Visceral Fat Accumulation Is an Important Determinant of PAI-1 Levels in Young, Nonobese Men and Women
Modulation by Cross-Sex Hormone Administration


Abstract—Increased plasminogen activator inhibitor type-1 (PAI-1) levels, leading to impaired fibrinolysis, are associated with increased visceral fat in middle-aged and obese subjects. It is unknown, however, whether this association is independent of other disturbances clustered in the insulin resistance syndrome. We analyzed this association in young, nonobese transsexual men and women before and after administration of cross-sex steroids, which potentially influence many elements of the insulin resistance syndrome, including PAI-1 levels and visceral fat accumulation. We assessed the visceral fat area (by MRI); total body fat; insulin sensitivity (with a glucose clamp technique); and plasma levels of PAI-1, insulin, and triglycerides in young (<37 years old), nonobese (body mass index <28 kg/m²), healthy men (n=18) and women (n=15) before and after 12 months of cross-sex hormone administration. Men were treated with ethinyl estradiol 100 µg/d plus cyproterone acetate 100 mg/d, and women were treated with testosterone esters 250 mg IM every 2 weeks. At baseline, only visceral fat area was significantly correlated with plasma PAI-1 levels in both men (r=0.57, P=0.03) and women (r=0.59, P=0.03). In multivariate linear regression analysis, this association was independent of total body fat, insulin sensitivity, and plasma levels of triglycerides and insulin. After 12 months of cross-sex hormone administration, the plasma PAI-1 levels were no longer correlated with visceral fat (which had increased). We conclude that in young, nonobese men and women, visceral fat area is an important determinant of plasma PAI-1 levels. After cross-sex hormone administration, this association was no longer demonstrable. (Arterioscler Thromb Vasc Biol. 1998;18:1716-1722.)

Key Words: plasminogen activator inhibitor type-1 ■ visceral fat accumulation ■ insulin sensitivity ■ sex hormones
visceral fat depots.\textsuperscript{30,31} It is unknown whether the shifts in PAI-1 levels during sex-steroid administration are partly mediated by quantitative changes in visceral fat accumulation or by other elements of the insulin resistance syndrome.

The aim of this study was to examine the association between plasma PAI-1 levels and visceral fat accumulation in young, nonobese subjects. We aimed particularly to examine whether this association was independent of other variables clustered in the insulin resistance syndrome. Furthermore, because we selected transsexual subjects for these studies, we were able to reassess this association after 12 months of cross-sex hormone administration, which is known to influence many elements of the insulin resistance syndrome, including PAI-1 levels and visceral fat accumulation. Transsexuals, being different from members of their own genital sex from an endocrine\textsuperscript{12–14} or metabolic\textsuperscript{22,27,31} viewpoint, provide us with the opportunity to study the effects of high-dose sex steroid administration in young, nonobese subjects. We therefore consider transsexuals as a valid model for a study of the regulation of PAI-1 levels.

Methods

Patients

Eighteen male-to-female (M→F) transsexuals with a median age of 27 years (range, 18 to 37) and 15 female-to-male (F→M) transsexuals with a median age of 23 years (range, 16 to 33) were recruited between October 1993 and April 1996 and agreed to participate in the study. Subjects with a body mass index (BMI) (weight/height\textsuperscript{2}) >28 kg/m\textsuperscript{2} were excluded. All subjects were judged to be clinically healthy on the basis of medical history, physical examination, and routine laboratory tests. In particular, hypertension, diabetes mellitus, and evidence of cardiovascular, liver, or endocrine diseases were not noted. None reported intake of hormones (such as oral contraceptives) or medications known to affect sex-steroid or lipid metabolism or insulin sensitivity. Ten M→F transsexuals and 7 F→M transsexuals were smokers. Before the start of hormone therapy, all F→M transsexuals had regular menstrual cycles (28 to 31 days).

We measured the variables reflecting insulin resistance (glucose and insulin levels and glucose utilization), plasma lipids (triglyceride and HDL cholesterol levels), fat distribution (BMI, WHR, total body fat, abdominal subcutaneous fat, and visceral fat area), and mean arterial pressure at baseline and again after 12 months of cross-sex hormone administration. For logistical reasons, some measurements were not obtained in all subjects. Mean arterial pressure was measured directly, not calculated, with an automatic device (BP-8800, Colin) at baseline and after 12 months of cross-sex hormone administration after the patient had rested for at least 15 minutes (mean of 4 recordings repeated every 3 minutes). M→F transsexuals were treated with ethinyl estradiol 100 μg/d (Lynoral, Organon) in combination with the antiandrogen cyproterone acetate 100 mg/d (Androcur, Schering) to counteract the effects of testosterone. F→M transsexuals were treated with testosterone esters (Sustanon, Organon) 250 mg every 2 weeks intramuscularly. Informed consent was obtained from all subjects, and the study was approved by the Ethical Review Board of the Hospital Vrije Universiteit.

Blood Sampling and Analysis

In F→M transsexuals, blood was drawn at baseline between days 3 and 9 of the menstrual cycle during the follicular phase, and during hormone treatment, within 5 to 9 days after the most recent testosterone injection. Blood samples were obtained between 9:30 and 10:30 AM after a 12-hour fast. 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 luminometry assays. Immunoradiometric assays were used to measure serum levels of sex hormone–binding globulin (SHBG) and insulin (Bio-source Diagnostics). Plasma levels of glucose, triglycerides, and HDL cholesterol were measured by using standard laboratory methods. Blood was also collected into evacuated tubes (DiaMute H CTAD, Becton Dickinson). Samples were immediately placed on ice and centrifuged at 3500 g for 30 minutes at 4°C. 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 (Thrombostick tPA and Thrombostick PAI-1, Organon Teknika), and tPA antigen was evaluated by using an in-house enzyme immunoassay.\textsuperscript{35}

Measurement of Fat Distribution

The lean body mass (LBM) and the total body fat were estimated at baseline and after 12 months of cross-sex hormone treatment by bioelectrical impedance analysis (BIA 101/S, RJL Systems).\textsuperscript{36} In addition, body circumferences were measured in duplicate with a flexible plastic tape at the level of the abdomen (midway between the lower rib margin and the iliac crest) and the hip (over the greater trochanters) to calculate the WHR. Areas of abdominal subcutaneous and visceral fat depots were assessed by MRI technique in 15 M→F and 14 F→M transsexuals. The procedure of image analysis has been described in detail elsewhere.\textsuperscript{31} Repeated measurements were obtained by using the same MRI and scanning parameters. An inversion recovery pulse sequence was used, and appropriate scanning parameters were chosen to obtain good image contrast between adipose and other tissues. Three transverse images were taken at the abdominal level: 1 at the anatomic marker (lower edge of the umbilicus) and 1 above and 1 below this position (slice thickness, 10 or 12 mm, depending on the imager used). The image-analyzing computer program (developed by our Department of Biomedical Engineering) is based on a “seed-growing” procedure. In short, after a seed point is placed in the abdominal subcutaneous or the visceral fat depot, it can be circumscribed by selection of a pixel intensity range. The intensity range is selected for each image separately according to the pixel intensity histogram. The areas of the circumscribed abdominal subcutaneous and visceral fat depots were calculated by converting the number of pixels to square centimeters, and the mean of the 3 abdominal images was taken. To reduce variability, all measurements were performed by a single experienced observer. The intraobserver coefficients of variation were 2.3% for abdominal subcutaneous fat and 9.8% for visceral fat.

Hyperinsulinemic Euglycemic Clamp

Insulin sensitivity was measured at baseline and after 12 months of cross-sex hormone treatment by using a glucose clamp technique.\textsuperscript{37} Two intravenous catheters were placed in contralateral antecubital or antebraachial veins of each arm, 1 for blood withdrawal and the other for insulin and glucose infusion. The insulin solution for intravenous infusion was prepared by adding 0.5 mL human insulin (100 IU/mL; Velosulin, Novo Nordisk A/S) to 45 mL of 0.9% NaCl to a final insulin concentration of 1 IU/mL. The insulin infusion rate was calculated per kilogram of LBM. The procedure consisted of a 2-hour period with the insulin infusion rate at 62.5 μUI/kg LBM per hour. The clamp procedure was started 30 minutes after cannulation (0 minutes). After the start of the insulin infusion, arterial blood glucose levels were measured every 5 minutes with the use of a Yellow Springs Instruments glucose analyzer (glucose oxidase method), and the 20% glucose infusion rate was adjusted to maintain blood glucose concentration at the fasting level (ie, mean blood glucose level from −30 to 0 minutes). Blood samples for the determination of insulin levels were collected every half hour. In the second hour, the glucose disposal rate was calculated from the steady-state glucose infusion rate, the LBM, and the mean insulin level, ie, M/I value=100×mg glucose/kg LBM per min per pmol insulin per L).

Statistical Analysis

Data are given as mean±SD. Variables with a skewed distribution (abdominal subcutaneous fat area, total body fat, and plasma levels
of PAI-1, uPA, and triglycerides) were logarithmically transformed before analysis to normalize their distributions. Student’s t test for independent samples and an ANCOVA was used to compare baseline differences between men and women. In the M→F and F→M groups separately, the ANOVA test for repeated measures or the t test for paired samples were used to explore the effects of cross-sex hormones on plasma PAI-1 levels and other variables of interest. Baseline values, values after 12 months of treatment, and proportional changes between baseline and 12 months were correlated by using Spearman’s correlation coefficient. Both groups were pooled in a multiple linear regression analysis to further explore interrelationships between the logarithmically transformed plasma PAI-1 level and elements of the insulin resistance syndrome. Interaction terms were included to test whether the associations of the plasma PAI-1 level and the visceral fat area differed between men and women or before and after cross-sex hormone administration. When endocrine measurements were below the lower limit of detection, the value of that lower limit was used for statistical calculations (for LH 0.3 IU/L, for FSH 0.5 IU/L, for 17β-estradiol 90 pmol/L, and for testosterone 1.0 nmol/L). Two-sided P<0.05 was considered statistically significant. The software used was SPSS for Windows 7.0.

Results

Determinants of PAI-1 Levels at Baseline

All subjects were eugonadal at baseline by clinical and laboratory criteria. Baseline characteristics are presented in Table 1. Plasma PAI-1 levels were statistically significantly higher in women than in men (P=0.049), a difference that disappeared (P=0.93) after controlling for total body fat (higher in women) in an ANCOVA. Plasma tPA and uPA levels were not statistically significantly different between men and women (P=0.63 and P=0.50). Plasma PAI-1 levels were positively and significantly correlated with plasma tPA levels in men (r=0.72, P=0.001) and women (r=0.63, P=0.01), negatively with serum SHBG levels in men (r=-0.50, P=0.05), and negatively with serum 17β-estradiol levels in women (r=-0.55, P=0.03).

The PAI-I level was correlated significantly with the visceral fat area in men (r=0.57, P=0.03) and with the visceral fat area and total body fat in women (r=0.59, P=0.03 and r=0.70, P=0.006) but not with WHR or insulin sensitivity (Figure 1). Also, no significant correlations were found with levels of glucose, insulin, triglycerides, HDL cholesterol, and mean arterial pressure (Table 1).

![Figure 1](http://atvb.ahajournals.org/)

**Figure 1.** Scatterplots of data for 16 men and 14 women at baseline with plasma PAI-1 levels on a logarithmic scale. The visceral fat area (using an MRI technique) was significantly correlated with plasma PAI-1 levels in men (r=0.57, P=0.03) and women (r=0.59, P=0.03). The logarithmically transformed total body fat was significantly correlated with plasma PAI-1 levels in women (r=0.70, P=0.006). No correlations were found between PAI-1 levels and WHR or insulin sensitivity for men and women separately or when the data were pooled in the analysis.

| TABLE 1. Baseline Characteristics in Men and Women |
|-----------------|--------|--------|
| Variable        | Men (n=18) | Women (n=15) |
| PAI-1 antigen, ng/mL | 19.0±13.6 | 28.8±19.5 |
| BMI, kg/m²       | 20.9±2.7  | 21.5±3.0  |
| WHR             | 0.82±0.04 | 0.79±0.05 |
| LBM, kg         | 57.2±9.0  | 43.4±6.0  |
| Total body fat, kg | 9.5±2.6  | 19.8±4.9  |
| Abdominal subcutaneous fat area, cm² | 85±40 | 163±90 |
| Visceral fat area, cm² | 41±16 | 38±13 |
| Glucose, mmol/L  | 5.0±0.7  | 5.0±0.6  |
| Insulin, pmol/L | 39±15  | 47±24  |
| Insulin sensitivity | 2.28±0.72 | 2.47±0.97 |
| (100×mg glucose per kg LBM per min per pmol insulin/L) | |
| Triglycerides, mmol/L | 0.8±0.6 | 0.8±0.5 |
| HDL cholesterol, mmol/L | 1.1±0.2 | 1.3±0.3 |
| Mean arterial pressure, mm Hg | 88±9 | 81±7 |

Data are mean±SD.
cholesterol, BMI, abdominal subcutaneous fat area, and mean arterial pressure (data not shown).

A multiple linear regression analysis was performed on pooled data from all subjects, with the plasma PAI-1 level as the dependent variable and biological sex, age, total body fat, and the visceral fat area forced into the model. The visceral fat area was the only variable that added significantly to the association with plasma PAI-1 levels after controlling for other possible confounding variables (Table 2). The use of the M value instead of the M/I value did not lead to different results.

Effects of Cross-Sex Hormone Administration

After estrogen and antiandrogen administration to M→F transsexuals, serum levels of testosterone decreased to undetectable levels. The ethinyl estradiol administered could not be detected by the assay used, but the biological effects of estrogens were reflected in a large increase of serum levels of SHBG. After testosterone administration to F→M transsexuals, serum levels of testosterone increased markedly while serum 17β-estradiol levels decreased. In 2 F→M subjects the serum 17β-estradiol levels decreased to below the lower limit of detection. The serum level of SHBG decreased, reflecting the biological effects of androgens. Serum levels of LH and FSH were not significantly suppressed (Table 3).

Ethinyl estradiol plus cyproterone acetate administration to men during a 12-month period was associated with decreases in plasma levels of PAI-1 by 62% (Figure 2), tPA by 53%, and uPA by 37% (Table 3). Testosterone treatment of women during the same 12 months was not associated with changes in PAI-1 (Figure 2) or tPA levels; however, uPA levels increased by 28% (Table 3). This increase in uPA level was only apparent after 12 months. There was a positive correlation between the proportional changes in plasma levels of PAI-1 and tPA in the men (r=0.77, P<0.001) and in the women (r=0.70, P=0.004). After 12 months of treatment, the plasma levels of PAI-1 and tPA were significantly correlated in M→F (r=0.58, P=0.01) and F→M (r=0.69, P=0.005) transsexuals, similar to the situation before hormone administration.

In men treated with ethinyl estradiol plus cyproterone acetate, significant increases were found in the fasting insulin levels (by 38%), the HDL cholesterol level (by 18%), the BMI (by 5%), the WHR (by 1%), the total body fat (by 38%), the abdominal subcutaneous fat area (by 54%), and the visceral fat area (by 17%). Insulin sensitivity (M/I value) and mean arterial pressure did not change significantly. In women treated with testosterone, plasma levels of glucose, insulin, and triglycerides; insulin sensitivity; and mean arterial pressure did not change significantly. The HDL cholesterol level decreased by 23%. The BMI did not change significantly, but the LBMI increased by 9%, the WHR increased by 3%, the total body fat decreased by 24%, the abdominal subcutaneous fat area decreased by 18%, and the visceral fat area increased by 18%, all significantly.

Proportional changes in plasma PAI-1 levels were not correlated with any of the proportional changes of the

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**TABLE 2. Standardized Regression Coefficients From Multiple Linear Regression Analysis of Plasma PAI-1 Levels in Relation to Visceral Fat Area and Possible Confounding Variables in Pooled Data for Men and Women at Baseline**

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variables</th>
<th>Standardized Coefficient, β*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma PAI-1 level, ng/mL</td>
<td>Biological sex, men/women</td>
<td>0.47</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Plasma insulin level, pmol/L</td>
<td>−0.08</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Insulin sensitivity (100×glucose per kg LBM per min per pmol insulin/L)</td>
<td>−0.18</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Plasma triglyceride level, mmol/L</td>
<td>0.01</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Total body fat, kg</td>
<td>0.07</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Visceral fat area, cm²</td>
<td>0.59</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Multiple R² (F=3.4, P=0.02)</td>
<td>0.52</td>
<td>..</td>
</tr>
</tbody>
</table>

*Standardized coefficients (β) are given to enable comparison of the relative strengths of associations. These are partial regression coefficients when all variables are expressed in standardized (z score) form. Unstandardized coefficients (B) are not given because of their complex interpretation due to the use of logarithmically transformed plasma PAI-1, plasma triglyceride, and total body fat values.

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**TABLE 3. Endocrine and Fibroinolytic Variables Before and After 4 and 12 Months of Cross-Sex Hormone Administration in Transsexual Subjects**

<table>
<thead>
<tr>
<th>M→F (n=18)</th>
<th>Baseline</th>
<th>4 Months</th>
<th>12 Months</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol, pmol/L</td>
<td>99±15</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Testosterone, nmol/L</td>
<td>23±6</td>
<td>1±0</td>
<td>1±0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LH, IU/L</td>
<td>3.3±2.2</td>
<td>0.3±0.1</td>
<td>0.3±0.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FSH, IU/L</td>
<td>2.8±2.4</td>
<td>0.5±0.1</td>
<td>0.6±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SHBG, IU/L</td>
<td>36±12</td>
<td>237±52</td>
<td>250±41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PAI-1 antigen, ng/mL</td>
<td>19.0±13.6</td>
<td>6.4±2.8</td>
<td>7.3±1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tPA antigen, ng/mL</td>
<td>9.7±3.7</td>
<td>4.8±2.3</td>
<td>4.6±1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>uPA antigen, ng/mL</td>
<td>1.01±0.27</td>
<td>0.55±0.16</td>
<td>0.64±0.13</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F→M (n=15)</th>
<th>Baseline</th>
<th>4 Months</th>
<th>12 Months</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol, pmol/L</td>
<td>160±63</td>
<td>129±34</td>
<td>129±33</td>
<td>0.04</td>
</tr>
<tr>
<td>Testosterone, nmol/L</td>
<td>1.5±0.5</td>
<td>29±10</td>
<td>34±12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LH, IU/L</td>
<td>3.7±1.5</td>
<td>2.5±2.9</td>
<td>2.3±2.1</td>
<td>0.17</td>
</tr>
<tr>
<td>FSH, IU/L</td>
<td>4.6±1.5</td>
<td>3.7±2.0</td>
<td>3.3±2.2</td>
<td>0.14</td>
</tr>
<tr>
<td>SHBG, IU/L</td>
<td>63±31</td>
<td>28±13</td>
<td>25±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PAI-1 antigen, ng/mL</td>
<td>28.8±19.5</td>
<td>24.8±12.0</td>
<td>24.3±13.3</td>
<td>0.49</td>
</tr>
<tr>
<td>tPA antigen, ng/mL</td>
<td>8.9±3.1</td>
<td>10.0±4.1</td>
<td>10.1±3.8</td>
<td>0.45</td>
</tr>
<tr>
<td>uPA antigen, ng/mL</td>
<td>0.97±0.33</td>
<td>0.98±0.29</td>
<td>1.24±0.27</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are mean±SD.

*M→F transsexuals were treated with ethinyl estradiol, which is not detected by the 17β-estradiol assay used.
elements of the insulin resistance syndrome, including visceral fat area. For values obtained after 12 months of cross-sex hormone treatment, plasma PAI-1 levels were not correlated with any of these variables except for mean arterial pressure, which was correlated positively with plasma PAI-1 levels in M→F transsexuals (r = 0.70, P = 0.004). A multiple linear regression analysis in data pooled from all subjects with the plasma PAI-1 level as the dependent variable and the biological sex and the visceral fat area forced into the model showed that the visceral fat area had lost the association with the plasma PAI-1 (b = 0.16, P = 0.13). When 12-month values were compared with baseline values in a linear regression analysis, the association between the plasma PAI-1 level and the visceral fat area was weakened significantly in M→F (P < 0.001) and nonsignificantly in F→M (P = 0.16; Figure 3) transsexuals.

Discussion

An increased plasma PAI-1 level, an important cause of impaired fibrinolysis, is a risk factor for CVD.1–3 We studied the relation between plasma PAI-1 levels and the visceral fat area. The strengths of our study are the inclusion of only healthy, nonobese men and women under the age of 37 years and the use of “gold standard” techniques to assess the visceral fat mass and insulin sensitivity. In previous studies, correlations have been found between plasma PAI-1 levels and the visceral fat area measured by means of CT in middle-aged men and women7 and in middle-aged men including obese subjects.8,9 Our findings show this association to exist even in healthy, young, nonobese subjects by the use of an accurate MRI technique. This raises the possibility that visceral fat accumulation is directly connected to CVD through increased PAI-1 levels.1–3 Indeed, increasing mortality due to CVD is already apparent with increasing BMI in nonobese and mildly overweight women (BMI <29 kg/m²)38 and is also apparent in obesity, especially if the depot of fat is visceral.39–42

The insulin resistance syndrome includes a cluster of metabolic and hemodynamic disturbances, ie, insulin resistance, hyperinsulinemia, glucose intolerance, dyslipidemia including hypertriglyceridemia, visceral fat accumulation, hypertension, and hypofibrinolysis, which increase the risk of CVD.17 Many studies in healthy subjects have suggested that beside visceral fat accumulation, other elements of the insulin resistance syndrome are associated with increased plasma PAI-1 levels. PAI-1 levels were reported to be positively correlated with increased insulin levels,9,10,12–14,16,43 decreased insulin sensitivity,10,13,14,16,43 increased plasma triglyceride levels,11,12,14,15,42 and increased blood pressure.16,43 However, these associations could well be indirect and, at least in part, depend on the relationship between PAI-1 and visceral fat, because visceral fat is associated with insulin resistance44 and increased glucose, insulin, and triglyceride levels.40,41 This concept is supported by our data showing that the association between plasma PAI-1 levels and visceral fat area was independent of insulin sensitivity and plasma levels of insulin and triglycerides. Moreover, some previous studies may have lacked sufficient sensitivity to show the influence of visceral fat area.
fat, because WHR or BMI was used in multivariate analysis as an estimate of intra-abdominal fat.4,5,11-14,19 These measures are reasonable but imprecise estimates of visceral fat, as illustrated by our finding of no relation between PAI-1 and WHR or BMI in the face of a strong relation between PAI-1 and MRI-estimated visceral fat mass.

In our young, nonobese subjects, PAI-1 levels were higher in women than in men. However, this sex difference disappeared after adjustment for total body fat. Previous large population-based studies in healthy men and premenopausal women found similar PAI-1 activity levels45 and higher PAI-1 levels in men versus women.46 The assumption, based on previous studies,23-25,47,48 that oral estrogen plus antiandrogen administration would decrease PAI-1 levels and that testosterone administration would not change PAI-1, was indeed confirmed by our data. Furthermore, uPA levels had increased after 12, but not after 4, months of testosterone administration in women.

In both groups, the correlation between the visceral fat area and plasma PAI-1 level at baseline had disappeared after 12 months of sex-steroid hormone administration. This was especially clear in M→F transsexuals, but in this group the administered sex steroids had a substantially larger effect. What might explain this dissociation? First, sex steroid-induced shifts in metabolic variables that are included in the insulin resistance syndrome could be involved in PAI-1 metabolism. This concept is not supported by our data, because proportional changes in PAI-1 levels were not correlated with proportional changes in insulin sensitivity or plasma levels of insulin, glucose, triglycerides, and HDL cholesterol. Second, changes in hepatic clearance might be relevant. The parallel decrease in PAI-1, tPA, and uPA in M→F transsexuals is consistent with a clearance effect, because PAI-1, tPA, and uPA share a major hepatic clearance pathway.49,50 This idea is further supported by the observation that oral, but not transdermal, administration of estrogens reduces plasma PAI-1 levels.24,25 A clearance effect, however, appears less likely to explain the changes in visceral testosterone administration that we observed.51 PAI-1 synthesis can take place in hepatocytes and adipocytes.6-8 Therefore, shifts could have occurred directly in the secretion of PAI-1 by adipocytes or indirectly in the secretion of substances influencing the hepatic secretion of PAI-1.5,52 In this respect, it is noteworthy that the cytokines interleukin-6 and tumor necrosis factor-α and the acute-phase reactant C-reactive protein were not associated with plasma PAI-1 levels either before or after 12 months of hormone administration (data not shown). We have, however, not studied portal concentrations of these cytokines or free fatty acids, because the topography of the portal vein makes these studies difficult.53

Our data indicate that in young, nonobese men and women, the visceral fat area is an important determinant of plasma PAI-1 level, independent of insulin sensitivity and plasma levels of insulin and triglycerides. Visceral fat may be directly linked to a low fibrinolytic activity and thereby to an increased risk of CVD. After oral estrogen and antiandrogen administration to men, plasma levels of PAI-1, tPA, and uPA decreased substantially, and after testosterone administration to women, only plasma uPA levels increased. After administration of cross-sex hormones, the association between plasma PAI-1 and the ensuing increases in visceral fat area was no longer demonstrable. This dissociation could not be explained by changes in elements of the insulin resistance syndrome, but whether it should be ascribed to changes in hepatic clearance of PAI-1 or changes in adipocyte metabolism directly or indirectly involved in PAI-1 synthesis remains to be elucidated.

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References

1722 PAI-1, Visceral Fat, and Insulin Sensitivity

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