

EFFECTS OF HORMONE-REPLACEMENT THERAPY ON FIBRINOLYSIS IN POSTMENOPAUSAL WOMEN

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ABSTRACT

Background Plasma levels of plasminogen-activator inhibitor type 1 (PAI-1), an essential inhibitor of fibrinolysis in humans, increase in women after menopause, and this may contribute to the risk of cardiovascular disease. We studied the effects of hormone-replacement therapy on PAI-1 levels.

Methods In a randomized, crossover study, we investigated the effects of oral conjugated estrogen (0.625 mg per day) in 30 postmenopausal women and transdermal estradiol (0.1 mg per day) in 20 postmenopausal women, either alone or in combination with medroxyprogesterone acetate (2.5 mg daily) for one month, on plasma PAI-1 antigen levels. Degradation products of cross-linked fibrin (D-dimer) were measured in serum as an index of fibrinolysis.

Results PAI-1 levels were inversely associated with D-dimer levels at base line ($r = -0.540$, $P = 0.002$). Conjugated estrogen, both alone and in combination with medroxyprogesterone acetate, reduced mean (\pm SD) plasma levels of PAI-1 from 32 ± 34 ng per milliliter to 14 ± 10 ng per milliliter ($P < 0.001$) and from 31 ± 29 ng per milliliter to 15 ± 11 ng per milliliter ($P = 0.003$), respectively; there was a significant inverse correlation between pretreatment PAI-1 levels and the degree of reduction in these levels during therapy ($r = -0.631$, $P < 0.001$ for conjugated estrogen; $r = -0.507$, $P = 0.004$ for combined therapy). The degree of reduction in PAI-1 levels was associated with increases in D-dimer levels both when conjugated estrogen was given alone ($r = -0.572$, $P = 0.001$) and when combined hormone therapy was given ($r = -0.541$, $P = 0.002$). Transdermal estradiol caused no significant changes in PAI-1 levels from base-line values.

Conclusions Conjugated estrogen, alone or combined with progestin therapy, reduced PAI-1 levels by approximately 50 percent in postmenopausal women and was associated with enhanced systemic fibrinolysis. These findings may partly explain the protective effect of hormone-replacement therapy with respect to coronary artery disease. (N Engl J Med 1997;336:683-90.)

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CARDIOVASCULAR disease results in more deaths among women in the United States than any other disease. Several prospective, observational studies have suggested that postmenopausal women who take estrogen have a lower rate of cardiovascular events than untreated women.^{1,2} The estrogen compound used most often

by women in these studies was a preparation of conjugated estrogen from equine sources. Orally administered estrogens raise the levels of high-density lipoprotein (HDL) cholesterol and lower the levels of low-density lipoprotein (LDL) cholesterol,^{3,4} producing a lipid profile similar to that of premenopausal women. However, the effects of estrogen on lipoproteins may not be solely responsible for the cardiovascular benefits of estrogen therapy.⁵

Plasminogen-activator inhibitor type 1 (PAI-1), an essential antagonist of fibrinolysis in humans, rapidly and specifically inhibits both tissue plasminogen activator and urokinase plasminogen activator.⁶ PAI-1 has been shown by immunohistochemical analysis and in situ hybridization to be present in endothelial and smooth-muscle cells of histologically normal arteries; increased quantities are present in all cellular components of atheromatous arteries.⁷ Several studies have found an association between increased plasma levels of PAI-1 and a higher risk of atherosclerosis and its ischemic manifestations.⁸⁻¹⁶ Higher levels of PAI-1 were noted in postmenopausal women than in premenopausal women in the Framingham Offspring Study¹⁷; this increased level may in part account for the increasing risk of atherosclerosis and its clinical consequences after menopause.

Several studies outside the United States have found that estrogen therapy reduces PAI-1 levels in postmenopausal women¹⁸⁻²⁰; evidence of the differential effects of estrogen alone and estrogen combined with a progestin and confirmatory evidence of the associated enhancement of fibrinolysis have not been studied, however. In contrast to these findings, activation of coagulation pathways has been detected in postmenopausal women treated with conjugated estrogen.²¹ Furthermore, three recent studies reported a higher risk of venous thromboembolism in postmenopausal women receiving hormone therapy than in nonusers of estrogen.²²⁻²⁴ We therefore examined the effect of hormone-replacement therapy on fibrinolysis in post-menopausal women, comparing different routes of administration and compar-

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ing the effects of estrogen alone with those of estrogen combined with a progestin compound. The study was randomized, with a crossover design, and the personnel performing the laboratory assays were unaware of the patients' identity and the sequence of the studies.

METHODS

Study Population and Design

Two groups of healthy postmenopausal women participated in this study, all of whom had plasma 17β -estradiol levels below 50 pg per milliliter (184 pmol per liter). The 30 women in the first group (mean [\pm SD] age, 55 ± 5 years) were randomly assigned to begin one month of treatment with either 0.625 mg of oral conjugated estrogen daily or 0.625 mg of conjugated estrogen and 2.5 mg of medroxyprogesterone acetate daily. After a one-month washout period, each woman received the other therapy for one month. The 20 women in the second group (age, 56 ± 5 years) were randomly assigned to begin one month of treatment with either 0.1 mg of transdermal estradiol daily or 0.1 mg of estradiol and 2.5 mg of medroxyprogesterone acetate daily, with a one-month washout period before receiving the other therapy for one month. None of the women had taken any cholesterol-lowering drugs, estrogen, or antioxidant-vitamin supplements during the two months preceding the study. The study was approved by the institutional review board of the National Heart, Lung, and Blood Institute, and all participants gave written informed consent.

Laboratory Assays

Both before and at the end of each one-month treatment period, blood samples for laboratory assay were obtained from an antecubital vein between 8 a.m. and 9 a.m. after an overnight fast; the samples were immediately coded so that the investigators performing the laboratory assays would be blinded to the women's identity and the sequence of treatments. Total cholesterol and triglycerides in the serum were quantified by automated enzymatic techniques. HDL cholesterol was measured after other lipoproteins were precipitated with dextran sulfate. LDL cholesterol levels were estimated with the formula of Friedewald et al.²⁵ Serum lipoprotein(a) was measured by an immunoturbidimetric assay (Incstar, Stillwater, Minn.) with a limit of detection of 5.8 mg per deciliter.²⁶ Plasma estrone and 17β -estradiol levels were measured by radioimmunoassay. PAI-1 antigen levels were determined by a sandwich enzyme-linked immunosorbent assay (Biopool, Ventura, Calif.) according to the method of Declerck et al.²⁷

After we determined that oral estrogen therapy significantly reduced PAI-1 levels, we assessed the hemostatic importance of this finding by measuring serum levels of D-dimer, a product of the degradation of cross-linked fibrin by plasmin, with use of an enzyme-linked immunosorbent assay (Asserachrom D-Di, Diagnostica Stago, Asnieres-sur-Seine, France). Serum insulin levels were measured with a microparticle enzyme immunoassay on an IMx instrument (Abbott Laboratories, Abbott Park, Ill.).

The oxidation of LDL isolated from plasma by sequential ultracentrifugation was measured, after the addition of copper chloride (final concentration, $5\ \mu\text{mol}$ per liter), with use of a spectrophotometric technique similar to that described by Esterbauer et al.,²⁸ as reported elsewhere.²⁹ In order to assess the reproducibility of this assay, we isolated LDL from plasma in seven paired blood samples and performed measurements of the oxidation of LDL in all samples. The correlation coefficients for the length of time to the start of oxidation and the maximal rate of oxidation in the paired samples were 0.980 and 0.967, respectively.

Statistical Analysis

Data are expressed as means \pm SD. Paired t-tests were used to compare values determined to be normally distributed before and after each treatment and the changes in those values in response

to treatment. Unpaired t-tests were used to compare the levels of PAI-1 in women with levels of lipoprotein(a) below the level of detection in our assay (5.8 mg per deciliter) with those in women with measurable lipoprotein(a) levels before and after each treatment; we also compared the changes in these levels in response to treatment. Pearson correlation-coefficient analysis was used to assess the associations between values determined to be normally distributed. The Wilcoxon signed-rank test and Spearman correlation-coefficient analysis were used when the data were determined not to be normally distributed. The three comparisons of the effects of unopposed estrogen and combined hormone therapy on PAI-1 levels, on the length of time to the beginning of oxidation of LDL, and on lipoprotein(a) levels were designated as primary comparisons before the study began. All other comparisons were considered secondary. Therefore, P values lower than the Bonferroni-adjusted α ($0.05 \div 3 = 0.017$) were considered to indicate statistical significance with respect to the primary hypotheses. No adjustments were made for secondary hypotheses.

RESULTS

Base-line values in the treatment groups before each treatment period were compared, and no significant differences were found (Tables 1 and 2). To assess the possibility of a carryover effect from the initial treatment phase to the next treatment phase, we compared the base-line values before the first treatment period with those before the second treatment period. No significant differences were found.

Effects of Treatment with Conjugated Estrogen

After one month of therapy with oral conjugated estrogen alone, plasma levels of estrone increased from 24 ± 12 pg per milliliter (89 ± 44 pmol per liter) at base line to 130 ± 76 pg per milliliter (481 ± 281 pmol per liter); plasma levels of 17β -estradiol increased from 20 ± 13 pg per milliliter (73 ± 48 pmol per liter) to 75 ± 30 pg per milliliter (275 ± 110 pmol per liter) ($P<0.001$ for both comparisons; Table 1). Conjugated estrogen lowered LDL cholesterol levels by 14 ± 9 percent and increased HDL cholesterol levels by 19 ± 13 percent ($P<0.001$ for both comparisons with pretreatment values). Conjugated estrogen decreased lipoprotein(a) levels from 29.9 ± 22.5 mg per deciliter to 26.7 ± 20.2 mg per deciliter ($P=0.07$) in the 22 women in whom lipoprotein(a) values could be measured (i.e., those with levels ≥ 5.8 mg per deciliter). After one month of therapy with both conjugated estrogen and medroxyprogesterone acetate, plasma levels of estrone increased from 22 ± 12 pg per milliliter (81 ± 44 pmol per liter) to 113 ± 60 pg per milliliter (418 ± 222 pmol per liter); plasma levels of 17β -estradiol increased from 16 ± 7 pg per milliliter (59 ± 26 pmol per liter) to 68 ± 32 pg per milliliter (250 ± 117 pmol per liter) ($P<0.001$ for both comparisons). These levels were similar to those achieved with conjugated estrogen alone ($P=0.79$ and $P=0.66$, respectively). Combined therapy lowered LDL cholesterol levels by 13 ± 11 percent ($P<0.001$ for the comparison with pretreatment values), a reduction similar to that achieved with

TABLE 1. EFFECTS OF ORAL CONJUGATED ESTROGEN, ALONE OR IN COMBINATION WITH MEDROXYPROGESTERONE ACETATE.

VARIABLE	CONJUGATED ESTROGEN ALONE		COMBINATION THERAPY	
	BASE LINE	AFTER THERAPY	BASE LINE	AFTER THERAPY
mean \pm SD				
Hormones (pg/ml)*				
Estrone	24 \pm 12	130 \pm 76†	22 \pm 12	113 \pm 60†
17 β -estradiol	20 \pm 13	75 \pm 30†	16 \pm 7	68 \pm 32†
Lipids (mg/dl)‡				
Triglycerides	103 \pm 59	120 \pm 63†	110 \pm 69	108 \pm 54
Total cholesterol	231 \pm 36	224 \pm 32†	238 \pm 39	221 \pm 35†
HDL cholesterol	58 \pm 15	69 \pm 17†	60 \pm 16	65 \pm 15†§
LDL cholesterol	152 \pm 31	131 \pm 27†	155 \pm 33	134 \pm 29†
Lipoprotein(a)	29.9 \pm 22.5	26.7 \pm 20.2	32.2 \pm 22.8	25.6 \pm 16.1†
PAI-1 (ng/ml)	32 \pm 34	14 \pm 10†	31 \pm 29	15 \pm 11†
D-dimer (ng/ml)	441 \pm 501	551 \pm 579	530 \pm 654	503 \pm 460
Insulin (μ U/ml)¶	10.4 \pm 9.6	8.1 \pm 4.6	8.3 \pm 6.2	8.7 \pm 6.2
LDL oxidation				
Lag time (min)	74 \pm 9	76 \pm 10	76 \pm 11	75 \pm 12
Maximal rate (change in OD/hr)	1.05 \pm 0.15	1.03 \pm 0.17	1.07 \pm 0.19	1.02 \pm 0.15

*To convert values for estrone to picomoles per liter, multiply by 3.699; to convert values for 17 β -estradiol to picomoles per liter, multiply by 3.671.

†P<0.05 for the comparison with the base-line value.

‡To convert values for triglycerides to millimoles per liter, multiply by 0.01129; to convert values for cholesterol to millimoles per liter, multiply by 0.02586.

§P<0.05 for the comparison with the value after therapy with conjugated estrogen alone.

¶To convert values for insulin to picomoles per liter, multiply by 6.945.

||OD denotes optical density.

TABLE 2. EFFECTS OF TRANSDERMAL ESTRADIOL, ALONE OR IN COMBINATION WITH MEDROXYPROGESTERONE ACETATE.

VARIABLE	ESTRADIOL ALONE		COMBINATION THERAPY	
	BASE LINE	AFTER THERAPY	BASE LINE	AFTER THERAPY
mean \pm SD				
Hormones (pg/ml)*				
Estrone	25 \pm 15	86 \pm 43†	27 \pm 16	61 \pm 25†
17 β -Estradiol	15 \pm 9	117 \pm 49†	15 \pm 8	116 \pm 54†
Lipids (mg/dl)‡				
Triglycerides	102 \pm 62	96 \pm 47	100 \pm 49	93 \pm 41
Total cholesterol	227 \pm 33	222 \pm 32	231 \pm 28	218 \pm 26†
HDL cholesterol	62 \pm 16	62 \pm 13	62 \pm 16	58 \pm 13
LDL cholesterol	145 \pm 28	141 \pm 26	149 \pm 24	141 \pm 23
Lipoprotein(a)	26.5 \pm 20.3	28.0 \pm 24.8	27.4 \pm 22.5	29.3 \pm 22.2
PAI-1 (ng/ml)	20 \pm 12	22 \pm 12	17 \pm 11	19 \pm 14
LDL oxidation				
Lag time (min)	76 \pm 13	84 \pm 15†	78 \pm 16	84 \pm 16
Maximal rate (change in OD/hr)§	1.09 \pm 0.19	1.05 \pm 0.21	1.05 \pm 0.22	1.02 \pm 0.27

*To convert values for estrone to picomoles per liter, multiply by 3.699; to convert values for 17 β -estradiol to picomoles per liter, multiply by 3.671.

†P<0.05 for the comparison with the base-line value.

‡To convert values for triglycerides to millimoles per liter, multiply by 0.01129; to convert values for cholesterol to millimoles per liter, multiply by 0.02586.

§OD denotes optical density.

conjugated estrogen alone ($P=0.78$). Combined therapy increased HDL cholesterol levels by an average of 10 ± 12 percent from pretreatment values ($P<0.001$), a lesser degree than conjugated estrogen alone ($P=0.004$). Combined therapy decreased lipoprotein(a) levels from 32.2 ± 22.8 mg per deciliter to 25.6 ± 16.1 mg per deciliter ($P=0.02$) in the 21 women with measurable values at base line. After one month of conjugated estrogen or combined therapy, there was no increase over pretreatment values in the length of time to the beginning of oxidation of LDL or the maximal rate (Table 1).

After one month of therapy with conjugated estrogen, plasma levels of PAI-1 decreased from 32 ± 34 ng per milliliter at base line to 14 ± 10 ng per milliliter ($P<0.001$) (Fig. 1). After one month of therapy with conjugated estrogen combined with medroxyprogesterone acetate, plasma levels of PAI-1 decreased from 31 ± 29 ng per milliliter to 15 ± 11 ng per milliliter ($P=0.003$). There were no significant differences in the effects of therapy with conjugated estrogen and combined therapy on PAI-1 levels ($P=0.508$) or in the degree of reduction in PAI-1 levels from base line ($P=0.952$). During both therapy with conjugated estrogen and combined therapy, there were significant inverse correlations between pretreatment PAI-1 levels and the degree of change in those levels after treatment ($r=-0.631$, $P<0.001$; and $r=-0.507$, $P=0.004$,

respectively). There were no correlations between the degree of increase in estrone or estradiol levels and the degree of change in PAI-1 levels during conjugated estrogen therapy ($r=-0.018$ for estrone and $r=-0.088$ for estradiol) or during combined therapy ($r=0.127$ and $r=0.133$, respectively). The degree of change in the PAI-1 levels correlated inversely but weakly with the degree of change in HDL cholesterol levels during conjugated estrogen therapy ($r=-0.423$, $P=0.02$), but not during combined therapy ($r=-0.156$). The relative changes in PAI-1 levels correlated weakly with the relative changes in LDL cholesterol levels during combined therapy ($r=0.370$, $P=0.044$), but not during treatment with conjugated estrogen alone ($r=-0.096$). The degree of change in PAI-1 levels did not correlate with the degree of change in triglyceride levels during treatment with conjugated estrogen ($r=0.159$) or combined therapy ($r=0.086$).

The 22 women with lipoprotein(a) levels ≥ 5.8 mg per deciliter before treatment with conjugated estrogen had significantly lower base-line PAI-1 levels than the 8 women with undetectable lipoprotein(a) levels (19.8 ± 12.8 vs. 66.0 ± 51.1 ng per milliliter, $P<0.001$). The degree of reduction in PAI-1 levels in these 22 women during therapy with conjugated estrogen was less than the reduction in the 8 women with undetectable lipoprotein(a) levels at base line (20.2 ± 40.7 percent vs. 51.0 ± 40.0 per-

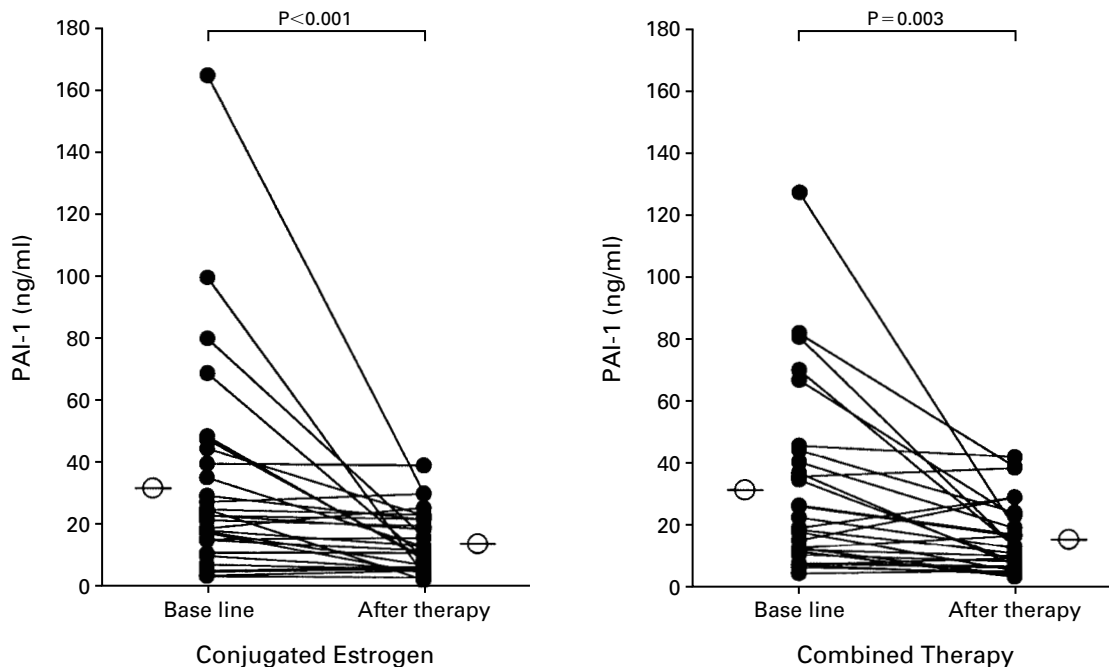


Figure 1. Changes in Plasma Levels of PAI-1 before and after Therapy with Oral Conjugated Estrogen and before and after Combined Therapy with Conjugated Estrogen and Medroxyprogesterone Acetate.

Mean values are identified by open circles.

cent), although the difference was not statistically significant ($P=0.08$). The 21 women with lipoprotein(a) levels ≥ 5.8 mg per deciliter before combined therapy had slightly lower base-line PAI-1 levels than the 9 women with undetectable lipoprotein(a) levels (24.3 ± 22.3 vs. 45.6 ± 38.3 ng per milliliter, $P=0.066$). The degree of reduction in PAI-1 levels in these 21 women during combined therapy was less than the reduction in the 9 women who had undetectable lipoprotein(a) levels at base line (27.1 ± 49.3 percent vs. 33.6 ± 37.5 percent), although the difference was not statistically significant ($P=0.73$). Changes from base line in PAI-1 levels correlated weakly with changes in lipoprotein(a) levels during treatment with conjugated estrogen alone ($r=0.373$, $P=0.088$), but not during combined therapy ($r=-0.264$, $P=0.247$).

Before the first treatment period, D-dimer levels in serum correlated inversely with PAI-1 levels ($r=-0.540$, $P=0.002$), but not with lipoprotein(a) levels ($r=-0.186$, $P=0.322$). After therapy with conjugated estrogen alone, the degree of reduction in PAI-1 levels correlated significantly with the degree of increase in D-dimer levels ($r=-0.572$, $P=0.001$) (Fig. 2). Similarly, after combined therapy, the degree of reduction in PAI-1 levels correlated significantly with the degree of increase in D-dimer levels ($r=-0.541$, $P=0.002$) (Fig. 2). There was no correlation between the degree of increase in D-dimer levels and the degree of decrease in lipoprotein(a) levels, whether the women received con-

jugated estrogen alone ($r=-0.263$, $P=0.246$) or combined therapy ($r=0.269$, $P=0.234$).

Before the first treatment period, insulin levels in serum correlated weakly with PAI-1 levels ($r=0.353$, $P=0.056$). After therapy with conjugated estrogen, the degree of reduction in PAI-1 levels also correlated weakly with the degree of decrease in insulin levels ($r=0.356$, $P=0.058$). No such association was observed during combined therapy ($r=-0.002$).

Effects of Treatment with Estradiol

After one month of therapy with transdermal estradiol, plasma levels of estrone increased from 25 ± 15 pg per milliliter (92 ± 55 pmol per liter) at base line to 86 ± 43 pg per milliliter (318 ± 159 pmol per liter); plasma estradiol levels increased from 15 ± 9 pg per milliliter (55 ± 33 pmol per liter) to 117 ± 49 pg per milliliter (430 ± 180 pmol per liter) ($P<0.001$ for both comparisons; Table 2). After one month of estradiol combined with medroxyprogesterone acetate, plasma levels of estrone increased from 27 ± 16 pg per milliliter (100 ± 59 pmol per liter) to 61 ± 25 pg per milliliter (226 ± 92 pmol per liter); plasma estradiol levels increased from 15 ± 8 pg per milliliter (55 ± 29 pmol per liter) to 116 ± 54 pg per milliliter (426 ± 198 pmol per liter) ($P<0.001$ for both comparisons) — levels similar to those achieved with estradiol alone ($P=0.166$ and $P=0.814$, respectively). Neither estradiol alone nor combined therapy caused a significant change in the level of any lipoprotein, except for a 5 percent

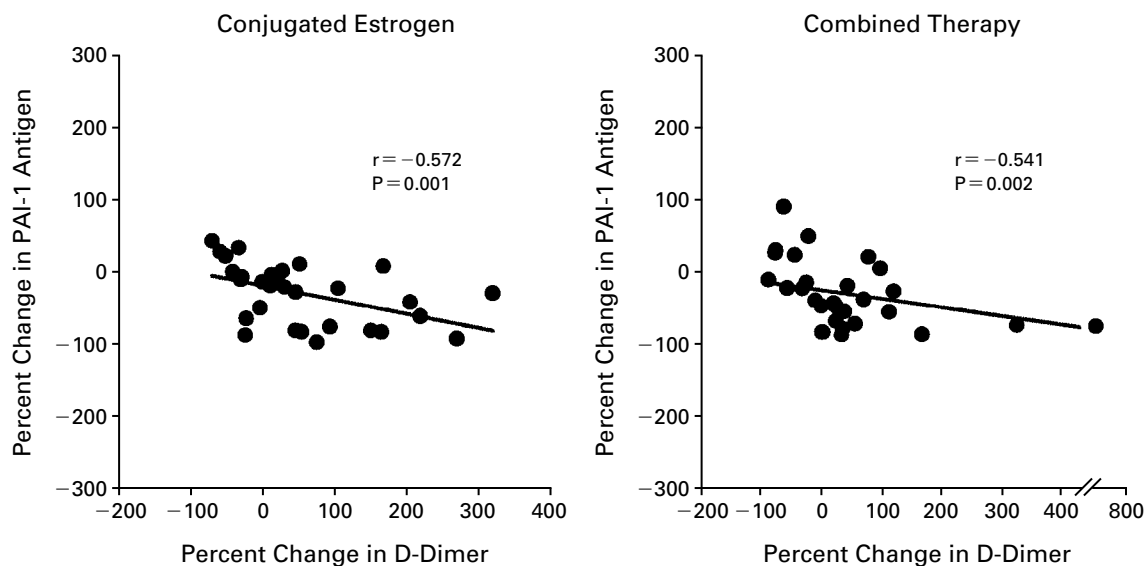


Figure 2. Scatter Plots Showing the Correlation between the Percent Change in PAI-1 Antigen Levels and the Percent Change in D-Dimer Levels after Therapy with Oral Conjugated Estrogen Alone and after Combined Therapy with Conjugated Estrogen and Medroxyprogesterone Acetate.

The line shows the predicted regression line.

reduction in total cholesterol with combined therapy (Table 2). Estradiol therapy prolonged the time to the onset of oxidation of LDL over the pretreatment value (from 76 ± 13 to 84 ± 15 minutes, $P=0.024$). Combined therapy slightly prolonged the time to the onset of oxidation (from 78 ± 16 to 84 ± 16 minutes, $P=0.084$). There was no significant change from base line in PAI-1 levels after therapy with transdermal estradiol alone ($P=0.608$) or combined therapy ($P=0.527$) (Table 2 and Fig. 3).

DISCUSSION

In our study, the daily administration of 0.625 mg of conjugated estrogen, either alone or in combination with 2.5 mg of medroxyprogesterone acetate, a commonly prescribed progestin, for one month to postmenopausal women had effects on LDL and HDL cholesterol levels that were similar to those in women who received the same treatments in the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial.³⁰ Conjugated estrogen reduced PAI-1 levels by 50 percent, with the greatest reduction occurring in women with the highest base-line PAI-1 levels. Despite concern that progestins might interfere with biologic effects of estrogen, as demonstrated for HDL cholesterol in the PEPI trial, the effect of conjugated estrogen on PAI-1 levels in our study was not diminished by the simultaneous administration of medroxyprogesterone acetate. With either type of hormone-replacement therapy, the degree of reduction in PAI-1 levels was significantly associated with the degree of increase in the levels of D-dimer,

a product of the degradation of cross-linked fibrin by plasmin, thus providing evidence of enhanced fibrinolysis. In contrast to oral therapy, the transdermal administration of estrogen did not change PAI-1 levels.

Reports of hormonal effects on PAI-1 from observational studies and from treatment trials conducted outside the United States have been contradictory. In the Framingham Offspring Study,¹⁷ postmenopausal women who received estrogen therapy had lower levels of PAI-1 antigen than those who did not receive therapy (13.0 vs. 19.5 ng per milliliter, $P<0.001$). The difference in PAI-1 levels between the women taking unopposed estrogen and those taking a combination of estrogen and progestin was of borderline significance after adjustment for the covariates (11.3 vs. 15.4 ng per milliliter, $P=0.04$). However, in the Atherosclerosis Risk in Communities Study,³¹ the levels of PAI-1 antigen were not significantly lower in current estrogen users than in nonusers. Scarabin et al.³² reported that 21 postmenopausal women receiving estrogen therapy, 19 of whom also took a progestin compound, had levels of PAI-1 activity that were lower, though not significantly so, than those in 99 postmenopausal women who were not taking estrogen therapy. Sporn et al.¹⁸ found that one month of therapy with the combination of estradiol (2 mg per day) and either norethindrone acetate or megestrol acetate reduced levels of PAI-1 activity by approximately 40 percent in 45 postmenopausal women. Van Wersch et al.¹⁹ showed that six months of treatment with a

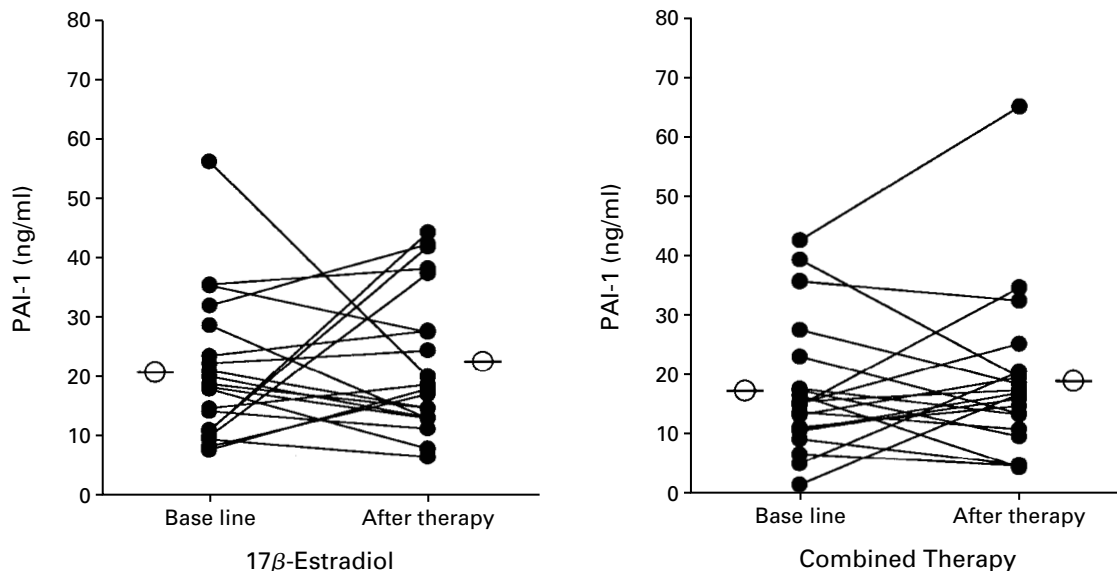


Figure 3. Changes in Plasma Levels of PAI-1 before and after Therapy with Transdermal Estradiol Alone and before and after Combined Therapy with Transdermal Estradiol and Medroxyprogesterone Acetate.

Mean values are identified by open circles.

synthetic steroid with estrogenic, progestogenic, and androgenic properties reduced levels of PAI-1 antigen from 68 to 47 ng per milliliter in 30 postmenopausal women. Kroon et al.²⁰ reported that 23 postmenopausal women taking 0.625 mg of conjugated estrogen per day for six weeks had about a 50 percent decrease in levels of PAI activity over pretreatment values (from 16.29 to 8.39 U per milliliter), whereas there was no change in the levels in 23 postmenopausal women treated with 0.05 mg of transdermal estradiol per day.

In order to investigate the mechanism of the effect of conjugated estrogen on PAI-1, we measured three variables that have been shown to stimulate the synthesis of PAI-1 and its release in endothelial-cell tissue cultures: lipoprotein(a) levels,³³ oxidation of LDL,³⁴ and insulin levels.³⁵ In our study, however, women with measurable levels of lipoprotein(a) actually had lower pretreatment PAI-1 levels than women in whom lipoprotein(a) could not be detected with our assay. Although conjugated estrogen, alone or combined with medroxyprogesterone acetate, reduced lipoprotein(a) levels by approximately 10 percent, we found no statistically significant or consistent correlation between the effects of these therapies on PAI-1 levels and lipoprotein(a) levels. In our study, PAI-1 levels were significantly reduced by the use of an estrogen that had no antioxidant effect (conjugated estrogen) and were unaffected by the use of estradiol, an estrogen that protects LDL from oxidation.^{29,36} We did find a weak correlation between insulin levels and PAI-1 levels at base line. However, fasting insulin levels did not change as a result of either therapy with conjugated estrogen or combined therapy; this finding is consistent with the results of the PEPI trial,³⁰ despite the significant reductions in PAI-1 levels. Furthermore, we did not find statistically significant or consistent correlations between the degree of reduction in PAI-1 levels and the degree of change in insulin levels during hormone therapy. Thus, in postmenopausal women, the effects on PAI-1 levels of conjugated estrogen, either alone or in combination with medroxyprogesterone acetate, may largely be independent of changes in factors that stimulate the endothelial release of PAI-1.

The observation that oral, but not transdermal, administration of estrogen reduced PAI-1 levels in both our study and that of Kroon et al.²⁰ suggests that the hepatic effects of estrogen regulate PAI-1 synthesis, clearance, or both in important ways. Several studies have shown that the liver is a major source of PAI-1.³⁷⁻³⁹ Thus, the presence of estrogen in sufficiently high concentrations in the portal circulation, after absorption from the gut, may inhibit the synthesis of PAI-1, although no mechanism for such an effect has been proposed. Alternatively, in vivo studies have shown that the liver is the chief organ responsible for the clearance of tissue plasmino-

gen activator-PAI-1 complexes,^{40,41} a process that is mediated by an LDL receptor-related protein that also binds α_2 -macroglobulin.⁴²⁻⁴⁴ The up-regulation of LDL receptors in association with oral estrogen use, which accounts in part for the reduction in LDL cholesterol levels,⁴⁵ may be associated with a comparable up-regulation or increased activity of the LDL receptor-related protein.

Although significant reductions in PAI-1 levels were detected in our study after oral hormone-replacement therapy, some women had no change in PAI-1 or D-dimer levels; this was especially true of those with relatively low pretreatment PAI-1 values. In these women, the procoagulant effects of hormone therapy, as reported previously from a study²¹ using the same estrogen preparation and dose of estrogen that were used in our study, may negate much of the cardiovascular benefit of hormone therapy or even increase the risk of thromboembolic events. Accordingly, a determination of the net hemostatic effects of estrogen may serve to identify women who are more or less likely to benefit from hormone-replacement therapy. This hypothesis will require prospective study.

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REFERENCES

1. Stampfer MJ, Colditz GA, Willett WC, et al. Postmenopausal estrogen therapy and cardiovascular disease: ten-year follow-up from the Nurses' Health Study. *N Engl J Med* 1991;325:756-62.
2. Stampfer MJ, Colditz GA. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. *Prev Med* 1991;20:47-63.
3. Rijkema AHM, van der Sanden AA, Ruijs AHC. Effects of postmenopausal oestrogen-progestogen replacement therapy on serum lipids and lipoproteins: a review. *Maturitas* 1990;12:259-85.
4. Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. *N Engl J Med* 1991;325:1196-204.
5. Bush TL, Barrett-Connor E, Cowan LD, et al. Cardiovascular mortality and noncontraceptive use of estrogen in women: results from the Lipid Research Clinics Program Follow-up Study. *Circulation* 1987;75:1102-9.
6. Sprengers ED, Kluff C. Plasminogen activator inhibitors. *Blood* 1987;69:381-7.
7. Lupu F, Bergonzelli GF, Heim DA, et al. Localization and production of plasminogen activator inhibitor-1 in human healthy and atherosclerotic arteries. *Arterioscler Thromb* 1993;13:1090-100.
8. Meade TW, Ruddock V, Stirling Y, Chakrabarti R, Miller GJ. Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. *Lancet* 1993;342:1076-9.
9. Salomaa V, Stinson V, Kark JD, Folsom AR, Davis CE, Wu KK. Association of fibrinolytic parameters with early atherosclerosis: the ARIC Study. *Circulation* 1995;91:284-90.
10. Olofsson BO, Dahlen G, Nilsson TK. Evidence for increased levels of plasminogen activator inhibitor and tissue plasminogen activator in plasma of patients with angiographically verified coronary artery disease. *Eur Heart J* 1989;10:77-82.
11. Ridker PM, Vaughan DE, Stampfer MJ, Manson JE, Hennekens CH. Endogenous tissue-type plasminogen activator and risk of myocardial infarction. *Lancet* 1993;341:1165-8.
12. Hamsten A, de Faire U, Walldius G, et al. Plasminogen activator in-

- hibitor in plasma: risk factor for recurrent myocardial infarction. *Lancet* 1987;2:3-9.
13. Cortellaro M, Cofrancesco E, Boschetti C, et al. Increased fibrin turnover and high PAI-1 activity as predictors of ischemic events in atherosclerotic patients: a case-control study. *Arterioscler Thromb* 1993;13:1412-7.
 14. Ridker PM, Hennekens CH, Stampfer MJ, Manson JE, Vaughan DE. Prospective study of endogenous tissue plasminogen activator and risk of stroke. *Lancet* 1994;343:940-3.
 15. Keber I, Keber D. Increased plasminogen activator inhibitor activity in survivors of myocardial infarction is associated with metabolic risk factors of atherosclerosis. *Haemostasis* 1992;22:187-94.
 16. Juhan-Vague I, Pyke SDM, Alessi MC, Jespersen J, Haverkate F, Thompson SG. Fibrinolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *Circulation* 1996;94:2057-63.
 17. Gebara OCE, Mittleman MA, Sutherland P, et al. Association between increased estrogen status and increased fibrinolytic potential in the Framingham Offspring Study. *Circulation* 1995;91:1952-8.
 18. Sporong T, Mattson LA, Samsioe G, Stigendal L, Hellgren M. Haemostatic changes during continuous oestradiol-progestogen treatment of postmenopausal women. *Br J Obstet Gynaecol* 1990;97:939-44.
 19. van Wersch JWJ, Ubachs JMH, van den Ende A, van Enk A. The effect of two regimens of hormone replacement therapy on the haemostatic profile in postmenopausal women. *Eur J Clin Chem Clin Biochem* 1994;32:449-53.
 20. Kroon U-B, Silfverstolpe G, Tengborn L. The effects of transdermal estradiol and oral conjugated estrogens on haemostasis variables. *Thromb Haemost* 1994;71:420-3.
 21. Caine YG, Bauer KA, Barzegar S, et al. Coagulation activation following estrogen administration to postmenopausal women. *Thromb Haemost* 1992;68:392-5.
 22. Daly E, Vessey MP, Hawkins MM, Carson JL, Gough P, Marsh S. Risk of venous thromboembolism in users of hormone replacement therapy. *Lancet* 1996;348:977-80.
 23. Jick H, Derby LE, Myers MW, Vasilakis C, Newton KM. Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopausal oestrogens. *Lancet* 1996;348:981-3.
 24. Grodstein F, Stampfer MJ, Goldhaber SZ, et al. Prospective study of exogenous hormones and risk of pulmonary embolism in women. *Lancet* 1996;348:983-7.
 25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.
 26. Levine DM, Sloan B-J, Donner JE, Lorenz JD, Heinzerling RH. Automated measurement of lipoprotein(a) by immunoturbidimetric analysis. *Int J Clin Lab Res* 1992;22:173-8.
 27. Declercq PJ, Alessi MC, Verstreken M, Kruithof EKO, Juhan-Vague I, Collen D. Measurement of plasminogen activator inhibitor 1 in biologic fluids with a murine monoclonal antibody-based enzyme-linked immunosorbent assay. *Blood* 1988;71:220-5.
 28. Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of in vitro oxidation of human low density lipoprotein. *Free Radic Res* 1989;6:67-75.
 29. Sack MN, Rader DJ, Cannon RO III. Oestrogen and inhibition of oxidation of low-density lipoproteins in postmenopausal women. *Lancet* 1994;343:269-70.
 30. The Writing Group for the PEPI Trial. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. *JAMA* 1995;273:199-208.
 31. Shahar E, Folsom AR, Salomaa VV, et al. Relation of hormone-replacement therapy to measures of plasma fibrinolytic activity. *Circulation* 1996;93:1970-5.
 32. Scarabin P-Y, Plu-Bureau G, Bara L, Bonithon-Kopp C, Guize L, Samama MM. Haemostatic variables and menopausal status: influence of hormone replacement therapy. *Thromb Haemost* 1993;70:584-7.
 33. Etingin OR, Hajjar DP, Hajjar KA, Harpel PC, Nachman RL. Lipoprotein (a) regulates plasminogen activator inhibitor-1 expression in endothelial cells: a potential mechanism in thrombogenesis. *J Biol Chem* 1991;266:2459-65.
 34. Kugiyama K, Sakamoto T, Misumi I, et al. Transferable lipids in oxidized low-density lipoprotein stimulate plasminogen activator inhibitor-1 and inhibit tissue-type plasminogen activator release from endothelial cells. *Circ Res* 1993;73:335-43.
 35. Schneider DJ, Sobel BE. Augmentation of synthesis of plasminogen activator inhibitor type 1 by insulin and insulin-like growth factor type 1: implications for vascular disease in hyperinsulinemic states. *Proc Natl Acad Sci U S A* 1991;88:9959-63. [Erratum, *Proc Natl Acad Sci U S A* 1992;89:1148.]
 36. Keaney JF Jr, Shwaery GT, Xu A, et al. 17 β -Estradiol preserves endothelial vasodilator function and limits low-density lipoprotein oxidation in hypercholesterolemic swine. *Circulation* 1994;89:2251-9.
 37. Kooistra T. The use of cultured human endothelial cells and hepatocytes as an in vitro model system to study modulation of endogenous fibrinolysis. *Fibrinolysis* 1990;4:Suppl 2:33-9.
 38. Quax PHA, van den Hoogen CM, Verheijen JH, et al. Endotoxin induction of plasminogen activator and plasminogen activator inhibitor type 1 mRNA in rat tissues in vivo. *J Biol Chem* 1990;265:15560-3.
 39. Sawdey MS, Loskutoff DJ. Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo: tissue specificity and induction by lipopolysaccharide, tumor necrosis factor- α , and transforming growth factor- β . *J Clin Invest* 1991;88:1346-53.
 40. Beebe DP, Aronson DL. Turnover of human tissue plasminogen activator (tPA) in rabbits. *Thromb Res* 1986;43:663-74.
 41. Korninger C, Stassen J-M, Collen D. Turnover of human extrinsic (tissue-type) plasminogen activator in rabbits. *Thromb Haemost* 1981;46:658-61.
 42. Bu G, Williams S, Strickland DK, Schwartz AL. Low density lipoprotein receptor-related protein/ α_2 -macroglobulin receptor is an hepatic receptor for tissue-type plasminogen activator. *Proc Natl Acad Sci U S A* 1992;89:7427-31.
 43. Orth K, Madison EL, Gething M-J, Sambrook JF, Herz J. Complexes of tissue-type plasminogen activator and its serpin inhibitor plasminogen activator inhibitor type 1 are internalized by means of the low density lipoprotein receptor-related protein/ α_2 -macroglobulin receptor. *Proc Natl Acad Sci U S A* 1992;89:7422-6.
 44. Nykjaer A, Petersen CM, Moller B, et al. Purified α_2 -macroglobulin receptor/LDL receptor-related protein binds urokinase-plasminogen activator inhibitor type-1 complex: evidence that the alpha 2-macroglobulin receptor mediates cellular degradation of urokinase receptor-bound complexes. *J Biol Chem* 1992;267:14543-6.
 45. Windler EE, Kovanen PT, Chao YS, Brown MS, Havel RJ, Goldstein JL. The estradiol-stimulated lipoprotein receptor of rat liver: a binding site that membrane mediates the uptake of rat lipoproteins containing apoproteins B and E. *J Biol Chem* 1980;255:10464-71.