Elevated Levels of Acute-Phase Proteins and Plasminogen Activator Inhibitor-1 Predict the Development of Type 2 Diabetes

The Insulin Resistance Atherosclerosis Study

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Elevated serum levels of acute-phase proteins, indicating chronic subclinical inflammation, have been associated with cardiovascular disease as well as the insulin resistance syndrome. Chronic inflammation may also be a risk factor for developing type 2 diabetes. We studied the concentrations of C-reactive protein (CRP), fibrinogen, and plasminogen activator inhibitor-1 (PAI-1) in 1,047 nondiabetic subjects in relation to incident diabetes within 5 years in the Insulin Resistance Atherosclerosis Study. Subjects with diabetes at follow-up (n = 144) had higher baseline levels of fibrinogen (mean ± SD; 287.8 ± 58.8 vs. 275.1 ± 56.0 mg/dl; P = 0.013) as well as of CRP (median [interquartile range]; 2.40 [1.29, 5.87] vs. 1.67 mg/l [0.75, 3.41]; P = 0.0001) and PAI-1 (24 [15, 37.5] vs. 16 ng/ml [9, 27]; P = 0.0001) than nonconverters. The odds ratio (OR) of converting to diabetes was significantly increased with increasing baseline concentrations of the inflammatory markers. In contrast to PAI-1, the association of CRP and fibrinogen with incident diabetes was significantly attenuated after adjustment for body fat (BMI or waist circumference) or insulin sensitivity (S_I), as assessed by a frequently sampled intravenous glucose tolerance test. In a logistic regression model that included age, sex, ethnicity, clinical center, smoking, BMI, S_I, physical activity, and family history of diabetes, PAI-1 still remained significantly related to incident type 2 diabetes (OR [95% CI] for 1 SD increase: 1.61 [1.20–2.16]; P = 0.002). Chronic inflammation emerges as a new risk factor for the development of type 2 diabetes; PAI-1 predicts type 2 diabetes independent of insulin resistance and other known risk factors for diabetes. ***Diabetes*** **51:1131–1137, 2002**

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Received for publication 10 May 2001 and accepted in revised form 17 December 2001.

AIR, acute insulin response; CRP, C-reactive protein; CV, coefficient of variation; IGT, impaired glucose tolerance; IRAS, Insulin Resistance Atherosclerosis Study; NGT, normal glucose tolerance; OR, odds ratio; PAI, plasminogen activator inhibitor; S_I, insulin sensitivity index.

Several experimental and prospective epidemiological studies have shown an association of elevated serum levels of acute-phase proteins, indicating chronic subclinical inflammation, with cardiovascular disease (reviewed in Lagrand et al. [1]). It has been hypothesized that atherosclerotic cardiovascular disease and type 2 diabetes arise from a "common soil" (2,3), and chronic inflammation may be such a candidate (4,5). Inflammatory markers, such as high white cell count, high fibrinogen, or low albumin (6), and markers of hemostasis, such as factor VIII (7), have been related to the development of type 2 diabetes. Adipose body mass may be an important mediator to explain these relations (6,7), but pathophysiological mechanisms remain elusive, and no data on the role of insulin resistance are available. This is of particular interest because decreased insulin sensitivity has been linked to incident type 2 diabetes (8), as well as increased levels of inflammatory proteins (9,10) and markers of hemostasis (11,12) and fibrinolysis (11,13).

In the present study, we investigated the relation of C-reactive protein (CRP), fibrinogen, and plasminogen activator inhibitor 1 (PAI-1) to incident type 2 diabetes during a 5-year period in the Insulin Resistance Atherosclerosis Study (IRAS). Furthermore, we sought to clarify the role of insulin resistance, as directly measured using a frequently sampled intravenous glucose tolerance test.

RESEARCH DESIGN AND METHODS

The Insulin Resistance Atherosclerosis Study (IRAS) is a multicenter, epidemiological study aiming to explore relationships among 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 (14). The IRAS protocol was approved by local institutional review committees, and all subjects gave informed consent.

A total of 1,625 individuals participated in the IRAS. This report includes data on 1,047 subjects, who were nondiabetic at the baseline examination and in whom CRP, PAI-1, and fibrinogen were measured. Subjects were investigated twice following the same protocol. The mean follow-up duration was 5.2 years (range 4.5–6.6). Each of the two IRAS examinations required two visits. Patients were asked before each visit to fast for 12 h, to abstain from heavy exercise and alcohol for 24 h, and to refrain from smoking on the morning of the examination. Race and ethnicity were assessed by self-report. Family history of type 2 diabetes and physical activity were assessed using standard interviewing procedures. A positive family history of diabetes was defined as parents and/or siblings having type 2 diabetes. Smoking status was dichotomized into "none" and "past" or "current" using a standard questionnaire.

TABLE 1
Descriptive data stratified by converters to type 2 diabetes (DM) versus nonconverters (Non-DM) in the IRAS

	DM	Non-DM	*P* value
n	144	903	
Ethnicity (NHW, Blacks, His)	37/28/35	41/26/33	NS
Glucose tolerance (NGT/IGT)	32/68	71/29	0.001
Hypertension (yes/no)	45/55	30/70	0.001
Sex (F/M)	60/40	56/44	NS
Age (years)	56.0 ± 7.8	54.6 ± 8.5	0.047
BMI (kg/m^2)	31.1 ± 6.3	28.0 ± 5.5	0.0001
Waist circumference (cm)	95.5 ± 13.1	89.8 ± 12.7	0.0001
Fasting insulin (pmol/l)	133.4 ± 154.3	88.6 ± 69.6	0.0007
Fasting glucose (mg/dl)	106.6 ± 12.0	97.5 ± 10.8	0.0001
$S_I \times 10^{-4}$ ($min^{-1} \cdot \mu U^{-1} \cdot ml^{-1}$)	1.26 ± 1.56	2.31 ± 2.06	0.0001
Fibrinogen (mg/dl)	287.8 ± 58.8	275.1 ± 56.1	0.013
CRP (mg/l)	2.40 (1.29–5.87)	1.67 (0.75–3.41)	0.0001
PAI-1 (ng/ml)	24 (15–37.5)	16 (9–27)	0.0001

Data are means ± SD, and median (interquartile range). *P* values are for χ^2, *t* test, or Kruskal-Wallis test as appropriate. NHW, non-Hispanic whites, His, Hispanics.

Physical activity was categorized into five groups according to the frequency of vigorous activity (from rarely/never to five times a week). Alcohol consumption was assessed using a standard questionnaire and categorized into three groups. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or current use of antihypertensive medication.

Assessment of glucose tolerance and insulin sensitivity. A standard 75-g oral glucose tolerance test was performed, and glucose tolerance status was based on the World Health Organization criteria (15). A frequently sampled intravenous glucose tolerance test (16) with minimal model analysis (17) 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_I), was calculated by mathematical modeling methods (MINMOD, version 3.0). Acute insulin response (AIR) was calculated as the mean of 2- and 4-min insulin concentrations after glucose administration.

Measures of body fat and body composition. Height was recorded to the nearest 0.5 cm, and weight was measured to the nearest 0.1 kg. BMI was calculated as weight/height^2 (kg/m^2) and was used as an estimate of overall adiposity. Girth measurements were estimated as the average of duplicate measures (taken to the nearest 0.5 cm using a steel tape). Waist circumference was considered an estimate of visceral fat mass and measured on bare skin during mid-respiration at the natural indentation between the 10th rib and the iliac crest (minimum waist).

Laboratory measurements. Glucose, triglyceride, HDL cholesterol, free fatty acids, and insulin levels were measured using standard methods. CRP was measured by in-house ultrasensitive competitive immunoassay (antibodies and antigens from Calbiochem, La Jolla, CA) with an interassay coefficient of variation (CV) of 8.9% (18). Fibrinogen was measured in citrated plasma with a modified clot-rate assay using the Diagnostica STAGO ST4 instrument, as described previously (19). This was based on the original method of Clauss (20) with a CV of 3.0%. PAI-1 was also measured in citrated plasma (21), using a two-site immunoassay that is sensitive to free PAI-1 but not to PAI-1 complexed with t-PA (22). The citrate sample was centrifuged for a minimum of 10 min at 3,000 *g* (or a corresponding combination of time and centrifugal force) to make certain that there was no contamination from platelet PAI-1; the CV was 14%. Samples for fibrinogen, PAI-1, and CRP were frozen and stored at −70°C at the centers no longer than 90 min after blood drawing. Frozen samples were shipped on a monthly basis to the Laboratory for Clinical Biochemistry Research, University of Vermont (R.P.T.), where measurements were performed.

Statistical analysis. Statistical analyses were performed using the SAS statistical software system (SAS, Cary, NC). Table 1 shows descriptive data stratified by conversion status to type 2 diabetes (%; mean ± SD; median [interquartile range]). Differences between groups were calculated using *t* test, χ^2, or Kruskal-Wallis test (CRP and PAI-1). Spearman rank correlations were performed (Table 2).

Next, logistic regression analyses with incident type 2 diabetes as the dependent variable were performed. Odds ratios (OR) indicating a 1 SD increase of the variables of interest were calculated. For these analyses as well as the interaction models, logarithmically transformed values of CRP and PAI-1 were used because the distribution of the residuals from the fitted models became normally distributed after log transformation. To make units of change in the logistic regression models better comparable between the log-transformed variables (CRP and PAI-1) and fibrinogen, we also performed a logistic regression model with logarithmically transformed fibrinogen values (model A only). Because age, sex, and smoking were related to the outcome variables in previous reports and/or the IRAS population, these covariates were considered in the analyses (demographic model). Further potential confounding covariates, such as fasting insulin, BMI, waist, and S_I, were included separately in logistic regression models. These analyses were also stratified by sex, ethnicity, BMI (median as cut-point value), and diabetic status at baseline (normal [NGT] versus impaired glucose tolerance [IGT]; Table 3). Additional analyses were performed with fasting glucose, AIR, and hypertension as covariates in the overall population only. In an extended logistic regression model, demographic covariates (age, sex, ethnicity, clinical center, and smoking) along with known risk factors for type 2 diabetes (S_I, BMI, family history of type 2 diabetes, physical activity) were included simultaneously to assess whether the variable of interest is independently related to incident diabetes. This model was performed only with PAI-1 as an independent variable, because the effect of the two other variables of interest (CRP and fibrinogen) was attenuated and no longer statistically significant after considering S_I and/or BMI separately in the models. Additional models were tailored, including as covariates alcohol consumption, fasting free fatty acids, triglycerides, HDL cholesterol, and antibodies against oxidized LDL.

Furthermore, to assess the effects of sex, ethnicity, BMI, and diabetic status at baseline on the relation of CRP, PAI-1, and fibrinogen with incident diabetes, we performed interaction analyses. We included in separate models interaction terms for sex × log CRP, sex × log PAI-1, sex × fibrinogen, and

TABLE 2
Spearman correlation analysis (unadjusted) of measures of body fat and metabolic variables with CRP, fibrinogen, and PAI-1*

	CRP	Fibrinogen	PAI-1
BMI	0.43	0.27	0.34
SBP	0.21	0.15	0.15
DBP	0.13	0.08†	0.19
Waist circumference	0.33	0.20	0.39
Fasting insulin	0.34	0.19	0.43
Fasting glucose	0.14	0.06‡	0.23
S_I	−0.39	−0.21	−0.38
AIR	0.16	0.12	0.18

*Data partly reported in Festa et al. (9). All $P < 0.0001$, except †$P < 0.01$ and ‡P = NS. SBP, systolic blood pressure; DBP, diastolic blood pressure.

TABLE 3
Logistic regression analysis for log PAI-1 with incident type 2 diabetes as the dependent variable stratified by diabetic status

Covariates	NGT		IGT	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
A. Demographic	2.42 (1.67–3.52)	0.0001	1.38 (1.03–1.83)	0.03
B. Demographic + fasting insulin	2.16 (1.46–3.20)	0.0001	1.31 (0.97–1.75)	0.07
C. Demographic + S_I	2.13 (1.43–3.19)	0.0002	1.26 (0.94–1.70)	0.13
D. Demographic + BMI	2.14 (1.45–3.16)	0.0001	1.26 (0.93–1.71)	0.14
E. Demographic + waist	2.15 (1.44–3.21)	0.0002	1.34 (0.99–1.82)	0.06
F. "Extended model"	2.13 (1.29–3.54)	0.003	1.47 (0.98–2.21)	0.06

Demographic model (model A) includes as covariates age, sex, clinical center, and smoking. Extended model includes as covariates age, sex, clinical center, smoking, ethnicity, S_I, BMI, family history of diabetes, and physical activity.

so forth. These models were adjusted for age, sex, smoking, and clinical center, analogous to the demographic logistic regression model.

Finally, the incidence of diabetes (%) was calculated by quartiles of CRP, fibrinogen, and PAI-1, and differences were assessed using the Mantel-Haenszel χ^2 test (Fig. 1). This was also done for PAI-1 stratified by diabetic status (Fig. 2).

$P < 0.05$ (two-sided) was considered statistically significant.

RESULTS

Subjects who converted to type 2 diabetes had higher baseline levels of fibrinogen, CRP, and PAI-1 (Table 1). A significant association of the three variables of interest with BMI, waist circumference, fasting insulin, fasting glucose, and S_I was found (except for the association of fibrinogen with fasting glucose; Table 2).

There was a linear increase in incident diabetes with increasing quartiles of CRP (6.9, 12.1, 16.2, and 19.9% in quartiles 1–4, respectively), PAI-1 (6.6, 10.4, 15.6, and 23.1% in quartiles 1–4, respectively), and fibrinogen (10.9, 13.7, 13.5, and 16.9% in quartiles 1–4, respectively; Fig. 1), which was significant for CRP and PAI-1 ($P = 0.001$, respectively) and of borderline significance for fibrinogen ($P = 0.06$).

Logistic regression analyses. PAI-1 showed a strong and consistent relation to incident diabetes. The relation was somewhat weaker for CRP and fibrinogen. Using logarithmically transformed fibrinogen values yielded similar results compared with using fibrinogen values on a linear scale; model A: OR (95% CI): log fibrinogen 1.24 (1.02–1.50) vs. 1.21 (1.01–1.44), $P = 0.03$ for both. All three proteins were related to incident diabetes after adjusting for age, sex, smoking, and clinical center (Fig. 3; model A). The relation was only slightly attenuated after adjusting for fasting insulin (model B). When S_I or BMI was included in the model, the relation was significantly attenuated for CRP and fibrinogen, whereas PAI-1 remained significantly related to incident diabetes (models C–E).

These findings indicate that the relation of CRP and fibrinogen to the development of diabetes is confounded or mediated by insulin resistance and/or body mass. By contrast, the relation of PAI-1 to incident diabetes was independent of these covariates. In an extended logistic regression model, including as covariates age, sex, ethnicity, smoking, clinical center, BMI, S_I, physical activity, and family history of diabetes, PAI-1 still remained significantly related to incident type 2 diabetes (OR, 1.61 [95% CI, 1.20–2.16]; $P = 0.0017$; Fig. 3*A*, model F). Adding fasting glucose as an additional covariate to model F modestly attenuated the relation (OR, 1.32 [95% CI, 1.01–1.72]; $P = 0.043$).

Additional models were tailored to investigate the impact of other possible confounding covariates. Alcohol consumption was significantly (inversely) related to fibrinogen but not to CRP or PAI-1 levels (data not shown). Adding alcohol consumption as an additional independent variable did not change the results of the logistic regression models (model A, for log CRP: OR, 1.44 [95% CI, 1.20–1.72], $P = 0.0001$; for fibrinogen: OR, 1.21 [95% CI, 1.01–1.44], $P = 0.039$, and for log PAI-1: OR, 1.98 [95% CI, 1.60–2.45], $P = 0.0001$). Furthermore, we performed a logistic regression model for PAI-1, including as covariates (in addition to variables included in model F) fasting free fatty acids, triglycerides, HDL cholesterol, and antibodies against oxidized LDL (IgG) (all of which were related to PAI-1 in univariate Spearman correlation analysis, $P = 0.0001$ for all). The OR was slightly lower as compared with model F, but the relation of PAI-1 to incident diabetes remained significant (OR, 1.48 [95% CI, 1.14–1.91], $P = 0.0029$).

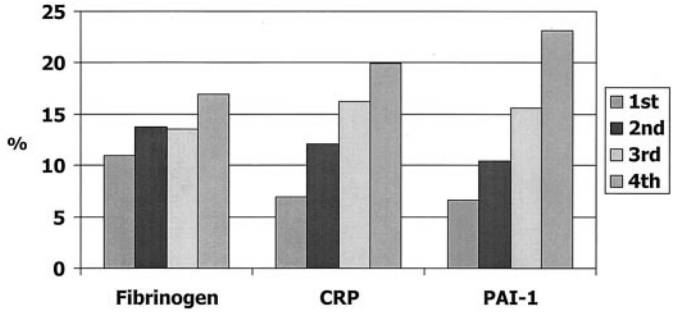

FIG. 1. Incidence of type 2 diabetes stratified by quartiles of inflammatory proteins. *P* values for χ^2 were 0.001 for PAI-1 and CRP and 0.06 for fibrinogen.

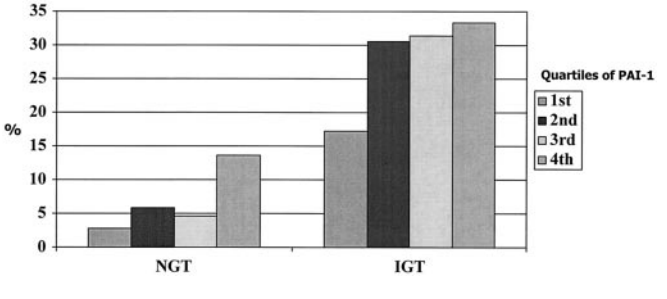

FIG. 2. Incidence of type 2 diabetes by quartiles of PAI-1 in subjects with NGT and IGT at baseline. Incidence rates were 2.7, 5.8, 4.7, and 13.6% in NGT and 17.2, 30.6, 31.3, and 33.3% in IGT. *P* for χ^2 in NGT = 0.001, and $P = 0.02$ in IGT.

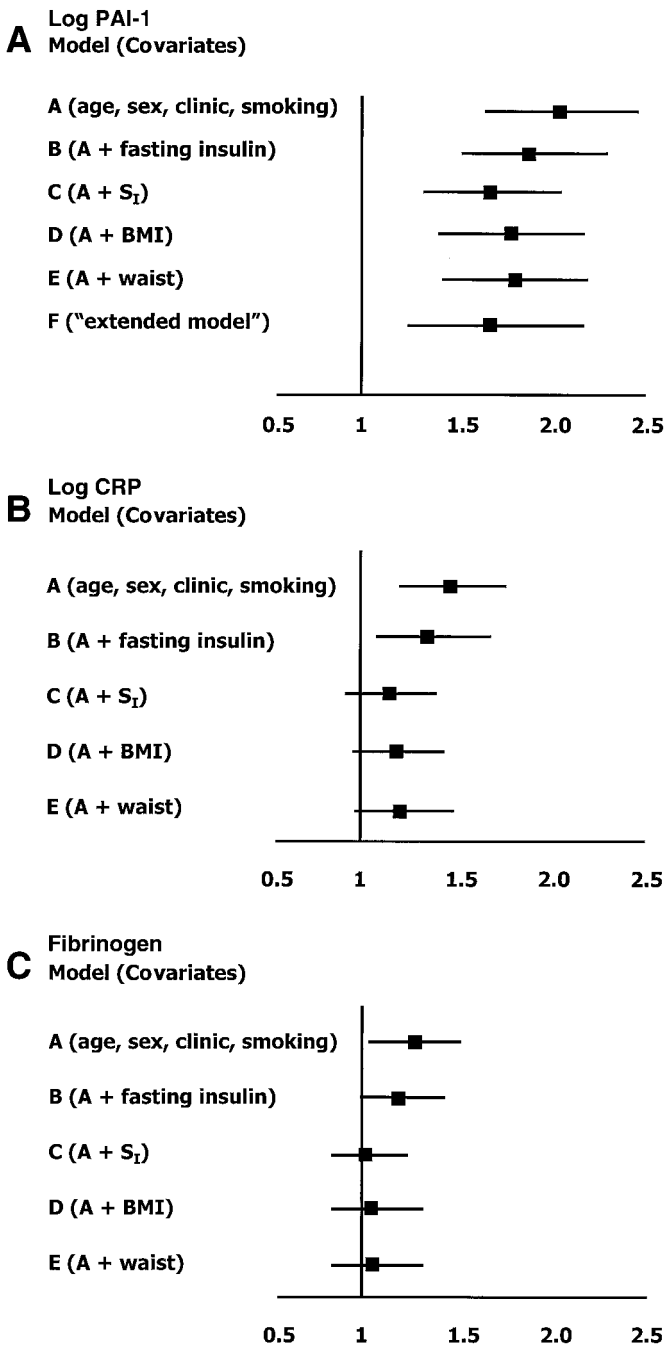

FIG. 3. Risk of developing type 2 diabetes (logistic regression analysis). *A*: ORs and 95% CIs for a 1 SD increase in levels of log PAI-1 at baseline. ORs (95% CI) were 1.99 (1.60–2.45) for model A, 1.80 (1.45–2.25) for model B, 1.60 (1.27–2.01) for model C, 1.69 (1.35–2.12) for model D, 1.71 (1.36–2.16) for model E, and 1.61 (1.20–2.16) for model F (extended model includes as covariates age, sex, clinical center, smoking, ethnicity, S_I, BMI, family history of diabetes, and physical activity). $P = 0.0001$ for all, except model F ($P = 0.002$). *B*: ORs and 95% CIs for a 1 SD increase in levels of log CRP at baseline. ORs (95% CI) for CRP were 1.44 (1.20–1.72; $P = 0.0001$) for model A, 1.34 (1.11–1.61; $P = 0.003$) for model B, 1.12 (0.91–1.38; $P = 0.3$) for model C, 1.17 (0.95–1.43; $P = 0.14$) for model D, and 1.20 (0.98–1.47; $P = 0.08$) for model E. *C*: ORs and 95% CIs for a 1 SD increase in levels of fibrinogen at baseline. ORs (95% CI) for fibrinogen were 1.21 (1.01–1.44; $P = 0.03$) for model A, 1.17 (0.97–1.40; $P = 0.10$) for model B, 1.01 (0.83–1.22; $P = 0.9$) for model C, 1.02 (0.85–1.24; $P = 0.8$) for model D, and 1.03 (0.85–1.25; $P = 0.7$) for model E.

Finally, we included AIR or hypertension (separately) in a demographic model (as shown in Fig. 3, model A). The ORs were comparable to those seen in the demographic models (without including AIR or hypertension), being 1.65 (95% CI, 1.35–2.01; $P = 0.0001$) for log CRP, 1.29 (95% CI, 1.07–1.56; $P = 0.007$) for fibrinogen, and 2.19 (95% CI, 1.74–2.76; $P = 0.0001$) for log PAI-1, for models including AIR, and 1.43 (95% CI, 1.18–1.73; $P = 0.0003$) for log CRP, 1.18 (95% CI, 0.98–1.43; $P = 0.075$) for fibrinogen, and 1.96 (95% CI, 1.57–2.44; $P = 0.0001$) for log PAI-1 for models including hypertension. Thus, hypertension or differences in AIR did not explain the relation of CRP, fibrinogen, or PAI-1 to incident diabetes.

Subgroup analyses. The relation of PAI-1 to incident diabetes was stronger in subjects with NGT than in subjects with IGT ($P = 0.02$ for interaction term; Table 3). As shown on Fig. 2, subjects with NGT presenting with PAI-1 levels in the highest quartile had a comparable risk of developing diabetes to that in subjects with IGT in the lowest quartile for PAI-1 levels.

By contrast, no interaction of glucose tolerance status was found for CRP and fibrinogen. In the demographic model (model A), the ORs (95% CI) in subjects with NGT and IGT for log CRP were 1.20 (0.88–1.63; $P = 0.3$) and 1.25 (0.97–1.62; $P = 0.09$), respectively, and for fibrinogen were 1.09 (0.79–1.50; $P = 0.6$) and 1.14 (0.91–1.43; $P = 0.3$), respectively.

The relation of CRP and fibrinogen but not PAI-1 to incident diabetes was somewhat stronger in lean than in obese individuals (Table 4); however, the respective interaction terms failed to reach statistical significance ($P = 0.3$, 0.4, and 0.7 for fibrinogen, CRP, and PAI-1, respectively). All other interaction analyses were also nonsignificant, indicating that the relation of the three variables of interest with incident diabetes was consistent in men and women, in lean and obese subjects, and across the three ethnic groups of the IRAS (data not shown).

DISCUSSION

In the present article, we have shown a significant relation of CRP, fibrinogen, and PAI-1 to the development of type 2 diabetes. The relation was consistent in men and women, in lean and obese subjects, and across the three ethnic groups of the IRAS. The association of PAI-1 to incident diabetes was particularly strong and independent of several other known risk factors for type 2 diabetes.

Recently, an association of inflammatory markers with the development of type 2 diabetes was reported in the Atherosclerosis Risk in Communities study population (6), which was significantly attenuated after accounting for differences in body mass. This is in agreement with the present study. Furthermore, we have shown that insulin resistance, as directly measured, attenuated the relation of CRP and fibrinogen to incident diabetes, suggesting a central role for body fat and insulin resistance, at least partly mediating these relations. By contrast, the association of PAI-1 to the development of diabetes was independent of both body fat and insulin resistance. This suggests that PAI-1, known to be related to features of the insulin resistance syndrome, including body fat and insulin resistance (11–13), may be a very early risk marker of the insulin resistance syndrome and eventually type 2 diabetes. Alternatively, enhanced PAI-1 expression may indicate a pathophysiological pathway distinct from the insulin resistance syndrome. In young, nondiabetic, nonobese

TABLE 4
Logistic regression analysis with incident type 2 diabetes as the dependent variable stratified by BMI [below (lean) and above (obese) the median = 27.4 kg/m^2]

	Lean		Obese	
Covariates	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Log CRP				
A. Demographic	1.47 (1.06–2.05)	0.021	1.10 (0.86–1.41)	0.5
B. Demographic + fasting insulin	1.46 (1.05–2.05)	0.027	1.04 (0.81–1.34)	0.8
C. Demographic + S_I	1.25 (0.88–1.79)	0.22	0.93 (0.70–1.22)	0.6
Log PAI-1				
A. Demographic	2.02 (1.39–2.93)	0.0002	1.67 (1.27–2.20)	0.0003
B. Demographic + fasting insulin	1.89 (1.30–2.75)	0.0009	1.56 (1.18–2.08)	0.0022
C. Demographic + S_I	1.72 (1.17–2.54)	0.0063	1.48 (1.10–2.00)	0.0098
Fibrinogen				
A. Demographic	1.32 (0.94–1.85)	0.11	1.00 (0.80–1.26)	0.9
B. Demographic + fasting insulin	1.34 (0.96–1.88)	0.09	0.98 (0.78–1.24)	0.9
C. Demographic + S_I	1.12 (0.78–1.59)	0.5	0.89 (0.70–1.14)	0.9

*Demographic model includes as covariates age, sex, clinical center, and smoking.

offspring of patients with type 2 diabetes, PAI-1 activity was elevated but unrelated to plasma insulin concentrations (23), suggesting that in a population at risk of developing diabetes, enhanced PAI-1 expression may be prevalent independent of insulin resistance. In contrast to BMI and S_I, fasting glycemia modestly attenuated the relation of PAI-1 to incident diabetes. It may be noteworthy that previous reports (including the present one) have shown a weaker cross-sectional relation of PAI-1 to glycemia as compared with body weight, insulinemia, and insulin resistance. It has been suggested that glucose (as well as insulin) may regulate PAI-1 gene expression in vascular smooth muscle cells (24). However, it should be noted that the associations as shown do not necessarily reflect causality.

The relation of PAI-1 to incident diabetes was stronger in subjects with NGT. In subjects with IGT, the relation may be confounded by prevailing features of the insulin resistance syndrome, which are related to both PAI-1 levels (11–13) and incident diabetes (2). This latter finding may be of particular clinical relevance by offering the potential to identify a population with a high risk of developing type 2 diabetes (high PAI-1 levels) among subjects with an a priori low risk (NGT).

Mechanisms that link chronic inflammation and increased PAI-1 expression to the development of diabetes remain elusive. It can be assumed, however, that any effects may be exerted via *1*) insulin secretion, *2*) insulin resistance, or *3*) both.

It has been shown recently that in subjects with type 2 diabetes, autoimmunity (as indicated by elevated levels of GAD65 and IA-2 autoantibodies) was associated with elevated levels of CRP and fibrinogen (25). Thus, one might speculate that subjects who present with increased levels of inflammatory proteins in the prediabetic state may in fact represent subjects who will eventually develop late-onset type 1 diabetes, with a deterioration of insulin secretion as the primary defect (26). In the IRAS population, autoantibodies against islet-related antigens have not been measured. Autoimmunity against these antigens, such as GAD, is considered to reflect predisposition to type 1 diabetes in general (27). Given that the IRAS population represents a population with a low expected prevalence of type 1 diabetes, relative to the expected prevalence of type 2 diabetes, it can be assumed that the proportion of antibody-positive (versus antibody-negative) subjects in our diabetic population is low, and therefore it is unlikely that antibody positivity may explain our results. Also, in contrast to what might be expected, we found a positive association of the three proteins with acute insulin secretion, and including AIR into the regression models did not change the results significantly. This argues against a significant role of (first-phase) insulin secretion for the relation of inflammatory markers and PAI-1 to incident diabetes. However, we acknowledge that insulin concentrations in response to an intravenous glucose load reflect first-phase insulin secretion at best, providing no information on second-phase insulin secretion. Therefore, subtle alterations of insulin secretion not reflected by the technique used in this study may still be involved.

Second, there is an ever-growing body of evidence derived from cross-sectional analyses linking chronic inflammation and the insulin resistance syndrome, including decreased insulin sensitivity itself (9,10). Several mechanisms may explain such a relation, such as the hypersecretion of proinflammatory cytokines (interleukin-6, tumor necrosis factor-α) from adipose tissue (28,29), which exert major stimulatory effects on the synthesis of acute-phase proteins (30), including PAI-1 (31), or enhanced expression of inflammatory proteins by counteracting the physiological effect of insulin on hepatic acute-phase protein synthesis (32), as a result of decreased insulin sensitivity.

Third, additional factors (or combinations thereof) that were not measured in the present study and that potentially affect both insulin secretion and insulin resistance may contribute to the relation of PAI-1 to incident diabetes. These factors include stimulation of PAI-1 expression by angiotensin II alone (33) or in combination with adrenal steroids (34). Other such factors include genetic factors and thinness at birth. Serum concentrations of PAI-1 are in part genetically determined, and several polymorphisms of the PAI-1 gene have been identified (35). Common genes may exist that confer the risk of developing both enhanced PAI-1 expression and type 2 diabetes. Small size at birth has been associated with the insulin resistance syndrome

(36) as well as cardiovascular disease (37) in adult life. Recently, it was shown that birth weight correlated inversely with PAI-1 levels in elderly men (38). Thus, one might speculate that PAI-1 predicts diabetes by virtue of being an integral part of the "small baby syndrome."

PAI-1 levels as measured in the present study reflect free and latent uncomplexed PAI-1, and not PAI-1 complexed with t-PA. PAI-1, as estimated with the assay we used, associates with insulin in a manner similar to total PAI-1 and most likely reflects PAI-1 as a weak acute-phase reactant. In addition, we have demonstrated that PAI-1, as assessed with this assay, seems to play a major role in regulating plasmin generation and in mediating the association between obesity and plasmin generation (39). Therefore, we believe that PAI-1 levels assessed this way are meaningful with respect to both PAI-1 regulators and PAI-1 effects.

Both PAI-1 levels (40) and, as shown more recently, CRP levels (1) predict the development of atherosclerotic disease. On the basis of the results of the present study, it is tempting to speculate that the common antecedent that has been postulated for both atherosclerosis and the insulin resistance syndrome/type 2 diabetes (2,3) may in fact be chronic inflammation and/or PAI-1 overexpression. If this proves to be the case, then PAI-1 would represent an ideal target for therapeutic interventions that aim to decrease the risk of both cardiovascular disease and type 2 diabetes. Previous studies have shown that measures that potentially reduce the risk of incident type 2 diabetes, such as reduction of body weight and increased physical activity (41), also have the potential to reduce PAI-1 levels (42,43). Also, treatment with metformin has been shown to decrease PAI-1 levels (44), and studies are under way to investigate the potential of this drug to prevent type 2 diabetes (45). It is interesting that treatment with an ACE inhibitor has been shown to decrease not only PAI-1 levels (46) and cardiovascular disease (47) but also in two recent randomized controlled trials the incidence of type 2 diabetes (47,48). However, because of the design of these studies (incident diabetes as a secondary end point), these results need to be interpreted with caution.

In conclusion, we have shown that chronic inflammation precedes the development of type 2 diabetes. High PAI-1 levels in subjects with NGT may help to identify a high-risk population with the potential to prevent both atherosclerotic disease and type 2 diabetes, two major causes of premature morbidity and mortality, by targeting its common antecedent.

ACKNOWLEDGMENTS

This work was supported by the National Heart, Lung and Blood Institute (Grants Hl47887, Hl47889, Hl47890, Hl47892, Hl47902, Hl55208, and R01 Hl58329) and the General Clinic Research Centers Program (Grants Ncrr Gcrc, M01 Rr431, and M01 Rr01346).

We thank Drs. Desire Collen and Paul DeClerck for reagents for the PAI-1 assay and Florence Keating, Julia Valiere, Sarah Nightingale, and Elizabeth Macy for technical assistance.

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