Comparison of Plasminogen Activator Inhibitor-1 Concentration in Insulin-Resistant Versus Insulin-Sensitive Healthy Women

Fahim Abbasi, Tracey McLaughlin, Cindy Lamendola, Izabella Lipinska, Geoffrey Tofler, Gerald M. Reaven

Abstract—The primary goal of this investigation was to see whether plasminogen activator inhibitor-1 (PAI-1) concentrations varied as a function of differences in insulin-mediated glucose disposal in 2 groups of healthy women matched for every other variable that might play a role in regulation of PAI-1. For this purpose, we recruited 32 healthy women, divided on the basis of their steady-state plasma glucose (SSPG) concentrations during the insulin suppression test into an insulin-resistant (SSPG=216±12 mg/dL, n=16) and an insulin-sensitive (94±6 mg/dL, n=16) group. PAI-1 antigen concentrations were significantly higher (26±4 versus 14±3 ng/mL, P<0.02) in the insulin-resistant group. In addition, fasting plasma insulin (18±3 versus 11±2 μU/mL, P<0.02) and triglyceride (160±19 versus 93±10 mg/dL, P<0.001) concentrations were higher in the insulin-resistant individuals, whereas HDL concentrations were lower (44±3 versus 58±3 mg/dL, P<0.005). However, the 2 groups were essentially identical in terms of age, menopausal status, hormone replacement therapy, body mass index (BMI), ratio of waist-to-hip girth, and blood pressure. When the experimental population was considered as 1 group, there were statistically significant correlations between PAI-1 antigen and the following variables: adjusting for differences in age and BMI, SSPG (r=0.56, P<0.001); triglyceride (r=0.39, P<0.05); and HDL cholesterol (r=−0.65, P<0.001) concentrations. Finally, multiple regression analysis revealed the major determinants of PAI-1 to be insulin resistance, or insulin concentration, and HDL cholesterol. These results: 1) demonstrate that PAI-1 concentrations are higher in healthy, insulin-resistant women as compared with insulin-sensitive individuals, independent of differences in BMI or ratio of waist-to-hip girth; and 2) provide another mechanism by which insulin-resistant individuals are at increased thrombotic cardiovascular risk.

Key Words: PAI-1 ■ insulin resistance ■ HDL cholesterol ■ hyperinsulinemia ■ triglycerides

Reports published over the last several years have presented a substantial amount of evidence of an association between plasma insulin concentrations and plasminogen activator inhibitor-1 (PAI-1) activity.1–4 Perhaps the most compelling evidence in this context came from the report of the European Concerted Action on Thrombosis and Disabilities (ECAT) Angina Pectoris Study.4 The results of the ECAT Study of 1484 patients with angina pectoris demonstrated an independent relationship between hyperinsulinemia and PAI-1 levels in individuals with evidence of coronary atherosclerosis. Given the very close relationship in nondiabetic individuals between insulin resistance and hyperinsulinemia,5,6 it has been suggested that the elevated levels of PAI-1 are a reflection of insulin resistance.1–4 On the other hand, results of studies in which insulin resistance has been measured directly have not universally confirmed the assumed relationship between insulin resistance and PAI-1 level.7–10 Indeed, 3 of these studies7–9 have been unable to demonstrate an independent relationship between insulin resistance and PAI-1, with the only positive report10 based on an experimental population of 70-year-old men. It is not clear why a disparity seems to exist concerning the consistency of the relationship of PAI-1 to plasma insulin concentration as compared with insulin resistance. However, several of the reports have been based on analysis of data collected in large population-based studies,5–10 not research efforts specifically initiated to evaluate the relationship between insulin resistance and PAI-1 levels. In some instances, the population was composed of individuals who were hypertensive6 or obese and often glucose intolerant.7 Finally, all of the publications relied on the use of multivariate analysis to take into account the wide differences in values of both the demographic and metabolic variables that were evaluated for their relationship to PAI-1. Although this can be a useful approach, it is based on many assumptions as to the quantitative nature of the relationships between the variables in question and certainly

Received August 27, 1998; revision accepted March 31, 1999.
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can be confounded by differences in the intra- and interindividual variability of each of the variables.

Given evidence of the association between impaired fibrinolytic activity and thrombotic cardiovascular risk,\textsuperscript{11,12} an attempt to clarify the relationship between insulin resistance and elevations of PAI-1 seemed to be a worthwhile goal. The current study was initiated for this purpose and was based on a protocol aimed at avoiding some of the confounding variables present in previous studies. Specifically, we limited enrollment to healthy individuals and used selection criteria that minimized or avoided differences in age, gender, obesity, etc.

Methods

Thirty-two healthy nondiabetic women were selected for this study. They were recruited from a larger group that responded to a newspaper advertisement indicating our interest in studying the relationship between insulin resistance and risk factors for coronary heart disease. To enter the study, women had to be in good general health, with a body mass index (BMI) between 20 to 33 kg/m\textsuperscript{2}, a normal medical history and physical examination, normal values on a routine hematological survey and chemical screening battery, and a nondiabetic glucose tolerance test by National Diabetes Data Group criteria.\textsuperscript{13} The population was subdivided into 2 groups—insulin-sensitive and insulin-resistant—on the basis of the results of their insulin suppression test as described below.

Patients were admitted to the General Clinical Research Center of Stanford Medical Center after informed consent had been obtained. Insulin-mediated glucose disposal was estimated by a modification\textsuperscript{14} of the insulin suppression test,\textsuperscript{13} as validated by our laboratory.\textsuperscript{16} After an overnight fast, an IV catheter was placed in each of the patients’ arms. Blood was sampled from 1 arm for measurement of plasma glucose and insulin concentrations, and the contralateral arm was used for administration of test substances. Somatostatin was administered (250 \(\mu\)g/h in a solution containing 2.5% [w/v] human serum albumin) to suppress endogenous insulin secretion. Simultaneously, insulin and glucose were infused at rates of 25 \(\mu\)U/(m\textsuperscript{2}·min) and 240 mg/(m\textsuperscript{2}·min), respectively. Blood was sampled every 30 minutes until 150 minutes into the study and then every 10 minutes until 180 minutes had elapsed. The 4 values obtained from 150 to 180 minutes were averaged and considered to represent the steady-state plasma glucose (SSPG) and steady-state plasma insulin (SSIPI) concentrations achieved during the infusion. Because SSPI concentrations are comparable in all individuals, SSSIP concentrations provide a direct estimate of insulin-mediated glucose disposal in each individual; the lower the SSPG, the more insulin-sensitive the individual. Volunteers were separated into 2 groups using an SSPG concentration of 150 mg/dL as the cut point.

This somewhat arbitrary value was chosen based on unpublished data showing that 1/3 of ~300 healthy volunteers will have an SSPG concentration >150 mg/dL. With this criterion, we created 2 equal groups of 16.

Aliquots of the blood specimens obtained on the morning of the insulin suppression test were also obtained to measure concentrations of triglycerides (TG), LDL cholesterol, and HDL cholesterol concentrations, as described previously.\textsuperscript{17} Finally, an aliquot was also taken to measure PAI-1 antigen concentration.\textsuperscript{18} All blood samples were collected in citrate tubes, centrifuged at 4°C immediately, and plasma stored in aliquots at −70°C until used for the various analyses. Plasma for PAI-1 measurements were shipped by overnight express under dry ice to G.T.’s laboratory in Boston for measurement.

Results are expressed as mean±SEM. Means of 2 groups were compared with Student’s nonpaired \(t\) test and Kruskal-Wallis 1-Way ANOVA. Pearson’s product-moment correlations and partial correlation coefficients were calculated to determine relations between variables of interest. Finally, multiple regression analysis was performed using different models (see Results) with dependent variable being PAI-1 antigen concentration. Statistical analyses were conducted using Systat 7.0 package for Windows.

**Table 1. Characteristics of Insulin-Sensitive and Insulin-Resistant Women (Mean±SEM)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Insulin Sensitive (n=16)</th>
<th>Insulin Resistant (n=16)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSPG (mg/dL)</td>
<td>94±6</td>
<td>216±12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (y)</td>
<td>52±2</td>
<td>52±3</td>
<td>NS</td>
</tr>
<tr>
<td>Post menopausal</td>
<td>10</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>Hormone replacement therapy (yes/no)</td>
<td>7/3</td>
<td>6/3</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>29.1±1.1</td>
<td>29.4±1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.80±0.04</td>
<td>0.82±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>91±3</td>
<td>92±2</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin ((\mu)U/mL)</td>
<td>11±2</td>
<td>18±3</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>93±10</td>
<td>160±19</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>58±3</td>
<td>44±3</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>121±10</td>
<td>118±8</td>
<td>NS</td>
</tr>
<tr>
<td>PAI-1 (mg/mL)</td>
<td>14±3</td>
<td>26±4</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

**MAP** indicates mean arterial pressure (1/3 pulse pressure+diastolic blood pressure); NS, not significant.
coefficients between PAI-1 concentration and the variables that were significantly different in Table 1. The results of this analysis are given in Table 2 and show that the PAI-1 concentration was significantly ($P<0.05$) correlated with age, BMI, SSPG concentration, fasting insulin, TG, and HDL cholesterol concentrations. When partial correlation coefficients between PAI-1 and the metabolic variables were determined, adjusted for differences in age and BMI, it can be seen in Table 2 that significant relationships continued to exist between PAI-1 concentrations and SSPG, insulin, TG, and HDL cholesterol concentrations. Parenthetically, the significant simple correlation coefficient ($r=0.39, P=0.03$) between BMI and PAI-1 was no longer significant when adjusted for differences in SSPG concentration ($r=0.16, P=0.41$).

To further define the relationship between PAI-1 concentrations and the variables related to it, multiple regression analysis was used. Table 3 presents the relationship between PAI-1 and age, BMI, WHR, MAP, and SSPG. It can be seen that only SSPG was significantly related to PAI-1, with a standardized regression coefficient of 0.65, $P<0.001$. Because fasting insulin and SSPG concentrations are highly correlated, we did not enter them in the same model. However, when insulin concentrations replaced SSPG in the model, the standardized regression coefficient was quite similar (0.74, $P<0.001$). Furthermore, the $r^2$ values for the models with either SSPG or insulin entered were essentially identical, being 0.64 and 0.57, respectively.

The addition of TG to the model shown in Table 3 had essentially no effect. However, when HDL cholesterol was entered, the results shown in Table 4 indicate that the strength of the relationship between SSPG and PAI-1 was greatly weakened. An essentially identical result was seen when fasting insulin replaced SSPG in the model shown in Table 4. Finally, when SSPG was replaced with HDL cholesterol in the model shown in Table 3, HDL cholesterol was independently related to PAI-1, with a standardized regression coefficient of $-0.74, P<0.001$. The $r^2$ for this model was 0.63, ie, essentially identical to the value when SSPG, rather than HDL cholesterol, was entered.

### Discussion

The primary goal of the investigation was to see whether PAI-1 concentrations varied as a function of differences in insulin-mediated glucose disposal in 2 groups of healthy women matched for every other variable thought to play a role in regulation of PAI-1. As such, the results presented were unequivocal in that PAI-levels were higher in insulin-resistant as compared with insulin-sensitive women, despite the fact that the 2 groups were identical in terms of age, menopausal status, hormone replacement therapy, overall obesity (BMI), regional fat distribution (WHR), and blood pressure. Thus higher PAI-levels in insulin-resistant women cannot be attributed to differences in any of the above potentially confounding variables. This point of view is in contrast to the findings of Nagi et al,7 Mykkanen et al,8 and Toft and associates,9 all 3 groups coming to the conclusion that obesity, not insulin resistance, was a determinant of PAI-1. There are several possible reasons for the disparity in experimental results. For example, the subjects studied by Nagi et al9 were generally obese and included individuals with diabetes and impaired glucose tolerance, and Toft et al8 studied only patients with hypertension. In contrast, we excluded individuals with any known disease. In addition, the method10 used to quantify insulin resistance by Mykkanen and associates8 has a much weaker correlation in glucose-tolerant individuals ($r=0.53, P<0.05$) to the hyperinsulinemic clamp technique for measuring insulin-mediated...
glucose disposal than the approach\textsuperscript{16} we used (\(r=0.9, P<0.001\)). Although these differences may be important ones, we believe the most likely explanation for the disparity in results is the fundamental difference in the experimental protocol of the various studies. Specifically, at the outset of our study, we purposefully recruited 2 groups of volunteers, totally disparate on the basis of insulin-mediated glucose disposal but similar in terms of BMI and WHR. In contrast, the conclusion of the other research groups that these measures of obesity were independent determinants of PAI-1 was based on multiple regression analysis and in 2 instances\textsuperscript{8,9} involved a random selection of nondiabetic volunteers from previously conducted population-based studies initiated for other reasons. However, even when we applied multiple regression analysis to our entire population, we were unable to confirm an independent relationship between PAI-1 and either BMI or WHR (see Tables 3 and 4). On the other hand, the study population was chosen to evaluate the relationship between insulin resistance and PAI-1. It is certainly possible that the relationship between obesity and PAI-1 would have been greater if the study population had a greater range of obesity.

Although we were unable to discern any relationship between measures of obesity and PAI, our results in other respects are consistent with previous publications. In this respect, our findings resemble most closely those of Byberg and associates,\textsuperscript{10} who found that insulin sensitivity was a statistically significant determinant of PAI-1 activity, independent of TG, BMI, and WHR in men. In addition, similar to the findings of Nagi et al\textsuperscript{9} and Mykkanen et al,\textsuperscript{8} we found that in women, PAI-1 was significantly related to both HDL cholesterol concentration and/or insulin concentration. Parenthetically, given the close association between insulin resistance and hyperinsulinemia,\textsuperscript{5,6} it seems somewhat surprising that insulin concentration, but not insulin action, was independently related to PAI-1 in the studies of Mykkanen and associates.\textsuperscript{8} Again, this may be the confounding effect that exists when 2 closely related variables are entered into multiple regression models.

It should be emphasized that our conclusion that the relationship between insulin resistance and PAI-1 concentrations is independent of obesity does not rule out the possibility that obesity, per se, may contribute to an increase in PAI-1 concentrations. Indeed, the results in Table 1 documented a simple correlation coefficient between BMI and PAI-1 that was statistically significant (\(r=0.39, P=0.03\)). The fact that it was no longer significant when adjusted for differences in SSPG suggests that the relationship between PAI-1 and insulin resistance is closer than that between PAI-1 and obesity. However, our experimental population was limited to 32 subjects, and our inability to discern a relationship between BMI and PAI-1 may represent a type II error. Consequently, we would like to reiterate that our findings do not negate the possibility that BMI and PAI-1 are also significantly related.

In conclusion, PAI-1 concentrations were higher in insulin-resistant than in insulin-sensitive women, and this difference was seen despite the fact that the 2 groups were essentially identical in terms of BMI and WHR. Three metabolic variables seemed to be closely related to PAI-1 concentrations—insulin resistance, plasma insulin concentration, and HDL cholesterol concentration. The 3 variables that were most closely related to PAI-1 are themselves highly correlated. As such, we would be loath to speculate as to which of these was “independently” related to PAI-1. However, we are not reluctant to conclude that PAI-1 levels were higher in insulin-resistant as compared with insulin-sensitive women and that this was not because the insulin-resistant women were more obese. As such, elevated PAI-1 concentrations appear to be another reason why insulin-resistant individuals are at increased risk for coronary heart disease.

Acknowledgment

Supported by Research Grants (HL-08506 and RR-00070) from the National Institutes of Health.

References


15. Abbasi et al. PAI-1 Concentration and Insulin Resistance

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doi: 10.1161/01.ATV.19.11.2818
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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