Differential Effects of Oral and Transdermal Estrogen Replacement Therapy on Endothelial Function in Postmenopausal Women

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Background—We determined whether the vascular effects of estradiol depend on the route of administration by comparing the effects of oral estradiol and transdermal placebo, transdermal estradiol and oral placebo, and transdermal placebo and oral placebo on in vivo endothelial function in 27 postmenopausal women.

Methods and Results—Endothelial function was assessed from blood flow responses to intrabrachial artery infusions of endothelium-dependent (7.5 and 15 μg/min acetylcholine) and endothelium-independent (3 and 10 μg/min of sodium nitroprusside) vasodilators at 0, 2, and 12 weeks. In the oral estradiol group, the increase in flow above basal during infusion of the low dose of acetylcholine at 0, 2, and 12 weeks averaged 6.0±0.8, 6.9±0.8, and 11.3±1.2 (P<0.01 versus 0 and 2 weeks) mL·dL⁻¹·min⁻¹ at 0, 2, and 12 weeks. The percentage increases versus 0 weeks averaged 21±14% at 2 and 120±34% at 12 weeks. During the high-dose acetylcholine infusion, the increase in flow above basal averaged 8.6±1.3, 10.2±1.5, and 15.1±1.8 (P<0.05 versus 0 weeks) mL·dL⁻¹·min⁻¹, respectively. The percentage increases versus 0 weeks averaged 22±10% at 2 weeks and 119±46% at 12 weeks. In the oral estradiol group, endothelium-independent vasodilatation also improved significantly, but less markedly than endothelium-dependent responses. In the transdermal and placebo groups, all vascular responses remained unchanged. Oral but not transdermal estradiol also induced significant decreases in LDL cholesterol and Lp(a) concentrations and an increase in HDL cholesterol within 2 weeks.

Conclusions—We conclude that oral but not transdermal estradiol induces potentially antiatherogenic changes in in vivo endothelium-dependent vasodilatation and lipid concentrations. (Circulation. 2000;102:2687-2693.)

Key Words: atherosclerosis • blood flow • endothelium

Observational studies suggest that postmenopausal hormone replacement therapy, including various estrogen preparations with or without progestin, approximately halves the risk of cardiovascular disease in women.¹ A recent prospective study in postmenopausal women with coronary artery disease failed, however, to demonstrate benefits of estrogen plus progestin on cardiovascular events.² Whether the possible cardioprotective effects of estrogen preparations depend on the route of estrogen administration is currently unknown.

Several mechanisms have been suggested to contribute to the cardiovascular effects of oral estradiol. These include oral estradiol–induced increases in HDL cholesterol and decreases in LDL cholesterol³ and lipoprotein (a) [Lp(a)]⁴ concentrations. Compared with oral estradiol, transdermal estradiol appears to have no⁵ or only marginal⁶ effects on plasma lipid and lipoprotein concentrations. Estrogen replacement therapy could also directly affect blood vessels. In postmenopausal women, short-term infusions of estradiol potentiate endothelium-dependent vasodilatation in coronary arteries⁷,⁸ and forearm resistance vessels,⁹ but it is unclear whether this acute estradiol effect is responsible for enhanced endothelium-dependent function during long-term therapy.¹⁰,¹¹ In none of the previous studies have effects of oral and transdermal estradiol on endothelial function been compared, and no study addressed the question of whether the acute effects of estradiol are responsible for enhanced endothelial function during long-term therapy. Because both oral and transdermal estradiol induce stable serum estradiol concentrations within a few days,¹² we reasoned that if direct vascular effects of estradiol were to be responsible for improvements in endothelial function, maximal enhancement should be observed already after 2 weeks, with no further improvement during continued therapy in either group.

Received March 21, 2000; revision received July 12, 2000; accepted July 14, 2000.

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In the present study, we compared the effects of oral and transdermal estradiol on endothelial function in vivo. We chose the clinically most commonly used doses of estradiol, a patch delivering 50 μg/d and a daily 2-mg estradiol tablet, which both effectively relieve postmenopausal symptoms and were estimated to increase peripheral free estradiol concentrations similarly. A total of 27 healthy postmenopausal women were randomized to receive either oral or transdermal estradiol or placebo, and they underwent invasive measurements of endothelial function after 0, 2, and 12 weeks of treatment.

**Methods**

**Subjects and Study Design**

**Screening Visit 1 (Internist)**

Postmenopausal women were recruited by a newspaper advertisement. To be acceptable for the study, the subjects had to fulfill the following inclusion criteria: (1) natural amenorrhea for ≥12 months or age ≥52 years, (2) follicle-stimulating hormone (FSH) >30 U/L, (3) no contraindications for estrogen treatment, (4) no evidence of any disease on the basis of history and physical examination and standard laboratory tests (blood counts, serum creatinine, electrolyte concentrations, liver function tests, and ECG), and (5) use of no medications, including vitamins and antioxidants.

**Screening Visit 2 (Gynecologist)**

A total of 36 women visited the gynecologist. A gynecological history was obtained, and a gynecological examination, including transvaginal ultrasound, was performed. To be included in the study, the patients had to fulfill the following criteria: (1) no suspicion of malignancy, (2) no myomas >4 cm, (3) no endometrial pathology, (4) endometrial thickness <6 mm, (5) and normal ovaries and breasts.

Of the 36 women attending the first gynecological examination, 4 had bleeding after the first screening visit and were excluded from the study. Another 3 patients were excluded from the study because of myomas, and 2 patients did not wish to continue the study. The patients gave written informed consent to participate in the study. The experimental protocol was designed and performed according to the principles of the Helsinki Declaration and was approved by the ethical committee of the Helsinki University Central Hospital.

**In Vivo Endothelial Function Tests at 0, 2, and 12 Weeks**

Endothelial function was assessed in forearm resistance vessels after an overnight fast by measurement of forearm blood flow responses to intra-arterial infusions of endothelium-dependent (acetylcholine, ACh) and endothelium-independent (sodium nitroprusside, SNP) vasodilators, as previously described in detail. Current smokers refrained from smoking for 12 hours before the study. An indwelling cannula was inserted in an antecubital vein for blood sampling. A 27-gauge unmounted steel cannula (Coopers Needle Works) connected to an epidural catheter was inserted into the left brachial artery. All drugs were infused at a constant rate of 1 mL/min in the following sequence: normal saline, SNP (Nitropress, Abbott Laboratories) 3 (low dose) and 10 (high dose) μg/min, and ACh (Miochol, OMJ Pharmaceuticals) 7.5 (low dose) and 15 (high dose) μg/min. Each dose was infused for 6 minutes, and infusion of each drug was separated by infusion of normal saline for 18 minutes, during which time blood flow returned to basal values. Forearm blood flow was recorded simultaneously in the infused (experimental) and control arms as previously described. The percentage changes in blood flow responses induced by treatment were calculated from the mean ± SEM of individual changes, which were defined as 100 × (flow after−flow before)/flow before treatment.

**Other Measurements**

Serum total cholesterol and triglycerides and LDL and HDL cholesterol concentrations were measured as previously described. Serum concentrations of Lp(a) were determined by use of the Pharmacia Apolipoprotein (a) RIA assay system, and FSH and sex hormone–binding globulin (SHBG) (AutoDELFI SHBG, Wallac) by fluoro-immunometric assays. Serum estradiol (Estradiol-2, Sorin Biomedica), estrone (Estrone-RIA, Bühlman), and testosterone
Statistical Analyses
All data are expressed as the mean±SEM. Group, dose, and group times dose effects of the vasoactive agents on forearm blood flow were analyzed by ANOVA for repeated measures. Vertical pairwise contrasts were performed with the unpaired t test and simple correlations with Spearman’s correlation coefficient.

Results
Physical and Biochemical Characteristics
At baseline, the groups were matched for biological and menopausal ages, body weight, blood pressure, and serum concentrations of FSH (Table 1). Body weight, body composition, and blood pressure remained unchanged during the 12-week treatment period in all groups (data not shown). At baseline, 5 women in the transdermal and 6 women in the oral groups were suffering from increased sweating or hot flushes. These symptoms were absent at the end of the study.

Serum total estradiol concentrations were below the limit of detection in the placebo group (<20 pmol/L) at 0, 2, and 12 weeks. In the oral estradiol group, serum total estradiol concentrations increased from <20 pmol/L at baseline to 378±29 pmol/L at 2 weeks and 423±45 pmol/L at 12 weeks (P<0.01 for treatment effect). In the transdermal estradiol group, serum total estradiol concentrations increased from <20 pmol/L at baseline to 156±26 pmol/L at 2 weeks and 216±31 pmol/L at 12 weeks (P<0.001 for treatment effect). Serum total estradiol concentrations were significantly higher in both the transdermal (P<0.05 and P<0.01 at 2 and 12 weeks versus placebo) and oral (P<0.001 at 2 and 12 weeks versus placebo) estradiol groups than in the placebo group and higher in the oral than the transdermal estradiol group at 2 (P<0.001) and 12 (P<0.01) weeks. Serum SHBG concentrations remained unchanged in the placebo (49±10 versus 48±9 nmol/L, 0 versus 12 weeks) and the transdermal estradiol (58±4 versus 65±7 nmol/L, 0 versus 12 weeks) groups but increased in the oral estradiol group by 133%, from 72±11 to 168±11 nmol/L at 12 weeks (P<0.001, Figure 1). Because of the increase in serum SHBG concentrations, free estradiol concentrations were similar in the oral (3.17±0.36 pmol/L) and transdermal (3.09±0.49 pmol/L) groups at 12 weeks (Figure 1). Serum estrone concentrations remained unchanged in the placebo and transdermal estradiol groups (Figure 2) but increased 10-fold in the oral estradiol group, from 238±34 pmol/L at baseline to 2727±316 pmol/L at 2 weeks and 2947±421 pmol/L at 12 weeks (P<0.001 for treatment effect). Serum total testosterone concentrations remained unchanged in the oral (0.99±0.02 versus 0.74±0.05 nmol/L, 0 versus 12 weeks), transdermal (1.13±0.17 versus 1.23±0.13, respectively), and placebo (1.07±0.12 versus 1.01±0.09, respectively) groups. Also due to an increase in serum SHBG concentrations, serum free testosterone concentrations decreased in the oral estradiol group from 11±1 pmol/L at baseline to 4±1 pmol/L at 12 weeks (P<0.001) and remained unchanged in the transdermal (14±2 versus 14±2 pmol/L, respectively) and placebo (15±2 versus 14±2 pmol/L, respectively) groups.

Serum Lipids and Lipoproteins and Apoproteins
In the oral estradiol group, LDL cholesterol decreased by 9%, from 3.6±0.2 mmol/L at baseline to 3.2±0.1 mmol/L (P<0.05) at 2 weeks, and remained at this level until 12 weeks. Also in the oral estradiol group, HDL cholesterol increased by 19% at 2 weeks and remained increased until 12 weeks. Lp(a) decreased significantly in the oral estradiol group at 2 and 12 weeks (P<0.01 versus baseline). In the transdermal and placebo groups, all lipid concentrations remained unchanged for the 12-week period (Table 2).

Endothelial Function
In the oral estradiol group, basal flow increased slightly, although not significantly, during the 12-week treatment period from 1.5±0.3 to 1.7±0.2 mL·dL⁻¹·min⁻¹ at 2 weeks (P=NS) and to 2.1±0.1 mL·dL⁻¹·min⁻¹ at 12 weeks (P<0.10). Total (basal and ACh-stimulated) flow during infusion of the low dose of ACh increased from 7.6±0.9 at 0 weeks to 8.9±0.9 mL·dL⁻¹·min⁻¹ at 2 weeks and by 92±26% to 13.0±1.1 mL·dL⁻¹·min⁻¹ at 12 weeks (P<0.01 versus 0 and 2 weeks).
Total flow during infusion of the high dose of ACh increased from 10.2±1.4 at baseline to 12.3±1.6 mL·dL⁻¹·min⁻¹ at 2 weeks and by 110±37% to 17.9±1.8 mL·dL⁻¹·min⁻¹ at 12 weeks (P<0.01 versus 0 and 2 weeks) (Figure 3). In the oral estradiol group, the increase in flow above basal during infusion of the low dose of ACh averaged 6.0±0.8, 6.9±0.8, and 11.3±1.2 (P<0.01 versus 0 and 2 weeks) mL·dL⁻¹·min⁻¹ at 0, 2, and 12 weeks, and during the high-dose ACh infusion, 8.6±1.3, 10.2±1.5, and 15.1±1.8 (P<0.05 versus 0 weeks) mL·dL⁻¹·min⁻¹, respectively. The percentage increases in flow above basal compared with 0 weeks averaged 16±12% and 50±18% at 2 and 12 weeks during the low-dose SNP infusion and 28±12% and 64±19% during the high-dose SNP infusion in the oral estradiol group. In the transdermal group, there were no significant changes in total flows during infusion of the low-dose (8.2±0.6 versus 10.2±0.9 versus 9.3±0.7 mL·dL⁻¹·min⁻¹, 0 versus 2 versus 12 weeks, P=NS) or the high-dose (10.8±1.0 versus 13.1±1.8 versus 13.1±1.2 mL·dL⁻¹·min⁻¹, 0 versus 2 versus 12 weeks, P=NS) SNP (Figure 3). Similarly, in the placebo group, total flow remained unchanged during infusion of both the low-dose (10.8±1.7 versus 9.1±2.2 versus 10.1±1.2 mL·dL⁻¹·min⁻¹, 0 versus 2 versus 12 weeks, P=NS) and the high-dose (13.6±2.3 versus 11.7±1.8 versus 12.2±1.7 mL·dL⁻¹·min⁻¹, 0 versus 2 versus 12 weeks, P=NS) SNP (Figure 3).

To determine the possible causes of enhanced endothelial function during estrogen therapy, simple correlation coefficients (Spearman) were calculated between changes in metabolic parameters and those of endothelium-dependent and -independent vasodilatation. None of the lipid or hormone concentrations correlated with measures of blood flow within the individual groups (data not shown). In the oral estradiol group, the correlation coefficient between the change in SHBG and the change in the blood flow response to the high-dose ACh was 0.60 (P=0.10, NS). Figure 2 shows estradiol and estrone concentrations and the change in endothelium-dependent vasodilatation as a function of time. Neither the time course nor the fold change in endothelium-dependent vasodilatation paralleled the hormone concentrations, implying that short-term effects of estradiol or estrone were not responsible for improved endothelium-dependent vasodilatation in the oral estradiol group.

Discussion

The present study is, to the best of our knowledge, the first to compare the effects of oral and transdermal estradiol and of
placebo oral and transdermal preparations on endothelial function in vivo. We found that oral and transdermal estradiol have different effects on endothelial function. Oral estradiol markedly improved endothelium-dependent and, to a lesser extent, endothelium-independent blood flow in forearm resistance vessels. In contrast, transdermal estradiol therapy had no significant effects on endothelial function. Oral estradiol also induced favorable and stable lipid changes within 2 weeks, whereas transdermal estradiol had no effect on lipids or lipoproteins.

We chose the clinically most commonly used doses of estradiol, a patch changed twice a week that delivers 50 mg/d and a daily tablet containing 2 mg estradiol. Both preparations produced symptomatic relief, although serum total estradiol concentrations were 2-fold higher in the oral than the transdermal estradiol group. The measured concentrations are consistent with previous data, which generally show higher total estradiol concentrations with oral than with transdermal estradiol, despite similar clinical efficacy. In previous studies, the impact of changes in binding protein concentrations or free hormone concentrations have not been considered. This is surprising, because ≥2 studies have shown oral but not transdermal estradiol to increase SHBG concentrations, which will decrease free estradiol concentrations. In the present study, consistent with similar clinical efficacy, serum free estradiol concentrations, calculated on the basis of concentrations of serum SHBG, total testosterone, estrone, and estradiol, were identical between the groups. Despite this, endothelium-dependent vasodilatation increased >100% in the oral estradiol group but was virtually unchanged in the transdermal group (Figure 3). These negative data are in keeping with recent studies showing no effect of estradiol patches delivering 50 or 100 µg/d on endothelial function. The latter studies, however, lasted 3 and 8 weeks, respectively, which is intermediate in time between the lack of effect of estradiol in the present study at 2 weeks and the significant effect at 12 weeks. The small, albeit significant, decrease in free testosterone concentrations could also have contributed to the beneficial effects of oral estradiol on endothelial function.

Acute administration of estradiol improves endothelium-dependent vasodilatation in humans. The total estradiol concentrations in the acute studies have been 3 to 4 times those of mean total estradiol concentrations of a normal menstrual cycle and those in the present study, or corresponded to maximal follicular-phase concentrations of premenopausal women. The

<p>| TABLE 2. Serum Lipid and Lipoprotein Concentrations |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Oral Estradiol Group | Transdermal Estradiol Group | Placebo Group |</p>
<table>
<thead>
<tr>
<th></th>
<th>0 Wk</th>
<th>2 Wk</th>
<th>12 Wk</th>
<th>0 Wk</th>
<th>2 Wk</th>
<th>12 Wk</th>
<th>0 Wk</th>
<th>2 Wk</th>
<th>12 Wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total triglycerides,* mmol/L</td>
<td>1.42±0.24</td>
<td>1.25±0.15</td>
<td>1.64±0.33</td>
<td>1.03±0.09</td>
<td>1.04±0.10</td>
<td>1.24±0.18</td>
<td>1.26±0.21</td>
<td>1.49±0.25</td>
<td>1.38±0.17</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.61±0.23</td>
<td>5.38±0.15</td>
<td>5.60±0.12</td>
<td>5.61±0.29</td>
<td>5.84±0.32</td>
<td>5.85±0.28</td>
<td>5.77±0.32</td>
<td>5.56±0.27</td>
<td>5.98±0.35</td>
</tr>
<tr>
<td>LDL</td>
<td>3.57±0.17</td>
<td>3.19±0.08‡</td>
<td>3.28±0.14</td>
<td>4.16±0.24</td>
<td>3.87±0.26</td>
<td>3.89±0.20</td>
<td>3.85±0.31</td>
<td>3.33±0.27</td>
<td>3.78±0.35</td>
</tr>
<tr>
<td>HDL*</td>
<td>1.48±0.11</td>
<td>1.74±0.11‡</td>
<td>1.72±0.08§</td>
<td>1.63±0.09</td>
<td>1.60±0.08</td>
<td>1.59±0.09</td>
<td>1.38±0.10</td>
<td>1.45±0.10</td>
<td>1.54±0.12</td>
</tr>
<tr>
<td>Lp(a),*† mg/dL</td>
<td>26 (11, 30)</td>
<td>21§ (6, 23)</td>
<td>14¶ (5, 15)</td>
<td>24 (10, 39)</td>
<td>26 (9, 39)</td>
<td>22 (8, 39)</td>
<td>29 (14, 43)</td>
<td>19 (14, 43)</td>
<td>34 (13, 42)</td>
</tr>
</tbody>
</table>

*Log-transformed for statistical analyses.
†Median, interquartile range (25%, 75%). Other data are shown as mean±SEM.
‡P<0.05 and §P<0.01 baseline vs 2 weeks.
¶P<0.05 and †P<0.01 baseline vs 12 weeks.
Acute effects of estradiol have been observed in minutes, which makes it likely that the observed dilatory responses have been due to estradiol per se and not to estradiol metabolites. In the present study, endothelial function was markedly improved by oral estradiol at 12 but not at 2 weeks, whereas total and free estradiol concentrations were maximal already at 2 weeks (Figure 2). This result is in keeping with data demonstrating doubling of postischemic vasodilatation between 1 and 6 months of oral hormone replacement therapy. If acute effects of estradiol alone had been responsible for the improved endothelial function, it should have improved similarly at 2 and 12 weeks. These time-course data are, to the best of our knowledge, novel and support the conclusion that changes in vascular function during estradiol replacement therapy cannot be attributed to acute vascular effects of estradiol.

Antiatherogenic changes in serum lipids by oral but not transdermal estradiol represent another possibility to explain the beneficial effects of oral estradiol on endothelial function. In the present study, oral estradiol decreased LDL cholesterol by 9% and increased HDL cholesterol by 20%. These data are in line with previous studies that reported 6% to 28% reductions in LDL cholesterol and 2% to 19% increases in HDL cholesterol with unopposed estrogen. Also, consistent with previous data, use of transdermal estradiol 100 μg/d for 6 weeks had no effect on lipid concentrations. In the study by Gerhard et al., use of transdermal estradiol 100 μg/d for 6 weeks had no effect on lipid concentrations. In the study by Gerhard et al., use of transdermal estradiol 100 μg/d for 6 weeks had no effect on lipid concentrations. In the study by Gerhard et al., use of transdermal estradiol 100 μg/d for 6 weeks had no effect on lipid concentrations.

Estradiol may also protect LDL from oxidation, a possibility not explored in the present study. Taken together, it is possible that the several antiatherogenic changes in lipids caused by estradiol were responsible for enhanced endothelial function. A limitation of our study is the relatively small number of subjects studied. Although we did not find statistically significant associations between changes in serum lipids or hormones and endothelial function, such knowledge could be found in a larger cohort. Therefore, these data should be interpreted with caution and reproduced.

In conclusion, 2 mg of oral estradiol markedly enhances endothelium-dependent and, to a lesser extent, endothelium-independent vasodilatation, whereas 50 μg/d of transdermal estradiol, which produces a similar increase in free estradiol, has no effect on vascular function in healthy postmenopausal women. The oral estradiol–induced increase in endothelium-dependent vasodilatation cannot be explained by acute estradiol effects. This is because serum estradiol concentrations were maximal at 2 weeks and remained at this concentration until 12 weeks, whereas endothelium-dependent vasodilatation improved significantly and by 119% only after 12 weeks. Thus, long-term effects of oral estradiol, such as the several antiatherogenic changes in lipids and lipoproteins, could be major mediators.

Acknowledgments

This study was supported by grants from the Academy of Finland (Drs Yki-Järvinen and Vehkavaara) and Liv och Hälsa (Dr Virkamäki). The authors gratefully acknowledge Kati Tuomola and Sari Haapanen for excellent technical assistance and Kaj Blomberg, PhD, for calculating serum free estradiol concentrations.

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Circulation. 2000;102:2687-2693
doi: 10.1161/01.CIR.102.22.2687

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/102/22/2687

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