the start of menopause, the duration of treatment, and the time of sampling. Although there are several reports relating the effect of ERT and HRT on PAI-1, few measured vWF. No other study measured vWF at one month, but more likely measured it at 3 to 4, 6, 12, and 36 months. Two studies collected samples as early as three months; however, one used micronized estradiol plus 1 or 2 mg dydrogestosterone (35) and found that vWF decreased, and the other used CEE plus 5 mg medrogestone (12) and found no effect on vWF.

Thus, it is possible that oral CEE exerts very early effects, increasing vWF antigen after one month, which may then dissipate over time with no effects found on vWF levels by three months to one year. We found that one month of transdermal estradiol had no effect on vWF antigen levels, a finding consistent with the results of a study of transdermal estradiol in 28 postmenopausal hysterectomized subjects after four months of therapy (42).

Our finding that oral, but not transdermal, estrogen increases plasma vWF antigen after one month of therapy is noteworthy in that vWF is not only a marker of endothelial dysfunction and enhanced atherothrombotic activity, but also is a marker of inflammation (36,41). In this regard, recent reports have discovered that HRT initially increases levels of C-reactive protein (CRP), an inflammatory marker that is a potent prognosticator of an increased risk of cardiovascular events in both women and men (36,37,42,50). Indeed, HRT appears to increase high-sensitivity CRP within four to eight months of the initiation of therapy. Therefore, oral CEE may cause an early increase in both inflammation and hemostasis, as evidenced by early elevations in both vWF and CRP, which result in an early increase in cardiovascular events. Furthermore, our findings that oral CEE, but not transdermal estradiol, induced the aforementioned effects is intriguing considering that the proinflammatory cytokine, interleukin-6, which is the primary stimulant of hepatic CRP (51), is also a potent inducer of vWF (52).

Oral CEE increased HDL cholesterol and decreased LDL cholesterol and total cholesterol, even in subjects who were overweight (Table 2), which corresponds to previous studies demonstrating that oral CEE has beneficial effects on the lipid profile (53,54). However, we also found that oral CEE concomitantly increases triglycerides, as evidenced by previous studies (53,54). In addition, it should be noted that, despite decreasing total cholesterol and LDL cholesterol, oral CEE actually decreases the LDL particle size, increases the very low density lipoprotein size, and only minimally reduces the concentration of apolipoprotein B (53,54). Furthermore, our study demonstrates that trans-
<table>
<thead>
<tr>
<th>Study (Ref.)</th>
<th>Design; Number of Subjects (n); and Age (years)</th>
<th>Time of Sampling</th>
<th>Oral Estrogen</th>
<th>Transdermal Estrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andersen et al. (38)</td>
<td>Active treatment and reference group; n = 60; age 40–60 years</td>
<td>Baseline and 3, 6, and 12 months</td>
<td>Estradiol valerate, 2 mg/day (cont or cyc) + CA, 1 mg/day</td>
<td>Not administered</td>
</tr>
<tr>
<td>Boschetti et al. (39)</td>
<td>Random allocation; n = 40</td>
<td>Baseline and 2, 4, and 12 months</td>
<td>CEE, 0.625 mg (3 one-week cycles) + MPA, 10 mg/day (third week)</td>
<td>Transdermal estradiol, 0.05 mg/day (3 one-week cycles) + MPA, 10 mg/day (third week)</td>
</tr>
<tr>
<td>Chen et al. (40)</td>
<td>n = 41</td>
<td>Baseline and 3, 6, and 12 months</td>
<td>Estradiol valerate, 2 mg/day (cont or cyc) + CA, 1 mg/day</td>
<td>Estradiol, 1.5 mg + MPA, 5 mg</td>
</tr>
<tr>
<td>Cushman et al. (36)</td>
<td>PEPI: randomized double-blind, placebo-controlled; n = 383; mean age 56 years</td>
<td>Baseline and 12 and 36 months</td>
<td>CEE, 0.625 mg; CEE + MPA, 10 mg/day (cyc); CEE + MPA, 2.5 mg/day (cont); CEE + MP, 200 mg/day (cyc)</td>
<td>Not administered</td>
</tr>
<tr>
<td>Cushman et al. (41)</td>
<td>Cardiovascular health study: population-based, longitudinal; n = 290; mean age 73 years</td>
<td>Annually</td>
<td>CEE (92%); CEE + MPA (cont or cyc)</td>
<td>Not administered</td>
</tr>
<tr>
<td>DeMitrio et al. (42)</td>
<td>Hysterectomized women; n = 28; age range 44–65 years (mean 47)</td>
<td>Baseline and 4 months</td>
<td>Not administered</td>
<td>17-beta-estradiol, 50 µg/24 h</td>
</tr>
<tr>
<td>Gilbert et al. (11)</td>
<td>n = 12 oral, n = 12 transdermal</td>
<td>Baseline and 3–4 and 12 months</td>
<td>Estradiol valerate, 2 mg/day + MPA, 2.5 mg/day</td>
<td>Estradiol, 0.5 mg + MPA, 2.5 mg/day</td>
</tr>
<tr>
<td>Gottsater et al. (43)</td>
<td>Part 1: double-blind, randomized trial for 3 months; part 2: open study for 9 months; postmenopausal women; n = 51; age range 53–54 years</td>
<td>Baseline and 3 and 12 months</td>
<td>Estradiol valerate, 2 mg + MPA, 10 mg for 10 days every 3 months</td>
<td>Not administered</td>
</tr>
<tr>
<td>Hoibraaten et al. (44)</td>
<td>Estrogen in women with atherosclerosis study: open randomized trial; n = 118; age &lt;71 years</td>
<td>Baseline and 3 and 12 months</td>
<td>Not administered</td>
<td>17-beta-estradiol, 50 µg/24 h + MPA, 5 mg (cyc)</td>
</tr>
<tr>
<td>Koh et al. (5)</td>
<td>Randomized, crossover; n = 50 (30 oral, 20 transdermal); mean age for oral 55 years, mean age for transdermal 56 years</td>
<td>Baseline and 1 month</td>
<td>CEE, 0.625 mg/day or CEE, 0.625 mg/day + MPA, 2.5 mg</td>
<td>Estradiol, 0.1 mg/day or estradiol, 0.1 mg/day + MPA, 2.5 mg</td>
</tr>
<tr>
<td>Kroon et al. (10)</td>
<td>Crossover; n = 23; age =65 years</td>
<td>Baseline and 6 weeks</td>
<td>CEE, 0.625 mg/day</td>
<td>17-beta-estradiol, 0.05 mg/day</td>
</tr>
<tr>
<td>Lindoff et al. (45)</td>
<td>Open, prospective trial with active treatment vs. control; n = 42 treated, n = 18 untreated</td>
<td>Baseline</td>
<td>CEE, 0.625 mg/day</td>
<td>17-beta-estradiol, 50 µg/24 h + MPA, 5 mg (cyc)</td>
</tr>
<tr>
<td>Lauer et al. (46)</td>
<td>Placebo-controlled; n = 26; age range 55–80 years</td>
<td>Baseline and 3 months</td>
<td>CEE, 0.625 mg/day</td>
<td>Not administered</td>
</tr>
<tr>
<td>Scarabin et al. (15)</td>
<td>Open, randomized trial with control group; n = 45; age 45–64 years</td>
<td>Baseline and 6 months</td>
<td>17-beta-estradiol, 2 mg/day + MP, 200 mg/day (cyc)</td>
<td>17-beta-estradiol gel, 2.5 mg/day + MP, 200 mg/day (cyc)</td>
</tr>
<tr>
<td>Seljeflot et al. (47)</td>
<td>Postmenopausal women with coronary artery disease; n = 98; age range 59–71 years</td>
<td>Baseline and 3 and 12 months</td>
<td>Not administered</td>
<td>17-beta-estradiol, 50 µg/day + sequential MPA, 5 mg for 14 days every third month</td>
</tr>
<tr>
<td>Shahar et al. (13)</td>
<td>ARIC study cohort with case control; n = 59; age 45–64 years</td>
<td>Baseline</td>
<td>Estrogen or estrogen-progestin combination; type not specified</td>
<td>Not specified</td>
</tr>
<tr>
<td>Teede et al. (48)</td>
<td>Double-blind, placebo-controlled; n = 42; age 50–75 years</td>
<td>Baseline and 6 weeks</td>
<td>Estradiol, 2 mg + continuous norethisterone acetate, 1 mg</td>
<td>Not administered</td>
</tr>
<tr>
<td>van Baal et al. (35)</td>
<td>Prospective, randomized, open; n = 27; mean age 52 years</td>
<td>Baseline and 3, 12, and 15 months</td>
<td>Micronized estradiol, 1 mg + dydrogesterone, 5–10 mg or micronized estradiol, 2 mg + dydrogesterone</td>
<td>Not administered</td>
</tr>
<tr>
<td>Vehkavaara et al. (49)</td>
<td>Randomized, placebo-controlled; n = 27; age ≥52 years; mean age 55 years</td>
<td>Baseline and 2 and 12 weeks</td>
<td>Estradiol, 2 mg</td>
<td>Estradiol, 50 µg/day</td>
</tr>
<tr>
<td>Winkler et al. (12)</td>
<td>n = 42</td>
<td>Baseline and 3 months</td>
<td>CEE, 0.6 mg + medrogestone, 5 mg (cyc)</td>
<td>17-beta-estradiol 50 µg/day</td>
</tr>
</tbody>
</table>

ARIC = Atherosclerosis Risk In Communities study; CA = cyproterone acetate, anti-androgen, progestational compound with androgen receptor blocking capacity; CEE = conjugated equine estrogen; cont = continuous; cyc = cyclical; MP = micronized progesterone; MPA = medroxyprogesterone acetate; PEPI = Postmenopausal Estrogen/Progestin Interventions study.
dermal estradiol has no significant effect on lipids. We found a positive correlation between the oral CEE-induced increase in vWF and HDL cholesterol. The mechanism underlying this interesting observation merits further investigation.

**Study limitations.** Of the 48 subjects randomized, 38 (79%) completed the trial and 10 (21%) discontinued treatment because of adverse events or because they were deemed no longer eligible or noncompliant. In subjects taking ERT for the first time, continuation of ERT is sporadic, and the current subjects parallel this experience. In the Massachusetts Women's Health Survey, among patients taking ERT for the first time, 20% stopped taking the drug within nine months and 10% used it on an intermittent basis (55). In a survey from The North American Menopause Society, among 833 women, 8% who had used ERT or HRT in the past stopped taking it, and among 58% who had never used it, 14% never tried it, even though it was prescribed (56). A second limitation is that the number of subjects assigned to transdermal estradiol and placebo is relatively small. The rationale for the randomization scheme, which favored oral administration, was the consideration that the majority of postmenopausal women treated with hormones use CEE, and furthermore, oral CEE was used in the HERS and ERA study, as well as the Women's Health Initiative. Thus, the findings reported here may provide insight into the mechanisms of benefit and risk, as well as the time course of markers, following administration of CEE in the postmenopausal years.

**Conclusions.** One month of oral CEE, but not transdermal estradiol, significantly increases the plasma levels of vWF antigen. Taken together, these data suggest that oral CEE may have very early deleterious effects promoting atherothrombosis by increasing a hemostatic factor, which encompasses the interaction of endothelial dysfunction, platelet aggregation, and inflammation.

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**REFERENCES**


