

Unopposed Estrogen Increases Total Plasma Factor VII, but not Active Factor VII

A Short-term Placebo-controlled Study in Healthy Postmenopausal Women

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Key words

Postmenopausal, estrogen, factor VII, postprandial

Summary

Estrogen therapy may increase the risk of arterial thromboembolism, at least in the short term. In a randomized, double-blind and placebo-controlled study in 25 healthy postmenopausal women (52.5 ± 2.8 years), we therefore examined the short-term effect of unopposed estrogen on the fasting and fat-load-stimulated plasma levels of total factor VII versus active factor VII. Plasma total factor VII was measured by use of a chromogenic assay; plasma active FVII by a recently developed method using truncated tissue factor. As compared to placebo, 8 weeks of oral 17β-estradiol (2 mg daily) increased the mean fasting and postprandial plasma levels of total factor VII by 17 and 21% points, respectively (both $P < 0.01$), but did not affect the fasting and/or postprandial plasma levels of active factor VII (mean change both 0.05 ng/mL; $P > 0.35$). Furthermore, the change in the fasting level of total factor VII after therapy was not associated with the change in the fasting level of active factor VII ($r = 0.27$; $P = 0.21$). These findings argue against the idea that elevated levels of total factor VII underlie an increased risk of arterial thromboembolism in postmenopausal women using unopposed estrogen replacement.

Introduction

Multiple observational studies suggest that postmenopausal hormone replacement therapy (HRT) is associated with a pronounced decrease of the risk of cardiovascular events (1). However, the recently published report of the Heart and Estrogen/progestin Replacement Study (HERS), the first randomized, controlled trial with clinical cardiovascular end-points (2), has questioned the cardioprotective effect of HRT. In that study, during an average follow-up of 4.1 years, treatment with oral conjugated equine estrogens continuously combined with medroxyprogesterone acetate did not reduce the overall rate of cardiovascular events in postmenopausal women with established coronary disease. The risk of cardiovascular events was increased in the first year of hormone substitution, and was decreased in the fourth and fifth year.

We hypothesized that a potential mechanism that may help to understand these overall null results may be an adverse effect of estrogen on blood coagulation. Factor VII (FVII) initiates the coagulation cascade by binding to tissue factor, a surface-bound protein present in atheromatous plaques and in subendothelial tissue (3). However, following stimulation by cytokines (4, 5) and/or modified LDL (6), tissue factor may also be expressed on endothelial cells, on macrophages and on monocytes. Epidemiologic data on the relevance of factor VII as a risk factor for cardiovascular disease are inconclusive. In the First Northwick Park Heart Study the level of FVII coagulant activity was found to be associated with the risk of arterial thrombotic disease. In that study, the FVII coagulant activity level independently predicted the risk of coronary heart disease (7). Other studies, however, only found a trend (8), or did not find an association between the factor FVII level and cardiovascular risk (9).

In a cross-sectional analysis in healthy subjects, age-adjusted levels of FVII were found to be lower in young women compared to men, in premenopausal compared to postmenopausal women, and in postmenopausal women using combined HRT compared to postmenopausal women not using HRT (10). In other cross-sectional analyses, however, the FVII level was found to be lower in postmenopausal women using combined HRT as compared to non-HRT-users, but was found to be higher in women using unopposed estrogen replacement (11, 12). Prospective studies on the use of HRT and FVII have also yielded conflicting results, which may be due to the regimen used and the use of different FVII assays. FVII may be measured as total circulating FVII, either by an antigen assay or by a chromogenic assay, or as active FVII, using a recently developed method (13) which measures the fraction of FVII which has already been activated in vivo.

The aim of our study was to assess the effects of unopposed estrogen replacement on the fasting plasma level of total and active FVII. Additionally, we wished to assess the effect of unopposed estrogen on fat-load-stimulated FVII activation (14–18). Therefore, 26 healthy postmenopausal women were randomly assigned to an 8 weeks' treatment period with either 17β-estradiol 2 mg daily orally or placebo. Before and following treatment, we measured levels of total and active FVII, both in the fasting state as well as following a standardized fat-rich meal.

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Table 1 General characteristics of the 25 healthy postmenopausal women at baseline

	Placebo (n = 13)	E2 (n = 12)	P-value
Age (years)	52.9 ± 2.8	52.3 ± 2.7	0.97
Current smokers (%)	23	23	0.92
FSH (U/L)	60 ± 22	53 ± 19	0.37
E_2 (pmol/L)	25 (21; 30)	35 (24; 50)	0.09
BMI (kg/m^2)	25.2 ± 3.2	27.6 ± 4.8	0.17
DBP (mmHg)	83 ± 6	82 ± 9	0.61
SBP (mmHg)	123 ± 11	125 ± 15	0.66
TG (mmol/L)	1.0 (0.7; 1.2)	0.9 (0.7; 1.1)	0.75
t-chol (mmol/L)	6.3 ± 1.0	5.8 ± 1.2	0.27
HDL-c (mmol/L)	1.8 ± 0.5	1.7 ± 0.5	0.79
LDL-c (mmol/L)	4.1 ± 0.9	3.8 ± 1.2	0.37
glucose (mmol/L)	4.2 ± 0.9	3.8 ± 0.9	0.28

Values are presented as means ± SD or, for non-normally distributed variables, as geometric means (95% CI). E2: 17β-estradiol; BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure; TG: triglycerides; t-chol: total cholesterol; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol.

Subjects, Materials and Methods

Subjects

Twenty-six healthy postmenopausal women of Caucasian origin were included in the study. Postmenopausal status was defined as a serum estradiol (E_2) of ≤ 95 pmol/l and FSH of ≥ 30 U/L as well as, for non-hysterectomized women (n = 11), an amenorrhoea of ≥ 12 months. Major exclusion criteria were: known or suspected estrogen-dependent carcinoma, known thromboembolic disease, diabetes mellitus, a history of excessive alcohol ingestion, changes of body weight greater than 4 kg during the last 4 weeks, intake of lipid-lowering drugs, and intake of female sex hormones during the past 3 months. Women with a serum creatinine > 100 μmol/L, alanine aminotransferase > 100 U/L, alkaline phosphatase > 300 U/L, fasting plasma glucose level > 6.8 mmol/L and/or total triglyceride level > 2.2 mmol/L were also excluded. Participants were randomly assigned to 8 weeks of treatment with either 17β-estradiol 2 mg orally per day or placebo. To monitor treatment adherence, subjects returned the study medication which was then counted. The mean treatment adherence was 96% (minimum 70%; maximum 100%), there was no statistically significant difference between the two treatment groups. The study had a double-blind design and was conducted on an outpatient basis according to the principles of the Declaration of Helsinki, and was approved by the medical ethical committee of the University Hospital Vrije Universiteit. Informed consent was obtained from all volunteers after oral and written information was supplied.

General Procedures

At baseline we assessed current smoking status (yes/no) and the body mass index (BMI; kg/m^2). Diastolic and systolic blood pressure were measured after 15 min of supine rest. After a 10 h overnight fast subjects ingested, between 0800 and 1030 a.m, 500 ml of a standardized, manufactured liquid meal tolerance test (Boehringer Mannheim, Mannheim, Germany). The liquid formula, served at room temperature, had a nutritive value of 1017 kcal and consisted of 32.5 g protein, 75 g carbohydrates, 10 g alcohol, 55.5 g fat and 0.96 g cholesterol. The fat was composed of 31.7 g (57%) saturated, 17.2 g (31%) mono-unsaturated, 1.5 g (3%) poly-unsaturated and 5.1 g (9%) nonspecific fatty acids. To minimize intra-individual variation, every subject was asked to record diet, alcohol use, number of cigarettes smoked and physical activities on the day preceding the meal tolerance tests. The day preceding the second test (i. e. following treatment), subjects were asked to mimic the day preceding the first test as closely as possible. After ingestion of the meal, the subjects fasted for 6 h but were permitted to drink water ad libitum. Throughout the test period, physical activity was minimized. Peripheral venous blood samples were collected before and 5 and 6 h following ingestion of the standardized meal. As an estimate of the mean postprandial level, the 5 and 6 h levels were averaged.

Metabolic Measurements

Blood samples for the determination of total FVII and active FVII were collected into siliconized tubes (Vacutainer, Becton Dickinson) containing 0.13 mmol/L trisodium citrate. The samples were centrifuged immediately at 3,500 RPM for ten min at ambient temperature. Then the plasma was ultracen-

Table 2 Plasma levels of total and active FVII under fasting conditions and following a fat-rich meal, before and following an 8 week treatment period with either placebo or 17β-estradiol

		Before treatment			Following treatment		
		actual value		mean difference	actual value		mean difference
fasting total FVII (%)	Placebo	83 ± 16	(13)	-	83 ± 13	(12)	-
	E2	92 ± 13	(11)	9 (-3; 21) §	112 ± 18	(12)	28 (15; 42) § ** †
pp. total FVII (%)	Placebo	85 ± 19	(12)	-	83 ± 16	(13)	-
	E2	93 ± 12	(12)	7 (-6; 21) §	110 ± 20	(11)	27 (11; 42) § * †
fasting active FVII (ng/mL)	Placebo	0.35 ± 0.26	(13)	-	0.37 ± 0.26	(12)	-
	E2	0.31 ± 0.11	(12)	-0.05 (-0.21; 12)	0.38 ± 0.14	(12)	0.01 (-0.17; 0.18)
pp. active FVII (ng/mL)	Placebo	0.48 ± 0.30	(10)	-	0.49 ± 0.36	(11)	-
	E2	0.54 ± 0.22	(12)	0.06 (-0.17; 0.29)	0.57 ± 0.23	(11)	0.08 (-0.18; 0.35)

For the actual values, values are means ± SD (n); for the differences in the E2 group versus the Placebo group, values are means (95% CI). E2: 17β-estradiol; pp.: postprandial (average level at 5 and 6 hours postprandially). §: % points; *: $P = 0.001$, E2 vs Placebo; **: $P < 0.001$, E2 vs Placebo; †: $P < 0.01$, mean difference after versus before treatment.

Table 3 Effect of a fat-rich meal on the plasma level of total and active FVII in 25 healthy postmenopausal women treated with either 17β-estradiol or placebo

		Before treatment			Following treatment		
		% change		mean difference	% change		mean difference
total FVII (%)	Placebo	4 (-6; 14)	(12)	-	-1 (-12; 10)	(12)	-
	E2	2 (-9; 13)	(11)	-2 (-16; 12)	-1 (-8; 7)	(11)	0.5 (-12; 13)
active FVII (ng/mL)	Placebo	36 (8; 65)	(10)	-	22 (3; 41)	(10)	-
	E2	77 (42; 113)	(12)	41 (-1; 84)	54 (20; 88)	(11)	32 (-5; 69)

Values are mean percentages of change from the fasting level to the averaged postprandial level at 5 and 6 hours (95% CI) (n). E2: 17β-estradiol.

trifuged at 11,000 RPM for 3 min. Without delay, aliquots of plasma were transferred to plastic tubes, snap-frozen (plasma for determination of active FVII only) and stored at -80° C until analysis. At the time of assay, plasma samples were transferred to a water-bath at 37° C for 5 min and then handled at room temperature. All samples from one subject were analyzed in random order using a single batch. Total FVII levels were determined in a single run using a chromogenic method (Coaset FVII), according to the manufacturers' instructions (Chromogenix, Mölndal, Sweden). The intra-assay coefficient of variation (CV) was < 5.4%. Active plasma FVII levels were assayed in two runs, using a truncated recombinant human tissue factor, as described by Morrissey and colleagues (13). The lower limit of detection was 0.20 ng/mL; the intra-and interassay CV were < 11%.

The serum level of estradiol (E2) was measured by a double antibody radioimmunoassay (RIA) (Sorin Biomedica, Saluggia, Italy). The lower limit of detection was 18 pmol/L, the intra- and interassay CV were 4 and 11%, respectively. The serum level of FSH was measured by a luminiscense immunometric assay (Amerlite, Amersham, United Kingdom), intra- and interassay CV 5%. Serum creatinine, alanine aminotransferase and alkaline phosphatase were measured using standard laboratory methods, and plasma glucose was measured using a glucose hexokinase method. Serum levels of total cholesterol (t-chol) and triglycerides (TG) were measured by standard enzymatic colorimetric assays. The serum level of high-density lipoprotein cholesterol (HDL-c) was measured after precipitation of the apo B-containing lipoproteins with phosphotungstic acid/magnesium chloride (Boehringer Mannheim, Mannheim, Germany) and the serum level of low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula.

Statistics

Data are given as mean ± SD. Variables with a skewed distribution (E2 and TG) were logarithmically transformed to normalize their distributions. For these variables, data are given as the geometric mean with the 95% CI. To compare differences within one treatment group, paired t-tests were used. To compare differences between the placebo and 17β-estradiol group, unpaired t-tests were used. The plasma level of active FVII was below the detection limit of 0.20 ng/mL in 13 of the 150 samples. For samples collected under fasting conditions, undetectable levels of active FVII (n = 5) were set at 0.10 ng/mL. As following ingestion of a fat-rich meal, active FVII is known to increase, for samples collected 5 and/or 6 h postprandially, undetectable levels of active FVII were omitted. No undetectable postprandial plasma active FVII levels were found in subjects with a fasting level of active FVII > 0.20 ng/mL. Pearson correlation analysis was used to examine whether a therapy-associated change in the fasting level of total FVII was related to a therapy-associated change in the fasting level of active FVII. To adjust for possible confounders, ANOVAs and partial correlation analyses were performed with either smoking status, age, the plasma E2 level, plasma FSH level, or the BMI as a covariate. Statistical significance was defined as a two-tailed P-value of P < .05. All analyses were performed using the Statistical Package for the Social Sciences (SPSS / windows 6.1).

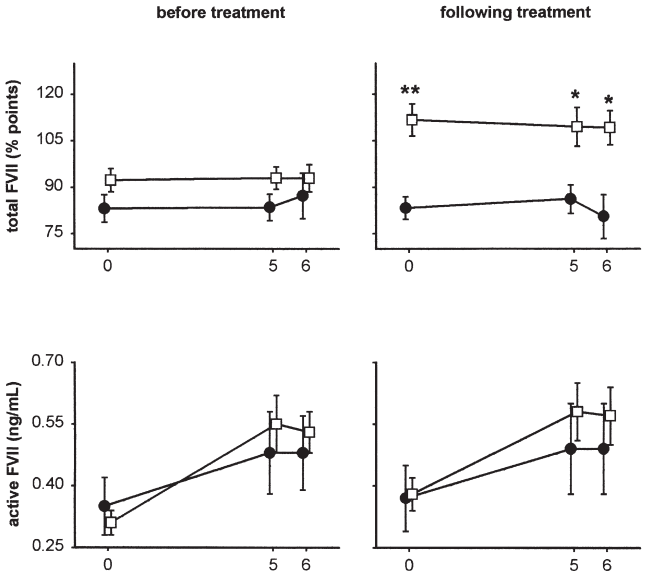

Fig. 1 Mean (SEM) plasma levels of total FVII and active FVII in 25 healthy postmenopausal women, before and 5 and 6 hours following ingestion of a standardized fat-rich meal, before and following an 8 weeks' treatment period with either 17β-estradiol (□) or placebo (●). Unpaired t-tests are used to test for significances between the 17β-estradiol and placebo group. **: P < 0.001; *: P < 0.01

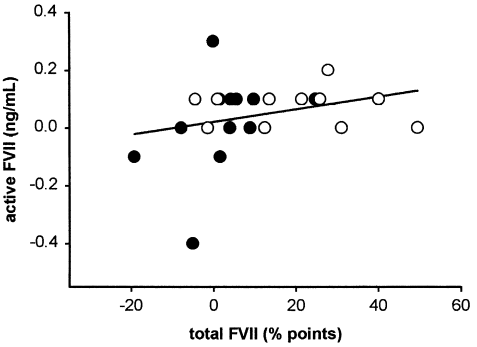

Fig. 2 Correlation between the change in the fasting plasma levels of total FVII and active FVII, following an 8 weeks' treatment period with either 17β-estradiol (□) or placebo (●). Pearson coefficient of correlation: r = 0.27, P = 0.21

Results

One participant dropped out of the study because of a hospital admission for varicose veins. Data in this study are therefore based on 25 women. Before therapy, there were no significant differences between the placebo (n = 13) and 17β-estradiol (n = 12) group in any of the general characteristics (Table 1). Also, there were no significant differences between the two groups in the total FVII and/or active FVII levels, neither under fasting conditions, nor following provocation by the fat-rich meal (Table 2, Fig. 1). The averaged percentual postprandial increase in the active FVII level was less pronounced in the placebo group than in the 17β-estradiol group, both before and following treatment. These differences, however, did not reach statistical significance (Table 3).

As compared to placebo, 17β-estradiol increased the mean level of E2 by 410 pmol/L (95% CI 169; 650 pmol/L, P = 0.003), increased the mean TG level by 0.27 mmol/L (95% CI 0.04; 0.50 mmol/L, P = 0.02) and decreased the mean level of LDL-c by 0.57 mmol/L (95% CI -0.91; -0.23, P = 0.002).

Ingestion of the fat-rich meal did not affect the level of total FVII (Table 3, Fig. 1). Whereas before treatment there was no significant difference in the fasting and/or postprandial total FVII level, following treatment, both the fasting and postprandial total FVII level were significantly higher in the 17β-estradiol group than in the placebo group (P < 0.001 and P = 0.001, respectively) (Table 2, Fig. 1). In addition, the mean treatment-induced, absolute changes in the fasting and postprandial level of total FVII differed significantly between both groups (P < 0.01; Table 2).

Ingestion of a fat load increased the active FVII level (Table 3, Fig. 1). However, as in the pretreatment situation, following 8 weeks of treatment, the fasting and postprandial level of active factor VII did not differ significantly between both groups (Table 2). The mean treatment-induced, absolute changes in the fasting and postprandial level of total FVII did not differ significantly between both groups ($P > 0.35$). In addition, compared to placebo, 17β-estradiol did not affect the postprandial increase in the active FVII level (Table 3). Finally, in the 17β-estradiol group, as compared to placebo, the mean percentual change in the fasting level of total FVII (18% [95% CI 3; 33%]) tended to be higher than the mean percentual change in the fasting level of active FVII (-2% [95% CI -39; 34%]; $P = 0.07$).

Fig. 2 shows that the therapy-associated change in the fasting level of total FVII did not correlate significantly with the therapy-associated change in the fasting level of active FVII, either in the whole group ($r = 0.27$, $P = 0.21$), or in the 17β-estradiol or placebo groups separately ($r = -0.04$, $P = 0.90$ and $r = 0.40$, $P = 0.20$, respectively). Adjustment for age, the plasma FSH and E2 level, smoking status, or the BMI did not affect any of the results (data not shown). Finally, the procedure of testing (parametric versus nonparametric) did not materially affect the results (data not shown).

Discussion

This is the first prospective, placebo-controlled study on unopposed estrogen therapy and FVII activation. In a group of 25 healthy postmenopausal women, as compared to placebo, 8 weeks of oral 17β-estradiol treatment increased the fasting and postprandial plasma level of total FVII, but did not affect the fasting and/or postprandial plasma level of active FVII. Furthermore, the change in the fasting level of total FVII after therapy was not associated with the change in the fasting level of active FVII.

Although many observational studies have found postmenopausal HRT to reduce the risk of cardiovascular disease (1, 19), estrogen replacement is associated with an adverse effect on blood coagulation. In the first 4 to 6 months of treatment, especially, postmenopausal HRT increases the risk of venous thromboembolism (2, 19, 20). On the basis of results of clinical trials (2, 21), however, it may be hypothesized that estrogen therapy also increases the risk of arterial thromboembolism, at least in the short term. In the present study, we therefore examined the short-term effect of unopposed estrogen on FVII activation in healthy postmenopausal women. Activated FVII initiates the coagulation cascade and even small increases in the release of active FVII may lead to a significant formation of thrombin and therefore to an increased risk of arterial occlusion. However, only recently has an assay selective for active FVII become available (13). So far, most studies determined the coagulant activity of FVII (FVIIc), which reflects an assay-dependent part of active FVII, but also an unknown part of the FVII zymogen.

Data on the effect of postmenopausal HRT on FVII are conflicting and seem to be importantly affected by the regimen used. Only one study has prospectively investigated the effect of HRT on active FVII (22). In that study, 45 postmenopausal women received estradiol, either orally or transdermally, cyclically combined with a progestogen, or no treatment. Six months of treatment did not affect the plasma levels of FVII antigen, FVII coagulant activity and/or active FVII. Other studies that investigated the effects of combined estrogen/progestogen regimens have shown either a decrease (23–27) or no effect (25, 26, 28) on the plasma FVII antigen and/or FVII coagulant activity levels. Most, although not all (29), studies on the effect of unopposed estrogen replacement, however, have shown an increase in the plasma FVII antigen and/or FVII coagulant activity level (28, 30, 31). In the present study, we found that unopposed 17β-estradiol increased the plasma level of total FVII but did not affect the plasma level of active FVII. In addition, the increase in the level of total FVII was not associated with a change in the level of active FVII. These results therefore suggest that the estrogen-associated increase in the level of total FVII is not accompanied by an increased activation of FVII.

Ingestion of a fat-rich meal transiently stimulates FVII activation (14–18). Therefore, we additionally examined the effect of 17β-estradiol on the postprandial total FVII and active FVII response. In agreement with previous studies (18), ingestion of the standardized fat-rich meal did not affect the 5 and 6 hour level of total FVII but substantially increased the 5 and 6 hour level of active FVII. Administration of 17β-estradiol, however, did not affect these fat-load-stimulated total FVII and/or active FVII responses. As most of our lives are spent in a postprandial state, it is of interest that the estrogen-induced increase in the total FVII plasma level was not accompanied by an increased in vivo FVII activation either in the fasting or the postprandial state.

We did not examine polymorphisms of the gene coding for FVII, which may be a limitation of our study. The FVII genotype has been associated with FVII plasma levels (32–34). Goal of our study, however, was to examine the effect of unopposed estrogen on FVII activation. So far, it has not been established whether polymorphisms of the FVII gene modulate the effect of 17β-estradiol. Nevertheless, we cannot entirely exlude the possibility that our results are affected by differences in the proportion of FVII polymorphisms between the placebo and 17β-estradiol group.

In conclusion, in this short-term, double-blind and placebo-controlled study in a group of healthy postmenopausal women, 17β-estradiol increased the level of total FVII, both in the fasting state as well as following stimulation by a standardized fat-rich meal. In contrast, 17β-estradiol did not affect the fasting and postprandial level of active FVII. Longterm data are needed to investigate the relevance of active FVII to the prediction of CHD risk. However, our results suggest that, although 17β-estradiol increases the level of total FVII, this increase is not accompanied by an increased FVII activation. Therefore, these findings argue against the idea that elevated levels of total FVII underlie an increased risk of arterial thromboembolism in postmenopausal women using unopposed estrogen replacement.

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