Relative Contribution of Insulin and Its Precursors to Fibrinogen and PAI-1 in a Large Population With Different States of Glucose Tolerance

The Insulin Resistance Atherosclerosis Study (IRAS)

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Abstract—Hyperinsulinemia is associated with the development of coronary heart disease. However, the underlying mechanisms are still poorly understood. Hypercoagulability and impaired fibrinolysis are possible candidates linking hyperinsulinemia with atherosclerotic disease, and it has been suggested that proinsulin rather than insulin is the crucial pathophysiological agent. The aim of this study was to investigate the relationship of insulin and its precursors to markers of coagulation and fibrinolysis in a large triethnic population. A strong and independent relationship between plasminogen activator inhibitor-1 (PAI-1) antigen and insulin and its precursors (proinsulin, 32-33 split proinsulin) was found consistently across varying states of glucose tolerance (PAI-1 versus fasting insulin [proinsulin], \( r = 0.38 \) \( r = 0.34 \)) in normal glucose tolerance; \( r = 0.42 \) \( r = 0.43 \) in impaired glucose tolerance; and \( r = 0.38 \) \( r = 0.26 \) in type 2 diabetes; all \( P < 0.001 \). The relationship remained highly significant even after accounting for insulin sensitivity as measured by a frequently sampled intravenous glucose tolerance test. In a stepwise multiple regression model after adjusting for age, sex, ethnicity, and clinic, both insulin and its precursors were significantly associated with PAI-1 levels. The relationship between fibrinogen and insulin and its precursors was significant in the overall population \( (r = 0.20 \) for insulin and proinsulin; each \( P < 0.001 \)) but showed a more inconsistent pattern in subgroup analysis and after adjustments for demographic and metabolic variables. Stepwise multiple regression analysis showed that proinsulin (split products) but not fasting insulin significantly contributed to fibrinogen levels after adjustment for age, sex, clinic, and ethnicity. Decreased insulin sensitivity was independently associated with higher PAI-1 and fibrinogen levels. In summary, we were able to demonstrate an independent relationship of 2 crucial factors of hemostasis, fibrinogen and PAI-1, to insulin and its precursors. These findings may have important clinical implications in the risk assessment and prevention of macrovascular disease, not only in patients with overt diabetes but also in nondiabetic subjects who are hyperinsulinemic. (Arterioscler Thromb Vasc Biol. 1999;19:562-568.)

Key Words: non–insulin-dependent diabetes mellitus ■ plasminogen activator inhibitor-1 ■ fibrinogen ■ insulin ■ proinsulin

Considerable epidemiological evidence indicates that hyperinsulinemia is associated with the development of coronary heart disease, and a hallmark of atherosclerosis. Hyperinsulinemia has been related to insulin resistance. The relationship between insulin resistance, as measured by a frequently sampled intravenous glucose tolerance test, and atherosclerosis, as determined by carotid ultrasound, was recently reported in a large, multiethnic population. In this study (Insulin Resistance Atherosclerosis Study [IRAS]), an inverse association between insulin sensitivity and the intimal-medial thickness of the carotid artery was demonstrated both in Hispanics and in non-Hispanic whites. However, the mechanisms linking hyperinsulinemia to atherosclerosis are still poorly understood, and the direct effects of insulin and/or its indirect effects via established cardiovascular risk factors have been proposed. Hyperinsulinemia and insulin resistance are associated with factors known to increase cardiovascular risk, namely, glucose intolerance, dyslipidemia, and hypertension. More recently, additional factors have been related to the insulin resistance syndrome, such as hypercoagulability and hypofibrinolysis. Fibrinogen is a strong and independent predictor of myocardial infarction and stroke in nondiabetic subjects, and high fibrinogen levels have been associated with the risk of...
macrovascular complications in diabetic patients.\textsuperscript{19,20} An independent relationship between fibrinogen and insulin has been demonstrated previously in nondiabetic subjects.\textsuperscript{21–23} However, data on a possible relationship in subjects with impaired glucose tolerance (IGT) or type 2 diabetes are scarce.

High levels of plasminogen activator inhibitor type 1 (PAI-1) have been consistently associated with increased insulin concentrations\textsuperscript{24} and decreased insulin sensitivity.\textsuperscript{22,25,26} Juhan-Vague et al\textsuperscript{27} have suggested that increased PAI-1 levels may be a link between insulin resistance and coronary heart disease.

Many of the studies describing the association between insulin resistance and/or hyperinsulinemia with cardiovascular disease and its risk factors have been limited by the fact that insulin was measured with an assay that cross-reacts with proinsulin. Temple et al\textsuperscript{28} have suggested that proinsulin and 32-33 split proinsulin may comprise the majority of circulating immunoreactive insulin in subjects with type 2 diabetes. Recent data suggest that proinsulin rather than insulin might determine PAI-1 expression in diabetic and nondiabetic subjects.\textsuperscript{29} Experimental data support this hypothesis, showing a direct stimulation of PAI-1 synthesis by proinsulin and proinsulin split products.\textsuperscript{30,31} Proinsulin has been related to cardiovascular risk factors, such as dyslipidemia and hypertension, in diabetic\textsuperscript{32} and nondiabetic\textsuperscript{23,33} subjects, whereas there are few data on the association of proinsulin and/or its split products with the hemostatic system, especially in subjects with IGT or type 2 diabetes. Currently, no information exists about the impact of insulin sensitivity on the association of insulin and its precursors with hemostasis.

The aim of our study was to investigate the relationship of insulin and its precursors (intact proinsulin, proinsulin split products) with markers of coagulation and fibrinolysis (fibrinogen and PAI-1) in a large, triethnic population across different states of glucose tolerance.

Methods

Study Subjects

The IRAS is a multicenter epidemiological study aiming to explore relationships between insulin resistance, cardiovascular risk factors, and disease across different ethnic groups and varying states of glucose tolerance. A full description of the design and methods of the IRAS has been published previously.\textsuperscript{34} In brief, this study was conducted at 4 clinical centers. Clinical centers in Oakland and Los Angeles, Calif, studied non-Hispanic whites and African-Americans recruited from Kaiser Permanente, a nonprofit health maintenance organization. Clinical centers in San Antonio, Tex, and the San Luis Valley, Colo, studied non-Hispanic whites and Hispanics recruited from 2 ongoing population-based studies (the San Antonio Heart Study\textsuperscript{35} and the San Luis Valley Diabetes Study\textsuperscript{36}). Recruitment was tailored to yield approximately equal numbers of participants with regard to ethnicity, sex, and glucose tolerance categories (type 2 diabetes, IGT, and normal glucose tolerance [NGT]). The IRAS protocol was approved by local institutional review committees, and all subjects gave informed consent.

This report includes data on 1551 subjects in whom proinsulin (intact and split products), PAI-1, and fibrinogen levels were assessed. Demographic and metabolic data of the study subjects are shown in Table 1. Race and ethnicity were assessed by self-report. Hispanic ethnicity was defined by the US census question: Are you of Spanish or Hispanic descent? Height and weight were measured by following a standardized protocol. Body mass index (BMI; weight/height\textsuperscript{2} [kg/m\textsuperscript{2}]) was used as an estimate of overall adiposity. The IRAS examination required 2 visits. Patients were asked before each visit to fast for 12 hours, to abstain from heavy exercise and alcohol for 24 hours, and to refrain from smoking on the morning of the examination. Blood was collected in the fasting state for measurements of all reported parameters on the same day that the oral glucose tolerance test (OGTT) was performed.

For the OGTT, a 75-g glucose load (Orangedex, Customs Laboratories) was administered over a period of \textless;10 minutes. Blood was drawn immediately before ingestion and 2 hours after the glucose load. Glucose tolerance status was based on the World Health Organization criteria.\textsuperscript{37}

Laboratory Measurements

Glucose and insulin levels in all samples were measured at the central IRAS laboratory at the University of Southern California, Los Angeles, Calif. Plasma glucose was measured with the glucose oxidase technique on an automated autoanalyzer (Yellow Springs Equipment Co). Insulin was measured using the dextran-charcoal radioimmunoassay.\textsuperscript{38} This insulin assay cross-reacts with proinsulin. The split pair coefficient of variation (CV) for the insulin radioimmunoassay was 19% (n = 163).

Fasting serum intact proinsulin and 32-33 split proinsulin were determined from samples stored at \textminus;70°C for an average of 3.3 years (range, 35 to 44 months) by means of highly specific, 2-site monoclonal antibody–based immunoradiometric assays.\textsuperscript{39} The split-pair CV was 14% for proinsulin (n = 348) and 18% for 32-33 split proinsulin (n = 510). There was no detectable cross-reactivity of insulin or 32-33 split proinsulin in the intact-proinsulin assay. Insulin did not significantly cross-react in the assay for 32-33 split proinsulin, and the cross-reactivity of intact proinsulin in this assay was 84%. Assay values of 32-33 split proinsulin were corrected for this by subtracting the corresponding proinsulin cross-reactivity. The assay of 32-33 split proinsulin cross-reacts equally with 32-33, des-32, and des-31-32 split proinsulins. We used the term “32-33 split proinsulin” to indicate the sum of these 3 molecules, the majority of which are des-31-32 split proinsulin.\textsuperscript{40} The sensitivity limit of the intact-proinsulin and of the 32-33 split proinsulin assays was 1.25 pmol/L (3 SDs from zero). Intact proinsulin and 32-33 split proinsulin were determined at the laboratory of the Department of Clinical Biochemistry at Addenbrooke’s Hospital, Cambridge, UK.

Insulin sensitivity was assessed by a frequently sampled intravenous glucose tolerance test (FSIGT\textsuperscript{41}) with minimal model analysis.\textsuperscript{42} Two modifications of the original protocol were used. An injection of regular insulin, rather than tolbutamide, was used to ensure adequate plasma insulin levels for the accurate computation of insulin sensitivity across a broad range of glucose tolerance.\textsuperscript{43} This change was made because of the blunted or absent insulin response in diabetic subjects. In addition, the reduced sampling protocol (which required 12 rather than 30 plasma samples and...
TABLE 2. Correlation Analysis (Unadjusted) in the Overall Population and by Glucose Tolerance Status (P in Parentheses)

<table>
<thead>
<tr>
<th></th>
<th>BMI</th>
<th>Fasting Insulin</th>
<th>Proinsulin (Intact)</th>
<th>Proinsulin (Split)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall population</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1</td>
<td>0.38</td>
<td>0.47</td>
<td>0.46</td>
<td>0.50</td>
<td>−0.43</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.28</td>
<td>0.20</td>
<td>0.20</td>
<td>0.21</td>
<td>−0.24</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
</tr>
<tr>
<td>NGT (n=693)</td>
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</tr>
<tr>
<td>PAI-1</td>
<td>0.30</td>
<td>0.38</td>
<td>0.34</td>
<td>0.40</td>
<td>−0.36</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.23</td>
<td>0.20</td>
<td>0.16</td>
<td>0.17</td>
<td>−0.21</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
</tr>
<tr>
<td>IGT (n=348)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1</td>
<td>0.27</td>
<td>0.42</td>
<td>0.43</td>
<td>0.43</td>
<td>−0.27</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.24</td>
<td>0.07</td>
<td>0.15</td>
<td>0.17</td>
<td>−0.12</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
<td>(NS)</td>
<td>(&lt;0.006)</td>
<td>(&lt;0.002)</td>
<td>(&lt;0.03)</td>
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<tr>
<td>Type 2 diabetes (n=510)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1</td>
<td>0.30</td>
<td>0.38</td>
<td>0.26</td>
<td>0.37</td>
<td>−0.27</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.23</td>
<td>0.11</td>
<td>0.04</td>
<td>0.08</td>
<td>−0.15</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
<td>(&lt;0.02)</td>
<td>(NS)</td>
<td>(NS)</td>
<td>(&lt;0.002)</td>
</tr>
</tbody>
</table>

Multivariate models (partial Spearman correlations, multiple linear regression analyses) were tailored to account for possible confounders of relationships found in univariate analysis. Partial Spearman correlations were estimated after adjusting for (1) age, sex, ethnicity, clinic, and BMI (demographics); (2) demographics plus glucose tolerance status (glucose model); and (3) glucose model plus insulin sensitivity (Table 3). Probability values <0.05 (2-sided) were considered statistically significant. In these models, we tested for interactions between ethnicity and the independent variables of interest (insulin, proinsulin, split proinsulin, and insulin sensitivity). In several models we found significant ethnic interactions, and for these models we reestimated the correlations stratified by ethnicity as well. Because only the magnitude (and not the direction) of the relationship was different among ethnic groups, analyses with ethnic groups pooled together are presented in this report.

Stepwise linear regression models were then fit, including all of the variables of interest at the same time as independent variables to enable us to demonstrate the relative contribution of each of these variables to the outcome variables (PAI-1 and fibrinogen). The first model considered log values of PAI-1 as the dependent variable. Logarithmically transformed values of PAI-1 were used because the distribution of the residuals from the fitted models became normally distributed after log transformation. After age, sex, ethnicity, and clinic were forced into the model, the following independent variables were considered for the model: BMI, glucose tolerance status, insulin sensitivity, fasting insulin, proinsulin (intact), and proinsulin split products. Only variables that had a value of P<0.05 or less were considered in the final fitted model (Table 4). Because proinsulin and split proinsulin were highly correlated, we fit separate models that included 1 or the other variable (but not both) to avoid collinearity problems. A similar stepwise regression model was then fit by considering fibrinogen as the dependent variable (Table 6).

Results

Plasminogen Activator Inhibitor-1
PAI-1 antigen levels were significantly different by glucose tolerance status irrespective of sex, age, ethnic group, and clinic (19.1±0.8 versus 26.7±1.1 versus 32.5±0.9 ng/mL in subjects with NGT, IGT, and type 2 diabetes, respectively; P<0.001 for all comparisons). However, further adjustment for BMI weakened the differences (21.4±0.8 versus...
TABLE 4. Stepwise Regression Analysis With Log PAI-1 as the Dependent Variable

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>B</th>
<th>SE (B)</th>
<th>P</th>
<th>Partial R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.029</td>
<td>0.0036</td>
<td>0.0001</td>
<td>15.8%</td>
</tr>
<tr>
<td>S₁</td>
<td>-0.072</td>
<td>0.012</td>
<td>0.0001</td>
<td>5.8%</td>
</tr>
<tr>
<td>Proinsulin (split)</td>
<td>0.0060</td>
<td>0.0015</td>
<td>0.0001</td>
<td>2.4%</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>0.0010</td>
<td>0.0002</td>
<td>0.0001</td>
<td>0.9%</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>0.225</td>
<td>0.049</td>
<td>0.0001</td>
<td>0.6%</td>
</tr>
<tr>
<td>IGT</td>
<td>0.123</td>
<td>0.048</td>
<td>0.01</td>
<td>0.3%</td>
</tr>
<tr>
<td>R² for the model</td>
<td>36.0%</td>
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<td></td>
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</tbody>
</table>

Model B

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>B</th>
<th>SE (B)</th>
<th>P</th>
<th>Partial R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.032</td>
<td>0.0036</td>
<td>0.0001</td>
<td>15.8%</td>
</tr>
<tr>
<td>S₁</td>
<td>-0.075</td>
<td>0.012</td>
<td>0.0001</td>
<td>5.8%</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>0.0011</td>
<td>0.0002</td>
<td>0.0001</td>
<td>1.8%</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>0.250</td>
<td>0.050</td>
<td>0.0001</td>
<td>1.4%</td>
</tr>
<tr>
<td>IGT</td>
<td>0.123</td>
<td>0.048</td>
<td>0.01</td>
<td>0.3%</td>
</tr>
<tr>
<td>Proinsulin (intact)</td>
<td>0.0037</td>
<td>0.0018</td>
<td>0.04</td>
<td>0.2%</td>
</tr>
<tr>
<td>R² for the model</td>
<td>35.5%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B indicates the regression coefficient; SE (B), standard error of the regression coefficient. After adjustment for age, sex, ethnicity, and clinic, independent variables entered into the model were as follows: BMI, glucose tolerance status, insulin sensitivity (S₁), fasting insulin, and for model A, proinsulin (split) or model B, proinsulin (intact). Only variables significantly contributing to PAI-1 levels are shown.

25.6±1.1 versus 30.4±0.9 mg/dL in NGT, IGT, and type 2 diabetes, respectively; P=0.0016 for NGT versus IGT, P<0.001 for NGT versus type 2 diabetes, and P<0.001 for IGT versus type 2 diabetes. After further adjustment for insulin sensitivity the difference between NGT and IGT was no longer statistically significant (23.0±0.9 versus 25.0±1.1 versus 28.9±1.0 in NGT, IGT, and type 2 diabetes, respectively; P=NS for NGT versus IGT, P<0.001 for NGT versus type 2 diabetes, and P=0.0071 for IGT versus type 2 diabetes).

A strong correlation between PAI-1 antigen and insulin and its precursors was found consistently across all states of glucose tolerance (Table 2). After adjustment for age, sex, ethnic group, clinic, BMI, glucose tolerance status, and insulin sensitivity, the relationship weakened but remained still highly significant (Table 3). Adjustment for insulin sensitivity was included in the analysis based on univariate analysis revealing a significant inverse correlation of PAI-1 with insulin sensitivity (ie, S₁), consistent across different states of glucose tolerance (Table 2). Subgroup analysis by glucose tolerance status showed a somewhat stronger relationship in subjects with NGT (PAI-1 versus fasting insulin, r=0.21; versus proinsulin, r=0.24; and versus split products, r=0.27; P<0.001 and IGT (PAI-1 versus fasting insulin, r=0.27; versus proinsulin, r=0.35; and versus split products, r=0.31; P<0.001) compared with patients with type 2 diabetes (PAI-1 versus fasting insulin, r=0.20; P<0.001; versus proinsulin, r=0.12, P=0.011; and versus split products, r=0.19, P<0.001) after adjustment for age, sex, ethnicity, clinic, BMI, and insulin sensitivity.

Insulin and proinsulin (split products) independently and significantly contributed to PAI-1 levels in a stepwise multiple regression model (Table 4); however, the association was weaker compared with BMI and S₁. The association of split products with PAI-1 levels was somewhat stronger relative to that of fasting insulin with PAI-1 levels.

Correlation analysis showed that PAI-1 was significantly related to insulin as well as its precursors in all 3 ethnic groups studied (Table 5). Significant interactions of ethnicity were found for the relationship of log PAI-1 to fasting insulin (P<0.05), proinsulin (P<0.01), and proinsulin split products (P<0.001). However, because only the magnitude (and not the direction) of the relationship was different, only the data derived from pooled analyses are reported for PAI-1 as well as for fibrinogen.

Fibrinogen levels increased with impairment of glucose tolerance (271.4±2.1 versus 287.7±3.1 versus 293.8±2.7 mg/dL in subjects with NGT, IGT, and type 2 diabetes, respectively; P<0.001 for overall comparison). However, after adjustment for sex, age, clinic, and ethnic group, fibrinogen levels were no longer significantly different in IGT compared with type 2 diabetes (270.5±2.2 versus 282.6±3.1 versus 289.9±2.6 mg/dL in NGT, IGT, and type 2 diabetes; P=0.0013 for NGT versus IGT, P<0.001 for NGT versus type 2 diabetes, and P=NS for IGT versus type 2 diabetes), and further adjustment for BMI and S₁ abolished all differences between the glucose tolerance groups (277.8±2.4 versus 278.2±3.1 versus 282.8±2.8, P=NS for all comparisons).

Fibrinogen levels were positively related to insulin and its precursors in the overall study population (Table 2). The relationship was strongest in subjects with NGT and weaker or absent in subjects with IGT and type 2 diabetes (Table 2). Again, as for PAI-1, adjustment for S₁ was performed because of a significant inverse relationship between fibrinogen and S₁ in univariate analysis. Multiple adjustments as shown in Table 3 generally weakened the relationships, and after adjustment for age, sex, clinic, ethnic group, BMI, glucose tolerance status, and S₁, only intact proinsulin but not fasting insulin or split products remained significantly related to fibrinogen.
and proinsulin (split). Only variables significantly contributing to PAI-1 levels are shown.

Table 6. Stepwise Regression Analysis With Fibrinogen as the Dependent Variable

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>B</th>
<th>SE (B)</th>
<th>P</th>
<th>Partial R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>2.18</td>
<td>0.29</td>
<td>0.0001</td>
<td>6.9%</td>
</tr>
<tr>
<td>S</td>
<td>-2.17</td>
<td>0.90</td>
<td>0.003</td>
<td>0.54%</td>
</tr>
<tr>
<td>Proinsulin (split)</td>
<td>0.22</td>
<td>0.11</td>
<td>0.047</td>
<td>0.23%</td>
</tr>
</tbody>
</table>

B indicates the regression coefficient; SE (B), standard error of the regression coefficient. R² for the model = 15.2%. After adjustment for age, sex, ethnicity, and clinic, independent variables entered into the model were as follows: BMI, glucose tolerance status, insulin sensitivity (S), fasting insulin, and proinsulin (intact) or proinsulin (split). Only variables significantly contributing to PAI-1 levels are shown.

fibrinogen levels. Subgroup analysis by diabetic status after adjustment for age, sex, ethnicity, clinic, and BMI failed to demonstrate a significant relationship between fibrinogen and insulin and its precursors in subjects with type 2 diabetes (fibrinogen versus fasting insulin, r = 0.01; versus proinsulin, r = 0.003; and versus split products, r = 0.005; P = NS for all) and in subjects with IGT (fibrinogen versus fasting insulin, r = -0.06; versus proinsulin, r = 0.06; and versus split products, r = 0.09; P = NS for all). However, in subjects with NGT, the correlation still remained statistically significant (fibrinogen versus fasting insulin, r = 0.09, P = 0.017; versus proinsulin, r = 0.10, P = 0.0091; and versus split products, r = 0.09, P = 0.026).

Stepwise multiple regression analysis showed that proinsulin (split products) but not fasting insulin significantly contributed to fibrinogen levels after adjustment for age, sex, clinic, and ethnic group. This association was weaker compared with the association of BMI and S to fibrinogen levels (Table 6).

Correlation analysis revealed that fibrinogen was significantly related to insulin as well as to its precursors in all 3 ethnic groups studied (Table 5). Significant interactions of ethnicity were found for the relationship of fibrinogen to proinsulin (P < 0.005) and its split products (P < 0.01).

Discussion

In the present study, we have demonstrated a positive relationship between insulin and its precursors to PAI-1 and fibrinogen levels consistently across varying states of glucose tolerance. This effect was independent of S.

The associations of variables included in the insulin resistance syndrome, such as BMI, waist-to-hip ratio, triglyceride and HDL cholesterol levels, as well as insulin with PAI-1 levels, have been demonstrated previously in obese and nonobese healthy subjects, in patients with type 2 diabetes, and in coronary heart disease. These findings and the corresponding in vitro data led to the view of impaired fibrolysis as a possible link between insulin resistance and atherosclerotic disease, as indicated by increased plasma PAI-1 levels. The strongest evidence that hyperinsulinemia is prospectively and independently associated with the development of atherosclerosis, as judged by clinically significant coronary artery disease, is a recent study by Després et al., which used a radioimmunoassay for insulin that does not cross-react with proinsulin.

In vitro data show that both insulin and proinsulin and its conversion intermediates are able to stimulate PAI-1 synthesis. However, in those studies, relatively high, conceivably supraphysiological concentrations of insulin precursors were used. The availability and more widespread use of sensitive insulin assays recognizing proinsulin and its conversion intermediates have focused increasing interest on the relative contribution of insulin per se versus its precursors (proinsulin and its conversion intermediates) to atherosclerotic disease. The present study is the first investigation addressing the relationship of insulin and its precursors to PAI-1 levels in a large population of nondiabetic and diabetic subjects.

Similar to our findings, a relationship between insulin and its precursors to PAI-1 activity has been demonstrated recently in a large, healthy population from northern Sweden. In that study, in subjects with NGT, partial correlation analysis (adjusted for age and sex) showed a significant relation of both fasting insulin and fasting proinsulin to PAI-1 as well as fibrinogen. In a multivariate regression model, however, fasting proinsulin but not fasting insulin significantly predicted fibrinogen levels, whereas neither proinsulin nor insulin was predictive of PAI-1 activity. This is in contrast to the findings of the present study. The differences in the results of these 2 studies might be due to differences in the multivariable regression models. In the Swedish study, the regression model included independent variables not included in the present study (triglycerides, postload insulin, diastolic blood pressure, and smoking).

Data on the relationship of PAI-1 and/or fibrinogen and insulin, including its precursors, in subjects with IGT or type 2 diabetes are both scarce and inconsistent. Most previous studies are limited by a relatively small number of subjects. In a recent study by Gray et al., PAI-1 was correlated with intact and split proinsulin in nondiabetic (n = 76) and diabetic (n = 56) subjects but not with fasting insulin. In a triethnic population of diabetic (n = 261) and nondiabetic (n = 314) subjects, PAI-1 activity was significantly correlated with fasting insulin, proinsulin, and des-31,32-proinsulin, irrespective of ethnicity. In a small subgroup of subjects with IGT and type 2 diabetes (n = 19) of the Sweden MONICA Study, only proinsulin but not fasting insulin was correlated with PAI-1 activity. PAI-1 activity was correlated significantly only with 32-33 split proinsulin but not with fasting insulin or intact proinsulin in 51 subjects with type 2 diabetes. The results of our study add to the evidence that the deleterious effects of insulin, its precursors, and/or insulin resistance could be at least partly mediated through PAI-1, as suggested previously by Juhan-Vague et al. In addition, in our study a consistent relationship of PAI-1 to insulin, its precursors, and insulin sensitivity across varying states of glucose tolerance was shown, thus indicating that similar pathomechanisms might link hyperinsulinemia and atherosclerotic disease in nondiabetic and diabetic subjects.

This study showed that insulin and its precursors are related to PAI-1 independently of insulin sensitivity. Insulin sensitivity has been independently related to PAI-1 levels in the present study and previously in patients with type 2 diabetes and obese diabetic and nondiabetic subjects. Including S in the analysis made it possible to discern direct effects of insulin from indirect effects of insulin sensitivity, as derived from the finding that fasting insulin levels may also serve as a proxy for insulin sensitivity. In fact, the demonstrated relationships were independent of insulin sensitivity,
thus favoring the hypothesis that insulin per se is indepen- 
dently involved in PAI-1 metabolism. Furthermore, our 
results do not support the hypothesis that the link between 
the insulin resistance syndrome and atherosclerotic disease 
is mediated by insulin precursors rather than “true” insulin, 
as shown by univariate and multivariate analyses. These find-
ings are in keeping with in vitro data, suggesting a dose-
dependent and additive impact of both insulin and proinsulin 
on PAI-1 synthesis in endothelial cells.58

The relationship of fibrinogen with insulin and its prec-
cursors is less clear. There is still controversy as to whether 
fibrinogen levels are generally elevated in type 2 diabetic 
patients or only in those with macrovascular disease19,20,59 or 
in the presence of increased urinary albumin excretion, as a 
marker of macrovascular disease.60 However, an independent 
relationship between insulin levels and fibrinogen levels has 
been reported in nondiabetic subjects.21–23 Mohamed-Ali et 
al23 showed a strong correlation of proinsulin, its split 
products, and insulin to fibrinogen in a large number of 
subjects with NGT. According to the present study, fasting 
insulin and proinsulin do not seem to contribute to fibrinogen 
levels in IGT and type 2 diabetes, whereas a weak but 
significant relationship was found in subjects with NGT. This 
might be explained by the fact that glucose-tolerant subjects 
represent a population with a relatively low prevalence of 
preexisting atherosclerosis compared with subjects with IGT 
or type 2 diabetes. Additional mechanisms, such as inflam-
ination and oxidative stress, which are involved in enhanced 
fibrinogen synthesis and atherogenesis, may contribute more 
importantly to fibrinogen levels in subjects with preexisting 
atherosclerosis. Accordingly, in patients with type 2 diabetes, 
high fibrinogen levels have been related to the presence of 
macrovascular disease.20 At least in healthy subjects, insulin 
seems to act as a general risk marker, with fibrinogen being 
predictive of clinical macrovascular disease, such as coronary 
heart disease and stroke61 and peripheral vascular disease.62

In summary, we showed a strong and consistent relation-
ship of 2 crucial factors of hemostasis, fibrinogen and PAI-1, 
to insulin and its precursors. This association was only 
partially explained by insulin sensitivity. Our data provide no 
evidence that the demonstrated relationship is markedly 
stronger for proinsulin than for “true” insulin. Our findings 
may have important clinical implications in the risk assess-
ment and prevention of macrovascular disease, not only in 
patients with overt diabetes but also in nondiabetic subjects 
who are hyperinsulinemic. The implications might be even 
more important in light of recent data emphasizing the 
importance of potentially modifiable metabolic factors, as 
opposed to genetic factors, in determining PAI-1
expression.63

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References
1. Pyorälä K, Savolainen E, Kaukola S, Haapakoski J. Plasma insulin as 
coronal heart disease risk factor: relationship to other risk factors and 
predictive value during 9% year follow-up of the Helsinki Policeman 
2. Eschwege E, Richard JL, Thibault N, Ducimetiere P, Warvet JM, Claude 
JR, Rosselin GE. Coronary heart disease mortality in relation with 
diabetes, blood glucose and plasma insulin levels: the Paris Prospective 
3. Després JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, 
Lupien PJ. Hyperinsulinemia as an independent risk factor for ischemic 
4. Laakso M. How good a marker is insulin level for insulin resistance? 
5. Howard G, O’Leary DH, Zaccaro D, Haffner SM, Rewers M, Hamman R, 
Selby JV, Saad MF, Savage P, Bergman R. Insulin sensitivity and ath-
Fuchs Z. Hyperinsulinemia: a link between hypertension, obesity and 
8. Zavaroni I, Bonora E, Pagliara M, Dall’Aglio E, Luchetti L, Buonanno G, 
Bonati PA, Berganzani M, Gnedi L, Passeri M, Reaven G. Risk factors 
for coronary artery disease in healthy persons with hyperinsulinaemia and 
9. Orchard TJ, Becker DJ, Bates M, Kuller LH, Drash AL. Plasma insulin 
and lipoprotein concentrations: an atherogenic association? Am J Epi-
10. Haffner SM, Fong D, Hazuda HP, Pugh JA, Patterson JK. Hyperinsulin-
emia, upper body adiposity, and cardiovascular risk factors in non-
11. DeFronzo RA, Ferrannini E. Insulin resistance: a multifaceted syndrome 
responsible for NIDDM, obesity, hypertension, dyslipidaemia and ath-
12. Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, 
Graziedei L, Pedrinelli R, Brandi L, De Iutilacqua S. Insulin resistance in 
Relationship between lipoprotein levels and in vivo insulin action in 
14. Abbott WGH, Lillioja S, Young AA, Zawadzki JK, Yki-Jarvinen H, 
Christin L, Howard BV. Relationship between plasma lipoprotein con-
centrations and insulin concentrations and insulin action in an obese 
MP. Prospective analyses of the insulin resistance syndrome (syndrome 
18. Ernst E, Resch KL. Fibrinogen as a cardiovascular risk factor: a meta-
Diabetes, fibrinogen, and risk of cardiovascular disease: the Framingham 
20. Ganda OP, Arkin CH. Hyperfibrinogenemia: an important risk factor for 
21. Juhani-Vague I, Thompson SG, Jespersen J. Involvement of the hemo-
static system in the insulin resistance syndrome: a study of 1500 patients 
22. Landin K, Stigendal L, Eriksson E, Krotkiewski M, Risberg B, Tengborn 
L, Smith U. Abdominal obesity is associated with an impaired fibrinolytic 
activity and elevated plasminogen activator inhibitor-1. Metabolism. 
Association of proinsulin-like molecules with lipids and fibrinogen in 
in-diabetic subjects: evidence against a modulating role for insulin. 
Coxon D. Correlation between blood fibrinolytic activity, plasminogen 
activator inhibitor level, plasma insulin level, and relative body weight in 
25. Potter van Loon BJ, Kluft C, Radder JK, Blankenstein MA, Meinders AE. 
The cardiovascular risk factor plasminogen activator inhibitor type 1 activity 
and VLDL triglyceride concentration, insulin levels and insulin sensitivity: studies in
Insulin and Proinsulin Related to PAI-1 and Fibrinogen


Relative Contribution of Insulin and Its Precursors to Fibrinogen and PAI-1 in a Large Population With Different States of Glucose Tolerance: The Insulin Resistance Atherosclerosis Study (IRAS)
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