The long-term effect of oral and percutaneous estradiol on plasma renin substrate and blood pressure

C. Hassager, M.D., B. J. Riis, M.D., V. Strøm, M.D., T. T. Guyene, M.D., and C. Christiansen, M.D.

ABSTRACT The long-term effect of percutaneous and oral estrogen replacement therapy on blood pressure, plasma renin substrate, and serum estrogens was examined in a 2 year placebo-controlled study with 110 early postmenopausal women. The women were allocated to four treatment groups: (1) oral cyclical combination of 2 mg estradiol valerate and cyproterone acetate, (2) oral placebo, (3) percutaneous 17β-estradiol, supplemented by 200 mg oral progesterone during the second year, or (4) percutaneous placebo cream. Systolic and diastolic blood pressure remained unchanged in both hormone treatment groups, whereas the diastolic blood pressure tended to increase in both placebo groups. Plasma renin substrate increased during oral treatment with estradiol, but remained unchanged with percutaneous estradiol. No correlation was found between blood pressure and plasma renin substrate. During percutaneous administration of estradiol, the serum concentrations of estrone and estradiol continued to rise after 3 months and reached a plateau at 6 months of therapy. Serum estrone but not estradiol showed the same pattern during oral estradiol therapy. No further changes in any of the measured variables were observed in the women treated with percutaneous estradiol after addition of cyclical oral progesterone. We conclude that both oral and percutaneous treatment with estradiol may provide protection against the age-related increase in diastolic blood pressure observed in early postmenopausal women, and that the metabolic steady state is not attained until after 3 months of estradiol therapy.


ESTROGEN REPLACEMENT therapy effectively relieves menopausal subjective symptoms\(^1\) and prevents osteoporosis in postmenopausal women.\(^2\) Unopposed estrogen therapy increases the risk of endometrial cancer,\(^3\) whereas combined estrogen/gestagen therapy may eliminate this risk.\(^4\) The metabolic side effects of estrogen therapy are influenced by the type\(^5\) and dosage\(^6\) of estrogen used and by the route of administration.\(^7\)

Oral administration of estrogen is characterized by a considerable conversion of estradiol to estroge\(^n\)\(^8\) and a first-pass liver effect with induction of the hepatic protein synthesis.\(^5-7,\)\(^9\) This alteration in liver metabolism results in changes in the serum concentration of renin substrate, clotting factors and inhibitors, and high- and low-density lipoproteins, which, respective-ly, may influence the risk of hypertension\(^10\) and thromboembolic disease.\(^7,\)\(^9,\)\(^11\) Some of the suggested side effects of oral estrogen therapy. The change in serum lipoproteins seems, on the other hand, to be beneficial.\(^12\)

Percutaneous administration of estrogen avoids the first-pass liver effect and no induction of the hepatic protein synthesis seems to occur within the first 2 months of replacement therapy.\(^7\) However, in another part of the present long-term placebo-controlled study we recently demonstrated that after 6 months of treatment there was a measurable change in serum lipoproteins.\(^13\)

The aim of the present placebo-controlled study was to examine the long-term effect of percutaneous and oral administration of estradiol on blood pressure and plasma renin substrate, a sensitive marker of estrogenic actions on the liver.\(^7\)

Materials and methods

Subjects. This study was part of a large double-blind clinical controlled trial carried out at Glostrup Hospital from June 1983 to December 1985. The participants were selected as follows. Questionnaires were sent to all women in Copenhagen county
who were 45 to 54 years old; 7484 of 9836 were returned. From information about the last menstrual bleeding, earlier gynecologic operations, and drug intake, 558 women fulfilled the following entry criteria: (1) spontaneous cessation of menstrual bleeding within the proceeding 0.5 to 3 years, and (2) no treatment with drugs known to influence calcium or liver metabolism. The 558 women were invited to a meeting and 397 attended. After receiving complete information about the trial 293 gave their written consent to participate (Helsinki Declaration II). Their clinical history was taken and they were given a medical examination including a gynecologic examination with cytologic test, breast palpation, and measurement of resting supine blood pressure, and blood was sampled.

Two hundred seventy women entered the trial. All were free of past or present diseases known to influence calcium or liver metabolism or to contraindicate the medications used in the trial. By random numbers the participants were then allocated to one of the five treatment types, and thereafter, also by random numbers, they were further allocated to either active or placebo treatment. Here we report on data from four of the 10 treatment groups (n = 133). The first two groups received, blindly, either a cyclic combination of estrogen and cyproterone acetate (oral E2),* or a placebo tablet (oral placebo). The two other groups blindly received either percutaneous estradiol (percutaneous E2),† or placebo cream (percutaneous placebo). The code for the two percutaneous groups was broken at the end of the first year of treatment; all the women receiving percutaneous E2 continued with an oral supplement of 200 mg of micronized progesterone‡ from days 13 to 24. The three other groups continued as before. The study design was thus double-blind throughout the study for the oral drug groups. For the percutaneous drug groups, it was double-blind during the first year and single-blind during the second year. The technicians recording blood pressure were always blinded. All medication was taken in the morning. The participants were examined every 3 months for 2 years. The trial was approved by the Ethical Committee of Copenhagen County.

Trial compliance. Of the 133 women in the four groups reported here who entered the study, 110 completed the 2-year trial (83%). Five women in the oral E2 group dropped out (lack of time, n = 2; moving out of the area, n = 1; gain in weight, n = 1; development of varicose veins, n = 1), and six in the oral placebo group dropped out (lack of time, n = 2; unacceptable clinical symptoms, n = 4). In the percutaneous E2 group nine women dropped out (lack of time, n = 3; irregular bleeding, n = 4; aggravation of migraine, n = 1; skin irritation, n = 1), and in the percutaneous placebo group three women dropped out (lack of time, n = 2; development of thyrotoxicosis, n = 1). This left 32 women in the oral E2 group, 33 in the oral placebo group, 20 in the percutaneous E2 group, and 25 in the percutaneous placebo group. Only the data of these women were included in the calculations.

Methods. Blood pressure was measured once at each visit by a mercury manometer (not random zero, bladder size 13 cm × 22 cm) after 10 min of supine rest. Systolic and diastolic pressures were recorded to the nearest 5 mm Hg at the respective phases I and V of the Korotkoff sounds. We always used the same instrument.

Body weight was measured while subjects wore indoor clothes, but without shoes. Body height was measured in subjects without shoes in the erect position. The body composition of the soft tissue (lean body mass [LBM] and fat mass [FM]) was measured initially in all subjects by dual photon absorptiometry, as reported elsewhere. This method, which requires a 90 min rectilinear scanning procedure, has an accuracy of approximately 1 kg or 2.5% for LBM and that of 5% for FM measurement in a normal adult female. Blood samples were taken between days 16 and 22 of the cycle, i.e., during gestagen treatment. All participants were examined in the morning after an overnight fast and tobacco abstinence, 24 hr after the last hormone dose. All serum samples were stored at −20°C until they were assayed.

Serum estrone (E1) and serum estradiol (E2) were measured by radioimmunoassays described elsewhere. The intra-assay and interassay variations were, respectively, 5.6% and 10.8% for the E1 assay and 8.8% and 14% for the E2 assay.

Initially, at 1 and at 2 years, plasma renin substrate was measured in all but two subjects in the two percutaneous groups, and in a randomly selected subgroup (n = 13) from the oral E2 group. The initial clinical data from the subgroup were virtually similar to those from the oral E2 group. Renin substrate was determined by an indirect assay as the amount of angiotensin I liberated in the presence of excess renin and complete inhibition of angiotensinases. Plasma samples (25 μL, diluted 1:50) were incubated at pH 5.7 in the same mixture as that used for measurement of plasma renin activity in the presence of an excess of human renin (0.1 Goldblatt units). The concentration of renin substrate was expressed in terms of the angiotensin I released (nanograms of angiotensin I per milliliter of plasma) assuming 1 mole of angiotensin I per mole of renin substrate. Radioimmunoassay of angiotensin I has been reported in detail.

Statistical analysis. For each participant the pretreatment blood pressure and body weight were assigned a value of 100% and the subsequent measurements were expressed as a percentage of this value. The cumulated response was calculated in each participant as the mean of all measurements (except t0) during the 2 years of treatment. Student’s t test for paired data was used to test the significance of differences within groups, and Student’s t test for unpaired data was used for comparisons between two groups. One-way analysis of variance was used to compare more than two groups.

Results

Table 1 shows the relevant clinical data of the subjects in the four groups. Both hormone groups were initially comparable with their respective placebo group. No significant difference was found between the oral E2 group and the oral placebo group, or between the percutaneous E2 group and the percutaneous placebo group, with respect to any of the variables listed in table 1. Menopause was defined as the last menstrual bleeding followed by at least 6 months of amenorrhea.

E1 and E2. Figure 1 shows the changes in serum estrogens during the study. In both placebo groups serum E1 and E2 remained within the postmenopausal range throughout the study. In both hormone treated groups serum E1 was significantly increased after 3 months of treatment (p < .001). In both groups, however, serum E1 continued to rise, reaching the maximum level at 6 months of therapy, after which serum E1 remained virtually constant in both groups, with a
TABLE 1
Initial clinical data on the subjects in the four groups (mean [range])

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Oral E₂</th>
<th>Oral placebo</th>
<th>Percutaneous E₂</th>
<th>Percutaneous placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>32</td>
<td>33</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.5 (45-53)</td>
<td>50.3 (46-53)</td>
<td>50.3 (45-53)</td>
<td>50.5 (45-53)</td>
</tr>
<tr>
<td>Menopausal age (months)</td>
<td>15.9 (6-36)</td>
<td>20.7 (6-36)</td>
<td>15.0 (6-35)</td>
<td>19.3 (6-34)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.7 (49-88.3)</td>
<td>68.8 (50.7-111.8)</td>
<td>61.1 (50.1-76)</td>
<td>60.4 (41-78.3)</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>39.7 (32.1-50.4)</td>
<td>41.5 (31.2-62.8)</td>
<td>39.9 (33.9-45.5)</td>
<td>39.2 (32.3-45.3)</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>20.4 (9.5-38.6)</td>
<td>23.4 (11.1-45.7)</td>
<td>17.9 (5.7-30.2)</td>
<td>18.2 (7.1-31)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.2 (154-170.5)</td>
<td>162.7 (150.2-172)</td>
<td>163.9 (155.5-172.5)</td>
<td>163.1 (150-173.5)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>122 (95-165)</td>
<td>125 (95-160)</td>
<td>122 (90-155)</td>
<td>126 (100-165)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>75 (55-95)</td>
<td>75 (50-95)</td>
<td>76 (50-100)</td>
<td>76 (60-90)</td>
</tr>
</tbody>
</table>

higher value in the oral group than in the percutaneous group.

In the percutaneous E₂ group serum E₂ also increased significantly after 3 months of treatment (p < .001), reaching the maximum level at 6 months and remaining constant at the same level as serum E₁ thereafter. In the oral E₂ group the increase in serum E₂ was minimal.

Blood pressure. Figure 2 shows the course of the systolic and diastolic blood pressure for each of the four groups during the study. A significant difference between the placebo and hormone group was observed only twice, both times in the diastolic blood pressure in the oral drug groups. The correlations between the diastolic and systolic blood pressure and time were insignificant in both hormone treatment groups. For both placebo groups, the correlation between diastolic blood pressure and time was significant, (r = .162, p < .05 and r = .265, p < .001), whereas the relationship of systolic blood pressure vs time was significant only for the oral placebo group (r = .159, p < .05).

Body weight. The course of the body weight during the study is shown at the bottom of figure 2. Body weight did not change significantly in the two hormone treatment groups, but a slight significant increase (p < .01) was observed in both placebo groups. However, significant differences between the hormone treatment groups and their respective placebo groups were not

FIGURE 1. Serum E₁ and serum E₂ during 2 years of treatment with either percutaneous or oral estradiol, compared with placebo. Values are given as mean ± 1 SEM. n = 20 to 33 (see text). ● = estradiol treatment; ○ = placebo. Difference from placebo: initially NS, otherwise p < .001.

FIGURE 2. Changes in systolic and diastolic blood pressure and body weight during 2 years of treatment with either percutaneous or oral estradiol, compared with placebo. The results are given as percent of the initial value, mean ± SEM. n = 20 to 33 (see text). ● = estradiol treatment; ○ = placebo. Difference from placebo: * p < .05.

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observed at any time. There was no correlation between weight change and change in diastolic (r = .14, n = 110, NS) or systolic (r = .07, n = 110, NS) blood pressure (as determined with the cumulated responses during the second treatment year).

**Renin substrate.** Figure 3 demonstrates the changes in plasma renin substrate during the study. Initially, no significant difference between the three groups was found in plasma renin substrate. In the percutaneous E2 group, there was no significant difference from the initial value or from the placebo group at any time. In the oral drug group plasma renin substrate was significantly (p < .01) increased at 12 months of treatment, after which it remained unchanged. In the oral E2 subgroup (n = 13) we found a significant correlation (r = .66, p < .02) between the cumulated response of plasma renin substrate (mean of two examinations) and the cumulated response of E1 (mean of five examinations), but no correlation between the cumulated responses of plasma renin substrate and E2 (r = .22, NS).

**Discussion**

The present study comprised a representative sample of the Danish population of women who had recently experienced natural menopause. The two hormone groups and their respective placebo groups were well matched with regard to clinical data, blood pressure, and initial serum estrogen levels; the subgroup taken from the oral E2 group for determination of renin substrate similarly matched the group from which it was drawn. The relatively high initial serum E2 values observed in three of the four groups and the decrease in serum E2 in the oral placebo group were caused by a few participants having initial premenopausal levels of serum E2 because they had experienced menopause shortly before the study began.

During percutaneous estradiol therapy serum concentrations of E1 and E2 rose to the same level. In the oral E2 group E1 increased greatly, whereas E2 did not change significantly, partly, perhaps, because of the initial high value in this group. Other groups have found that serum E2 increases during oral administration of 2 mg estradiol valerate daily,7, 17 and after 3 months our oral E2 group had significantly higher serum E2 than the oral placebo group for the rest of the study period (p < .001). Therefore, after 3 months of treatment the E1/E2 ratio was far above 1 in the orally treated group, whereas in the percutaneously treated group, this ratio was approximately 1 and close to that observed during the follicular phase in premenopausal women.18 Other groups have reported similar E1/E2 ratios after 2 months of treatment.7, 17 The higher E1/E2 ratio after oral intake of E2 reflect the E2 oxidation, which takes place in the liver and in the intestine.8, 19, 20

Renin substrate is a protein produced in the liver,21 and plasma renin substrate is a sensitive marker of estrogenic action on the liver.6, 7 The unchanged plasma renin substrate during percutaneous E2 administration and the increased concentration during oral estradiol treatment in the present study confirm results of short-term nonplacebo-controlled studies.7, 9 Short-term (2 months) studies have also showed changes in lipoproteins and clotting factors after oral administration, but not after percutaneous administration, of estradiol.7, 17 In the present study, however, the serum levels of both estrogens in the percutaneous group and serum E1 in the oral group continued to rise during the first 6 months of treatment. Furthermore, we have recently reported that changes do occur in serum total cholesterol and low-density lipoprotein cholesterol during percutaneous estrogen therapy, but only after 6 months of therapy.13 Studies of the metabolic changes that occur during estrogen therapy should therefore probably run for at least 6 months.

The induction of hepatic protein synthesis occurs

**FIGURE 3.** Plasma renin substrate during 2 years of treatment with either percutaneous or oral estradiol, compared with placebo. Values are given as nanograms of angiotensin I released per milliliter plasma (see text) and are mean ± 1 SEM. • = placebo; ○ = percutaneous E2; □ = oral E2. Difference from placebo: ** p < .01.
more extensively after oral than after percutaneous administration of estradiol when given in comparable doses. There are three possible reasons for this difference. First, the first-pass effect in the liver results in a higher concentration of estrogens in the portal circulation than in the general circulation. Second, by the oral route estrogens are rapidly absorbed, with peak plasma concentrations within the first hours of administration, whereas absorption is prolonged by the percutaneous route. Third, orally administered E2 is oxidized to E1 to a greater extent than percutaneously administered E2. This oxidation occurs both in the intestinal tract and in the liver. E1 might have different actions on the liver than E2. Indeed, binding of E1 in mouse liver has been shown to be greater than that of E2 and E1 has been found twice as active as E2 on rat hepatocytes. In the oral E2 subgroup in the present study, the cumulated response of plasma renin substrate was significantly (p < .02) correlated with the cumulated response of serum E1, but not with the cumulated response of serum E2.

The effect of postmenopausal estrogen replacement therapy on blood pressure was unclear. Some studies show an increase in blood pressure level or in hypertension during estrogen replacement therapy, while others report a small but significant lowering of blood pressure and protection from hypertension during such therapy. In the present study we found no change in systolic blood pressure and a tendency toward a lowering of diastolic blood pressure during both percutaneous and oral estradiol therapy. Exactly the same response has been reported during oral estrogen replacement therapy with higher estradiol doses.

The blood pressure response in the present study could have been due to changes in body weight, but significant differences in body weight between the hormone groups and their placebo groups were not observed at any time. Furthermore, changes in blood pressure did not correlate with changes in body weight in the present study.

The increase in plasma renin substrate observed in the oral E2 group might, theoretically, cause development of hypertension. However, no significant correlation was found between plasma renin substrate and systolic or diastolic blood pressure in any of the groups, and none of the subjects developed hypertension over the 2 year period. However, some women may be hypersensitive to renin substrate and therefore develop hypertension.

Oral administration of micronized progesterone exerts little of no adverse effect on lipid metabolism and it does not influence bone or calcium metabolism.

In the present study, none of the measured variables (E1, E2, renin substrate, diastolic or systolic blood pressure) appeared to be influenced by the addition of progesterone.

References


Erratum

In the above article, a portion of the last heading in table 2 was missing. The corrected table is reprinted below:

TABLE 2
Statistical comparisons between infusion and baseline values

<table>
<thead>
<tr>
<th>Hemodynamic variable</th>
<th>Norepinephrine vs baseline</th>
<th>Norepinephrine plus epinephrine vs baseline</th>
<th>Norepinephrine plus epinephrine plus atropine vs baseline</th>
<th>Norepinephrine plus epinephrine plus atropine vs baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma norepinephrine</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma epinephrine</td>
<td>NS</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Heart rate</td>
<td>&lt;.05</td>
<td>NS</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Mean BP</td>
<td>&lt;.01</td>
<td>NS</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
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<tr>
<td>SBP</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
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<td>HR-SBP</td>
<td>NS</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>EDV</td>
<td>NS</td>
<td>&lt;.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ESV</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Stroke volume</td>
<td>NS</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>NS</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Systemic vascular resistance</td>
<td>NS</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Cardiac output</td>
<td>NS</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

BP = blood pressure; EDV = end-diastolic volume; ESV = end-systolic volume; HR = heart rate; SBP = systolic blood pressure.
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