Differential Effects of Oral and Transdermal Estrogen/Progesterone Regimens on Sensitivity to Activated Protein C Among Postmenopausal Women

A Randomized Trial

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Objective—Activated protein C (APC) resistance not related to the factor V Leiden mutation is a risk factor for venous thrombosis. Oral estrogen replacement therapy (ERT) has been reported to induce APC resistance. Little is known about the effect of transdermal estrogen.

Methods and Results—We enrolled 196 postmenopausal women who were randomly allocated to receive either 1 mg $17\beta$-estradiol orally (n = 63) or 50 $\mu$g $17\beta$-estradiol transdermally per day (n = 68), both associated with 100 mg progesterone daily or placebo (n = 65) for 6 months. An activated partial thromboplastin time (APTT)–based test and the effect of APC on thrombin potential (ETP) were used. Oral ERT induced an ETP-based APC resistance compared with both placebo ($P < 0.006$) and transdermal ERT ($P < 0.001$), but there was no significant effect of transdermal ERT compared with placebo ($P = 0.191$). There was no significant effect of ERT on the APTT-based APC sensitivity ratio. Prothrombin fragment 1+2 plasma levels were significantly higher after 6 months of treatment in women allocated to oral ERT compared with those on placebo and transdermal ERT and were positively and significantly correlated with changes in ETP-based APC sensitivity ratio.

Conclusions—Our data show that oral, unlike transdermal, estrogen induces APC resistance and activates blood coagulation. These results emphasize the importance of the route of estrogen administration. (Arterioscler Thromb Vasc Biol. 2003;23:lll-lll.)

Key Words: hormone replacement therapy ■ APC resistance ■ blood coagulation ■ randomized trial ■ factor V

Several observational studies$^1$–$^7$ found that oral estrogen replacement therapy (ERT) was associated with a 2-fold increased risk of venous thromboembolism (VTE). This finding was confirmed in 2 randomized clinical trials.$^8,^9$ Furthermore, consistent data provided evidence that oral ERT resulted in coagulation activation.$^{10-14}$ However, studies investigating the effect of transdermal estrogen on the thrombotic process are scarce.$^{15,15}$

Activated protein C (APC) resistance has recently emerged as a risk factor for venous thrombosis.$^{16,17}$ In most cases, this defect is related to the presence of the R506Q mutation in factor V (FV) Leiden.$^{18}$ However, an APC-resistant phenotype detected in the absence of FV Leiden is also an independent risk factor for venous thrombosis.$^{19}$ Observational studies$^{20-22}$ and 1 randomized trial$^{23}$ showed that oral ERT could induce an acquired APC resistance. Little is known about the effect of transdermal ERT on APC resistance. Therefore, we conducted a randomized, placebo-controlled trial that investigated the effect of both oral and transdermal estrogen/progesterone regimens on the anticoagulant response to APC and on coagulation activation.

Methods

Study Design and Setting

This study was a randomized, double-blind, placebo-controlled, parallel-group trial that took place at the Hôpital de la Cavale Blanche, Brest, France, between September 1999 and August 2001. Participants were followed up by regular visits, at randomization (baseline), and after 6 months. A medical review with use of a standardized questionnaire was conducted at baseline, and a medical examination was performed at each visit.

Subjects

Altogether, 196 postmenopausal women younger than 70 years were randomized. Postmenopausal was defined as follows: no natural...
menstruation for at least 6 months, no progesterone-induced men-
struation over 3 cycles (10 days on treatment followed by 18 days
off), or bilateral ovariectomy or hysterectomy with concentrations of
folic acid-stimulating hormone >40 IU/mL and estradiol <20 pg/mL.
A normal venous thrombosis result was also required. Exclusion criteria
were the following: gynecological cancer, cardiovascular disease
(heart valve disease, coronary heart disease, stroke, atrial fibrillation,
or uncontrolled arterial hypertension), previous venous thrombosis,
and liver insufficiency. Women who had used hormone replacement
therapy (HRT) or oral contraceptives in the previous 6 months
were excluded, as were women on antithrombotic treatment. Women
were recruited through gynecologist and endocrinologist practitioners
(list in Appendix) in the Brest area. The protocol was approved by the
local ethics committee. Written, informed consent was obtained from
all women. The study was carried out according to the Helsinki
Declaration and Good Clinical Practice.

Interventions
The participants were allocated to 1 of the following 3 groups: (1) 1
mg/d of oral 17β-estradiol, (2) 50 µg/d transdermal 17β-estradiol,
both combined with 100 mg/d oral, micronized progesterone on a
continuous basis, or (3) triple-dummy, equal-looking placebo. The
allocation schedule was computer generated with a random block size-stratified according to the recruitment procedure (endocrinolo-
gists or gynecologists). The Cavale Blanche Hospital Pharmacy
Department had the randomization list and received from the
manufacturers blister packs containing pills, capsules, and patches of
either active drugs or placebo, identical in appearance. The blister
packs were supplied in sequentially numbered, tamper-proof, and
similar-looking containers. The Pharmacy Department distributed
the containers and retained the trial codes, which were disclosed after
the study. The participants, investigators, and outcome assessors
remained unaware of the intervention assignment throughout the trial.
The women were requested to take tablets at bedtime, and a
continuous regimen was chosen to avoid regular bleeding in the 2
active treatment groups; thus, strong hints were minimized.

Blood Collection
Blood samples were drawn between 8 and 10 AM after an overnight
fast and a 10-minute rest. With regard to coagulation measurements,
venous blood (9 volumes) was collected in 5-mL evacuated tubes
(Vacutainer, Becton-Dickinson) containing 0.11 mol/L trisodium citrate
(1 volume). Platelet-poor plasma was obtained by 2 centrif-
ugation steps at 2500

 g for 15 minutes at 37°C followed by 15 minutes at 0°C, after which the clot
was defibrinated by incubation with reptilase (final concentration, 0.2 UB/mL; Diagnostica Stago) for 30 minutes at 37°C followed by 15 minutes at 0°C, after which the clot
was removed with a plastic spatula. Defibrinated plasma was used
within 3 hours after defibrination. Phospholipid vesicles (containing
dioleoyl-sn-glycero-3-phosphoserine, dioleoyl-sn-glycero-3-
phosphoethanolamine, and dioleoyl-sn-glycero-3-phosphocholine;
Avanti Polar Lipids; molar ratio of 20:20:60) were prepared as
described.25 The tissue factor factor in the reagent (Innovin,
Dade Behring) was quantified by using a tissue factor ELISA kit
(Imubind, American Diagnostica). Thrombin generation was started
at 37°C in plastic microtubes by adding 10 µL of a mixture containing the tissue factor, CaCl,
and phospholipid vesicles with or without APC (Hyphen) in 25 mmol/L HEPES, 175 mmol/L NaCl,
and 5 mg/mL bovine serum albumin (RelA7030, Sigma), pH 7.7, to
20 µL defibrinated plasma. This resulted in final concentrations of
0.43 ng/mL tissue factor, 16 mmol/L CaCl,
15.2 µmol/L phospho-
lipid, and, when present, 40 nmol/L APC (the APC concentration
used was selected to give a residual activity of ~10% in normal
plasma). After a 20-minute incubation, 100 µL of ice-cold, 50 mmol/L Tris buffer, pH 7.9, containing 175 mmol/L NaCl,
20 mmol EDTA, and 0.5 g/L bovine serum albumin was added. Next, 25 µL of the mixture was added to 100 µL of the same buffer
in a microtiter plate. After a 5-minute incubation at 37°C, 50 µL of
prewarmed 1.2 mmol/L S2238 (Biogenics) was added. The rate of change in absorbance at 405 nm (αc-macroglobulin–thrombin
amidolytic activity, αc-M-IIa) was determined at 37°C in a microtiter
plate reader (Dynatech MR5000, Dynex Technologies). Forty sub-
ject plasmas together with 3 samples of pooled, normal plasma were
tested in each series. The APC sensitivity ratio (APCsr) in each subject’s plasma was defined as the ratio of αc-M-IIa determined in
the presence and absence of APC. Each subject’s plasma was tested in
2 independent series. We used the mean APCsr unless they differed by >0.2. In this case, a third determination was performed.
The normalized APC sensitivity ratio (nAPCsr) was defined as the ratio of APCsr obtained for the subject’s plasma to the APCsr
obtained for the normal plasma pool. The plasma pool was collected from 40 healthy subjects (medical doctors and nurses) in the same
way as for postmenopausal women. Prothrombin fragment 1 + 2 was
assayed with a micro kit (Enzygnost F1+2, Dade Behring). The free
(FPS) and total (TPS) protein S antigen levels and the free (FTFP)
and total (TTFP) tissue factor pathway inhibitor levels were also
calculated as the change in APCsr from baseline by ANOVA.

Laboratory Investigations
At the time of assay, plasma samples were transferred to a 37°C
water bath for 5 minutes and then handled at room temperature.
Baseline and 6-month samples from the same subject were analyzed
within 3 hours after defibrination. Phospholipid vesicles (containing
the tissue factor, CaCl,
and total (TTFPI) tissue factor pathway inhibitor levels and the free (FTFP)
and total (TTFP) tissue factor pathway inhibitor levels were also measured with a commercially available kit (Asserachrom kits from
Diagnostica Stago).

Other Laboratory Measurements
High-molecular-weight DNA was isolated from lymphocytes by
phenol–chloroform extraction. Genotyping for the FV Leiden muta-
tion was performed as previously described.26 Serum estradiol was
quantified with an enzyme-linked fluorescent assay (VIDAS Esta-
diol II, BioMérieux). Serum folic acid-stimulating hormone was deter-
mined with a 1-step immunometric chemoluminescent assay on an
automated system (VITROS Eci, Ortho-Clinical Diagnostics).

Sample Size Estimation
With a 5% 2-sided α level, 60 subjects per group showed a
difference between groups of about two thirds SD for a normally
distributed variable with 95% statistical power.

Statistical Analysis
Data are presented as mean and SD. Treatment effects were
calculated as the change in APCsr from baseline by ANOVA.
Pairwise differences were assessed with a post hoc ANOVA with
Bonferroni adjustment. Variables with skewed distributions were
logarithmically transformed. Owing to the mechanistic study objec-
tives, a per-protocol analysis approach and an intention-to-treat analysis was performed. A 2-sided value of P <0.05 was considered statistically significant. The Spearman coefficient correlation was used to detect
any association between changes in APCsr and changes in hemo-
static variables. All statistical analyses were performed with the
SPSS 10.0 statistical software package (SPSS for Windows, SPSS Inc).

Results
Figure 1 shows the trial profile. Altogether, 196 women were
enrolled between September 7, 1999, and August 20, 2001: 63
were allocated to receive oral estradiol plus oral proges-
terone, 68 to receive transdermal estradiol plus oral proges-
terone, and 65 to receive placebo. Thirty-six women (18.4%) discontinue their treatment because of vaginal bleeding or
mastodynia (5 women in the oral group, 2 women in the transdermal group), hot flushes (3 women in the placebo group), or miscellaneous reasons (skin problem caused by an allergy, edema, headache, or nonreported reasons). Among these 36 women, 29 (14.8%) were lost to follow-up at 6 months and 7 attended the final visit. Overall, 167 women attended the 6-month visit, and 160 women completed the trial with effective intervention.

Table 1 shows the baseline characteristics of participants by treatment group. Women ranged in age from 43 to 69 years, with a mean age of 53.2 years at baseline. There was no significant imbalance between the groups with regard to age and cardiovascular risk factors (hypertension, diabetes, lipid profile, tobacco use, and body mass index). Seventeen women (8.7%) carried the FV Leiden mutation. Table 2 shows the changes in sex hormone levels by treatment group. Both active treatments significantly increased plasma estradiol levels and decreased follicle-stimulating hormone levels. Levels of both sex hormones remained unchanged in the placebo group.

According to the per-protocol analysis scheme, we analyzed only the 160 women who completed the trial and actually took the study drug. Table 3 shows values of hemostatic variables at baseline and after 6 months of follow-up, as well as mean changes by treatment group. Oral ERT significantly increased the ETP-based nAPCsr, ie, induced an APC resistance, compared with both placebo ($P=0.006$) and transdermal estrogen ($P<0.001$). Transdermal ERT had no significant effect on the ETP-based nAPCsr compared with placebo ($P=0.191$). ERT, either oral or transdermal, had no significant effect on the APTT-based APCsr. Similar results were found when the analysis was restricted to FV Leiden noncarriers (data not shown). The oral, unlike transdermal, estrogen/progesterone regimen significantly increased prothrombin fragment $1_2$ plasma levels compared with placebo. The change in prothrombin fragment $1_2$ level was correlated with the change in the ETP-based nAPCsr in FV Leiden noncarriers, in the whole population (Spearman coefficient of correlation, 0.264, $P=0.001$), and in the women allocated to oral estrogen (Spearman coefficient of correlation, 0.264, $P=0.001$).

### Table 1. Baseline Characteristics of Participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Oral Estradiol+ Progesterone</th>
<th>Estradiol Patch+ Progesterone</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>$53\pm4$</td>
<td>$52\pm4$</td>
<td>$54\pm4$</td>
</tr>
<tr>
<td>Duration of amenorrhea, months</td>
<td>$37\pm59$</td>
<td>$30\pm36$</td>
<td>$40\pm55$</td>
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<tr>
<td>Known and treated hypertension, n (%)*</td>
<td>7 (11.1)</td>
<td>10 (14.7)</td>
<td>12 (18.5)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg†</td>
<td>$124\pm14$</td>
<td>$124\pm19$</td>
<td>$125\pm19$</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg†</td>
<td>$72\pm11$</td>
<td>$74\pm11$</td>
<td>$73\pm9$</td>
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<tr>
<td>Diabetes, n (%)‡</td>
<td>2 (3.2)</td>
<td>4 (5.9)</td>
<td>8 (12.3)</td>
</tr>
<tr>
<td>Abnormal lipid profile, n (%)§</td>
<td>6 (9.5)</td>
<td>5 (7.3)</td>
<td>8 (12.3)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>8 (12.7)</td>
<td>13 (19.1)</td>
<td>14 (21.6)</td>
</tr>
<tr>
<td>Ever smoker, n (%)</td>
<td>10 (15.9)</td>
<td>17 (25.0)</td>
<td>9 (13.8)</td>
</tr>
<tr>
<td>Never smoker, n (%)</td>
<td>45 (71.4)</td>
<td>38 (55.9)</td>
<td>42 (64.6)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>$24.4\pm3.5$</td>
<td>$24.9\pm4.3$</td>
<td>$24.8\pm4.6$</td>
</tr>
<tr>
<td>Factor V Leiden carriers, n (%)</td>
<td>7 (11.1)</td>
<td>6 (8.8)</td>
<td>4 (6.2)</td>
</tr>
</tbody>
</table>

*Values are mean±SD or number (percentage).

*Hypertension includes women having a systolic blood pressure $\geq140$ mm Hg or a diastolic blood pressure $\geq90$ mm Hg at baseline and at 3 months, or women who were receiving antihypertensive drugs at baseline.

†BP women not taking antihypertensive drugs.

‡Diabetes includes women having a baseline plasma glucose level $\geq126$ mg/dL or women who were taking insulin or receiving oral treatment for diabetes.

§Abnormal lipid profile includes women having a baseline LDL cholesterol level $\geq130$ mg/dL or a triglycerides level $\geq200$ mg/dL or women who were receiving lipid-lowering drugs.
TABLE 2. Plasma Estradiol (E2) and FSH at Baseline and After 6 Months by Treatment Groups (Oral 17β-Estradiol [E2] 1 mg or Transdermal 17β-Estradiol [E2] 50 μg, Both Combined With Micronized Progesterone (P) 100 mg/d Continuously)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment</th>
<th>No.</th>
<th>Baseline</th>
<th>After 6 Months</th>
<th>Difference</th>
<th>P†</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>54</td>
<td>63±24</td>
<td>64±27</td>
<td>2±18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E2 oral + P</td>
<td>55</td>
<td>62±29</td>
<td>48±23</td>
<td>−31±110</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E2 patch + P</td>
<td>58</td>
<td>55±29</td>
<td>43±23</td>
<td>−15±24</td>
<td>0.001</td>
<td></td>
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<tr>
<td></td>
<td>Placebo</td>
<td>54</td>
<td>42±98</td>
<td>43±47</td>
<td>−3±83</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E2 oral + P</td>
<td>55</td>
<td>51±74</td>
<td>193±133</td>
<td>140±131</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E2 patch + P</td>
<td>58</td>
<td>47±41</td>
<td>70±63</td>
<td>25±68</td>
<td>0.008</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD.
*Analysis of variance.
†Comparison between active treatment and placebo.
‡Comparison between the two active treatments.

Statistical tests were performed after logarithmic transformation of the variables.

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of correlation, 0.368, P=0.011). The oral ERT significantly decreased FTPI levels compared with both placebo (P=0.001) and transdermal ERT (P=0.001). Transdermal ERT had no significant effect. Both oral and transdermal ERT significantly decreased TPS levels compared with placebo. Oral and transdermal ERT had no significant effect on TTTP and FSP levels. Changes in the ETP-based nAPCsr were not significantly correlated with changes in either FTPI or TPS levels in FV Leiden noncarriers allocated to oral ERT. The intention-to-treat analysis scheme showed similar results.

Discussion

The main finding of this randomized trial is that oral, unlike transdermal, ERT (1 mg orally or 50 μg transdermally 17β-estradiol, both plus 100 mg oral, micronized progesterone, given on a continuous basis over 6 months) significantly alters the effect of APC on thrombin generation (ETP-based assay), leading to an acquired APC resistance. Furthermore, the oral estrogen/progesterone treatment was associated with significantly higher plasma prothrombin fragment 1 + 2 levels, a marker for coagulation activity, than placebo or transdermal treatment, with changes in prothrombin fragment 1 + 2 levels being correlated with changes in the ETP-based nAPCsr.

Four randomized, placebo-controlled trials previously investigated the effect of ERT on APC resistance. All of these trials studied oral ERT23,27,28,30 but used different methods to measure APC resistance: the original APTT-based assay16 in the first 2 trials, a thrombin generation assay29 in the third trial,23 and the Staclot APC-R test in the last 1.30 In the first 2 trials,27,28 oral ERT had no significant effect on the APTT-based APC resistance at 3 months. Our data are consistent with these negative results. A lack of sensitivity of the APTT-based assay to detect ERT-induced modifications could explain these negative findings.24 In the EVTET trial,23 oral ERT significantly increased the ETP-based nAPCsr at 6 months. Our results confirm this finding. However, the size of the effect was smaller in our study. Several differences could explain this discrepancy. First, we evaluated a lower dose of estrogen (1 mg vs 2 mg) combined with micronized progesterone instead of norethisterone. Second, we enrolled healthy women. Finally, in our trial, ERT was initiated earlier in the course of menopause, and treatment effects might have been attenuated. In the fourth trial, which used the Staclot APC-R test, Demir et al30 reported that 0.625 mg conjugated equine estrogen plus 5 mg medroxyprogesterone acetate over 6 months resulted in an acquired APC resistance.

The mechanistic basis of APC resistance induced by oral treatment is not clear. It has been reported that changes in FSP23,31 and TFPI23 might have important roles in determining the sensitivity of plasma to APC. Our results do not confirm these previous findings. However, in agreement with several reports, we confirm that oral ERT significantly decreases TPS but does not change the FSP levels.32,33

The safety of HRT with regard to VTE is an important issue. Early studies of VTE risk among ERT users provided inconclusive results.2 More recently, observational studies showed consistent associations between current use of ERT and risk of VTE in postmenopausal women.1–7 These results have been clearly confirmed by randomized clinical trials.8,9,34 However, most of those studies investigated women who were using conjugated equine estrogens alone or combined with progestin. These results do not apply to users of transdermal ERT. Clinical data evaluating the influence of the route of estrogen administration are scarce. Two case-control studies reported no difference in VTE risk between users of oral and transdermal ERT.3,5 However, those results were based on 5 and 7 cases of VTE exposed to transdermal estrogen, and confidence intervals were wide. Therefore, data on the effect of transdermal estrogen on VTE risk remained inconclusive.

Biologic evidence supports a differential effect of oral versus transdermal estrogen on hemostasis. Randomized trials have shown that oral ERT increases plasma levels of prothrombin fragment F1 + 2,11,35 which is a marker for in vivo thrombin generation and which was recently related to the risk of recurrent VTE.9 Consistent data reported that transdermal ERT had no detrimental effect on coagulation,11,32,35 especially prothrombin fragment 1 + 2 plasma level, and our findings are in accordance with these results. Thus, oral ERT might impair the balance between procoagu-
TABLE 3. Comparison of Treatment Effects on Hemostatic Variables in a Per Protocol Analysis Involving the 160 Women Who Completed the Trial

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment</th>
<th>No.</th>
<th>Baseline</th>
<th>After 6 Months</th>
<th>Difference</th>
<th>P†</th>
<th>P‡</th>
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<tr>
<td>nAPCsr, ETP-based</td>
<td>Placebo</td>
<td>52</td>
<td>1.66±1.24</td>
<td>1.54±1.24</td>
<td>−0.12±0.72</td>
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<tr>
<td></td>
<td>E2 oral+P</td>
<td>53</td>
<td>1.61±1.35</td>
<td>1.89±1.63</td>
<td>0.27±0.87</td>
<td>0.006</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>E2 patch+P</td>
<td>55</td>
<td>1.67±1.34</td>
<td>1.27±1.02</td>
<td>−0.39±0.89</td>
<td>0.191</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P*</td>
<td></td>
<td>0.972</td>
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<td>APCsr, APTT-based</td>
<td>Placebo</td>
<td>52</td>
<td>2.28±0.26</td>
<td>2.31±0.27</td>
<td>0.025±0.177</td>
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<tr>
<td></td>
<td>E2 oral+P</td>
<td>53</td>
<td>2.27±0.24</td>
<td>2.25±0.24</td>
<td>−0.025±0.159</td>
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<td>E2 patch+P</td>
<td>55</td>
<td>2.32±0.25</td>
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<td>0.024±0.143</td>
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<td></td>
<td>P*</td>
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<td>0.582</td>
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<td>F1+2, nmol/L</td>
<td>Placebo</td>
<td>52</td>
<td>0.95±0.43</td>
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<td>0.82±0.19</td>
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<td>0.92±0.48</td>
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<td>P*</td>
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<td>Free TFPI, ng/mL</td>
<td>Placebo</td>
<td>52</td>
<td>13.38±3.09</td>
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<td>0.38±2.95</td>
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<td>E2 oral+P</td>
<td>53</td>
<td>13.27±3.65</td>
<td>10.46±2.79</td>
<td>−2.81±2.79</td>
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<td>E2 patch+P</td>
<td>55</td>
<td>13.12±3.37</td>
<td>12.52±3.06</td>
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<tr>
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<td>P*</td>
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<td>0.926</td>
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<td>Total TFPI, ng/mL</td>
<td>Placebo</td>
<td>52</td>
<td>70.32±14.31</td>
<td>65.34±17.83</td>
<td>−4.96±10.65</td>
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<td></td>
<td>E2 oral+P</td>
<td>53</td>
<td>74.85±20.14</td>
<td>68.92±18.60</td>
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<td>E2 patch+P</td>
<td>55</td>
<td>77.40±18.39</td>
<td>71.03±19.26</td>
<td>−6.38±11.10</td>
<td></td>
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<tr>
<td></td>
<td>P*</td>
<td></td>
<td>0.287</td>
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<tr>
<td>Free protein S, UI/L</td>
<td>Placebo</td>
<td>52</td>
<td>101.96±18.41</td>
<td>99.61±16.88</td>
<td>−2.34±13.32</td>
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<tr>
<td></td>
<td>E2 oral+P</td>
<td>53</td>
<td>101.30±16.63</td>
<td>96.67±14.65</td>
<td>−4.63±11.57</td>
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<td></td>
<td>E2 patch+P</td>
<td>55</td>
<td>103.17±20.39</td>
<td>96.36±17.24</td>
<td>−6.86±12.30</td>
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<tr>
<td></td>
<td>P*</td>
<td></td>
<td>0.863</td>
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<tr>
<td>Total protein S, UI/L</td>
<td>Placebo</td>
<td>52</td>
<td>121.43±20.27</td>
<td>121.75±17.50</td>
<td>0.32±17.86</td>
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<tr>
<td></td>
<td>E2 oral+P</td>
<td>53</td>
<td>117.11±18.50</td>
<td>106.38±16.00</td>
<td>−10.73±16.25</td>
<td>0.002</td>
<td>0.999</td>
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<tr>
<td></td>
<td>E2 patch+P</td>
<td>55</td>
<td>120.27±21.30</td>
<td>112.37±21.24</td>
<td>−7.90±14.49</td>
<td>0.031</td>
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<tr>
<td></td>
<td>P*</td>
<td></td>
<td>0.524</td>
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</table>

Values are mean±SD.
*Analysis of variance.
†Comparison between active treatment and placebo.
‡Comparison between the two active treatments.

Statistical tests were performed after logarithmic transformation of the variables, if necessary.

lant factors and antithrombotic mechanisms, whereas transdermal ERT appears to have little or no effect on hemostasis.

APC resistance has recently emerged as a risk factor for VTE.16,17 Although this phenotype is mostly related to the FV Leiden mutation,18 APC resistance detected in the absence of any thrombogenic mutation has also been shown to be an independent risk factor for VTE.17,19,36 Oral ERT alters APC resistance as measured by a thrombin generation assay; moreover, our data exhibited a correlation between prothrombin fragment 1+2 level and the ETP-based APCs. Taken together, these data provide a plausible biologic mechanism to the clearly demonstrated association between oral estrogen and venous thrombosis. In addition, our data provide further evidence that transdermal ERT has little or no effect on APC resistance. This result extends and strongly supports findings from observational21 and nonrandomized37 studies. There is now a strong body of biologic evidence that suggests a lower risk of VTE, if any, among users of transdermal ERT. However, these trials investigated the effect of transdermal ERT on biologic markers, and none used clinical end points. Whether transdermal ERT is a safe option for the relief of severe climacteric symptoms needs further investigation that our study might well stimulate.

In summary, our data show that oral, unlike transdermal, ERT induces an acquired APC resistance and activates blood coagulation. These results underline the potential importance of the route of estrogen administration in prescribing HRT.

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**References**


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for the SARAH Investigators

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