Progression of Plasminogen Activator Inhibitor-1 and Fibrinogen Levels in Relation to Incident Type 2 Diabetes

Andreas Festa, MD; Ken Williams, MS; Russell P. Tracy, PhD; Lynne E. Wagenknecht, DRPH; Steven M. Haffner, MD

Background—Several studies have shown that fibrinolytic and coagulation abnormalities as well as low-grade inflammation predict cardiovascular disease and type 2 diabetes. We studied in the Insulin Resistance Atherosclerosis Study the relation of incident diabetes to dynamic changes of plasminogen activator inhibitor-1 (PAI-1) and fibrinogen.

Methods and Results—After a follow-up of 5.2 years, diabetes developed in 140 (16.6%) of 843 individuals (57% women; mean age [range], 54.7 [40, 69] years) (converters versus nonconverters). Baseline and follow-up levels of PAI-1 and fibrinogen (demographically and smoking adjusted) were higher in converters versus nonconverters (mean [SE]): at baseline, 23.7 ng/mL (1.5) versus 14.5 (0.4) and 286.2 mg/dL (4.8) versus 273.6 (2.1); at follow-up, 45.3 ng/mL (3.2) versus 25.9 (0.8) and 292.0 mg/dL (5.6) versus 275.2 (2.5); all P<0.05. In a demographically and smoking-adjusted logistic regression model, the change in PAI-1 was related to incident diabetes (OR for a 1-SD change [CI], 1.75 [1.37, 2.22]; P<0.001) after adjusting for baseline PAI-1 levels. After further adjusting for insulin sensitivity (S) or waist, change in PAI-1 remained significantly related to incident diabetes (OR, 1.66 [1.28, 2.15], and 1.64 [1.28, 2.10]; P<0.001). In contrast, change in fibrinogen was not significantly related to incident diabetes.

Conclusions—Progression of PAI-1 levels over time, in addition to high baseline PAI-1 levels, is associated with incident diabetes. PAI-1 levels (but not fibrinogen) further increase with the rising glucose levels and the development of diabetes. These findings extend the current knowledge on the relation of fibrinolysis and coagulation abnormalities to the development of type 2 diabetes. (Circulation. 2006;113:1753-1759.)

Key Words: diabetes mellitus ■ epidemiology ■ fibrinogen ■ inflammation ■ plasminogen activator inhibitor-1

Several studies have shown a relation of low-grade inflammation to cardiovascular disease (CVD).1,2 and, more recently, to insulin resistance cross-sectionally3,4 and to incident type 2 diabetes prospectively5-8; similar relations have been demonstrated for markers of coagulation/fibrinolysis, to CVD,5,10 insulin levels,10,11 insulin resistance, as directly measured,4 and to incident diabetes.5,7,8 Typically, in these epidemiological studies, a single, cross-sectional spot measure was investigated in relation to prevalent or incident disease.

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To our knowledge, no studies have investigated the relation of dynamic changes of markers of fibrinolysis and coagulation to clinical end points (including CVD and diabetes), either in epidemiological studies or in controlled clinical studies.

Therefore, we studied the relation of dynamic changes of plasminogen activator inhibitor-1 (PAI-1) and fibrinogen (FIB) to incident diabetes in healthy, nondiabetic individuals in the Insulin Resistance Atherosclerosis Study (IRAS).
measured by using standard methods, and hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg or current use of antihypertensive medication. Subjects were asked to bring in all prescribed medications at both the baseline and follow-up examinations. Pharmacological treatments were assessed by using these medications and responses to a medical history survey.

A standard 75-g oral glucose tolerance test was performed, and diabetes was defined by the oral glucose tolerance test, using World Health Organization criteria or by the use of diabetes medication. Among nondiabetic subjects at baseline, 19 were taking oral hypoglycemic medication at follow-up, and an additional 1 was taking oral hypoglycemic medication plus insulin. A frequently sampled intravenous glucose tolerance test with minimal model analysis was performed to assess insulin sensitivity. 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. In addition, the reduced sampling protocol (which required 12 rather than 30 plasma samples) was used because of the large number of subjects. Insulin sensitivity, expressed as the insulin sensitivity index (S), was calculated by mathematic modeling methods (MINMOD, version 3.0 [1994], Los Angeles, Calif, courtesy of Richard Bergman, PhD). Height, weight, and girth measurements and laboratory measurements were performed by using standard methods, as described previously.

Fibrinogen was measured (coefficient of variation [CV], 3.0%) in citrated plasma with a modified clot-rate assay, using the Diagnostica STAGO ST4 instrument (Diagnostica, Inc, Parsippany, NJ). PAI-1 samples were collected in chilled citrate coated tubes, which were centrifuged for a minimum of 10 minutes at 3000g (or a corresponding combination of time and centrifugal force) to make certain that there was no contamination from platelet PAI-1, and samples were frozen and stored at −70°C at the centers not later than 90 minutes after blood drawing. PAI-1 was measured by using a two-site immunoassay that is sensitive to free PAI-1 but not to PAI-1 complexed with t-PA; the CV was 14%. Frozen samples were measured at the Laboratory for Clinical Biochemistry Research, University of Vermont. Validation studies were done to investigate agreement between the Linco Adipokine Panel A Active PAI-1 assay (Lincro Research Immunoassay, St. Charles, Mo) with the assay used in the IRAS. For 23 citrated plasma samples, the Linco Adipokine Panel A Active PAI-1 assay showed moderate to high agreement with the in-house/Laboratory for Clinical Biochemistry Research PAI-1 antigen enzyme immunoassay. $R^2$ was 0.746 (n = 23). The mean CV between sample replicates was 25.5%. The same PAI-1 assay was used for the baseline and follow-up analyses; IRAS I was assayed in 1993 to 1994, using a robotic system to implement a standard immunoassay; IRAS II was assayed in 1998 to 1999, using the same immunoassay but done manually. Although a control sample assessed by manual approach was approximately 15% higher than the original sample (by robotic system), we believe that higher PAI-1 levels found at follow-up were mainly due to increased age and obesity.

### Statistical Analyses

To analyze whether changes in PAI-1 and FIB occur before the onset of diabetes, we mainly used two different statistical methods; first, we compared the measures in prediabetic individuals with those individuals who remained nondiabetic at follow-up by using ANCOVA, and second, we analyzed the changes in the measures between baseline and follow-up in relation to incident diabetes (dependent variable) by using logistic regression analyses. In addition, Spearman correlation analysis was used to assess cross-sectional and coincidental associations. All statistical calculations were performed in SAS version 8.0 (SAS Institute, Cary, NC). Log-transformed values were used in all ANCOVA and logistic regression analyses of PAI-1 levels because they appeared to be more normally distributed with the transformation than without. All

<table>
<thead>
<tr>
<th>TABLE 1. Baseline Clinical Characteristics (Percentage or Mean [SD]) Stratified by Follow-Up Diabetes Status: The Insulin Resistance Atherosclerosis Study</th>
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<tbody>
<tr>
<td><strong>Non-Diabetic at</strong></td>
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<tr>
<td><strong>Follow-Up (Non-DM)</strong></td>
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<tr>
<td>Ethnicity, %</td>
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<tr>
<td>Black</td>
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<tr>
<td>Hispanic</td>
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<td>Non-Hispanic white</td>
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<td>IGT, %</td>
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<td>Age, y</td>
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<td>BMI, kg/m²</td>
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<td>Waist circumference, cm</td>
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<td>Fasting glucose, mg/dL</td>
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<td>Fasting insulin, µU/mL</td>
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<td>HOMA-IR, mmOL·µU/L·min/L</td>
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<td>S, $10^{-1}$, µM·min$^{-1}$·µU$^{-1}$·mL$^{-1}$</td>
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<tr>
<td>C-reactive protein, mg/L</td>
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<td>Fibrinogen, µg/dL</td>
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<td>PAI-1, ng/mL</td>
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Probability values are from comparing percentages by $x^2$ tests or means by t test. $P<0.05$ in bold type.

*Impaired glucose tolerance (2-hour glucose ≥7.78 mmol/L [140 mg/dL]).
†Blood pressure ≥140/90 or taking antihypertensive medication.
‡Log-transformed for analysis; back-transformed for presentation.
In a demographically and smoking-adjusted logistic regression model, ΔPAI-1 was related to incident diabetes after adjusting for baseline PAI-1 levels (odds ratio [OR], 95% confidence interval [CI]: 1.75 [1.37, 2.22]; P<0.0001). After further adjusting for SI or waist, ΔPAI-1 remained significantly related to incident diabetes (OR, 1.64 [1.28, 2.10]; P<0.001). In a model adjusted for age, sex, ethnicity, smoking, baseline PAI-1, IGT, SI, waist, HDL cholesterol, and triglycerides (Figure 2A, model F), ΔPAI-1 was significantly correlated to BMI, waist, SI, fasting glucose, and CRP levels.4,7 The ΔPAI-1 was correlated to changes in BMI, waist, fasting glucose, and 2-hour glucose. ΔPAI-1 was only modestly correlated to the change in insulin sensitivity, as directly measured (ΔSI; r = -0.07, P<0.05), and somewhat more strongly correlated to insulin resistance as assessed by homeostasis model assessment (ΔHOMA-IR; r = 0.22, P<0.0001). However, the change in FIB (ΔFIB) showed no marked correlation with the change in any of the metabolic variables (Table 2).

Results

Baseline Characteristics, Unadjusted

After a follow-up of 5.2 years, diabetes developed in 140 of 843 individuals (16.6%) (see Table 1). As expected, risk factors for diabetes prevailed in converters (DM) versus nonconverters (N-DM), including higher age, higher body mass (body mass index [BMI] and waist), higher insulin resistance (lower SI), and higher levels of inflammatory proteins (C-reactive protein [CRP], FIB, PAI-1).

PAI-1 and Fibrinogen Levels at Baseline and Follow-Up

Table 1 shows unadjusted PAI-1 and FIB levels at baseline. In a model adjusted for demographic covariates and smoking, baseline and follow-up levels of PAI-1 and FIB were higher in DM versus N-DM (mean [SE]): at baseline, 23.7 ng/mL (1.5) versus 14.5 (0.4) (P<0.001) for PAI-1 and 286.2 mg/dL (4.8) versus 273.6 (2.1) (P=0.016) for FIB; at follow-up: 45.3 ng/mL (3.2) versus 25.9 (0.8) (P<0.001) for PAI-1 and 292.0 mg/dL (5.6) versus 275.2 (2.5) (P=0.006) for FIB. To assess potential confounding by glucose-lowering therapy, adjusted means from an ANCOVA to compare change (Δ)PAI-1 levels (adjusting for oral hypoglycemic medication/insulin in addition to age, ethnicity, gender, smoking, and baseline PAI-1) were calculated; means were for DM, 25.8 ng/mL (2.96), versus N-DM, 16.0 (1.34) (P=0.0034). This indicates that higher ΔPAI-1 levels in DM versus N-DM were unaffected by the use of oral hypoglycemic drugs at follow-up.

Spearman Correlation Analyses

Previous reports have shown that at baseline, PAI-1 and FIB were significantly correlated to BMI, waist, Ss, fasting glucose, 2-hour glucose, and CRP levels.4,7 The ΔPAI-1 was correlated to changes in BMI, waist, fasting glucose, and 2-hour glucose. ΔPAI-1 was only modestly correlated to the change in insulin sensitivity, as directly measured (ΔSI; r = -0.07, P<0.05), and somewhat more strongly correlated to insulin resistance as assessed by homeostasis model assessment (ΔHOMA-IR; r = 0.22, P<0.0001). However, the change in FIB (ΔFIB) showed no marked correlation with the change in any of the metabolic variables (Table 2).

Incidence of Type 2 Diabetes Stratified by Tertiles of PAI-1 Levels at Baseline and Median Split of ΔPAI-1

Both baseline levels of PAI-1 as well as ΔPAI-1 were related to incident diabetes (see Figure 1).

Logistic Regression Analyses

In a demographically and smoking-adjusted logistic regression model, ΔPAI-1 was related to incident diabetes after adjusting for baseline PAI-1 levels (odds ratio [OR], 95% confidence interval [CI]: 1.57 [1.37, 2.22]; P<0.0001). After further adjusting for SI or waist, ΔPAI-1 remained significantly related to incident diabetes (OR, 1.66 [1.28, 2.15], and 1.64 [1.28, 2.10]; P<0.001). In a model adjusted for age, sex, ethnicity, smoking, baseline PAI-1, IGT, SI, waist, HDL cholesterol, and triglycerides (Figure 2A, model F), ΔPAI-1 was significantly correlated to BMI, waist, SI, fasting glucose, and CRP levels.4,7 The ΔPAI-1 was correlated to changes in BMI, waist, fasting glucose, and 2-hour glucose. ΔPAI-1 was only modestly correlated to the change in insulin sensitivity, as directly measured (ΔSI; r = -0.07, P<0.05), and somewhat more strongly correlated to insulin resistance as assessed by homeostasis model assessment (ΔHOMA-IR; r = 0.22, P<0.0001). However, the change in FIB (ΔFIB) showed no marked correlation with the change in any of the metabolic variables (Table 2).

Incidence of type 2 diabetes stratified by tertiles of PAI levels at baseline and median split of ΔPAI-1. Probability values are from Cochran-Mantel-Haenszel test.
remained significantly related to incident diabetes (OR, 1.61 [1.23, 2.11]).

Further multivariate analyses were performed; a model replacing waist with BMI (model A plus BMI) yielded an OR of 1.33 (1.11, 1.58; P < 0.002). A model including age, gender, ethnicity, smoking, baseline PAI-1, IGT, insulin sensitivity (SI), triglycerides, HDL cholesterol, and BMI yielded an OR of 1.25 (1.04, 1.51; P < 0.02). To further characterize whether changes in body weight contribute to the relation of ΔPAI-1 to incident diabetes, ΔBMI was introduced as an additional covariate. In a model adjusting for age, sex, ethnicity, smoking, baseline PAI-1, IGT, SI, triglycerides, HDL cholesterol, and ΔBMI, ΔPAI-1 remained significantly related to incident diabetes (OR, 1.25 [1.03, 1.51]; P = 0.025). This result does not differ from the OR obtained from the same model without the addition of ΔBMI as a covariate (OR: 1.25 [1.04, 1.51]; P < 0.02). When the original model A is stratified by median split of ΔBMI (< or ≥ +0.38 kg/m²), the ΔPAI-1 OR remains highly significant in each strata (data not shown). Thus, the relation of ΔPAI-1 levels to incident diabetes was independent from ΔBMI and statistically significant, irrespective of whether waist or BMI was used as a measure of body weight.

Model A plus CRP at baseline yielded an OR of 1.41 (1.19, 1.67; P < 0.0001); model A plus fasting glucose yielded an OR of 1.32 (1.10, 1.57; P = 0.0022); adjustment for age, gender, ethnicity, smoking, baseline PAI-1, IGT, insulin sensitivity (SI), triglycerides, HDL cholesterol, BMI, CRP, systolic blood pressure, and fasting glucose yielded an OR of 1.23 (1.02, 1.49; P = 0.028). Thus, adjustment for (baseline) CRP levels as well as systolic blood pressure and fasting glucose did not significantly affect the relation of ΔPAI-1 levels to incident diabetes. Treatment at baseline and follow-up, including aspirin, statins, and...
antihypertensive medication, was introduced into multivariate models by using six categorical variables; model A plus aspirin, statins, and antihypertensive medication yielded an OR of 1.43 (1.20, 1.70; P<0.0001). Thus, the use of non–glucose-lowering medication did not affect the relation of ΔPAI-1 levels to incident diabetes.

In contrast, ΔFIB was not significantly related to incident diabetes (Figure 2B).

Change in PAI-Levels Stratified by Glucose Tolerance Status at Baseline and at Follow-Up
First, subjects with normal glucose tolerance (NGT) at baseline had a smaller increase of ΔPAI-1 than subjects with IGT at baseline. Second, deteriorating glucose tolerance status at follow-up (NGT-IGT diabetes) was related to increasing ΔPAI-1 levels (see Figure 3).

Heterogeneity Analyses
There were no significant interactions of gender, age or ethnicity, baseline glucose tolerance status, or obesity on the relation of ΔPAI-1 to incident diabetes, indicating that the relation of ΔPAI-1 to incident diabetes was consistent across the respective subgroups (odds ratios shown in Figure 4).

Discussion
In nondiabetic, healthy individuals, progression of PAI-1 levels over time was associated with incident diabetes after adjusting for demographics, smoking, and baseline PAI-1 levels. This association was independent of common risk factors for diabetes, including body weight and insulin resistance, as directly measured. Both fibrinogen and PAI-1 levels are elevated in prediabetic subjects; however, only PAI-1 levels further increase with the rising glucose levels and the development of diabetes.

Cross-sectional analyses have shown a relation of PAI-1 to fasting insulin and proinsulin, other components of the insulin resistance syndrome, including obesity, and to insulin resistance, as directly measured. Previous studies have also shown a relation of increased levels of coagulation factor VIII, fibrinogen, and PAI-1 to incident diabetes. Additionally, based on longitudinal analyses, we have shown in the IRAS that the relation of PAI-1 to incident diabetes was independent of common risk factors for diabetes. In this latter report, baseline levels of PAI-1, FIB, and CRP were investigated in relation to incident diabetes. By contrast, the current study is the first to investigate dynamic changes of PAI-1 and FIB over time. Our findings indicate that those healthy individuals are at increased risk for development of diabetes who have an enhanced hypo-fibrinolytic/proinflammatory/procoagulant state, as evidenced by both high prevalent levels of PAI-1 and FIB (and other inflammatory proteins, as shown in previous studies), as well as an increased progression of PAI-1 levels over time (as shown in the present report).

This finding has two important implications. First, from a pathophysiological perspective, it adds to our knowledge on the contribution of inflammation to the development of diabetes. Second, from a clinical perspective, based on these findings one might speculate that therapies aiming at lowering PAI-1 levels might decrease diabetes incidence. This, however, needs to be substantiated by results of a randomized, controlled trial.

We found a relation of incident diabetes to changes in PAI-1 levels but not to changes in FIB levels. Although PAI-1 has been termed an acute-phase protein (defined as one whose plasma concentration increases/decreases by at least 25% during inflammatory disorders), elevated PAI-1 levels are commonly considered indicative of compromised fibrinolysis rather than reflecting inflammation. Classic acute-phase proteins, namely CRP and FIB, as opposed to PAI-1, appear to follow distinct pathways with respect to the pathophysiology of diabetes. A close interrelation of CRP and PAI-1 has been confirmed experimentally in a study showing that CRP results in a time- and dose-dependent increase in PAI-1 expression in human aortic endothelial cells. As is the case for PAI-1 and, to a lesser degree for FIB, CRP has been related to insulin resistance and the increased incidence of diabetes. Thus, changes in PAI-1 with changing glucose tolerance might also reflect changes in CRP. Unfortunately, in the IRAS, CRP levels have only been measured at baseline and not at follow-up. Therefore, we are not able to provide CRP levels at follow-up, and, hence, we were unable to include these data in our analyses.

How could a relation of PAI-1 to incident diabetes be explained? First, a common genetic background may underlie both PAI-1 expression as well as type 2 diabetes risk. Second, PAI-1 might be causally involved in the pathophysiology of type 2 diabetes. Finally, various factors have been identified that affect both PAI-1 expression and diabetes incidence (such as body weight, insulin resistance, glucocorticoids, triglycerides, and free fatty acids), suggesting that high PAI-1 levels might be a marker (rather than a causative factor) of the underlying disease process. For example, experimental studies have shown that glucose (as well as insulin) regulate PAI-1 gene expression. This observation suggests that increasing glycemia (in subjects transitioning from NGT to IGT, or NGT or IGT to
diabetes, respectively) might promote PAI-1 gene expression and result in disproportionally elevated and increasing circulating PAI-1 plasma levels. Multivariate analyses were performed to adjust for potential confounders that have been shown to affect both PAI-1 levels and the development of diabetes. Respective covariates included lipids, glycemia, insulin resistance, and body weight. Although these analyses showed that these covariates did not noticeably affect the relation of ΔPAI-1 to incident diabetes, we would like to point out that we were unable to adjust for additional confounding pathways, namely adipokines and reduced nitric oxide production.

Results from animal studies lend support to a causative role for PAI-1 in the development of obesity and insulin resistance. In a high-fat-high-carbohydrate diet-induced obesity model, obesity and insulin resistance were completely prevented in PAI-1−/– mice. Accordingly, genetically obese and diabetic (ob/ob) mice lacking the PAI-1 gene (PAI-1−/−) exhibited reduced adiposity and amelioration of the diabetic phenotype. Interestingly, these effects were paralleled by a reduction of adipose tissue tumor necrosis factor-α expression. Tumor necrosis factor-α is a mediator of both insulin resistance and PAI-1 expression.

Clinical studies have investigated dynamic changes of PAI-1 in response to various nonpharmacological and pharmacological interventions, including diet, lifestyle intervention, weight reduction, treatment with metformin, a thiazolidinedione, postmenopausal hormone replacement, angiotensin-converting enzyme (ACE) inhibitors, and an angiotensin II receptor blocker. Some of these interventions have also been shown to reduce the risk for development of diabetes. The main putative mechanisms by which this preventive effect is being achieved are reductions of body weight and the ensuing improved insulin resistance or improved insulin resistance alone. In the present study, the change in PAI-1 levels was related to incident diabetes, independent of increased insulin resistance and increased body weight and independent of changes in BMI over time (ΔBMI). Therefore, from an intervention standpoint, attenuating an “enhanced and accelerated” hypofibrinolytic/proinflammatory state might add to a diabetes-preventive effect beyond the respective effects on body weight and/or insulin resistance.

Clinical studies that discovered a (largely unexpected) reduction in diabetes incidence lend further support to this hypothesis. Post hoc and/or secondary end point analyses from large, randomized trials, investigating primarily CVD end points, have shown a reduction in diabetes incidence in response to various medications. Interestingly, many drugs that have been shown a reduction in diabetes incidence in response to various randomized trials, investigating primarily CVD end points, have proved insulin resistance or improved insulin resistance achieved are reductions of body weight and the ensuing improvement in BMI over time (ΔBMI). In the present study, the change in PAI-1 levels was related to incident diabetes, independent of increased insulin resistance and increased body weight and independent of changes in BMI over time (ΔBMI). Therefore, from an intervention standpoint, attenuating an “enhanced and accelerated” hypofibrinolytic/proinflammatory state might add to a diabetes-preventive effect beyond the respective effects on body weight and/or insulin resistance.

In summary, findings of the present study extend current knowledge on the relation of fibrinolysis and coagulation to the development of type 2 diabetes. Finally, if elevated PAI-1 is a risk factor for coronary heart disease, a further increase in PAI-1 with development of diabetes mellitus might lead to a more marked increase in risk of coronary heart disease in diabetes mellitus compared with prediabetic or IGT subjects.

Acknowledgments

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Disclosures

Andreas Festa is an employee of Eli Lilly and Co, Vienna Austria. Ken Williams, Russell P. Tracy, Lynne Wagenknecht, and Steven M. Haffner report no conflicts.

References

Progression of PAI-1 and Incident Type 2 Diabetes


**CLINICAL PERSPECTIVE**

The role of chronic, subclinical inflammation in the development of cardiovascular disease (CVD) and type 2 diabetes has been a major focus of epidemiological and clinical research over the past several years. This is the first study investigating serial changes in plasminogen activator inhibitor-1 (PAI-1) and fibrinogen levels in relation to incident type 2 diabetes. We observed in the nondiabetic population of the IRAS (Insulin Resistance Atherosclerosis Study) that the change in PAI-1 levels over time (follow-up: 5.2 years) was related positively to incident diabetes. Our observations are consistent with the notion that those healthy individuals who are at increased risk of developing diabetes experience an enhanced hypofibrinolytic/proinflammatory/procoagulant state, as evidenced by both high prevalent levels of PAI-1 and fibrinogen (and other inflammatory proteins, as shown in previous studies), as well as an increased progression of PAI-1 levels over time (as shown in the present study). From a pathophysiological perspective, our observations provide new data on the tracking of PAI-1 levels with the development of diabetes. Additional studies are warranted to confirm our findings.