Plasma PAI-1 Levels Are More Strongly Related to Liver Steatosis Than to Adipose Tissue Accumulation

Marie-Christine Alessi, Delphine Bastelica, Alenka Mavri, Pierre Morange, Bruno Berthet, Michel Grino, Irene Juhan-Vague

Objective—Because obesity and insulin resistance (IR) are strongly associated with liver steatosis (LS), we investigated the relation between the degree of LS and plasminogen activator inhibitor-1 (PAI-1) in ob/ob mice, in C57/BL6 mice with alcoholic LS, and in severely obese humans.

Methods and Results—In both mouse models, plasma PAI-1 levels were associated with PAI-1 expression in the liver and with the degree of LS. Liver PAI-1 antigen was associated with the tumor necrosis factor receptor-II (TNFRII) antigen, whereas association with TNF antigen content was found in ob/ob mice only. No significant correlation between plasma PAI-1 and PAI-1 expression in adipose tissue of ob/ob mice was observed. Furthermore, the relation between plasma PAI-1 levels and body weight was positive in ob/ob mice but negative in C57/BL6 mice (both P<0.001). In humans, PAI-1 levels were correlated with the degree of LS, and 26% of plasma PAI-1 activity was independently explained by LS and serum insulin levels.

Conclusions—Plasma PAI-1 levels are more closely related to fat accumulation and PAI-1 expression in the liver than in adipose tissue. In steatotic liver, PAI-1 antigen content is associated with those of TNF and TNFRII. Therefore, we suggest that TNF pathway dysregulation in LS could be involved in increased plasma PAI-1 in obesity with IR.

(Arterioscler Thromb Vasc Biol. 2003;23:1262-1268.)

Key Words: liver steatosis  ■  PAI-1  ■  adipose tissue  ■  insulin resistance

Plasminogen activator inhibitor type 1 (PAI-1) is the main inhibitor of fibrinolysis. PAI-1 modulates the development of atherosclerosis in mice, and an elevated plasma PAI-1 concentration is predictive for myocardial infarction in humans. Interestingly, the predictive value of circulating PAI-1 levels is highly dependent on the insulin resistance syndrome.

Despite several efforts in the last few years, the mechanism of increased plasma PAI-1 concentration in insulin resistance associated with android obesity is not completely understood. PAI-1 is expressed in murine as well as in human adipose tissue, and its expression in adipose tissue is correlated positively with body mass index (BMI). Human visceral adipose tissue expresses more PAI-1 than does subcutaneous adipose tissue. Furthermore, PAI-1 expression in only abdominal, but not in femoral subcutaneous adipose tissue, is associated with the features of insulin resistance. Therefore, it has been postulated that in the insulin resistance syndrome with central obesity, abdominal adipose tissue is an important source of plasma PAI-1. Of note, an increase in plasma PAI-1 is also observed in lipodystrophy associated with antiretroviral treatment in HIV patients. These patients typically have prominent, peripheral fat wasting and maintained or decreased visceral fat depots and are insulin resistant. Interestingly, the difference in plasma PAI-1 levels between HIV patients and healthy controls was independent of HIV infection status and was not affected after adjustment for visceral fat estimation but was rather explained by metabolic disturbances such as hyperinsulinemia. Recently, a close relation between plasma PAI-1 levels and the amount of liver fat was described in these patients.

Indeed, besides insulin resistance, nonalcoholic liver steatohepatitis (NASH) is a common feature of android obesity and lipodystrophy. Patients with NASH have increased serum levels of γ-glutamyltransferase (γ-GT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Hepatic enzyme activity is associated with plasma PAI-1 levels in hyperlipemic as well as in normolipemic subjects. Furthermore, in ob/ob mice, which usually develop fatty liver, and in rabbits fed a high-fat diet, an increased PAI-1 expression was observed in liver, and PAI-1 is produced by cultured, human hepatoma HepG2 cells and by freshly

Received March 7, 2003; revision accepted April 17, 2003.
From the Laboratory of Hematology (M.-C.A., D.B., A.M., P.M., I.J.-V.), EPI 99[bhypen]36, Faculty of Medicine, Marseille, France; the Department of Angiology (A.M.), University Medical Centre, Ljubljana, Slovenia; the Department of Gastric Surgery (B.B.), CHU Timone, Marseille, France; the Service d’Endocrinologie (M.G.), Maladies Métaboliques et Nutrition, CHU Nord, Marseille, France; and the Centre d’Investigation Clinique (M.-C.A., P.M., I.J.-V.), AP-HM INSERM 95-02, Hôpital Ste. Marguerite, Marseille, France.
Correspondence to Pr M.-C. Alessi, Laboratoire d’Hématologie, EPI 99, 27, Bd Jean Moulin, 13385 Marseille Cedex 5, France. E-mail Marie-Christine.Alessi@medecine.univ-mrs.fr.
isolated human hepatocytes and is increased under insulin stimulation.24–26 These data suggest that the liver could be involved in plasma PAI-1 regulation. However, data on the relation between plasma PAI-1 levels and NASH are scanty.

In this study, the relation between PAI-1 and liver steatosis was evaluated in the murine model of genetically obese, insulin-resistant ob/ob mice with NASH and in the murine model of alcohol-induced liver steatosis. Also, the relation between PAI-1 and NASH was studied in a population of morbidly obese humans.

Methods

Murine Models

Genetically Obese, Insulin-Resistant ob/ob Mice

Eight-week-old male ob/ob mice (n=10; Elevage, Janvier, France) were fed a high-fat, liquid diet (47% carbohydrate, 35% fat, 18% protein; Lieber-DeCarli Control, BioServ) ad libitum for 5 weeks. Mice were euthanized by cervical dislocation after an overnight fast, always at the same time of day. Blood was collected by retro-orbital puncture into tubes with anticoagulant (0.01 mol/L trisodium citrate) and centrifuged for 15 minutes at 3000g to obtain plasma. Liver, intra-abdominal (epididymal), and subcutaneous (inguinal) adipose tissue territories were removed, weighed, and immediately frozen in LN₂.

Alcohol-Induced Liver Steatosis in C57/BL6 Mice

Eight-week-old female C57/BL6 mice (n=15; Elevage, Dépré, France) were fed an ethanol–high-fat liquid diet, which provided 1 kcal/mL and contained 36% ethanol, 35% fat, 18% protein, and 11% carbohydrate (Lieber-DeCarli Regular Ethanol Deyts Inc). Control C57/BL6 mice (n=15) were pair-fed a control diet in which ethanol was replaced isocalorically with maltose dextrin (Lieber-DeCarli Regular Control Deyts Inc). Euthanasia and blood sampling were performed as described for ob/ob mice. The liver only was collected, because the amount of adipose tissue territories was negligible. All experiments in animals were performed in accordance with the guiding principles of the ethics committee of the Faculty of Medicine, Marseille, France.

Morbidly Obese Humans

Forty morbidly obese subjects (BMI>35 kg/m²), 33 women and 7 men, with a mean age of 40 years (range, 23 to 59 years), were recruited at the Gastric Surgery Department, University Hospital Timone, Marseille, France. They had been admitted to the department for treatment of their obesity by gastroplasty. In the control group, there were 28 lean, apparently healthy volunteers, 21 women and 7 men, with a mean age of 40 years (range, 24 to 56 years). Ten obese subjects had type 2 diabetes, and 8 were being treated with hypoglycemic drugs, none with insulin. Four obese subjects were receiving antihypertensive drugs. None of the subjects had a history of a liver disease, alcohol abuse, or thromboembolic disease or suffered from any ongoing disease (eg, infection, cancer). In all obese subjects, gastropathy was performed and liver biopsy specimens were taken as a routine part of the operative procedure. Tissue samples were immediately embedded in paraffin. Blood samples were taken between 7:30 and 9 AM. Platelet-poor plasma was obtained after centrifugation for 30 minutes at 2000g and 4°C and stored at −70°C until analyzed. Informed, written consent was obtained from all subjects, and the study was conducted in conformance with the Helsinki Declaration.

Methods

Studies in Mice

Immunological Determination

PAI-1 antigen was determined by a home-made ELISA,27 and tumor necrosis factor-α (TNF) and TNF receptors I and II (TNFRI and TNFRII, respectively) were determined by specific ELISAs (R&D Systems). Values were expressed per milliliter of plasma or per total organ. In mice with alcohol-induced liver steatosis, adjustment per body weight was performed because of differences in body weight between the intervention and control groups.

Proteins, RNA, DNA, and Lipid Extractions

Tissue proteins were extracted as previously described.28 Total RNA from 200 to 250 mg adipose tissue or 30 to 40 mg liver was extracted by using a commercially available kit (RNeasy Mini Kit, Qiagen). The integrity of the RNA was confirmed by electrophoresis in ethidium bromide–containing agarose gels, and the RNA concentration was determined spectrophotometrically. DNA was extracted as proposed by Maniatis et al,29 except that two chloroform extractions were performed instead of one. The DNA concentration was determined spectrophotometrically. The method of Folch et al30 was used for lipid extraction.

Reverse Transcription and Relative Real-Time Polymerase Chain Reaction

Reverse transcription (RT) was performed on 2 μg total RNA, as previously described.31 Then the samples were diluted twice (for PAI-1 determination) or 1/1000 (for 18S) in sterile water. Amplification was performed in 25 μL on a thermal cycler (Abbott Prisma 7700, Perkin-Elmer). The polymerase chain reaction (PCR) conditions were as follows: 2 minutes at 50°C; 10 minutes at 95°C, followed by 40 cycles of a two-step PCR denaturation at 95°C for 15 seconds and annealing extension at 60°C for 60 seconds. Each sample contained 5 μL cDNA in 1× fluorescence fluid (SYBRGreen PCR Master Mix, Applied Biosystems) and 1 μL of each primer (400 nmol/L, Invitrogen). A control without cDNA was performed for each experiment. Primer express software (Applied Biosystems) was used to design primers for PAI-1 mRNA and 18S rRNA (housekeeping gene). For PAI-1 mRNA quantification, primers were 5'-ACA GCC TTT GTC ATC TCA GCC-3' (forward) and 5'-CGG AAC CAC AAA GAG AAA GGA-3' (reverse) (GenBank accession No. M33960). The primers for 18S rRNA were 5'-CTA CCA CAT CCA AGG AAC GCA-3' (forward) and 5'-TCT TTC GTC ACC TCC CCG-3' (reverse) (GenBank accession No. X00686). With the use of this technology, several PCR cycles are required before the specific fluorescence signal emerges above background fluorescence. The number of cycles (Cₚ) required to generate a threshold of 0.04 fluorescence units was determined in triplicate for each sample. The results of PAI-1 were normalized to those of 18S rRNA and expressed as a percentage of the sample chosen in each tissue; that was considered as 100% (user bulletin No. 2, Applied Biosystems).

Estimation of Liver Steatosis

Liver steatosis was estimated by determination of hepatic triglyceride content.

Study in Morbidly Obese Humans

Immunological and Biochemical Analyses

Plasma PAI-1 antigen and PAI-1 activity were assayed with commercially available kits (Asserachrom PAI-1, Diagnostica Stago, and Chromolize, Biopool International, respectively). HDL cholesterol, triglycerides, γ-GT, AST, and ALT concentrations were determined by routine biochemical methods. Insulin was assayed with an ELISA (Insulin Kit, Dako).

Estimation of Liver Steatosis

Human liver sections (5 μm) were mounted on slides (Superfrost Plus, CML), deparaffinized (MicroClear), and counterstained with Harris hematoxylin. Total and steatosis surfaces were automatically estimated using an image analyser (Samba 2005 TTIN-Alcatel). Steatosis surface was defined as the surface of empty holes within the tissue. The mean surface analyzed per subject was 1.89 mm² (range, 0.61 to 5.19 mm²). Results were expressed as a percentage of the total surface.
Statistical Analysis

In mice, data were expressed as mean ± SEM, and in humans, as the median and range between the 25th and 75th percentiles. Spearman’s correlation coefficient was used to examine relations among the variables. Differences in variables within the group of ob/ob mice were tested with the Wilcoxon test. Differences between C57/BL6 mice with and without alcoholic liver steatosis and differences between obese and lean subjects were tested by the Mann-Whitney U test. Stepwise multiple linear regression analysis was performed to evaluate the independence of associations. The independent variable was logarithmically transformed before being entered into the model. Significance was defined as P<0.05. Statistical analysis was performed with a software package (Statistica).

Results

Genetically Obese, Insulin-Resistant ob/ob Mice

Variables measured in ob/ob mice are shown in Table 1. PAI-1 antigen content in the liver was significantly higher than in subcutaneous or intra-abdominal fat (both P<0.05). Corresponding values for PAI-1 antigen expressed per gram of tissue were (mean ± SEM) as follows: 317.3±43.7, 162.9±11.5, and 181.1±7.9 ng/g, respectively. PAI-1 mRNA was detected in the liver as well as in subcutaneous and intra-abdominal fat (mean ± SEM, 109.2±27.6, 295.5±42.2, and 236.6±52.7 PAI-1 mRNA/18S rRNA as a percentage of control values, respectively). However, the method used for this determination (relative RT-PCR) does not allow comparison between these tissues, because the liver contained 25-fold more DNA per gram than did adipose tissue (data not shown).

A significant correlation was found between plasma PAI-1 levels and triglyceride content in the liver (r=0.82, P<0.004), PAI-1 antigen (r=0.65, P<0.05), and PAI-1 mRNA (r=0.70, P=0.02), and a positive association was also observed between plasma PAI-1 and body weight (Figure 1). Interestingly, liver PAI-1 mRNA expression was associated significantly and positively with liver steatosis (r=0.82, P<0.004), indicating that the degree of liver steatosis might influence PAI-1 expression in the liver.

In contrast to the relation obtained with variables measured in liver, there was no significant association between plasma PAI-1 levels and adipose tissue content of triglycerides, PAI-1 antigen (Figure 2), or PAI-1 mRNA. These results show that PAI-1 production in steatotic liver is more importantly connected with plasma PAI-1 level than PAI-1 production in adipose tissue.

Because it has been shown that TNF might be implicated in PAI-1 regulation in obesity,31 we quantified TNF and TNFRs antigens in the liver. We found a significant association between liver TNF or TNFRII and PAI-1 antigen content (r=0.80, P<0.01, and r=0.79, P<0.01, respectively). TNF antigen was associated with liver steatosis, with a borderline significance (r=0.61, P=0.06).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>42.5 ± 1.03</td>
</tr>
<tr>
<td>PAI-1 antigen</td>
<td></td>
</tr>
<tr>
<td>In plasma, ng/mL</td>
<td>27.2 ± 3.56</td>
</tr>
<tr>
<td>In subcutaneous fat, ng</td>
<td>39.1 ± 35.7</td>
</tr>
<tr>
<td>In intra-abdominal fat, ng</td>
<td>58.7 ± 35.9</td>
</tr>
<tr>
<td>In liver, ng</td>
<td>78.4 ± 138.8</td>
</tr>
<tr>
<td>Triglyceride content in liver, µmol</td>
<td>163.8 ± 27.58</td>
</tr>
<tr>
<td>TNF antigen in liver, pg</td>
<td>1.229 ± 0.194</td>
</tr>
<tr>
<td>TNFR1 antigen in liver, ng</td>
<td>15.46 ± 1.04</td>
</tr>
<tr>
<td>TNFR2 antigen in liver, ng</td>
<td>34.31 ± 4.59</td>
</tr>
</tbody>
</table>

Data in the liver and adipose tissue are expressed in ng or pg per organ.

Table 1. Variables Measured in ob/ob Mice

![Figure 1. Relations between plasma PAI-1 antigen (ag) and triglyceride, PAI-1 ag, and PAI-1 mRNA content in the liver and body weight in ob/ob and C57/BL6 mice. Open circles represent control mice and filled circles represent alcohol-fed mice. Variables determined in the liver of C57/BL6 mice are adjusted for body weight.](http://atvb.ahajournals.org/)

![Figure 2. Relation between plasma PAI-1 antigen (ag) and PAI-1 ag content of intra-abdominal (open circles) and subcutaneous (dark circles) adipose tissues in ob/ob mice.](http://atvb.ahajournals.org/)
Alcohol-Induced Liver Steatosis in C57/BL6 Mice

Development of liver steatosis in mice on the alcohol diet was verified by histological analysis (data not shown). Variables measured in C57/BL6 mice with and without alcohol-induced liver steatosis are shown in Table 2; an adjustment for body weight was performed, because the two groups differed in body weight. Plasma PAI-1 levels, liver content of PAI-1 antigen, PAI-1 mRNA, TNFRII antigen, triglycerides, and liver mass were significantly higher in mice with alcoholic liver steatosis than in control mice.

A significant correlation was found between plasma PAI-1 levels and liver content of triglycerides (r=0.37, P=0.05), PAI-1 antigen (r=0.37, P=0.05), and PAI-1 mRNA (r=0.78, P=0.0002; Figure 1). In contrast to findings in ob/ob mice, we assessed TNF and TNFRs in the liver. We found a significant association between liver TNFRII and PAI-1 antigen, PAI-1 mRNA, and triglyceride content (r=0.62, P<0.01; r=0.51, P<0.01; and r=0.43, P=0.02, respectively).

Study in Morbidly Obese Humans

As expected, higher levels of plasma PAI-1, insulin resistance markers, and liver enzymes were found in obese than in lean subjects (Table 3). In liver biopsies of obese subjects, computerized estimation of the steatosis surface had a median value of 2.38% (25th and 75th percentiles, 0.9% and 9.6%, respectively). Liver steatosis surface was correlated positively with serum AST (r=0.48, P<0.01), ALT (r=0.51, P<0.01), and γ-GT (r=0.38, P<0.02).

Positive correlations between plasma PAI-1 antigen and activity and some parameters of insulin resistance were

---

**TABLE 2. Variables Measured in C57/Bl6 Mice With and Without Alcoholic Liver Steatosis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>With Alcoholic Liver Steatosis</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (final), g</td>
<td>16.5±0.5</td>
<td>19.2±0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>∆ in BW during the study, g</td>
<td>-2.1±0.5</td>
<td>-1.7±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>PAI-1 antigen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In plasma, ng/mL</td>
<td>4.2±0.5</td>
<td>0.8±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>In liver, ng/g BW</td>
<td>43.0±2.7</td>
<td>36.4±1.9</td>
<td>0.04</td>
</tr>
<tr>
<td>PAI-1 mRNA in liver, ratio to 18S rRNA</td>
<td>249.8±41.9</td>
<td>90.3±15.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>1.01±0.03</td>
<td>0.84±0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride in liver, μmol/g BW</td>
<td>2.25±0.23</td>
<td>1.53±0.15</td>
<td>0.02</td>
</tr>
<tr>
<td>TNF antigen in liver, pg/g BW</td>
<td>0.76±0.21</td>
<td>0.43±0.18</td>
<td>NS</td>
</tr>
<tr>
<td>TNFR1 antigen in liver, pg/g BW</td>
<td>50.48±3.90</td>
<td>59.19±5.61</td>
<td>NS</td>
</tr>
<tr>
<td>TNFR2 antigen in liver, pg/g BW</td>
<td>196.5±16.26</td>
<td>92.7±7.73</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are shown as mean±SEM. Variables determined in liver are adjusted for body weight (BW).

**TABLE 3. BMI, Plasma PAI-1 Antigen and Activity, Markers of Insulin Resistance, Liver Enzymes in Morbid Obese and Lean Subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obese (N=40)</th>
<th>Lean (N=28)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>41.3 (38.6–45.9)</td>
<td>21.8 (20.7–23.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PAI-1 antigen, ng/mL</td>
<td>53.5 (32.5–91)</td>
<td>4.8 (2.5–6.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PAI-1 activity, IU/mL</td>
<td>21.5 (15.5–40.0)</td>
<td>3.2 (2.3–5.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin, μU/L</td>
<td>13 (9–18)</td>
<td>3.4 (2.4–5.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.00 (4.44–5.60)</td>
<td>4.65 (4.40–5.10)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.85 (1.50–2.31)</td>
<td>0.86 (0.63–1.20)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.99 (0.90–1.21)</td>
<td>1.45 (1.22–1.66)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>γ-GT, IU/mL</td>
<td>20 (14–29)</td>
<td>11.0 (8.0–13.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>21 (16–29)</td>
<td>12.0 (10.0–16.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>10.5 (7–16)</td>
<td>8.0 (5.0–10.0)</td>
<td>&lt;0.04</td>
</tr>
</tbody>
</table>

Data are shown as median and range between 25th and 75th percentiles.
TABLE 4. Spearman Correlation Coefficients Between Plasma PAI-1 (Activity and Antigen) and Markers of Insulin Resistance, Liver Enzymes and Steatosis Surface in Morbid Obese Subjects (n=40)

<table>
<thead>
<tr>
<th></th>
<th>PAI-1 act</th>
<th></th>
<th>PAI-1 ag</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>BMI</td>
<td>0.22</td>
<td>0.16</td>
<td>0.33</td>
<td>0.05</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.20</td>
<td>0.23</td>
<td>0.19</td>
<td>0.29</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.31</td>
<td>0.06</td>
<td>0.42</td>
<td>0.01</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.37</td>
<td>0.02</td>
<td>0.38</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.17</td>
<td>0.32</td>
<td>-0.19</td>
<td>0.30</td>
</tr>
<tr>
<td>AST</td>
<td>0.57</td>
<td>0.0001</td>
<td>0.50</td>
<td>0.002</td>
</tr>
<tr>
<td>ALT</td>
<td>0.28</td>
<td>0.08</td>
<td>0.11</td>
<td>0.54</td>
</tr>
<tr>
<td>γ-GT</td>
<td>0.32</td>
<td>0.04</td>
<td>0.08</td>
<td>0.64</td>
</tr>
<tr>
<td>Liver steatosis surface</td>
<td>0.52</td>
<td>0.0005</td>
<td>0.42</td>
<td>0.01</td>
</tr>
</tbody>
</table>

expected but were rather weak in this morbidly obese, insulin-resistant population (Table 4). Furthermore, we observed that plasma PAI-1 activity was more strongly associated with liver steatosis surface and with liver enzymes (mainly AST). A multiple linear regression analysis, with plasma PAI-1 activity as the dependent variable and insulin levels and liver steatosis surface as independent variables, showed significant and independent association between these three variables. Liver steatosis surface ($\beta=0.43$, $P=0.005$) and insulin levels ($\beta=0.36$, $P=0.02$) contributed to 26% of the variability of plasma PAI-1 activity.

Discussion

PAI-1 is involved in the atherothrombotic process. An elevated plasma PAI-1 level might be considered a risk factor for cardiovascular events, but its predictive value disappears after adjustment for insulin resistance parameters. In insulin resistance with android obesity, plasma PAI-1 levels are increased. Therefore, several recent studies that aimed to determine PAI-1 origin were focused on adipose tissue. Generally, adipose tissue was found to be a potential contributor to elevated plasma PAI-1 concentration; however, as a source of PAI-1, it cannot satisfactorily explain the increase in plasma PAI-1 in insulin resistance. In this study, we extended the knowledge on PAI-1 by showing that its plasma levels are closely related to the degree of liver steatosis, regardless of its etiology, suggesting that fat accumulation in the liver is more closely related to plasma PAI-1 levels than fat accumulation in adipose tissue.

It was previously shown that genetically obese ob/ob mice, which develop fatty liver early in life, have 2-fold higher PAI-1 expression in the liver than do control lean mice. Our study in ob/ob mice showed that plasma PAI-1 concentration is associated with fat accumulation in both adipose tissue (reflected by body weight) and liver (reflected by the degree of liver steatosis). PAI-1 antigen content in the liver was significantly higher than in the collected adipose tissue territories. Interestingly, plasma PAI-1 was associated with PAI-1 expression in the liver but not in adipose tissue. From these results, we concluded that the pathologic process of liver steatosis reflected systemic PAI-1 levels more importantly than adipose tissue accumulation. Our results in lean CB57/BL6 mice with alcohol-induced liver steatosis were in accordance with this concept. After we induced liver steatosis, there was a 2- to 3-fold increase in plasma PAI-1 levels and in liver PAI-1 expression compared with those values in the control group. Plasma PAI-1 levels were associated with fat accumulation in the liver as well as with PAI-1 expression in the liver. Importantly, we found a significant, negative correlation between body weight and plasma PAI-1. This further suggests that PAI-1 production in adipose tissue is no longer important for systemic PAI-1 in the presence of liver steatosis. In contrast to the model of ob/ob mice, in the model of alcohol-induced liver steatosis, no correlation between liver PAI-1 mRNA and the degree of liver steatosis was observed. This suggests that regulation of PAI-1 expression does not depend only on the accumulation of fatty acids in hepatocytes. An effect of alcohol on PAI-1 synthesis also could not be completely ruled off. Ethanol has been shown to decrease PAI-1 transcription in cultured human endothelial cells, and a 251-bp fragment of the PAI-1 gene promoter was involved in this phenomenon. However, short-term alcohol intake increased plasma PAI-1 levels in vivo, and long-term alcohol consumption was accompanied by increased plasma PAI-1 levels, but in these studies, the degree of liver steatosis was not measured.

In humans, only a few studies have revealed the relations between circulating PAI-1 and liver dysfunction, as assessed by increased liver enzyme activity. In our population of morbid obesity subjects, plasma PAI-1 was associated more strongly with AST than with ALT or γ-GT levels, suggesting a common triggering factor for AST release from damaged hepatocytes and elevated plasma PAI-1. The present study showed for the first time the association between histologically assessed degree of liver steatosis and plasma PAI-1 levels. In our population of patients with morbid obesity, this association appeared to be stronger than that observed between insulin resistance markers and circulating PAI-1. From results of the multivariate regression analysis, we found that both liver steatosis and insulin may regulate plasma PAI-1 levels in morbid obesity. Overall, these results support the possibility that in insulin resistance, a fatty liver is an important source of plasma PAI-1. Highly accordant with this hypothesis also are recent findings in HIV patients with antiretroviral therapy–associated lipodystrophy. The amount of liver fat, detected by magnetic resonance imaging, is significantly correlated with plasma PAI-1 antigen, and changes in the amount of liver fat after rosiglitazone treatment were associated with changes in plasma PAI-1 antigen levels. Furthermore, our hypothesis is further favored by evidence of an association between circulating PAI-1 and PAI-1 expression in the liver, but not in adipose tissue, in the mouse model. Unfortunately, we were unable to confirm this finding in obese humans, because storage of the liver tissue was not proceeded for mRNA detection.

The molecular basis of the relation between PAI-1 and NASH remains unknown. It has been shown that TNF hepatic expression is increased in ob/ob mice and also in mice with alcohol-induced liver disease, and it was purposed that TNF contributes to the mitochondrial dysfunction that is impli-
cated in the pathogenesis of NASH and alcoholic steatohepatitis.30 Furthermore, in ob/ob mice, inactivation of both TNFRs (TNFRI and TNFRII) or the administration of TNF-neutralizing antibodies significantly reduced plasma PAI-1 levels.31 In this study, we showed for the first time that PAI-1 antigen is associated with TNF and TNFRII antigens in steatotic liver. Thus, dysregulation of the TNF pathway in fatty liver accompanies increased liver PAI-1 expression. Strong correlations between liver TNFRII and PAI-1 antigen, found in both animal models of liver steatosis, suggest that in such conditions, TNF signaling of PAI-1 is mediated through TNFRII or that PAI-1 and TNFRII are simultaneously expressed. Additional experiments should be performed to delineate the role of TNFRs in PAI-1 regulation during NASH. Nevertheless, we speculate that TNF-induced PAI-1 synthesis in NASH is one of the mechanisms for increased plasma PAI-1 in obesity with insulin resistance.

There is another potential mechanism for increased plasma PAI-1 in obesity, which includes a direct effect of free fatty acids. Resistance to the antilipolytic action of insulin in peripheral tissues, particularly abdominal adipose tissue, results in an increased liver supply of free fatty acids and enhanced triglyceride deposition in the liver.17,43 Lipoproteins can induce PAI-1 production in endothelial cells and hepatocytes.42–44 Recently, it was shown that the PAI-1 promoter contains sequences able to bind free fatty acids or VLDL response elements.45,46 Thus, free fatty acids might induce PAI-1 gene expression in the liver directly.

In conclusion, this study shows a strong relation between plasma PAI-1 and the degree of liver steatosis, which is independent of body (and adipose tissue) mass. Our results suggest that a complex interaction between free fatty acid accumulation in hepatocytes and innate immunity may be responsible for increased circulating PAI-1 in obesity with insulin resistance.

Acknowledgments
This work was supported by grants from Institut National de la Santé et de la Recherche Médicale (INSERM—Centre d’Investigation Clinique, Hôpital St. Marguerite), the MENESR (Contrat Quadrennal), and the Fondation de France (No. 2001005208). D. Bastelica is a recipient from the Fondation pour la Recherche Médicale (FRM). We thank M. Verdier, O. Geel-Georgelin, and J-C. Coudeyre for excellent technical assistance.

References


Plasma PAI-1 Levels Are More Strongly Related to Liver Steatosis Than to Adipose Tissue Accumulation

Marie-Christine Alessi, Delphine Bastelica, Alenka Mavri, Pierre Morange, Bruno Berthet, Michel Grino and Irene Juhan-Vague

Arterioscler Thromb Vasc Biol. 2003;23:1262-1268; originally published online May 15, 2003; doi: 10.1161/01.ATV.0000077401.36885.BB

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/23/7/1262

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/