Impaired Procoagulant-anticoagulant Balance during Hormone Replacement Therapy?
A Randomised, Placebo-controlled 12-week Study

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Key words
Hormone replacement therapy, coagulation, fibrinolysis, postmenopausal

Summary
In this randomised, placebo-controlled 12-week study, sixty healthy postmenopausal women received either placebo (N = 16) or daily 2 mg micronised oestradiol, either unopposed (N = 16, E₂ group) or combined with a progestagen for 14 days of each cycle (N = 28, E₂+P group).

Results: As compared to placebo, plasma levels of AT III were reduced only in the E₂ group (~28%), plasma levels of protein C decreased only in the E₂+P group (~4%) and plasma levels of protein S decreased in both the E₂ and E₂+P group (~21%). In both the E₂ and E₂+P groups, the plasma levels of factor VII (antigen and activity) showed a borderline significant increase (~10%), whereas no significant change was observed in active factor VII. Plasma levels of tissue-type plasminogen activator (~22%), urokinase plasminogen activator (~25%) and plasminogen activator inhibitor type-1 (~43%) decreased in the E₂ and E₂+P groups, whereas those of plasminogen increased (~12%). Treatment was associated with an increase in levels of prothrombin fragment 1+2 (~31%), but levels of thrombin-antithrombin III complexes, and of plasmin-α₂-antiplasmin complexes and total fibrinogen degradation products did not change significantly.

Conclusion: Short-term E₂ and E₂+P treatment is associated with a shift in the procoagulant-anticoagulant balance towards a procoagulant state. A substantial proportion of women do not have a net increase in fibrinolytic activity. These data may be relevant in explaining the increased risk of venous thromboembolism associated with ERT and HRT, and possibly also in explaining the negative results of the Heart and Estrogen/progestin Replacement Study.

Introduction
Cardiovascular disease (CVD) is the most common cause of death in women above the age of 60 in western countries (1). Epidemiological data have suggested that the use of hormone replacement therapy (HRT) in healthy postmenopausal women is associated with a 40-50% decreased risk of cardiovascular events (2, 3). The effects of HRT on lipids and lipoproteins account for less than the half of the observed protection (4); other potential mechanisms are favourable effects on endothelial function, homocysteine and carbohydrate metabolism (5, 6).

Although the data from studies of HRT on risk factors and surrogate cardiovascular endpoints in general support the concept that HRT is protective, the first randomised trial so far (HERS), in women with a history of coronary heart disease, actually showed an increased risk of cardiovascular events in the first year of treatment (7). In addition, the risk of venous thromboembolism is reported to increase during HRT (7-10). One hypothesis to explain these findings is that HRT has procoagulant effects, especially in the short-term.

Among studies of oestrogen (ERT) (11-16) and combined hormone replacement therapy (12, 15-22) on coagulation and fibrinolysis, very few have been randomised (12, 13, 21) and placebo-controlled (11, 14, 17), and no studies have directly compared the effects of ERT with those of HRT.

Therefore, our aim was to evaluate, in a placebo-controlled, blinded study, the balance between markers of coagulation and fibrinolysis during short-term treatment with unopposed 2 mg micronised oestradiol (E₂) and 2 mg micronised oestradiol sequentially combined with a progestagen (E₂+P).

Materials and Methods

Subjects
Healthy, non-hysterectomised postmenopausal women were recruited through advertisements in the local newspapers; 65 women were enrolled in this 12-week study, which was performed at the outpatient clinic of the Department of Obstetrics and Gynaecology. Written informed consent was obtained from each participant before entry into the study. The protocol was approved by the Institutional Review Board of the University Hospital Vrije Universiteit.

Participants were 45-60 years old, smoked less than 15 cigarettes per day, were normotensive (<160/90 mmHg), had a body mass index ≤30 kg/m² and had been amenorrhoeic for 6 months to 5 years. Postmenopausal status was confirmed by FSH concentrations >20 IU/L and E₂ concentrations <150 pmol/L at the initial screening. None of the women had received HRT for at least 3 months before entering the study and none took cardiovascular medication. Exclusion criteria included a history of cardiovascular, metabolic, endocrinological, and (pre) malignant disease, as well as clinically relevant abnormalities in laboratory tests of haematological, renal and hepatic function. Women with plasma levels of cholesterol and triglycerides >8 mmol/L and >4 mmol/L, respectively, were also excluded.
Study Design

Eighteen participants, assigned by a computerised randomisation procedure, to either sequentially combined HRT (micronised 17β-oestradiol 2 mg daily plus either trimegestone 0.5 mg daily (Hoechst Marion Roussel, Romainville-Cedex, France) or dydrogesterone 10 mg daily (Femoston®, Solvay Pharmaceuticals, Weesp, The Netherlands), given for 14 days each of a 28-day cycle (E₂ +P group); or to unopposed micronised 17β-oestradiol 2 mg daily (E₂ group), or to placebo in a ratio of 2:1:1. The tablets of the sequentially combined HRT were put into capsules of identical appearance and organoleptic characteristics by Hoechst Marion Roussel (Paris, France). Unopposed oestradiol and placebo were manufactured as capsules of identical appearance and organoleptic characteristics by the pharmacist of the University Hospital Vrije Universiteit, Amsterdam. After three months of follow-up, women assigned to treatment with unopposed oestradiol were treated with dydrogesterone 10 mg daily for 14 days to induce a withdrawal bleed.

Blood Collection

At baseline and after 4 weeks (cycle 1; cycle day 24–28) and 12 weeks (cycle 3; cycle day 24–28) of follow-up, venous blood samples were taken between 8.00 a.m. and 10.00 a.m., with participants having rested in a supine position for 20 min. Blood was collected with a Vacutainer® (Becton Dickinson, Meyren, Cedex-France) system into tubes containing CTAD (Becton Dickinson, Meyren, Cedex-France), and into tubes containing trisodium citrate (1:9 vol/vol). The subjects had fasted for at least 10 h and had refrained from smoking for ≥10 h and from consuming alcohol for ≥24 h before sampling. The blood samples were immediately placed on ice and centrifuged at 3,000 g and 20° C. Plasma was divided into aliquots, snap-frozen and stored at -70° C until analysis.

Assays

All samples for a given parameter were assayed in a single run at the end of the study. The laboratory was blinded to the treatment code of each individual participant. We measured plasma levels of:

- anticoagulant proteins: antithrombin III (AT III) activity [Spectrolyse AT III (anti Xa) chromogenic assay kit; Biopool AB, Umea, Sweden], protein C antigen [by ELISA; Technoclone, Vienna, Austria] and protein S antigen, both total and free, [by ELISA; Thrombostika Protein S, Organon Teknika, Turnhout, Belgium];
- coagulant proteins: active factor VII [using a truncated recombinant human tissue factor, as described by Morisse et al. (23)], factor VII antigen [by ELISA; Asserachrom VII:Ag; Diagnostica Stago, Asnières-sur-Seine, France], and FVII activity [by a chromogenic assay; Chromogenix AB, Mölndal, Sweden];
- markers of net coagulation activity: prothrombin fragment 1+2 antigen [by ELISA; Enzygnost F1+2, Behringwerke AG, Marburg, Germany], and thrombin-antithrombin III complex antigen [by ELISA; Enzygnost TAT, Behringwerke AG, Marburg, Germany];
- profibrinolytic proteins: tissue-type plasminogen activator (t-PA) antigen [by ELISA; Enzygnost TPA, Taurus, Leiden, The Netherlands], urokinase plasminogen activator (u-PA) antigen [by home-made ELISA], and plasminogen activity [by a chromogenic assay (24)];
- the antifibrinolytic plasminogen activator inhibitor type-1 (PAI-1) antigen [by ELISA; Thrombostika PAI-1, Organon Teknika, Turnhout, Belgium]; and
- markers of net activation of fibrinolysis: plasmin-α₂-antiplasmin (PAP) complex antigen [by ELISA; Enzygnost PAP, Behringwerke AG, Marburg, Germany] and total fibrinogen degradation products (TDP) [by ELISA; Thrombostika TDP, Organon Teknika, Turnhout, Belgium].

Serum FSH was determined with a specific immunometrical (luminescence) assay (Amerlita, Amersham, Little Chalfont, UK). Serum oestradiol was quantified by using a double-antibody radioimmunoassay (Sorin Biomedica, Saluggia, Italy) after extraction with diethylther to eliminate possible cross-reactivity with oestrogen conjugates (25), with a lower limit of detection of 18 pmol/L. Serum total cholesterol was measured automatically (Boehringer Mannheim, Germany). For all assays the intra-assay coefficients of variation was 6.9% or less, except for the factor VIIa assay, for which it was 14%.

Statistics

Statistical analysis was performed using the Statistical Package for the Social Sciences PC + 4.0 (SPSS Inc., Chicago, Illinois). Values are given as mean ± SD or as median [range]. We compared baseline measurements between groups using standard parametric and non-parametric tests where applicable. Correlations between variables were calculated with Pearson’s or Spearman’s correlation coefficient. There was a significant baseline difference between the groups in body mass index (BMI; Table 1); therefore analyses of covariance (ANCOVA) for repeated measurements, with the baseline value of the variable under consideration and BMI as constant co-variates, were used for comparisons among the groups. A two-tailed P <0.05 was accepted as the level of significance. The analyses were based on 60 participants. Sixty-five participants were initially enrolled, from whom five women were excluded. Three women (two in the E₂+P group and one in the placebo group) dropped out before the measurement at 4 weeks because of lack of motivation, and two women in the E₂ group dropped out because sufficient serum could not be obtained (N = 1) and because of incorrect inclusion (hormonal status not postmenopausal, N = 1). Another three women dropped out between 4 and 12 weeks, two in the E₂+P group because of hypermenorrhoea (N = 1) and oedema (N = 1), and one in the placebo group because of social reasons. The last-observation-carried-forward procedure was applied for the missing values at 12 weeks.

Results

Baseline characteristics of the three groups are shown in Table 1. At baseline, BMI (Table 1), Protein S (total and free), factor VIIag, F1+2 (Table 2), PAI-1 and PAP (Table 3) differed significantly among the groups. BMI did not change during the 12-week study period.

At baseline and during the 3 months of follow-up, no statistical differences in descriptive characteristics and markers of coagulation and fibrinolysis were found between the dydrogesterone and trimegestone subgroups (data not shown). We therefore combined these groups in the analyses.

During the 12-week period, plasma levels of AT III were reduced in total protein S. There was an increase in factor VIIa and VIIch in both total protein S. There was an increase in factor VIIa and VIIch with treatment of E₂ or E₂+P was associated with a significant decrease in total protein S. There was an increase in factor VIIa and VIIch with treatment of E₂ or E₂+P, as compared to placebo (Table 2). In the E₂ and E₂+P groups, plasma levels of t-PA, u-PA and PAI-1 decreased, whereas plasminogen levels increased, as compared to placebo (Table 3).

Except for Protein C and AT III, none of the results differed significantly between the E₂ and E₂+P groups. The changes observed were not related to the duration of amenorrhoea or to smoking, when we used these variables as a constant covariate in the ANOVA (data not shown). Levels of free protein S, factor VIIa, TAT, PAP and TDP did not change significantly.

It was not possible to analyse F1+2 data with the ANCOVA model, because of their extremely skewed distribution. Therefore, we analysed the per centile and absolute change between baseline and 12 weeks with non-parametric tests. There was a significant change in F1+2 between
Table 1 Descriptive characteristics of the three groups at baseline

|                | E₂ group | E₂ + P group | Placebo group | P-value*  
|----------------|----------|--------------|---------------|-----------
| N              | 16       | 28           | 16            | 0.29      
| Age (years)    | 53.1 ± 2.8 | 52.1 ± 3.0   | 51.4 ± 3.2    |           
| Duration of amenorrhea (months) | 29.2 [5.8 - 69.1] | 18.8 [6.2 - 64.2] | 21.2 [8.2 - 55.6] | 0.51      
| Body mass index (kg/m²)       | 24.3 ± 2.9 | 26.5 ± 2.6   | 25.0 ± 3.1    | 0.04      
| Blood pressure: systolic (mmHg) | 119 ± 15    | 123 ± 14     | 121 ± 9       | 0.64      
| diastolic (mmHg)             | 66 ± 8     | 70 ± 10      | 70 ± 8        | 0.30      
| Smokers N (%)               | 6 (38)    | 4 (14)       | 2 (13)        | 0.12      
| Serum cholesterol (mmol/L)   | 6.0 ± 1.1  | 6.3 ± 1.0    | 6.2 ± 1.0     | 0.70      
| Serum FSH (U/L)             | 52.8 ± 20.0 | 51.6 ± 14.7  | 54.3 ± 22.9   | 0.88      
| Serum E₂ (pmol/L)           | 18 [18-213]| 18 [18-173]  | 18 [18-244]   | 0.92      

Note: values are given as mean ± SD, median [range] or as number (N) with percentage in parentheses

One-way ANOVA or Kruskal-Wallis or χ²-test for between-group differences

FSH = follicle stimulating hormone, E₂ = oestradiol, P = progesterone

Table 2 Plasma markers of markers of coagulation

|                | Baseline | 4 weeks | 12 weeks | %Δ† | P-value □  
|----------------|----------|---------|----------|-----|-----------
| Antithrombin III (% NPP) |          |         |          |     |           
| E₂             | 77 ± 19  | 59 ± 17 | 50 ± 19  | -11.6 (-64.0 to -16.0)% | <0.0001  
| E₂ + P         | 85 ± 17  | 72 ± 19 | 77 ± 17  | -11.7 (-26.5 to +4.1)  |           
| Placebo        | 72 ± 21  | 79 ± 26 | 78 ± 20  | -4.6 (-13.7 to +7.8)   |           
| Protein C (mg/L) |          |         |          |     |           
| E₂             | 3.3 ± 0.4 | 3.3 ± 0.3 | 3.3 ± 0.4 | -0.3 (-7.7 to +9.8) | 0.02      
| E₂ + P         | 3.3 ± 0.3 | 3.1 ± 0.3 | 3.1 ± 0.3 | -0.4 (-10.1 to +3.2)  |           
| Placebo        | 3.4 ± 0.4 | 3.4 ± 0.4 | 3.4 ± 0.5 | +0.3 (+0.1 to +5.4)   |           
| Protein S (total) (%NPP) |        |         |          |     |           
| E₂             | 104 ± 18 | 91 ± 19 | 86 ± 15  | -18.9 (-31.7 to +0.1)% | 0.005     
| E₂ + P         | 126 ± 22 | 110 ± 19 | 102 ± 17 | -17.2 (-29.3 to +4.2)% |           
| Placebo        | 103 ± 17 | 106 ± 17 | 105 ± 23 | +2.2 (+12.6 to +18.2) |           
| Protein S (free) (%NPP) |       |         |          |     |           
| E₂             | 36 ± 10 | 38 ± 12 | 37 ± 11  | -1.5 (-20.5 to +17.6) | 0.10      
| E₂ + P         | 51 ± 16 | 51 ± 13 | 47 ± 16  | -7.1 (-30.9 to +27.8) |           
| Placebo        | 41 ± 11 | 42 ± 9  | 46 ± 12  | +4.2 (+19.4 to +55.2) |           
| VⅪa (µg/L)    | 1.0 (0.3 - 2.1) | 1.1 (0.4 - 3.3) | 0.9 (0.4 - 4.8) | +7.1 (+6.3 to +70.0) | 0.55      
| E₂             | 1.0 (0.3 - 3.5) | 0.9 (0.3 - 3.2) | 1.0 (0.3 - 3.8) | 0.0 (20.0 to +436.2) |           
| E₂ + P         | 0.8 (0.3 - 1.4) | 0.9 (0.2 - 3.3) | 0.9 (0.5 - 3.1) | 0.0 (0.0 to +313.3) |           
| Placebo        | 0.8 ± 0.3 | 1.0 ± 0.0 | 0.9 ± 0.2 | -0.0 (+0.0 to +13.1) |           
| VⅪag (µg/L)   | 72 ± 15 | 81 ± 17 | 80 ± 13  | +10.3 (+4.2 to +31.6) | 0.06      
| E₂             | 90 ± 16 | 91 ± 16 | 92 ± 13  | -1.4 (-9.1 to +21.1)  |           
| E₂ + P         | 73 ± 13 | 74 ± 13 | 78 ± 17  | +11.0 (+8.3 to +14.0) |           
| Placebo        | 73 ± 13 | 74 ± 13 | 78 ± 17  | +11.0 (+8.3 to +14.0) |           
| VⅩhr (µNPP)   | 81 ± 19 | 93 ± 23 | 95 ± 21  | +13.3 (+1.3 to +23.2) | 0.05      
| E₂             | 67 ± 20 | 90 ± 30 | 90 ± 28  | +20.7 (+2.9 to +90.1) |           
| E₂ + P         | 82 ± 23 | 77 ± 20 | 82 ± 21  | +1.3 (-20.8 to +20.6) |           
| Placebo        | 51.6 ± 14 | 51.7 ± 14 | 51.7 ± 14 | -0.6 (-3.8 to -0.3)   |           
| FⅠ+2 (nmol/L) | 13.0 ± 18 | 13.0 ± 21 | 15.0 ± 9.0 | +16.5 (+11.6 to +77.4)% |       
| E₂             | 12.0 (7.4 - 43) | 15.0 (7.8 - 8.1) | 15.0 (10.4 - 46) | +26.0 (+5.2 to +57.1)% |           
| E₂ + P         | 15.0 (9.4 - 63) | 13.0 (10.3 - 5.3) | 13.0 (8.7 - 8.6) | +10.6 (-8.2 to -5.1) |           
| Placebo        | 3.7 ± 1.1 | 3.9 ± 1.5 | 4.4 ± 1.9 | +26.8 (+22.9 to +61.7)| 0.53      
| TAT (µg/L)     | 4.2 ± 1.0 | 4.6 ± 1.7 | 4.3 ± 1.4 | -9.4 (-20.9 to +9.2) |           
| E₂             | 4.2 ± 1.0 | 4.6 ± 1.7 | 4.3 ± 1.4 | -9.4 (-20.9 to +9.2) |           
| E₂ + P         | 3.8 ± 1.2 | 4.1 ± 2.1 | 3.2 ± 0.8 | +14.8 (+28.6 to +49.9)|           

VⅪag = factor VII antigen; VⅪa = active factor VII; VⅩhr = factor VII chromogenic activity; AT III = antithrombin III; NPP = normal pooled plasma; FⅠ+2 = prothrombin fragment 1+2; TAT = thrombin-antithrombin III complexes

† = %Δ = median (25-75th) percentile change at 12 weeks versus baseline

‡ = Determined by covariance analysis for repeated measurements for between-group differences with the baseline value of the variable under consideration and BMI as constant covariates (ANCOVA) and, when not normally distributed, after log transformation

§ = P <0.05 and §§ p <0.005 by one-way ANOVA or Kruskal-Wallis for between-group differences at baseline

* P <0.05, ** P <0.005 by Mann-Whitney test for between-group differences compared to placebo

¶ = No p-value given because of the extreme skewness

Values are given as means ± SD or as the median with the range in parentheses

Figure 1 shows that FⅠ+2 levels increased in 27 of 36 (75%) women receiving E₂ or E₂+P versus 3 of 15 (20%) of those receiving placebo (P = 0.0005). Levels of PAP complexes increased in 23 of 42 (54%)
women receiving E, or E + P versus 7 of 15 (47%) of those receiving placebo (P = 0.59). The change in F1+2 levels was not related to the change in factor VII (ag, chr or a) or to the change in AT III, protein S or protein C (data not shown).

There were no significant correlations between baseline levels of conventional cardiovascular risk factors (age, duration of amenorrhea, BMI, blood pressure, smoking habits, cholesterol, and E$_2$ levels) and baseline levels of coagulation and fibrinolysis markers, except for blood pressure and protein S (systolic: r = 0.38, P = 0.002; diastolic: r = 0.35, P = 0.005), and E$_2$ and AT III (r = –0.37, P = 0.004).

**Discussion**

Compared to placebo, 12 weeks of hormone replacement was associated with significant and substantial decreases in the plasma levels of AT III (E$_2$ group) and total protein S (both E$_2$ and E$_2$+P groups), and a small decrease in levels of protein C (E$_2$+P group). In both the E$_2$ and E$_2$+P groups, the plasma levels of factor VII (ag and chr) showed a borderline significant increase, whereas no significant change was observed in active factor VII. Plasma levels of t-PA, u-PA and PAI-1 decreased, whereas those of plasminogen increased. There was an in-

### Table 3 Plasma levels of markers of fibrinolysis

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4 weeks</th>
<th>12 weeks</th>
<th>%Δ†</th>
<th>P-value ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasminogen (% NPP) E$_2$</td>
<td>111 ± 26</td>
<td>124 ± 36</td>
<td>136 ± 30</td>
<td>+19.5 (7.8 to +32.6)**</td>
<td>0.008</td>
</tr>
<tr>
<td>E$_2$+P</td>
<td>116 ± 26</td>
<td>128 ± 27</td>
<td>115 ± 21</td>
<td>+2.9 (–4.4 to +16.7)</td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>125 ± 34</td>
<td>115 ± 14</td>
<td>113 ± 22</td>
<td>–2.1 (–26.3 to +11.2)</td>
<td></td>
</tr>
<tr>
<td>t-PA (µg/L)</td>
<td>E$_2$</td>
<td>14.3 ± 6.7</td>
<td>11.9 ± 5.9</td>
<td>10.3 ± 5.4</td>
<td>–24.3 (–36.5 to –11.5)**</td>
</tr>
<tr>
<td></td>
<td>E$_2$+P</td>
<td>14.9 ± 6.3</td>
<td>13.4 ± 5.5</td>
<td>13.2 ± 5.4</td>
<td>–8.1 (–32.6 to +29.7)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>11.5 ± 4.4</td>
<td>12.3 ± 4.5</td>
<td>13.1 ± 4.3</td>
<td>+7.3 (–9.7 to +39.3)</td>
</tr>
<tr>
<td>u-PA (µg/L)</td>
<td>E$_2$</td>
<td>3.2 ± 1.0</td>
<td>2.7 ± 1.0</td>
<td>2.5 ± 0.8</td>
<td>–21.0 (–26.1 to –16.1)**</td>
</tr>
<tr>
<td></td>
<td>E$_2$+P</td>
<td>2.9 ± 0.4</td>
<td>2.2 ± 0.4</td>
<td>2.2 ± 0.4</td>
<td>–22.9 (–28.3 to –15.4)**</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>2.8 ± 0.4</td>
<td>2.8 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>+3.2 (+2.0 to +10.6)</td>
</tr>
<tr>
<td>PAI-1 (µg/L)</td>
<td>E$_2$</td>
<td>34 (7-66)</td>
<td>17 (6-66)</td>
<td>13 (4-66)</td>
<td>–50.9 (–68.0 to –27.0)**</td>
</tr>
<tr>
<td></td>
<td>E$_2$+P</td>
<td>52 (10-96)</td>
<td>24 (7-66)</td>
<td>28 (8-66)</td>
<td>–33.7 (–67.9 to –16.6)**</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>34 (10-96)</td>
<td>25 (8-69)</td>
<td>32 (8-67)</td>
<td>+0.5 (–23.6 to +50.0)</td>
</tr>
<tr>
<td>PAP (µg/L)</td>
<td>E$_2$</td>
<td>471 ± 168</td>
<td>475 ± 145</td>
<td>475 ± 148</td>
<td>+1.4 (–11.0 to +30.8)</td>
</tr>
<tr>
<td></td>
<td>E$_2$+P</td>
<td>344 ± 137</td>
<td>438 ± 165</td>
<td>395 ± 199</td>
<td>–16.0 (–51.5 to +53.8)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>444 ± 118</td>
<td>441 ± 157</td>
<td>441 ± 124</td>
<td>–4.3 (–25.3 to +22.1)</td>
</tr>
<tr>
<td>TDP (µg/L)</td>
<td>E$_2$</td>
<td>531 (162-1399)</td>
<td>467 (274-1166)</td>
<td>489 (122-1044)</td>
<td>–15.6 (–50.9 to +26.5)</td>
</tr>
<tr>
<td></td>
<td>E$_2$+P</td>
<td>385 (228-4246)</td>
<td>464 (63-1146)</td>
<td>429 (72-1797)</td>
<td>+13.2 (+21.8 to +62.6)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>391 (109-1002)</td>
<td>447 (257-718)</td>
<td>437 (182-715)</td>
<td>+4.5 (+15.8 to +60.3)</td>
</tr>
</tbody>
</table>

NPP = normal pooled plasma; PAI-1 = plasminogen activator inhibitor-1; TDP = total fibrin(ogen) degradation products; t-PA = tissue-type plasminogen activator; u-PA = urokinase plasminogen activator; PAP = plasmin-α$_2$-antiplasmin complexes

† = Δ% = median (25-75th) percentile change at 12 weeks versus baseline
‡ = Determined by covariance analysis for repeated measurements for between-group differences with the baseline value of the variable under consideration and BMI as constant covariates (ANCOVA) and, when not normally distributed, after log transformation
§ = P <0.05 by one-way ANOVA or Kruskal-Wallis for between-group differences at baseline
** P <0.005 and *** P <0.005 by Mann-Whitney test for between-group differences compared to placebo
Values are given as means ± SD or as the median with the range in parentheses

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**Fig. 1** Scattergram of percentage change at 12 weeks, compared to baseline, in PAP and F1+2 in treated women (●) and women receiving placebo (○)

% change F1+2

% change PAP

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**Fig. 2** Scattergram of percentage change at 12 weeks, compared to baseline, in PAP and TDP

○ = women with a decrease in PAI-1 (versus baseline) of more than 50%
● = women with a decrease in PAI-1 (versus baseline) of less than 50%
PAP = plasmin-α$_2$-antiplasmin complexes; TDP = total degradation products; PAI-1 = plasminogen activator inhibitor-1
crease in levels of F1+2, but levels of TAT complexes, and of PAP complexes and TDP did not change significantly. Taken together, our results suggest that 12 weeks’ treatment with unopposed 2 mg micronised oestradiol or sequentially combined HRT may impair anticoagulant defences, and increase the net level of activity of the coagulation system. Although PAI-1 levels decreased, we found no evidence of an increase in net fibrinolytic activity.

A decrease in the coagulation inhibitors, AT III, protein C and protein S, has been associated with an increased risk of venous thromboembolism (26). Our results are in agreement with most (11, 13, 15, 17, 21, 27) but not all (14) earlier publications, and support the data found in epidemiological studies (8-10). Furthermore, we extend these previous observations in that we observed a decrease in these variables already shortly after the start of treatment.

Elevated levels of factor VII are an independent predictor of coronary artery disease (28, 29). Earlier studies on HRT and factor VII are not consistent (13, 17, 20, 30). We found a borderline increase in VII antigen and activity, but no change in active factor VII, suggesting that any increase in factor VII protein level need not have functional consequences. However, there were strong correlations between factor VII ag and chr on the one hand and active factor VII on the other, which were sustained during treatment (data not shown). Therefore, and because our study had relatively low power, the lack of increase in active factor VII should be interpreted with considerable caution.

Elevated levels of F1+2 and TAT complexes reflect a state of increased in vivo activation of the coagulation system (26). In our study, both treatment regimens, compared to placebo, showed no effect on TAT complexes and a significant increase in F1+2, of on average 31%. These observations are consistent with previous studies (11, 13, 20), but extend these observations by the direct comparison of the effects of ERT with those of HRT. A possible explanation for this oestrogen-associated difference in F1+2 and TAT complexes could be through increased clearance of TAT but not F1+2 by the liver as a result of up-regulation of the TAT clearance mechanism (38) by oestrogens, similar to the upregulation of t-PA clearance by oestrogens (39). Further investigations need to clarify this issue.

Our data showed that treatment with E2 or E2+P results in activation of the coagulation system. The change in F1+2 levels was not related to the change in factor VII (ag, chr or a) or the change in AT III, protein S or protein C. Thrombin generation, however, is a complex process regulated by a number of tissue and plasmatic factors. Further studies are necessary to investigate whether ERT- or HRT-associated changes in such factors can explain the increase in F1+2 observed.

Figure 1 shows that about 20% of the women receiving E2 or E2+P had a net increase in coagulation activity which was not accompanied by a net increase in fibrinolytic activity, as estimated from plasma levels of F1+2 and PAP complexes, respectively. This raises the possibility that a substantial percentage of women receiving ERT or HRT have a short-term imbalance of coagulation versus fibrinolysis.

As have others (12, 13, 17, 20, 31), we found a substantial decrease of PAI-1 levels both with E2 and with E2+P, without, however, a net increase in fibrinolytic activity, since overall levels of PAP complexes and TDP did not change, again in agreement with previous studies (12, 32). It must be stressed, however, that the relation between PAI-1 levels and t-PA activity is not linear but exponential, and that t-PA activity increases when PAI-1 levels decrease below a certain threshold (see figure 7 in ref. (33)). Indeed, in our study, levels of PAP complexes and of TDP increased significantly in those subjects in whom PAI-1 levels decreased by more than 50% or by more than 40 μg/L (Fig. 2). Only in this subgroup, therefore, did the decrease in PAI-1 translate into an increased fibrinolytic activity, which was also found in a small study of Koh et al. (34). The observed decrease in t-PA antigen is most likely due to two processes. Firstly, as demonstrated by Chandler et al. (35), a decrease in PAI-1 antigen will, because of the slower clearance of t-PA-PAI-1 complexes compared to free t-PA, result in lower plasma levels of t-PA (35, 36). Secondly, t-PA (and PAI-1) clearance may be increased due to up-regulation of a hepatic clearance receptor (37, 39). This analysis suggests therefore that, although PAI-1 levels will decrease in most women on HRT or ERT, fibrinolytic activity will increase in substantially fewer women, and this may be clinically relevant.

Our study had several limitations. The study was “blinded” for the laboratory, but the investigators knew whether participants were in the E2+P treatment arm or in the E2/placebo arm. It is doubtful, however, whether a truly double-blind study of HRT among non-hysterectomised postmenopausal women is possible, as withdrawal bleeds are present after one month in those on sequentially combined HRT. In addition, the E2 versus placebo comparisons were not affected by this issue.

In summary, we found that both short-term unopposed micronised oestradiol and sequentially combined HRT with oestradiol and progesterone/dydrogesterone in healthy postmenopausal women was associated with: 1) a decrease in coagulation inhibitor levels; 2) an increase of the activity of the coagulation system, which was not related to an increase in factor VII activity or the decrease in coagulation inhibitors; and 3) a decrease of the fibrinolysis inhibitor PAI-1, which, however, did not result in an overall net increase of fibrinolytic activity.

In conclusion, short-term ERT and HRT are associated with a shift in the procoagulant-anticoagulant balance towards a procoagulant state. A substantial proportion of women do not have a net increase in fibrinolytic activity. These data may be relevant in explaining the increased risk of venous thromboembolism associated with ERT and HRT (8-10), and possibly, together with our recent finding of increased inflammatory activity shortly after the start of ERT or HRT (40), also in explaining the disappointing results of the HERS trial (7). Further investigations are required to investigate if this procoagulant state is sustained also after long-term treatment.

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