Hyperinsulinemia, Hyperglycemia, and Impaired Hemostasis
The Framingham Offspring Study

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Type 2 diabetes mellitus and its precursor, impaired glucose tolerance (IGT), are associated with increased risk for morbidity and mortality from cardiovascular disease (CVD), particularly coronary heart disease. An increased prevalence of traditional risk factors for atherosclerosis (eg, hypertension, low high-density lipoprotein cholesterol [HDL-C] levels) occurs in type 2 diabetes and IGT, but these factors account for only half of the observed excess risk for CVD. Novel CVD risk factors, including insulin resistance and hyperinsulinemia, have been proposed to account for excess CVD risk in glucose intolerance. However, the role of hyperinsulinemia as an independent risk factor for CVD remains uncertain; its apparent effect could be due to other factors rather than to the direct toxicity of insulin itself.

The emerging role of acute thrombosis in the etiology of CVD suggests additional novel risk factors that could mediate risk associated with hyperinsulinemia. Biochemical markers of hypercoagulability, including elevated plasminogen activator inhibitor 1 (PAI-1) antigen, tissue-type plasminogen activator (tPA) antigen, von Willebrand factor (vWF) antigen, factor VII antigen, fibrinogen, and plasma viscosity, may represent such novel risk factors.

Context Increased risk for cardiovascular disease in persons with glucose intolerance (impaired glucose tolerance and type 2 diabetes mellitus) is not fully explained by concomitant elevations in traditional atherosclerosis risk factors. Hyperinsulinemia associated with glucose intolerance may increase risk directly, or its effect could be mediated through impaired hemostatic function.

Objective To evaluate associations between fasting insulin levels and hemostatic factors in subjects with normal and impaired glucose homeostasis.


Setting The population-based Framingham Offspring Study.

Subjects A total of 1331 men and 1631 women aged 26 to 82 years, without diagnosed diabetes or cardiovascular disease and classified as having normal glucose tolerance (80.2%) or glucose intolerance (impaired glucose tolerance and impaired fasting glucose combined, 15.2%; previously undiagnosed diabetes, 4.7%) using an oral glucose tolerance test.

Main Outcome Measures Trends across quintiles of fasting insulin in levels of plasminogen activator inhibitor 1 (PAI-1) antigen, tissue-type plasminogen activator (tPA) antigen, von Willebrand factor (vWF) antigen, factor VII antigen, fibrinogen, and plasma viscosity. We stratified analyses by sex and glucose tolerance status and adjusted hemostatic factor levels for obesity, lipid levels, and traditional cardiovascular disease risk factors.

Results Mean levels of all hemostatic factors (except for fibrinogen in men) increased across insulin quintiles among subjects with normal glucose tolerance (P<.001 for trend). Levels of PAI-1 and tPA antigens, but not other hemostatic factors, were higher comparing subjects with glucose intolerance with those with normal glucose tolerance (P<.001). Among subjects with glucose intolerance, levels of PAI-1 and tPA antigen in men and women (P<.01 for trend) and vWF antigen in men (P<.05 for trend) increased significantly across insulin quintiles, but levels of factor VII antigen, fibrinogen, and plasma viscosity did not increase.

Conclusions Elevated levels of fasting insulin are associated with impaired fibrinolysis and hypercoagulability in subjects with normal glucose tolerance. Hyperinsulinemia is associated primarily with impaired fibrinolysis in subjects with glucose intolerance. Excess risk for cardiovascular disease associated with hyperinsulinemia and glucose intolerance may be mediated in part by enhanced potential for acute thrombosis.

See also Patient Page.
levels of fibrinogen, factor VII, or von Willebrand factor (vWF), or of decreased fibrinolytic potential, including elevated levels of plasminogen activator inhibitor 1 (PAI-1) antigen or tissue-type plasminogen activator (tPA) antigen, are associated with increased risk for CVD among nondiabetic persons. A link between hyperinsulinemia and thrombotic potential might explain increased CVD risk in glucose intolerance, but the evidence supporting this hypothesis is limited to analysis of PAI-1 or fibrinogen levels among subjects with normal glucose tolerance or in small, selected groups of diabetic patients.

We evaluated the association between fasting hyperinsulinemia and plasma levels of PAI-1 antigen, tPA antigen, factor VII antigen, vWF antigen, fibrinogen, and viscosity, stratified by normal glucose tolerance or glucose intolerance, among subjects of the population-based Framingham Offspring Study. We hypothesized that elevated levels of fasting insulin would be associated with elevated levels of hemostatic factors, that these levels would be higher among subjects with glucose intolerance compared with those with normal glucose tolerance, independent of higher fasting insulin levels, and that these effects would all be independent of levels of obesity, lipids, and other CVD risk factors.

METHODS

Study Population

Participants in the Framingham Offspring Study, a long-term, community-based, prospective observational study of risk factors for CVD, are the children and spouses of the children of the original Framingham Heart Study cohort. From January 1991 through June 1995, during the fifth 4-year study cycle, 3799 participants underwent a standardized medical history taking and physical and laboratory examinations. Written informed consent was obtained from participants prior to examination. We analyzed data from 2962 participants after exclusion of 383 with missing information on fasting insulin levels, hemostatic factors, or glucose tolerance status and 454 with diagnosed diabetes or prevalent CVD or who received warfarin for anticoagulation. Excluded subjects were older (60 vs 53 years; \( P < .001 \)) and more likely to be male (21% men vs 14% women; \( P = .001 \)). We defined diagnosed diabetes as a fasting plasma glucose level of 126 mg/dL \( [7.0 \text{ mmol/L}] \) or more at any 2 previous examinations or by use of hypoglycemic drug therapy. Prevalent CVD, including coronary heart disease and stroke, was defined previously.

Height, weight, waist, and hip circumferences were measured with the subject standing. Obesity was defined as a body mass index (BMI) of 27.3 kg/m\(^2\) or more in women or 27.8 kg/m\(^2\) or more in men, corresponding to approximately 120% ideal body weight. Central obesity was defined as a waist-hip ratio of more than 0.9 in men or more than 1.0 in women, or as a waist circumference exceeding the sex-specific population median. An elevated triglyceride level was defined as more than 200 mg/dL \( [2.26 \text{ mmol/L}] \). Hypertension was defined as a blood pressure of more than 140/90 mm Hg on 2 measurements or report of antihypertensive medication use. Participants reporting smoking at least 1 cigarette per day during the previous year were classified as current smokers. Physical activity was assessed as a weighted sum of the proportion of a typical day spent sleeping and performing sedentary, slight, moderate, or heavy physical activities. Alcohol use was categorized as usual consumption of none, 1-7 oz, or 7 oz or more of alcohol per week. Aspirin use was categorized as none, any use 1 to 7 days preceding evaluation, or any use more than 7 days preceding evaluation. Estrogen replacement therapy among postmenopausal women was determined.

We drew blood samples after an overnight fast for glucose, insulin, lipid, and hemostatic factor measurements. We administered a 75-g oral glucose tolerance test to participants not known to have diabetes and interpreted results according to 1997 American Diabetes Association standards. Previously undiagnosed diabetes was defined as a fasting plasma glucose level of at least 126 mg/dL \( [7.0 \text{ mmol/L}] \) or a 2-hour postchallenge glucose level of at least 200 mg/dL \( [11.1 \text{ mmol/L}] \). Impaired fasting glucose (IFG) was defined as a fasting plasma glucose level of at least 110 mg/dL \( [6.1 \text{ mmol/L}] \) but less than 126 mg/dL \( [7.0 \text{ mmol/L}] \). Impaired glucose tolerance was defined as a 2-hour postchallenge glucose level of at least 140 mg/dL and less than 200 mg/dL \( [11.1 \text{ mmol/L}] \). In this analysis, IFG and IGT are combined and referred to as IFG/IGT. Hemostatic factor levels were similar comparing subjects with IFG to those with IGT. Normal glucose tolerance was defined as a fasting glucose level of less than 110 mg/dL \( [6.1 \text{ mmol/L}] \) and a 2-hour postchallenge level of less than 140 mg/dL \( [7.8 \text{ mmol/L}] \).

Laboratory Methods

Fasting plasma glucose levels were measured in fresh specimen with a hexokinase reagent kit. Glucose assays were run in duplicate; the intra-assay coefficient of variation (CV) was less than 3%. Hemoglobin A\(_1c\) was measured by high-performance liquid chromatography after an overnight dialysis against isotonic sodium chloride solution to remove the labile fraction. The mean (SD) for this assay among subjects with normal glucose tolerance in this population was 5.22% (0.6%) and the interassay and intra-assay CVs were less than 2.5%. The assay was standardized against and comparable with the glycosylated hemoglobin assay used in the Diabetes Control and Complications Trial. Fasting insulin levels were measured in EDTA plasma as total immunoreactive insulin and calibrated to serum levels for reporting purposes. Cross-reactivity of this assay with proinsulin at mid curve is approximately 40%, the intra-assay and interassay CVs ranged from 5.0% to 10.0% for concentrations reported here, and the lower limit of sensitivity was 1.1 µU/mL \( [7.9 \text{ pmol/L}] \). Hemostatic factor analyses were performed in blood anticoagulated with 3.8% trisodium citrate (9.1 vol/vol) and kept on crushed ice until centrifugation and separation.
of plasma at 2500g for 30 minutes at 4°C. Plasma aliquots were frozen and stored at −70°C for subsequent analysis. Enzyme-linked immunosorbent assay methods were used to measure levels of PAI-1 antigen (CV, 9.6%), tPA antigen (CV, 5.5%), factor VII antigen (CV, 3.0%), and vWF antigen (CV, 8.8%). Viscosity was measured using a digital viscometer (CV, 2.0%). Fibrinogen levels were measured with the Clauss method (CV, 2.6%). Fasting total plasma cholesterol and triglyceride levels were measured enzymatically, and the HDL-C fraction was measured after precipitation of low-density and very low-density lipoprotein cholesterol with dextran sulfate-magnesium. The Framingham laboratory participates in the lipoprotein cholesterol laboratory standardization program administered by the Centers for Disease Control and Prevention.

**Statistical Analysis**

We performed analyses using SAS Version 6 statistical software. Log transformation of levels of PAI-1 and tPA antigens, triglycerides, and physical activity normalized their distributions for statistical testing; we report their geometric means. We assessed statistical significance (defined as a 2-tailed P<.05) of trends across quintiles of fasting insulin level with linear regression models (for continuous variables) using insulin as an ordinal variable with the median insulin value in each quintile assigned as a score, or with the Mantel-Haenszel test of trend (for categorical variables). We used linear regression analysis of variance models to calculate least-square means and SEs for levels of hemostatic factors and other continuous covariates. We used age-adjusted linear regression analysis of variance models to test pairwise differences in fasting insulin levels among glucose tolerance categories. We then stratified analyses of hemostatic factor levels by sex and glucose tolerance category, with subjects classified into glucose tolerance category–specific quintiles of fasting insulin levels. We used multivariable regression models to control for effects of age alone or the combined effects of age, BMI, levels of HDL-C, total cholesterol, and fasting glucose (all modeled as continuously distributed); and hypertension, alcohol use, cigarette use, physical activity, aspirin use, and estrogen replacement therapy (all modeled as categorical). Additional multivariable models examined the effect of insulin independent of triglyceride levels. We tested trends across insulin quintiles in these models using the glucose tolerance category–specific median insulin value in each quintile as a score. We compared multivariable-adjusted mean hemostatic factor levels among subjects with glucose intolerance vs those with normal glucose tolerance using regression models including glucose tolerance status, fasting insulin quintile, and their first-order interaction.

**RESULTS**

**Population Characteristics**

The mean age of 1331 men (45% of the population) and 1631 women (55%) in the study population was 53 years (range, 26-82 years); 2375 (80.2%) had normal glucose tolerance and 587 (19.8%) had glucose intolerance. Men with normal glucose tolerance and glucose intolerance.

**Hemostatic Factor Levels**

The age-adjusted geometric mean (SE) fasting serum insulin levels were 6.5 (1.0) µU/mL (47 [7] pmol/L) in men with normal glucose tolerance, 11.8 (1.1) µU/mL (85 [8] pmol/L) in men with glucose intolerance (P<.001), 5.2 (1.0) µU/mL (37 [7] pmol/L) in women with normal glucose tolerance, and 8.6 (1.1) µU/mL (62 [8] pmol/L) in women with glucose intolerance (P<.001). To limit the confounding effect of glucose tolerance status on the relation between levels of insulin and hemostatic factors, we stratified all men and women by normal glucose tolerance and glucose intolerance. Among men, age-adjusted levels of all 6 hemostatic factors increased significantly across fasting insulin quintiles, with the exception of levels of fibrinogen and viscosity in glucose intolerance (TABLE 2). Among women, age-adjusted levels of all 6 hemostatic factors increased significantly across fasting insulin quintiles among those with either normal glucose tolerance or glucose intolerance (TABLE 3).

The associations of fasting hyperinsulinemia with markers of impaired fibrinolysis did not substantially change after adjustment for potential confounders. Multivariable-adjusted levels of PAI-1 and tPA antigens (FIGURE, top) among all subjects increased significantly across fasting insulin quintiles. Additional adjustment of triglyceride levels did not alter relationships between hyperinsulinemia and PAI-1 antigen among subjects in either glucose tolerance category. In these models, triglyceride levels were independent determinants of PAI-1 antigen levels among men in both glucose tolerance categories and among women with normal glucose tolerance (P<.001).

Multivariable adjustment did not substantially change associations of levels of vWF and factor VII antigens (FIGURE, center) with fasting hyperinsulinemia among subjects with normal glucose tolerance, but significantly weakened this association for vWF among women with glucose intolerance and for factor VII in all subjects with glucose intolerance. Only among glucose-intolerant men did multivariable-adjusted levels of vWF antigen increase significantly across fasting insulin quintiles. Multivariable adjustment of
levels of fibrinogen and viscosity (Figure, bottom) eliminated their associations with hyperinsulinemia among all subjects with glucose intolerance and, for fibrinogen, among men with normal glucose tolerance. Among women with normal glucose tolerance, multivariable-adjusted levels of fibrinogen increased significantly, albeit subtly, across fasting insulin quintiles. Further adjustment for triglyceride levels did not substantially change associations between fasting hyperinsulinemia and these markers of hypercoagulability.

Controlling for body fat distribution (rather than overall obesity) by replacement of BMI with either waist-hip ratio or waist circumference in multivariable models did not substantially change results for any hemostatic factor. Furthermore, overall obesity, central obesity, and elevated triglyceride levels did not modify the effect of fasting hyperinsulinemia on hemostatic factor levels, as indicated by a lack of consistently significant first-order interaction terms (eg, obesity × insulin quintile) in either glucose tolerance category.

Within the glucose intolerance category, multivariable-adjusted levels of all hemostatic factors were similar comparing subjects with IFG with those with IGT, and those with IFG/IGT combined with those with undiagnosed diabetes (all \(P > .05\) for pairwise comparisons). Compared with those with normal glucose tolerance, men with glucose intolerance had significantly higher mean (SE) multivariable-adjusted levels of PAI-1 antigen (25.4 [0.08] vs 18.5 [0.03] ng/mL; \(P < .001\)), as did women with glucose intolerance (21.2 [0.08] vs 15.1 [0.03] ng/mL; \(P < .001\)). Mean (SE) levels of tPA antigen were also higher comparing glucose intolerance with normal glucose tolerance among men (10.4 [0.05] vs 8.5 [0.02] ng/mL; \(P < .001\)) and women (8.6 [0.05] vs 7.0 [0.02] ng/mL; \(P < .001\)). Multivariable-adjusted levels of vWF antigen, factor VII antigen, fibrinogen, and viscosity were similar in both glucose tolerance categories for both men and women (\(P > .05\) for all) (Figure).

**COMMENT**

We found strong, positive associations between levels of fasting insulin and levels of PAI-1 antigen, tPA antigen, factor VII antigen, vWF antigen, fibrinogen, and viscosity among both men and women in the Framingham Offspring Study. These findings sug-

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### Table 1. Characteristics of 2962 Study Participants Stratified by Fasting Insulin Quintiles

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fasting Insulin Level, µU/mL</th>
<th>(P) Value</th>
<th>(P) Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0-3.5</td>
<td>3.6-5.6</td>
<td>5.7-8.5</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>571</td>
<td>586</td>
<td>607</td>
</tr>
<tr>
<td>Women, %</td>
<td>64.3</td>
<td>60.6</td>
<td>57.5</td>
</tr>
<tr>
<td>Age, y</td>
<td>53 (0.4)</td>
<td>53 (0.4)</td>
<td>54 (0.4)</td>
</tr>
<tr>
<td>Normal glucose tolerance, %</td>
<td>89.3</td>
<td>89.4</td>
<td>86.3</td>
</tr>
<tr>
<td>Impaired fasting glucose/impaired glucose tolerance, %</td>
<td>9.8</td>
<td>8.7</td>
<td>10.9</td>
</tr>
<tr>
<td>Undiagnosed diabetes mellitus, %</td>
<td>0.9</td>
<td>1.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.3 (0.1)</td>
<td>25.5 (0.2)</td>
<td>26.7 (0.2)</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>10.0</td>
<td>10.6</td>
<td>14.6</td>
</tr>
<tr>
<td>Physical activity score</td>
<td>34.5 (1.0)</td>
<td>34.2 (1.0)</td>
<td>34.2 (1.0)</td>
</tr>
<tr>
<td>Alcohol use, %</td>
<td>78.0</td>
<td>74.1</td>
<td>71.1</td>
</tr>
<tr>
<td>Current cigarette smoking, %</td>
<td>20.8</td>
<td>22.1</td>
<td>18.9</td>
</tr>
<tr>
<td>Aspirin use, %</td>
<td>52.9</td>
<td>52.3</td>
<td>54.7</td>
</tr>
<tr>
<td>Estrogen replacement therapy (women only), %</td>
<td>18.3</td>
<td>21.2</td>
<td>19.5</td>
</tr>
<tr>
<td>Fasting insulin level, µU/mL</td>
<td>1.6 (1.0)</td>
<td>4.3 (1.0)</td>
<td>6.7 (1.0)</td>
</tr>
<tr>
<td>Fasting glucose level, mg/dL</td>
<td>91.3 (0.4)</td>
<td>93.6 (0.8)</td>
<td>95.6 (0.5)</td>
</tr>
<tr>
<td>HbA1c ((n = 2269)), %</td>
<td>5.2 (0.03)</td>
<td>5.3 (0.04)</td>
<td>5.3 (0.03)</td>
</tr>
<tr>
<td>Total cholesterol level, mg/dL</td>
<td>197.8 (1.5)</td>
<td>200.2 (1.5)</td>
<td>206.8 (1.5)</td>
</tr>
<tr>
<td>HDL-C level, mg/dL</td>
<td>58.0 (0.6)</td>
<td>55.2 (0.6)</td>
<td>51.5 (0.6)</td>
</tr>
<tr>
<td>Triglyceride level, mg/dL</td>
<td>89.6 (1.0)</td>
<td>101.2 (1.0)</td>
<td>118.5 (1.0)</td>
</tr>
<tr>
<td>PAI-1 antigen level, ng/mL</td>
<td>11.6 (1.0)</td>
<td>13.7 (1.0)</td>
<td>16.1 (1.0)</td>
</tr>
<tr>
<td>tPA antigen level, ng/mL</td>
<td>6.2 (1.0)</td>
<td>6.9 (1.0)</td>
<td>7.9 (1.0)</td>
</tr>
<tr>
<td>Factor VII antigen, % control</td>
<td>95.2 (0.6)</td>
<td>99.0 (0.6)</td>
<td>99.2 (0.6)</td>
</tr>
<tr>
<td>vWF antigen, % control</td>
<td>119.4 (1.8)</td>
<td>119.0 (1.8)</td>
<td>122.1 (1.7)</td>
</tr>
<tr>
<td>Fibrinogen level, mg/dL</td>
<td>286.5 (2.2)</td>
<td>296.6 (2.2)</td>
<td>304.3 (2.2)</td>
</tr>
<tr>
<td>Viscosity, cP</td>
<td>1.2 (0.00)</td>
<td>1.2 (0.00)</td>
<td>1.2 (0.00)</td>
</tr>
</tbody>
</table>

*Data are given as mean (SE) except where noted. Geometric means are given for levels of fasting insulin, triglycerides, plasminogen activator inhibitor 1 (PAI-1) antigen, tissue-type plasminogen activator (tPA) antigen, and physical activity score. Ellipses indicate data not applicable; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; and vWF, von Willebrand factor. To convert insulin from µU/mL to pmol/L, multiply by 7.175. To convert glucose from mg/dL to mmol/L, multiply by 0.05551. To convert total cholesterol and HDL-C from mg/dL to mmol/L, multiply by 0.02586. To convert triglycerides from mg/dL to mmol/L, multiply by 0.01129. To convert fibrinogen from mg/dL to µmol/L, multiply by 0.02941.

†P values for trend across quintiles of fasting insulin level.
gest a mechanism for the observed increased risk for CVD associated with hyperinsulinemia.35,36 An enhanced propensity for acute thrombosis, rather than a direct effect of insulin itself, may increase risk for CVD. In this cohort, the prevalence of glucose intolerance and level of glycemia also increased with fasting insulin levels, potentially modifying associations between hyperinsulinemia and hemostasis. Most markers of impaired hemostasis were positively associated with fasting insulin levels among subjects with normal glucose tolerance, but among those with glucose intolerance (IFG/IGT and undiagnosed diabetes), only markers of impaired fibrinolysis (levels of PAI-1 and tPA antigens) were significantly associated with hyperinsulinemia.

Markers of hypercoagulability, with the exception of vWF antigen in men, did not show substantial associations with fasting hyperinsulinemia among subjects with glucose intolerance. Furthermore, subjects with glucose intolerance had higher PAI-1 and tPA antigen levels than those with normal glucose tolerance, even after accounting for higher fasting insulin levels. These findings suggest that increased risk for acute thrombosis associated with glucose intolerance may be mediated more by impaired fibrinolysis than by increased hypercoagulability, with fibrinolytic function in glucose intolerance further impaired by elevated fasting insulin levels.

**Glucose Intolerance, Hyperinsulinemia, and CVD**

The specific vasculopathic mechanisms underlying the close association between CVD and type 2 diabetes are incompletely understood. Hyperglycemia may contribute to atherogenesis through irreversible glycation of collagen and other vessel wall proteins,37 or through accelerated generation of reactive oxygen species, especially affecting low-density lipoprotein cholesterol and vascular endothelial cells.38 However, while clearly related to diabetic microvascular complications, the contribution of hyperglycemia to excess CVD in diabetes remains controversial. The few randomized controlled trials of intensive glycemic control in diabetes patients have not demonstrated significant reductions in CVD end points.39,40 Observational evidence linking glycemia and CVD in diabetes is also inconclusive.40 However, it is very clear that people with glucose intolerance but without clinically diagnosed diabetes are at increased risk for CVD.41,42 Focusing attention on the prediabetic state as a condition of heightened atherogenic risk.

Insulin resistance and compensatory hyperinsulinemia in prediabetes may be pathogenic mechanisms underlying risk for atherosclerosis.43 Hyperinsulinemia may theoretically contribute to CVD through diverse effects on vascular endothelial cells.43 However,
as is the case for hyperglycemia, the role of insulin as an independent CVD risk factor is uncertain.\textsuperscript{44} It may be that insulin resistance, rather than insulin levels per se, is the causative mechanism for CVD.\textsuperscript{45} Two recent reports support the role of insulin as an independent risk factor for coronary heart disease, but control for potential confounders in these studies was limited to effects of lipid fractions and other traditional CVD risk factors.\textsuperscript{33,34} Our data suggest that the apparent atherogenic effects of hyperglycemia or hyperinsulinemia may be mediated through novel factors predisposing to acute thrombosis.

### Impaired Hemostasis and CVD

The role of impaired hemostasis in the etiology of acute coronary syndromes was suggested by observations that myocardial infarction may occur in the absence of severe coronary artery stenosis.\textsuperscript{46} Elevated levels of PAI-1, in particular, appear to predispose to formation of acellular, thin-walled plaques particularly prone to acute rupture.\textsuperscript{47} Impaired hemostasis may promote unstable plaque formation as well as reflect ongoing endothelial injury and chronic inflammation accompanying atherosclerosis.\textsuperscript{48} Although hemostatic abnormalities could be the consequence rather than the cause of atherosclerosis, prospective studies have demonstrated that elevated markers of impaired hemostasis may precede myocardial infarction or stroke in apparently healthy subjects.\textsuperscript{8,9,49} Furthermore, elevated levels of PAI-1 and insulin have been associated with increased carotid artery intimal-media thickness in nondiabetic subjects, again suggesting a pathogenic link among impaired fibrinolysis, insulin resistance, and glucose intolerance in the preclinical state.\textsuperscript{50,51}

### Insulin and Impaired Hemostasis

Positive associations between levels of fasting insulin or insulin resistance and markers of impaired fibrinolysis (espe-
cially PAI-1) are consistent findings in studies of subjects with normal glucose tolerance. However, these associations may be partially explained by increased central fat distribution or triglyceride levels. Plasminogen activator inhibitor 1 production by hepatocytes, endothelial cells, and adipocytes (particularly those derived from omental fat) can be stimulated in vitro by glucose, insulin, or triglycerides, or in vivo by oral glucose loading or infusion of insulin combined with intravenous fat emulsion, but apparently not by insulin infusion alone. Our data clearly demonstrate a positive correlation between fasting insulin levels and impaired fibrinolytic potential among people with normal glucose tolerance, independent of intermediary effects of obesity or lipid levels. Because fasting insulin levels reasonably estimate insulin resistance measured by direct assessment, especially among subjects with normal glucose tolerance, our data support at least a partial role for insulin resistance mediating fibrinolysis.

The insulin resistance syndrome typically features glucose intolerance and elevated fasting insulin and triglyceride levels. Elevated levels of PAI-1 and tPA antigens associated with glucose intolerance, hyperinsulinemia, and hypertriglyceridemia support inclusion of impaired fibrinolysis as an additional feature of the insulin resistance syndrome. Elevated fibrinolytic factors are also correlated with elevated markers of inflammation and endothelial dysfunction, which has been hypothesized to cause insulin resistance and thereby be the common pathogenic mechanism underlying atherosclerosis, insulin resistance, and glucose intolerance. Our cross-sectional study design cannot resolve whether impaired fibrinolysis (reflecting a hemostatic and an endothelial abnormality) is a cause or a result of hyperinsulinemia. An additional limitation of our study is that disproportionately elevated levels of plasma proinsulin associated with insulin resistance may increase levels of PAI-1 antigen. Because our assay detects total immunoreactive insulin, unmeasured elevations in proinsulin may account for some of the elevations in PAI-1 antigen levels.

In contrast with markers of impaired fibrinolysis, markers of hypercoagulability (especially of fibrinogen or factor VII) have been inconsistently associated with glucose intolerance, and with endogenous hyperinsulinemia in nondiabetic subjects, or with exogenous insulin therapy in type 2 diabetes. Our data demonstrate strong, positive correlations between several markers of hypercoagulability and elevated fasting insulin levels in subjects with normal glucose tolerance, but no consistent association in those with glucose intolerance. Type 2 diabetes and IFG/IGT are states of increased thrombotic potential. Our data suggest that in these states, thrombotic risk may be mediated more by impaired fibrinolysis than by hypercoagulability, due in part to endogenous hyperinsulinemia and insulin resistance.

In conclusion, we demonstrated strong, positive correlations between fasting insulin levels and a wide variety of markers of impaired hemostatic function potentially linked to CVD among subjects with normal glucose tolerance. Among subjects with IFG/IGT and undiagnosed diabetes, hemostatic function was further impaired. Impaired fibrinolysis, reflecting increased thrombotic potential and endothelial dysfunction, may be an important mechanism linking hyperinsulinemia, insulin resistance, and increased risk for CVD in glucose intolerance. Prospective study is required to determine if accounting for levels of hemostatic factors enhances CVD risk prediction or explains the apparent atherogenic effects of blood levels of glucose or insulin. Modifying the fibrinolytic state by improving insulin sensitivity may offer a novel approach to reducing CVD risk, especially in people with impaired or diabetic glucose tolerance.

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