Comparison of the effects of tibolone and estrogen therapy on hemostasis in surgical menopause: a randomized, double-blind, placebo-controlled study

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Objective: To examine the effects of unopposed estrogen (E) and tibolone therapy on coagulation and natural anticoagulant systems in surgical menopause.

Design: A randomized, double-blind, placebo-controlled study.

Setting: University hospital clinic in Turkey.

Patient(s): Ninety healthy surgically postmenopausal women.

Intervention(s): Ninety surgically postmenopausal women were randomized into three groups: unopposed conjugated ET (0.625 mg/d, group 1), tibolone (2.5 mg/d, group 2), and identical tablets of placebo (group 3).

Main Outcome Measure(s): Effects on parameters in the clotting cascade at baseline and after 24 weeks of treatment.

Result(s): After 6 months, fibrinogen, lipoprotein (a), and factor VIIa were decreased, and activated partial thromboplastin time was increased significantly in the ET group compared with in the placebo group. However, tibolone significantly decreased only the serum levels of factor VIIa and factor IX and prolonged the activated partial thromboplastin time, compared with placebo group. In addition, conjugated ET caused a significantly greater decrease in serum fibrinogen level than did tibolone.

Conclusion(s): Neither E nor tibolone therapy led to activation of coagulation in the surgically menopausal women. Both preparations changed the overall hemostatic balance to a more fibrinolytic state. (Fertil Steril 2007;87:842–8. ©2007 by American Society for Reproductive Medicine.)

Key Words: Tibolone, estradiol, surgical menopause, hemostasis, coagulation, fibrinolysis.

Cardiovascular disease is the leading cause of death in women (1). The incidence of myocardial infarction in women increases dramatically after the menopause. The increase is at least in part a result of increasing age, but the role of the menopause itself is not so clear (2).

The evaluation of hemostatic differentiation is important during this time period. Many factors contribute to the formation of a thrombus. Mainly, it may result from an overload of local coagulatory activity. Hormone therapy (HT), estrogen (E) therapy (ET), and compounds with tissue-specific hormonal activity, which have been used to relieve vasomotor symptoms of the menopause and to prevent structural abnormalities, affect both coagulation and fibrinolytic activity (3).

Until recently, only a few epidemiological studies have observed no increased risk of venous thromboembolism (VTE) from E-containing preparations (4–8). However, recent findings from observational (9–12) and randomized controlled studies (13, 14) have indicated a slightly increased risk of VTE in HT users. There is still controversy about the effect of unopposed E on venous and arterial thrombosis because the Women’s Health Initiative unopposed E trial found no overall reduction in CHD risk.

In a subgroup analysis of subjects aged 50–59 years, a trend toward a lower risk of CHD was observed with E use vs. placebo (14). Another recent study suggested a lower coronary heart disease risk with conjugated equine E among women 50 to 59 years of age at baseline (15). Thus, the effect of E on CHD risk in younger postmenopausal women still is being debated.

Another alternative in postmenopausal therapy is tibolone, a synthetic steroid with estrogenic, androgenic, and...
progestogenic properties that relieves climacteric symp-
toms and prevents postmenopausal bone loss. The influ-
ence of tibolone on coagulation and fibrinolysis compared
with a placebo was assessed in some studies (16), and in
others it was compared with continuous combined HT (17,
18). To our knowledge, a comparison with conjugated E
in surgically menopausal younger women has not been
performed.

The present randomized placebo-controlled double-blind
study compares the effects of tibolone and unopposed ET on
hemostatic and coagulation variables in surgically meno-
pausal younger women.

MATERIALS AND METHODS
Study Design
A randomized double-blind placebo-controlled study was
conducted in Hacettepe University School of Medicine’s
Department of Obstetrics and Gynecology over 6 months to
examine the effects of E and tibolone on extrinsic and
intrinsic coagulation cascades and natural anticoagulation
systems.

The study was in full compliance with the Declaration of
Helsinki plus revisions and with local rules and regulations,
and institutional review board approval was obtained. The
protocol was approved by the local ethics committees, and
all subjects gave written, informed consent before partici-
pating.

Ninety postmenopausal women who had undergone hys-
terectomy and bilateral salpingo-oophorectomy because of
benign diseases were randomized prospectively into three
groups, and HT was given in the 1st week of the postoper-
ative period. Group 1 (n = 30) received conjugated E (Pre-
marin, 0.625 mg/d oral tablets; Wyeth, Istanbul, Turkey)
only, group 2 (n = 30) received tibolone (Livial, 2.5 mg/d
oral tablets; Organon, Istanbul, Turkey), and group 3 (n =
30) received placebo, based on computer-generated random
numbers. Tibolone and conjugated E tablets are not identi-
cal; therefore, a double-dummy method was used for double
blinding.

A sample size of 25 subjects in each group was planned to
have a power of 80% at an error level of 5% to detect
treatment effects or differences in hemostatic parameters
(factor VII, fibrinogen, antithrombin III) on the basis of
results from a previous study on hemostasis and tibolone
(18).

Patient Selection
The patients included were healthy menopausal women
45–50 years of age who had undergone hysterectomy and
bilateral salpingo-oophorectomy as a result of benign dis-
eases in the current clinic, and they gave written informed
consent.

The exclusion criteria were smoking; history of cerebro-
vascular, cardiovascular, or thromboembolic events; hyper-
tension (blood pressure: systolic, ≥140 mm Hg or diastolic,
≥90 mm Hg, based on the average of two or more properly
measured readings at each of two or more visits after an
initial screening); diabetes (fasting plasma glucose of ≥126
mg/dL, with confirmed diagnosis of diabetes on a subsequent
day by measurement of fasting plasma glucose criteria);
obesity (body mass index of ≥30 kg/m2); known hereditary
hyperlipidemia; use of medications known to alter lipopro-
tein or coagulation indices, including any hormonal prepar-
rations; natural menopause; significant systemic illnesses;
and acute or chronic infection. The use of medications that
may affect coagulation and/or lipoprotein levels was not
allowed.

Study Procedures
Blood levels of modified activated protein C resistance
(aPCR), antithrombin III (ATIII), fibrinogen, factor VIIa
(FVIIa), factor VIII (FVIII), factor IX (FIX), activated par-
tial thromboplastin time (aPTT), prothrombin time, thrombin
time, and lipoprotein (a) were measured just before and after
6 months after the medications were started.

Blood samples were collected in the fasting state between
8:00 and 10:00 AM. Blood was drawn with minimal stasis.
All samples for assessment of hemostatic parameters were
analyzed at the end of the study by the same laboratory
(Hematology Unit, Hacettepe University, Faculty of Medi-
cine Hospital), and assay procedures were followed for prep-
aration and storage.

A modified aPTT-based test (STA-staclot APC-R; Diag-
nostica Stago, Asnières, France), including steps of factor
V–deficient plasma predilution and normalization by
plasma, was used for aPCR detection. Antithrombin III ac-

tivity (STA-Stachrom ATIII) was assayed by the chromo-
genic substrate method. Prothrombin time, aPTT, TT, FVIII,
FIX, and FVIIa levels were determined by clotting assay.
Lipoprotein (a) levels were measured by commercially avail-
able ELISAs (DAKO, Glostrup, Denmark).

Medical follow-up was repeated at 6-week intervals; com-
pliance was checked by counting returned tablets and by
face-to-face questionnaire, and the patients were evaluated
clinically.

Two women from the conjugated E group, two from the
Tibolone group, and three from the control group left the
study. Eighty-three women completed the entire study. The
average compliance rate for regular drug use among women
who completed the entire study protocol was 97%, and that
of the least compliant participant was 88%.

Statistical Analysis
Data were expressed as means ± SD. The subjects who
dropped out were excluded from the analysis. Within the
analysis of the covariance model, the adjusted mean changes from the baseline within each group were estimated and statistically tested by a Student’s *t*-test or Wilcoxon signed-rank test and one-way analysis of variance. The statistical analysis was performed by using SPSS, version 10.0 for Windows (SPSS, Chicago, IL). Statistical significance was set at *P*<.05.

**RESULTS**

Eighty-three healthy surgically postmenopausal women (28 in the E, 28 in the tibolone, and 27 in the placebo group) completed the study. The median age of the patients was 46 years (range, 45–49 y). There were no significant differences in age, mean systolic or diastolic blood pressure, or body mass index among the groups (Table 1). There were no significant differences at the beginning of the study with respect to the mean hemostatic or coagulation parameters among the three groups.

Fibrinogen levels were reduced in all groups but only significantly in the E group (*P*<.01). Level of factor VIIa was significantly decreased in the E and tibolone groups (*P*<.01 and <.01, respectively) and was slightly increased in the placebo group. Levels of factor VIII and FIX were decreased in all groups. The reduction in FVIII was significant in the E group (*P*=.002), whereas the reduction in FIX was significant in the tibolone and E groups (*P*=.001 and .007, respectively).

Levels of AT-III were significantly increased in the E and tibolone groups (*P*<.01 and *P*=.042, respectively). The aPCR ratio and Lp(a) levels were slightly decreased in the E and tibolone groups. The level of Lp(a) was significantly increased in the placebo group (*P*=.008).

Levels of aPTT increased in all groups, significantly so in the E and tibolone groups (*P*<.001 and <.001, respectively). The PTT levels were significantly increased in the E and tibolone groups (*P*<.001 and <.001, respectively) and were slightly decreased in the placebo group. There was a slight increase in the thrombin time in all groups.

The adjusted mean changes in procoagulation, anticoagulation, anti-fibrinolytic, clotting pathway parameters between the tibolone, conjugated E, and placebo groups are compared in Tables 2 and 3. Compared with the placebo, conjugated ET significantly decreased the serum levels of fibrinogen, FVIIa, and Lp(a) and increased the serum levels of FVIII and prolonged the aPTT.

However, tibolone significantly decreased only the serum levels of FVIIa and FIX and prolonged the aPTT, compared with the placebo. In addition, conjugated ET caused a greater decrease in the serum fibrinogen level than did tibolone (Tables 2 and 3).

**DISCUSSION**

Venous thromboembolism, including thrombosis of the deep veins of the legs and embolism to the pulmonary arteries, is a serious and potentially fatal event. The risk is increased in persons who have undergone recent surgical procedures and in those with previous VTE, immobilization, fractures of the lower extremities, and inherited coagulation disorders (19).

The incidence of VTE in women increases after menopause (20), and evidence concerning the effect of HT on the risk of VTE is contradictory (4–12). We examined the effect of tibolone and conjugated E on hemostasis in surgically menopausal patients in whom immediate replacement therapy was started postoperatively.

Recent studies with oral ET and sequential HT showed fairly consistent effects toward procoagulation, which were characterized by an increase in FVIIa and a reduction in AT-III (21, 22). However, many recent studies have observed a dramatic risk of VTE from E-containing preparations (4–8); only a few of those did not observe an increased risk of VTE with E-containing preparations (9–13).

Furthermore, the coagulation effects of conjugated E were demonstrated to be dose dependent, and the variety of Es used may have had different potencies with regard to their effect on coagulation activity (23, 24). In the current study, E and tibolone replacement shifted the hemostatic parameters to a more fibrinolytic state with respect to some parameters over 6 months, but the number of women evaluated was far too small for any meaningful conclusion with regard to the actual occurrence of such a clinical thromboembolic event.
**TABLE 2**

Effects of tibolone and conjugated estrogen on procoagulation and anticoagulation parameters compared with the placebo.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estrogen (n = 28)</th>
<th>Tibolone (n = 28)</th>
<th>Placebo (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Adjusted mean ± SD change</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Procoagulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>357.53 ± 120.80</td>
<td>342.83 ± 109.12</td>
<td>391.90 ± 116.82</td>
</tr>
<tr>
<td>24 wk</td>
<td>272.73 ± 50.99</td>
<td>−84.80 ± 114.75</td>
<td>328.83 ± 104.51</td>
</tr>
<tr>
<td>FVIIa (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>126.03 ± 55.19</td>
<td>121.26 ± 36.67</td>
<td>113.18 ± 27.59</td>
</tr>
<tr>
<td>24 wk</td>
<td>89.78 ± 29.42</td>
<td>−33.43 ± 41.36</td>
<td>94.71 ± 17.54</td>
</tr>
<tr>
<td>FVIII (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>223.36 ± 48.73</td>
<td>215.6 ± 84.32</td>
<td>254.96 ± 87.49</td>
</tr>
<tr>
<td>24 wk</td>
<td>194.56 ± 30.17</td>
<td>−28.80 ± 45.79</td>
<td>197.53 ± 58.08</td>
</tr>
<tr>
<td>FIX (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>167.66 ± 33.57</td>
<td>175.16 ± 37.82</td>
<td>179.96 ± 34.26</td>
</tr>
<tr>
<td>24 wk</td>
<td>143.03 ± 42.18</td>
<td>−24.63 ± 46.45</td>
<td>135.79 ± 33.08</td>
</tr>
<tr>
<td>Anticoagulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aPCR</td>
<td>1.93 ± 0.35</td>
<td>1.92 ± 0.28</td>
<td>1.78 ± 0.31</td>
</tr>
<tr>
<td>ATIII (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>23.25 ± 1.81</td>
<td>23.67 ± 2.67</td>
<td>23.83 ± 1.59</td>
</tr>
<tr>
<td>24 wk</td>
<td>24.91 ± 1.33</td>
<td>1.66 ± 1.95</td>
<td>24.97 ± 2.22</td>
</tr>
</tbody>
</table>

Note: Values are expressed as mean ± SD. Gr 1 = group 1 (estrogen); Gr 2 = group 2 (tibolone); Gr 3 = group 3 (placebo). aPCR = Activated protein C resistance ratio

^a Values of P and 95% CI are the comparison of adjusted mean ± SD changes.

^b Statistically significant at P<.05.

### TABLE 3

Effects of tibolone and conjugated estrogen on the clotting pathway (intrinsic, extrinsic, and trombin), and antifibrinolytic parameters compared with the placebo.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estrogen (n = 28)</th>
<th>Tibolone (n = 28)</th>
<th>Placebo (n = 27)</th>
<th>Adjusted mean change</th>
<th>Mean ± SD</th>
<th>Adjusted mean change</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting pathway</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>PT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.45 ± 0.76</td>
<td>12.84 ± 0.65</td>
<td>13.71 ± 0.87</td>
</tr>
<tr>
<td>Baseline</td>
<td>12.45 ± 0.76</td>
<td>12.84 ± 0.65</td>
<td>13.71 ± 0.87</td>
<td>12.47 ± 1.12</td>
<td>.01 (Gr 1 vs. Gr 3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.547, 1.533</td>
<td></td>
</tr>
<tr>
<td>24 wk</td>
<td>13.36 ± 0.66</td>
<td>0.90 ± 0.70</td>
<td>13.71 ± 0.87</td>
<td>0.86 ± 0.95</td>
<td>.05 (Gr 2 vs. Gr 3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.63, 7.61</td>
<td></td>
</tr>
<tr>
<td>aPTT</td>
<td>27.02 ± 4.06</td>
<td>27.35 ± 5.39</td>
<td>27.1 ± 3.59</td>
<td>27.73 ± 4.02</td>
<td>.05 (Gr 1 vs. Gr 2)</td>
<td>-2.43, 3.577</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>27.02 ± 4.06</td>
<td>27.35 ± 5.39</td>
<td>27.1 ± 3.59</td>
<td>27.73 ± 4.02</td>
<td>.05 (Gr 1 vs. Gr 2)</td>
<td>-2.43, 3.577</td>
<td></td>
</tr>
<tr>
<td>24 wk</td>
<td>33.27 ± 3.65</td>
<td>6.25 ± 4.53</td>
<td>5.68 ± 6.49</td>
<td>5.68 ± 6.49</td>
<td>.05 (Gr 2 vs. Gr 3)</td>
<td>-1.28, 1.485</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>16.31 ± 2.14</td>
<td>16.28 ± 1.84</td>
<td>16.52 ± 1.53</td>
<td>16.52 ± 1.53</td>
<td>.05 (Gr 2 vs. Gr 3)</td>
<td>-.86, 1.422</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>16.31 ± 2.14</td>
<td>16.28 ± 1.84</td>
<td>16.52 ± 1.53</td>
<td>16.52 ± 1.53</td>
<td>.05 (Gr 2 vs. Gr 3)</td>
<td>-.86, 1.422</td>
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<tr>
<td>24 wk</td>
<td>16.93 ± 2.24</td>
<td>0.62 ± 3.24</td>
<td>17.08 ± 2.12</td>
<td>0.80 ± 2.52</td>
<td>.05 (Gr 1 vs. Gr 2)</td>
<td>-1.73, 1.377</td>
<td></td>
</tr>
<tr>
<td>Antifibrinolytic parameter (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>359.77 ± 207.75</td>
<td>385.03 ± 178.40</td>
<td>398.27 ± 181.52</td>
<td>.04 (Gr 1 vs. Gr 3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-159.86, -12.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>359.77 ± 207.75</td>
<td>385.03 ± 178.40</td>
<td>398.27 ± 181.52</td>
<td>.04 (Gr 1 vs. Gr 3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-159.86, -12.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 wk</td>
<td>291.07 ± 171.75</td>
<td>-68.70 ± 186.84</td>
<td>350.20 ± 176.98</td>
<td>-34.83 ± 120.21</td>
<td>.05 (Gr 2 vs. Gr 3)</td>
<td>-10.71, -4.345</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>415.97 ± 170.24</td>
<td>17.70 ± 33.96</td>
<td>.05 (Gr 1 vs. Gr 2)</td>
<td>-118.38, 5.64</td>
<td></td>
</tr>
</tbody>
</table>

Note: Values are expressed as mean ± SD. PT = prothrombin time; TT = thrombin time; Gr 1 = group 1 (estrogen); Gr 2 = group 2 (tibolone); Gr 3 = group 3 (placebo). aPCR = Activated protein C resistance ratio.

<sup>a</sup> Values of P and 95% CI are the comparison of adjusted mean ± SD changes.

<sup>b</sup> Statistically significant at P<.05.

Low-dose progestogens administered in progestogen-only contraceptives do not have clinically relevant effects on the hemostatic system (25), whereas some progestogens may indirectly affect hemostatic function by their vasoconstrictive effects on the arteries (26). However, androgens may increase fibrinolytic capacity and reduce platelet aggregation (27), but in high doses they may predispose to VTE (28, 29).

Because tibolone is a synthetic steroid with estrogenic, androgenic, and progestogenic properties, its intrinsic androgenic properties may play a role in the activation of profibrinolysis and inactivation of antifibrinolysis (17, 30). In this study, tibolone shifted hemostasis parameters toward a more fibrinolytic state, and our findings are comparable with those of other studies (9, 17). Moreover, ET showed a more powerful effect with respect to hemostasis parameters toward the fibrinolysis.

Fibrinogen and FVII play a major role in the coagulation cascade. The menopause itself causes changes in the levels of many coagulation factors. Some of the changes resulting from the menopause might be expected to lead to a greater risk of thrombosis, including increases in factor VII, FVIII, and fibrinogen (31).

In the current study, reverse effects in these factors for tibolone or E were observed. Fibrinogen levels rise after menopause, and most studies have found a reduction in fibrinogen levels with E alone or with combined HT (24, 32–36). In addition, a lower dose of ET (0.3 mg/d) also causes a reduction in fibrinogen levels (24). Such a reduction could be considered beneficial because of the reduced amount of substrate for fibrin formation and the associated reduction in blood viscosity. In our study, conjugated ET caused a significantly greater decrease in serum fibrinogen level than did tibolone, which suggests a protective effect against thrombosis.

Antithrombin III is a significant physiologic inhibitor of thrombin. Conflicting results regarding the effect of ET on blood ATIII levels have been obtained, with some studies (37, 38) reporting an increase in serum levels, and others, a decrease (17, 39). In addition, more recent studies have reported that a reduction in ATIII may be observed after either normal (0.625 mg/d) or lower (0.3 mg/d) doses of HT compared with tibolone (24, 40).

We observed a significant increase in ATIII levels in the E and tibolone groups. The increase in ATIII may be desirable for further regulation of coagulation cascade. Because all patients in our study were surgically menopausal, it may be speculated that an unknown factor secreted from postmenopausal ovary may be responsible for decreasing ATIII levels. In addition to the low fibrinogen, FVII, FVIII, and FIX levels in both groups, a significant increase in ATIII levels may further contribute to the regulation of coagulation.

Level of Lp(a) is an independent risk factor for premature coronary artery disease and cerebrovascular accidents (41, 42), and concentrations of this lipoprotein tend to increase after the menopause. A lower cardiovascular disease rate in women receiving ET has been shown (43, 44).

In our study, compared with the placebo group, a significant decrease in Lp(a) levels in the ET group was observed. However, compared with the placebo group, no significant change in Lp(a) levels in the tibolone group was observed, although a decreasing tendency of serum Lp(a) levels was found in the tibolone group, in agreement with the literature (17, 30, 38). In addition, with regard to decreasing Lp(a) levels, there was no significant difference between the tibolone and E groups.

Activated protein C serves as an anticoagulant that downregulates the procoagulant cascade by inactivating the FVa and FVIIIa (45). It has been claimed that HT may increase the risk of thrombosis mediated through factor V Leiden mutation, which in turn lowers the aPCR (46). In our study, after 6 months of therapy, the mean changes in the aPCR ratio did not differ significantly among the groups, although decreased levels were observed in the E and tibolone groups without reaching statistical significance.

Our results are similar to some previous hemostatic studies of tibolone and HT preparations (16, 17, 30, 38). However, in some studies, an interaction between HRT usage and VTE has been observed (9–14), even though most of those did not screen the baseline parameters for potential prethrombotic states, and in some individuals, the preexisting risk may be high, and HT may trigger thrombosis. For example, venodilatation may cause thromboembolism rather than coagulation activation.

Most women in the present study had a normal body mass index, because those with high body mass indexes (i.e., who were obese) were excluded. In addition, obesity is not a common problem in this country.

In conclusion, the present study showed that neither E nor tibolone led to activation of coagulation in surgically menopausal women who were started immediately on HT postoperatively, but both preparations changed overall hemostatic balance toward a more fibrinolytic state. Moreover, with respect to some parameters like fibrinogen, FVIIa, and ATIII, E had a more powerful effect.

REFERENCES


