Oral, but Not Transdermal, Administration of Estrogens Lowers Tissue-Type Plasminogen Activator Levels in Humans Without Affecting Endothelial Synthesis

E.J. Giltay, L.J.G. Gooren, J.J. Emeis, T. Kooistra, C.D.A. Stehouwer

Abstract—Oral estrogen administration decreases plasma levels of tissue-type plasminogen activator (tPA), which may be explained by a decrease in endothelial tPA synthesis, an increase in its hepatic clearance, or both. In the present study, we determined (1) differences between oral (ie, via the liver) ethinyl estradiol and transdermal (ie, systemic) 17β-estradiol administration on plasma antigen levels of tPA and plasminogen activator inhibitor type-1 before and after 4 months of hormone administration and (2) effects on endothelial tPA synthesis, by measuring the local increase in plasma tPA during venous occlusion of the upper extremity. Thirty transsexual males (median age 32 years, range 20 to 44 years) were randomly assigned to either oral ethinyl estradiol (n=15) or transdermal 17β-estradiol (n=15); both treatments included the antiandrogen cyproterone acetate (CA). Ten males were treated with CA alone. Seventeen transsexual females (median age 27 years, range 18 to 37 years) were treated with intramuscular testosterone esters. Only oral ethinyl estradiol plus CA but neither transdermal 17β-estradiol plus CA, nor oral CA, nor parenteral testosterone lowered plasma tPA and plasminogen activator inhibitor-1 (P<0.001 for both). tPA release during venous occlusion was not affected by oral ethinyl estradiol plus CA in males (P=0.52) or by parenteral testosterone in females (P=0.89). These data are consistent with a previous observation, in rodents, that the decrease in tPA after oral estrogen administration can be explained by an increase in hepatic tPA clearance, leaving endothelial tPA synthesis unchanged, and suggest that these mechanisms also explain the decrease in tPA in humans. (Arterioscler Thromb Vasc Biol. 2000;20:1396-1403.)

Key Words: tissue-type plasminogen activator ■ sex hormones ■ venous occlusion ■ endothelium ■ estrogen

Estrogens and testosterone are administered for a growing number of indications, including oral contraception and hormone replacement therapy in premenopausal and postmenopausal women and androgen substitution therapy in aging men. Their effects on the fibrinolytic system are still incompletely understood. Administration of estrogens via the oral route decreases tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) levels in males1–3 and females.4–9 By contrast, transdermal administration of estrogens in females is not associated with decreases in tPA and PAI-1 antigen levels.7–9

Because most studies focused on changes in plasma levels, it is not clear whether the decrease in tPA levels after oral administration of estrogens reflects an increase in tPA clearance, a decrease in its synthesis, or both. Because tPA is synthesized and released by the vascular endothelium10–15 and because the continuous deposition and dissolution of fibrin along the vessel wall would be directly and locally affected if endothelial synthesis of tPA changed, the difference between the 2 possibilities is of some clinical importance.

Circulating tPA, either in the free form or complexed with PAI-1, is rapidly cleared from the circulation by the liver,10,16 resulting in a half-life of 4 to 6 minutes for tPA antigen.10,17,18 Lansink et al19 have shown, in rodents, that ethinyl estradiol increases tPA clearance through upregulation of the mannose receptor, a hepatic clearance receptor for tPA. It may be inferred that this mechanism also accounts for the decrease in tPA levels in humans, provided that endothelial synthesis of tPA is not affected by estrogens. A relatively easy way of assessing basal12,13,15 production and release of tPA by the endothelium is venous occlusion of the upper extremity, which increases tPA antigen levels by eliminating its clearance.11–15

To dissect the potential contributions of clearance by the liver and release by the endothelium to changes in plasma tPA, we used 2 approaches. First, the effects of transdermal administration of 17β-estradiol were compared with those of oral ethinyl estradiol, both in combination with oral cyproterone acetate (CA). Previous studies have documented different metabolic effects, implicating hepatic mechanisms, of...
these 2 treatment regimens.20,21 Approximately 60% of orally administered ethinyl estradiol is inactivated by the liver via a first-pass effect through the enterhepatic circulation.22 The comparison of orally administered ethinyl estradiol (with a strong hepatic impact)23 to transdermally administered 17β-estradiol (with less hepatic effects)20,21 would enable differentiation between hepatic and nonhepatic effects. It has been well documented that oral ethinyl estradiol does (possibly compound-specifically) affect hepatic markers, such as sex hormone–binding globulin, corticosteroid-binding globulin, growth hormone–binding protein, insulin–like growth factor-I, and angiotensinogen.24–26 If dissimilar effects of the 2 treatment regimens were found, this would indicate that a hepatic mechanism is likely to be involved.

Second, the effects of venous occlusion were examined to document whether tPA synthesis by the endothelium changes during sex hormone administration. Venous occlusion increases tPA antigen levels in the blood in the occluded arm by eliminating the clearance process11–15 and provides a way of assessing basal production and release of tPA by the endothelium.10 We investigated transsexual subjects receiving standard cross-sex hormone administration, which achieves profound changes in the hormonal milieu as well as in plasma levels of tPA.2

### Methods

#### Patients

We included 40 male-to-female (M→F) transsexuals, with regular menstrual cycles (28 to 31 days) before cross-gender sex hormone administration. There was no evidence of hypertension, cardiovascular disease, thromboembolism, diabetes mellitus, or use of sex hormones.

Lean body mass and total body fat were estimated by bioelectric impedance analysis (BIA 101/S, RJL Systems, Detroit, Michigan).28 The manufacturer’s sex-specific equations were used, corresponding to the genetic sex of subjects. Smoking status (yes or no) and body mass index (BMI, weight/height²) were also assessed. Informed consent was obtained from all subjects, and the study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethical Review Committee of the University Hospital Vrije Universiteit.

#### Blood Sampling and Analysis

Each subject served as her or her own control, with samples drawn before and after hormone administration. In F→M transsexuals, blood was drawn at baseline between days 5 and 9 of the follicular phase of the menstrual cycle, because of potential fluctuations of measurements during the cycle.29 During testosterone treatment, blood was drawn within 5 to 9 days after the most recent testosterone injection. An intravenous catheter was placed in the antecubital vein of supine subjects after an overnight fast and 10 minutes of bed rest. Because of the travel time to our clinic, the time of blood sampling was between 8:30 AM and 1:30 PM. Within-subject time of sampling was, however, comparable before and after 4 months of hormone administration (mean 10:55 AM [95% CI 10:37 to 11:12 AM] versus mean 10:37 AM [95% CI 10:19 to 10:55 AM], respectively; P=0.10). The mean intraindividual variation was 50 minutes (95% CI 40 to 70 minutes).

Plasma tPA and PAI-1 levels were not associated with the time of sampling before or after hormonal treatment (data not shown). Blood was collected without a tourniquet into evacuated tubes (Diatube H CTAD, ie, citrate, theophylline, adenosine, and dipyridamole; Becton Dickinson). Samples were immediately placed on ice and centrifuged at 3500g for 30 minutes at 4°C to obtain platelet-poor plasma. Plasma was separated and snap-frozen within 1 hour and stored at −70°C until analysis. Plasma levels of tPA and PAI-1 antigen were measured by using commercially available enzyme immunoassay kits (Thrombonostika tPA and Thrombonostika PAI-1, Organon Teknika). von Willebrand factor antigen (vWF) was measured by a test for platelet adhesion (Ristocetin–Cowden test; Organon Teknika). von Willebrand factor antigen (vWF) was measured by an in-house enzyme immunoassay and expressed as a percentage of vWF levels in pooled normal plasma, which contained 1.03 IU of vWF per milliliter. Standardized radioimmunoassays were used to measure serum levels of 17β-estradiol and testosterone. Serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by immunometric luminescence assays. To test whether the oral or transdermal estrogen administration modes differed in their effects on peripheral androgen activity in the M→F transsexuals, we measured serum levels of 5α-androstan-3α 17β-diol glucuronide (Adiol G)30 by a radioim-

### TABLE 1. Baseline Characteristics of the 4 Study Groups

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Transdermal 17β-Estradiol* Plus CA (n=15)</th>
<th>F→M Transsexuals</th>
<th></th>
<th>M→F Transsexuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>22.8±2.7</td>
<td>22.8±2.7</td>
<td>22.8±2.7</td>
<td>22.8±2.7</td>
</tr>
<tr>
<td>Total body fat, kg</td>
<td>11.7 (10.0–13.7)</td>
<td>10.8 (9.0–13.0)</td>
<td>10.8 (9.0–13.0)</td>
<td>10.8 (9.0–13.0)</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>62.4±7.8</td>
<td>57.8±6.4</td>
<td>57.8±6.4</td>
<td>57.8±6.4</td>
</tr>
<tr>
<td>Smoking, n</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.18 (1.07–1.31)</td>
<td>1.11 (0.99–1.26)</td>
<td>1.11 (0.99–1.26)</td>
<td>1.11 (0.99–1.26)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.87 (0.68–1.11)</td>
<td>0.87 (0.63–1.22)</td>
<td>0.87 (0.63–1.22)</td>
<td>0.87 (0.63–1.22)</td>
</tr>
<tr>
<td>tPA, mg/mL</td>
<td>7.9 (5.6–11.0)</td>
<td>9.4 (6.4–13.9)</td>
<td>9.4 (6.4–13.9)</td>
<td>9.4 (6.4–13.9)</td>
</tr>
<tr>
<td>PAI-1, mg/mL</td>
<td>25.7 (19.5–33.8)</td>
<td>20.3 (12.5–33.0)</td>
<td>20.3 (12.5–33.0)</td>
<td>20.3 (12.5–33.0)</td>
</tr>
</tbody>
</table>

Values are mean±SD or n. For total body fat, HDL cholesterol, triglycerides, tPA, and PAI-1, geometric means (95% CI) are given. P value was determined by t test or χ² test comparing males treated with oral ethinyl estradiol vs transdermal 17β-estradiol.

*Randomized. †Nonrandomized.
munoassay (Diagnostic Systems Labs, Webster, Texas; reference range was 5.5 to 32 nmol/L in men and 1.3 to 6.4 nmol/L in women). Furthermore, we measured plasma levels of HDL cholesterol by using an enzymatic colorimetric method after phosphotungstic acid/magnesium chloride precipitation (Boehringer-Manheim) and triglycerides by using an enzymatic colorimetric method (Boehringer-Manheim). Changes in HDL cholesterol and triglyceride levels may serve as markers for the hepatic effects of oral compared with transdermal administration of estrogens.

**Venous Occlusion**

Venous occlusion studies were performed at baseline and after 4 months of cross-gender sex hormone administration in 12 M→F transsexuals treated with ethinyl estradiol plus CA and in 17 F→M transsexuals treated with testosterone esters. Posttreatment measurements were not obtained in 2 M→F transsexuals for logistical problems, and 1 M→F transsexual declined participation in the venous occlusion protocol. The venous occlusion test was performed by applying a cuff to the upper arm for 10 minutes midway between systolic and diastolic pressures. On the opposite arm of the intravenous catheter, the systolic and diastolic arterial pressures were assessed with an automatic oscillometric device (BP-8800, Colin). These arterial pressure measurements were repeated every 5 minutes and then averaged. The cuff pressure was maintained as exactly as possible, adjusted according to the blood pressure values measured at the opposite arm. Movements of the occluded arm were avoided to prevent outflow of blood from the occluded segment. Blood was sampled from venous drainage of the occluded arm. It can be calculated that venous occlusion increases tPA concentrations by the accumulation of basal tPA production and release from the endothelium, without invoking an increased rate of local tPA release.12,13,15

**TABLE 2. Laboratory and Metabolic Data Before and After 2 and 4 mo of Cross-Gender Sex Hormone Administration in 30 M→F Transsexuals, Randomized for Administration of Either Oral Ethinyl Estradiol or Transdermal 17β-Estradiol, Both in Combination With CA**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Baseline</th>
<th>2 mo</th>
<th>4 mo</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol, pmol/L</td>
<td>97±31</td>
<td>23±6‡</td>
<td>23±5‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Testosterone, nmol/L</td>
<td>22.9±6.3</td>
<td>1.1±0.1</td>
<td>1.0±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adiol G, mmol/L</td>
<td>23.1±13.4</td>
<td>...</td>
<td>2.9±1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LH, IU/L</td>
<td>3.2±1.6</td>
<td>0.3±0.0</td>
<td>0.3±0.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FSH, IU/L</td>
<td>3.4±2.0</td>
<td>0.5±0.0</td>
<td>0.5±0.0</td>
<td>0.003</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.8±2.7</td>
<td>...</td>
<td>23.2±1.8</td>
<td>0.34</td>
</tr>
<tr>
<td>Total body fat, kg</td>
<td>11.7 (10.0–13.7)</td>
<td>...</td>
<td>13.7 (11.9–15.8)</td>
<td>0.04</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>62.4±7.8</td>
<td>...</td>
<td>61.7±5.9</td>
<td>0.44</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.06 (0.93–1.21)</td>
<td>...</td>
<td>1.22 (1.11–1.34)</td>
<td>0.05</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.17 (0.85–1.60)</td>
<td>...</td>
<td>1.57 (1.35–1.81)</td>
<td>0.02</td>
</tr>
<tr>
<td>tPA, ng/mL</td>
<td>7.9 (5.6–11.0)</td>
<td>3.9 (2.5–6.0)</td>
<td>3.8 (2.5–5.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PAI-1, ng/mL</td>
<td>25.7 (19.5–33.8)</td>
<td>7.1 (5.2–9.8)</td>
<td>8.9 (6.4–12.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean±SD. For total body fat, HDL cholesterol, triglycerides, tPA, and PAI-1, geometric means (95% CI) are given.

*By ANOVA for repeated measurements or t test for paired samples.
†Comparison of the 2 groups by ANOVA for repeated measurements.
‡Ethinyl estradiol, which suppresses endogenous 17β-estradiol, cannot be detected in conventional 17β-estradiol assays.

**Statistical Analysis**

Variables with distributions that were skewed to the right (total body fat and plasma levels of HDL cholesterol, triglycerides, tPA antigen, and PAI-1 antigen) were logarithmically transformed before analysis to normalize their distributions, and antilogarithms of the transformed means were used to obtain geometric means. All data are given as (geometric) means (±SD or 95% CI). Student t tests for independent samples or χ² tests were used to compare baseline differences. ANCOVA was used for intergroup comparisons after adjusting for possible confounders. Baseline values were correlated with the Pearson correlation coefficient.

An ANOVA for repeated measurements or a Student t test for paired samples was used to analyze the effects of cross-gender sex hormones. An ANCOVA was used to compare effects of different treatment regimens and to adjust for potential covariates. Proportional changes, at 4 months, were correlated with the Spearman correlation coefficient. If values were below the lower limit of detection, the value of that lower limit was used for statistical analysis (1.0 nmol/L for testosterone, 0.3 IU/L for LH, and 0.5 IU/L for FSH). Venous occlusion data on PAI-1 levels were excluded from the analyses for 3 F→M transsexuals with levels, uncorrected for hemoconcentration after venous occlusion, above the upper limit of detection (ie, 96 ng/mL). One-sample t tests were used to analyze whether increases of tPA, PAI-1, and vWF during venous occlusion (ie, postocclusion values, adjusted for hemoconcentration, minus preocclusion values) were significantly different from zero. A 2-tailed P<0.05 was considered statistically significant. The software used was SPSS for Windows 8.0.

**Results**

**Pretreatment Values**

At baseline, all subjects were eugonadal by clinical and laboratory criteria. Baseline characteristics are presented in Table 1. Plasma PAI-1 antigen levels were correlated with those of tPA (r=−0.51, P<0.001). In men, testosterone levels were inversely correlated with tPA and PAI-1 antigen concentrations (r=−0.30, P=0.06 and r=−0.28, P=0.08, respectively).

**Effects of Sex Steroid Administration**

In M→F transsexuals at baseline, there were no significant differences between the 2 groups except for BMI, lean body mass, and triglyceride level, which were slightly, but not significantly, higher in males randomized to treatment.
TABLE 2. Continued

<table>
<thead>
<tr>
<th>Transdermal 17β-Estradiol Plus CA (n=15)</th>
<th>Baseline</th>
<th>2 mo</th>
<th>4 mo</th>
<th>P*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>81±16</td>
<td>183±116</td>
<td>183±131</td>
<td>0.008</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>20.9±6.4</td>
<td>1.5±1.7</td>
<td>1.0±0.3</td>
<td>&lt;0.001</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>27.5±9.5</td>
<td>...</td>
<td>9.1±4.8</td>
<td>&lt;0.001</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>2.5±1.6</td>
<td>2.8±8.5</td>
<td>0.5±0.5</td>
<td>0.002</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>3.7±3.8</td>
<td>2.2±6.0</td>
<td>0.5±0.1</td>
<td>0.08</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>20.9±2.6</td>
<td>...</td>
<td>21.9±2.5</td>
<td>0.007</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>10.8 (9.0–13.0)</td>
<td>...</td>
<td>13.1 (11.0–15.5)</td>
<td>0.001</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>57.8±6.4</td>
<td>...</td>
<td>58.7±6.3</td>
<td>0.27</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>1.20 (1.05–1.37)</td>
<td>...</td>
<td>1.08 (0.95–1.21)</td>
<td>0.004</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>0.87 (0.68–1.11)</td>
<td>...</td>
<td>0.76 (0.59–0.98)</td>
<td>0.26</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>9.4 (6.4–13.9)</td>
<td>8.7 (5.8–12.9)</td>
<td>7.9 (5.4–11.6)</td>
<td>0.45</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>20.3 (12.5–33.0)</td>
<td>19.0 (14.0–25.7)</td>
<td>19.0 (12.0–30.0)</td>
<td>0.91</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

with oral ethinyl estradiol compared with males randomized to treatment with transdermal 17β-estradiol (Table 1). The ethinyl estradiol administered could not be detected by the assay used, whereas percutaneous administration of 17β-estradiol increased its plasma levels up to values typical of the midfollicular phase in women (Table 2). Oral and transdermal estrogen administration decreased serum levels of testosterone, Adiol G, LH, and FSH, although the suppression was more gradual after transdermal 17β-estradiol. Effects of oral ethinyl estradiol compared with transdermal 17β-estradiol are shown in Table 2 and Figure 1. tPA and PAI-1 antigen levels decreased sharply in only the oral ethinyl estradiol group (P<0.001 and P<0.001, respectively, compared with the transdermal 49 7β-estradiol group). After correction for baseline BMI, lean body mass, and triglyceride level in an ANCOVA for repeated measures, the 2 regimens of estrogen administration still differed significantly in their effects on tPA and PAI-1 antigen levels (P=0.003 and P=0.001, respectively).

In the oral ethinyl estradiol group, PAI-1 antigen levels showed a larger decrease than did tPA antigen levels after 2 months (72% versus 51%, P=0.001) and after 4 months (65% versus 52%, P=0.07). Proportional changes, at 4 months, of tPA and PAI-1 antigen levels were positively associated in the oral ethinyl estradiol group (r=0.51, P=0.05) and, although less strongly, in the transdermal 49 7β-estradiol group (r=0.39, P=0.15). Proportional changes, at 4 months, of HDL cholesterol and triglycerides were not associated with proportional changes of tPA and PAI-1 antigen levels in either group (data not shown).

In the group treated with CA alone, there were significant decreases, after 4 months, in serum levels of testosterone and 17β-estradiol (P=0.001 and P<0.001, respectively), a slight suppression of FSH (P=0.07), and no change in LH (P=0.93). Plasma tPA and PAI-1 antigen levels did not change significantly on administration of CA alone (tPA from 5.8 ng/mL [95% CI 3.9 to 8.7 ng/mL] to 5.4 ng/mL [95% CI 4.2 to 7.0 ng/mL], P=0.50; PAI-1 from 14.8 ng/mL [95% CI 8.2 to 26.7 ng/mL] to 16.3 ng/mL [95% CI 11.2 to 23.9 ng/mL], P=0.65; Figure 1).

After testosterone administration to F→M transsexuals, serum levels of testosterone and Adiol G increased markedly, whereas serum levels of 17β-estradiol, LH, and FSH levels decreased (Table 3). tPA and PAI-1 antigen levels did not change significantly (Table 3 and Figure 1).

Venous Occlusion

Table 3 shows the effects of venous occlusion in M→F and F→M transsexuals at baseline and after 4 months of cross-gender sex hormone administration. tPA, in M→F and in F→M transsexuals, showed significant increases during venous occlusion before and after hormone administration (P<0.005 for all). In M→F and in F→M transsexuals, there were positive correlations between values of tPA before and after 10 minutes of venous occlusion; this was the case before (r=0.66, P=0.01 and r=0.94, P<0.001) and after (r=0.42, P=0.17 and r=0.90, P<0.001) 4 months of cross-gender sex hormone administration.

The increase in tPA during venous occlusion did not change after 4 months of oral ethinyl estradiol in M→F transsexuals (P=0.52, Table 3 and Figure 2) or after parental testosterone in F→M transsexuals (P=0.89, Table 3). Moreover, the proportional changes of tPA levels after 4 months, before versus after venous occlusion, were not associated, either in M→F or in F→M transsexuals (P=0.88 and P=0.97). Plasma PAI-1 (Table 3) and vWF antigen levels did not change significantly during venous occlusion either before or after 4 months of hormone administration in either treatment group (change in vWF [95% CI] was as follows: in M→F transsexuals, baseline −4.3% [−21.4% to 12.8%], after 4 months 1.3% [−14.5% to 17.1%]; in F→M transsexuals, baseline −0.7% [−9.0% to 7.6%], after 4 months 1.7% [−4.0% to 7.4%]; all P≥0.54).

Discussion

The present study sought to clarify the mechanism by which sex steroids affect circulating levels of tPA. Two lines of evidence support the concept that the decrease of tPA antigen
levels observed after oral administration of ethinyl estradiol plus CA is not related to a decreased endothelial tPA synthesis. First, tPA levels decreased after oral ethinyl estradiol (plus CA) but not after transdermal 17β-estradiol (plus CA) administration. Second, this decrease was not accompanied by a decrease in endothelial tPA synthesis during venous occlusion.

If estrogens affected tPA levels through a decreased endothelial synthesis, one would expect oral ethinyl estradiol and transdermal 17β-estradiol administration to have similar effects on tPA levels, but this was not found. An important difference between these 2 treatment regimens is their effect on liver metabolism, which is stronger with oral ethinyl estradiol than with transdermal 17β-estradiol. One explanation for the dissimilar effects of oral ethinyl estradiol and transdermal 17β-estradiol is the contrasting first-pass effect on the liver. After absorption from the intestine, estrogens enter the portal circulation and pass the hepatocyte before reaching other organs. It has been found that plasma levels of HDL cholesterol, triglyceride, and sex hormone–binding globulin increased after oral conjugated or oral natural 17β-estradiol but not after transdermal 17β-estradiol administration. Also, uniform and robust hepatic responses to 3 oral estrogens (ethinyl estradiol, conjugated estradiol, and estradiol valerate) have been reported, as measured by circulating levels of insulin-like growth factor-I, growth hormone–binding protein, and sex hormone–binding protein. A second explanation for a dissimilar hepatic impact may be the chemical difference between the 2 estrogenic compounds. Ethinyl estradiol may have a selectively enhanced hepatic impact, as evidenced by the finding that oral and transvaginal administration of ethinyl estradiol induced comparable changes in circulating levels of sex hormone–binding globulin, corticosteroid-binding globulin binding capacity, HDL cholesterol, and LDL cholesterol.

The venous occlusion test is thought to eliminate the effects of hepatic clearance and thus provide an index of endothelial tPA synthesis. Previous studies using venous occlusion observed an increase in tPA antigen levels or no change in tPA activity after oral and transdermal estrogen administration, but these studies did not correct for hemoconcentration and therefore cannot be interpreted.

The fact that we found no change in tPA concentrations after administration of CA alone, together with the differential effects of oral ethinyl estradiol versus transdermal 17β-estradiol administration (both plus CA), suggests that ethinyl estradiol, and not CA, was the cause of the decreased tPA level. Together, our findings indicate that a decreased tPA synthetic rate is an unlikely explanation for the decreased plasma tPA levels after oral administration of ethinyl estradiol. In line with this are the observations that incubation of human endothelial cells with ethinyl estradiol as well as testosterone in vitro did not affect the synthesis of tPA and, conversely, that subcutaneous injection of ethinyl estradiol in vivo in rodents did increase the plasma clearance rate of tPA via upregulation of the mannose receptor on liver endothelial cells. Thus, an increased hepatic clearance rate, combined with the lack of effect on endothelial release found in the present study, strongly supports the concept that oral ethinyl estradiol affects circulating levels of tPA through an enhanced hepatic clearance.

The exact source of PAI-1 is unknown, but there is evidence that circulating PAI-1 in vivo originates from hepatocytes or adipocytes, although endothelial cells are also capable of producing PAI-1, at least in vitro. Circulating PAI-1, like tPA, is rapidly cleared from the circulation by the liver, resulting in a half-life of 10 to 15 minutes for PAI-1 antigen, which would enable the venous occlusion test to differentiate between endothelial and alternative sources for PAI-1 release in vivo. We found no evidence of substantial PAI-1 secretion by endothelium of the upper extremity in males and females, both before or after hormone administration, in accordance with previous data. The differential effects of oral versus transdermal administration of estrogen-CA combinations on PAI-1 antigen levels may also point to an increased hepatic PAI-1 clearance, as found for tPA, although, alternatively, they may result from a decreased nonendothelial (possibly hepatic) PAI-1 production after oral estrogen administration.

In M-F transsexuals, before estrogen administration, PAI-1 and tPA antigen levels correlated inversely with testosterone levels, whereas in previous studies, testosterone levels correlated positively with tPA activity and inversely with PAI activity in men. Interestingly, oral administration of the androgenic steroid stanozolol has been shown to decrease tPA and PAI-1 antigen levels. This contrasts with...
TABLE 3. tPA and PAI-1 Antigen Levels Before and After Venous Occlusion Before and After 4 mo of Cross-Gender Sex Hormone Treatment in M→F Transsexuals Receiving Oral Ethinyl Estradiol Plus CA and F→M Transsexuals Receiving Parenteral Testosterone Esters

<table>
<thead>
<tr>
<th></th>
<th>M→F Transsexuals (n=12)</th>
<th>F→M Transsexuals (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>4 mo</td>
</tr>
<tr>
<td>17β-Estradiol, pmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before V0</td>
<td>100±34</td>
<td>24±5‡</td>
</tr>
<tr>
<td>Testosterone, nmol/L</td>
<td>22.9±6.3</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>Adiol G, nmol/L</td>
<td>23.7±14.0</td>
<td>3.1±1.8</td>
</tr>
<tr>
<td>LH, IU/L</td>
<td>3.2±1.6</td>
<td>0.3±0.0</td>
</tr>
<tr>
<td>FSH, IU/L</td>
<td>3.1±1.4</td>
<td>0.5±0.0</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.42±0.04</td>
<td>0.36±0.02</td>
</tr>
<tr>
<td>tPA, ng/mL Before V0</td>
<td>7.9 (5.3–11.9)</td>
<td>3.5 (2.3–5.4)</td>
</tr>
<tr>
<td>tPA, ng/mL After V0</td>
<td>20.8 (13.0–33.3)</td>
<td>15.4 (11.1–21.4)</td>
</tr>
<tr>
<td>tPA, ng/mL Change during V0</td>
<td>16.6 (5.3–27.8)§</td>
<td>13.0 (7.0–18.9)§</td>
</tr>
<tr>
<td>PAI-1, ng/mL Before V0</td>
<td>25.7 (18.5–35.7)</td>
<td>8.1 (6.2–10.6)</td>
</tr>
<tr>
<td>PAI-1, ng/mL After V0</td>
<td>26.0 (18.8–35.8)</td>
<td>8.1 (6.4–10.2)</td>
</tr>
<tr>
<td>PAI-1, ng/mL Change during V0</td>
<td>−0.2 (−3.8–3.5)</td>
<td>−0.2 (−1.2–0.8)</td>
</tr>
</tbody>
</table>

Values are mean±SD or geometric means (95% CI). V0 indicates venous occlusion. PAI-1 data are based on data derived from 14 F→M transsexuals only, because PAI-1 levels exceeded the upper limit of detection after V0 levels in 3 F→M transsexuals. Post V0 data are corrected for hemoconcentration.33,34

* By t test for paired samples.
† Comparison of the 2 groups by ANOVA.
‡ Ethinyl estradiol, which suppresses endogenous 17β-estradiol, cannot be detected in conventional 17β-estradiol assays.
§ P < 0.005.

the lack of effect of parenteral administration of testosterone esters in males47 and in females in our present and previous studies.1,2 This raises the possibility that distinct androgenic compounds have different effects on tPA and PAI-1 levels; alternatively, the route of administration of androgens may be relevant.

Prospective epidemiological data support the notion that estrogen replacement therapy alone or in combination with progestogen protects postmenopausal women against cardiovascular disease.48–50 Alterations in the fibrinolytic system, such as the decrease in plasma PAI-1 concentrations with oral, but not transdermal,7–9 administration of estrogens in...
postmenopausal women, are thought to contribute to this reduced cardiovascular risk. The effects of oral estrogens on plasma tPA and PAI-1 antigen levels are independent of the chemical compounds used: oral conjugated equine,6,7,9 natural,3,4 and synthetic1,2,4,5 estrogens all had similar effects, which suggests that a similar mechanism regulates these changes. Our finding of a lack of effect on the local tPA release by the endothelium may indicate that tPA and PAI-1 antigen alterations do not necessarily translate into an improved local fibrinolytic potential on oral estrogen administration.

In conclusion, the hepatic impact of orally administered ethinyl estradiol appears to be responsible for an enhanced hepatic clearance of tPA, which consequently decreases plasma levels of tPA antigen. Thus, an increased fibrinolytic potential after oral estrogen administration does not necessarily represent an improved endothelial function.

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Oral, but Not Transdermal, Administration of Estrogens Lowers Tissue-Type Plasminogen Activator Levels in Humans Without Affecting Endothelial Synthesis

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