Original Article

Hemostatic Markers of Endothelial Dysfunction and Risk of Incident Type 2 Diabetes

The Framingham Offspring Study

James B. Meigs,[1] Christopher J. O'Donnell,[2] Geoffrey H. Tofler,[3] Emelia J. Benjamin,[4] Caroline S. Fox,[5] Izabela Lipinska,[4] David M. Nathan,[6] Lisa M. Sullivan,[7] Ralph B. D'Agostino,[7] and Peter W.F. Wilson[8]

Endothelial dysfunction may precede development of type 2 diabetes. We tested the hypothesis that elevated levels of hemostatic markers of endothelial dysfunction, plasminogen activator inhibitor-1 (PAI-1) antigen, and von Willebrand factor (vWF) antigen predicted incident diabetes independent of other diabetes risk factors. We followed 2,924 Framingham Offspring subjects (54% women, mean age 54 years) without diabetes at baseline (defined by treatment, fasting plasma glucose ≥7 or 2-h postchallenge glucose ≥11.1 mmol/l) over 7 years for new cases of diabetes (treatment or fasting plasma glucose ≥7.0 mmol/l). We used a series of regression models to estimate relative risks for diabetes per interquartile range (IQR) increase in PAI-1 (IQR 16.8 ng/ml) and vWF (IQR 66.8% of control) conditioned on baseline characteristics. Over follow-up, there were 153 new cases of diabetes. Age- and sex-adjusted relative risks of diabetes were 1.55 per IQR for PAI-1 (95% CI 1.41–1.70) and 1.49 for vWF (1.21–1.85). These effects remained after further adjustment for diabetes risk factors (including physical activity; HDL cholesterol, triglyceride, and blood pressure levels; smoking; parental history of diabetes; use of alcohol, nonsteroidal anti-inflammatory drugs, exogenous estrogen, or hypertension therapy; and impaired glucose tolerance), waist circumference, homeostasis model assessment of insulin resistance, and inflammation (assessed by levels of C-reactive protein): the adjusted relative risks were 1.18 per IQR for PAI-1 (1.01–1.37) and 1.39 for vWF (1.09–1.77). We conclude that in this community-based sample, plasma markers of endothelial dysfunction increased risk of incident diabetes independent of other diabetes risk factors including obesity, insulin resistance, and inflammation. ***Diabetes*** **55:530–537, 2006**

Type 2 diabetes is increasingly common worldwide. Development of diabetes is related to that of atherosclerotic cardiovascular disease (CVD) (1). The etiologic interrelationship of type 2 diabetes with CVD suggests that many cases arise from a common antecedent, thought to be a "metabolic syndrome" of insulin resistance (2). This metabolic syndrome is characterized by central obesity, impaired glycemia, low HDL cholesterol level, and increased levels of triglycerides, blood pressure, markers of subclinical inflammation, and insulin resistance (3). The syndrome increases risk for both type 2 diabetes and CVD (4), but the specific mechanisms unifying its diverse pathophysiological effects remain uncertain.

Endothelial dysfunction is a mechanism that potentially unifies the etiology of type 2 diabetes and CVD. Arteriolar endothelial dysfunction could contribute to insulin resistance and lead to diabetes, while conduit arterial endothelial dysfunction leads to clinical CVD (5,6). Arterial endothelial dysfunction is a consistent antecedent of CVD (7). Whether endothelial dysfunction is an antecedent of type 2 diabetes is less well established. In epidemiological analyses, endothelial dysfunction has been assessed by elevated plasma levels of plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor (vWF), or cellular adhesion molecules (8–14); altered forearm blood flow or vasodilatation in the forearm skin in response to infusion or iontophoresis of acetylcholine (9,11,15); impaired flow-mediated vasodilatation of the brachial artery (16,17); or the presence of retinal arteriolar narrowing (18). By all these diverse measures, endothelial dysfunction has been a consistent finding in cross-sectional studies of patients with type 2 diabetes (8–10), in relatives of patients with type 2 diabetes, and in people with insulin resistance or

From the [1]General Medicine Division, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; the [2]Cardiology Division, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; the [3]Cardiology Department, Royal North Shore Hospital, Sydney, New South Wales, Australia; the [4]Evans Department of Medicine, Preventive Medicine Section, Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston, Massachusetts; the [5]Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; the [6]Department of Medicine, Diabetes Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; the [7]Department of Mathematics, Statistics, and Consulting Unit, Boston University, Boston, Massachusetts; and the [8]Department of Endocrinology, Diabetes, and Medical Genetics, Medical University of South Carolina, Charleston, South Carolina.

Address correspondence and reprint requests to James B. Meigs, MD, MPH, General Medicine Division, Massachusetts General Hospital, 50 Staniford St., 9th Floor, Boston, MA 02114. E-mail: jmeigs@partners.org.

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CRP, C-reactive protein; CVD, cardiovascular disease; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IQR, interquartile range; NGT, normal glucose tolerance; PAI-1, plasminogen activator inhibitor-1; vWF, von Willebrand factor.

pre-diabetes (11,12,15,16). Several recent studies (13,14, 17,18) have also shown associations of endothelial dysfunction with incident type 2 diabetes. However, these studies were not able to fully account for all important confounding factors, in particular insulin resistance and inflammation, leaving unresolved the independent role of endothelial dysfunction in the pathogenesis of type 2 diabetes.

Type 2 diabetes burdens society with poor health and high health care costs and is potentially preventable. Establishment of endothelial dysfunction as a fundamental precursor to type 2 diabetes may reveal new avenues for diabetes prevention and treatment (19). In this study, we tested the hypothesis that elevated levels of hemostatic markers of endothelial dysfunction, PAI-1 and vWF, predict incident type 2 diabetes in a large, population-based sample, independent of known risk factors for diabetes and CVD.

RESEARCH DESIGN AND METHODS

Participants in the Framingham Offspring Study, a 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 (20). Offspring subjects are primarily whites. Of 5,124 original offspring participants, 4,019 (78.4%) attended the fourth 4-year study cycle, and of these, 3,799 (94.5%) attended the fifth study cycle. During the fifth examination (baseline 1991–1995), the 3,799 participants fasted overnight and had a standardized medical examination, including a 2-h oral glucose tolerance test. A total of 2,924 subjects provided data for the present analysis, after exclusion of 429 with prevalent diabetes and 446 with missing exposure information. No subjects remaining in the analytic sample reported taking anticoagulants. Comparing the subjects included in the analysis with those excluded, included subjects were younger (aged 54 vs. 57 years, $P < 0.0001$), were less obese (BMI 27.1 vs. 28.6 kg/m2, $P < 0.0001$), and included more women (54 vs. 47%, $P = 0.0004$), and fewer had impaired fasting glucose (IFG)/impaired glucose tolerance (IGT) (32 vs. 39%, $P = 0.001$). Subjects were followed from baseline over a mean of 7 years for the incident development of diabetes. The institutional review board of Boston University approved the study protocol, and all subjects gave informed consent at each examination.

Clinical definitions. We defined diabetes at the baseline exam as a fasting plasma glucose level ≥7.0 mmol/l, a 2-h oral glucose tolerance test level of ≥11.1 mmol/l, or use of hypoglycemic drug therapy. We defined diabetes at follow-up as a fasting plasma glucose level ≥7.0 mmol/l or use of hypoglycemic drug therapy. Over 98% of diabetic case subjects in the Framingham population have type 2 diabetes (21). Baseline characteristics included height, weight, and waist circumference, measured with the subject standing. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Blood pressure was measured as the mean of two measurements with a mercury sphygmomanometer after the subject had been seated for at least 5 min; hypertension was defined as a blood pressure >130/85 mmHg or antihypertensive medication use. We defined low HDL cholesterol levels as <40 mg/dl in men and 50 mg/dl in women. Participants who reported smoking at least one cigarette per day during the year before the examination 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 in ounces per week. Participants were defined as using aspirin or nonsteroidal anti-inflammatory drugs if they reported any use during the week preceding evaluation. Estrogen replacement therapy was defined as present or absent among postmenopausal women. IFG/IGT was defined as a fasting glucose of 5.6–10.9 mmol/l and/or a 2-h oral glucose tolerance test glucose level of 7.8–10.9 mmol/l. A positive parental history of diabetes was based on self-report of diabetes in one or both parents (22). We defined CVD as coronary heart disease, stroke, or intermittent claudication as described previously (23). We measured insulin resistance using a surrogate measure, the homeostasis model assessment (HOMA-IR) defined as [(fasting glucose × fasting insulin)/22.5] (24). Laboratory methods for glucose, insulin, lipids, PAI-1, vWF, and C-reactive protein (CRP) assays have been previously published (10,25). Coefficients of variation were <3% for glucose, <10% for insulin, 9.9% for PAI-1 antigen, and 8.8% for vWF antigen, and the correlation coefficient for 36 replicate CRP measurements was 0.86.

Statistical analysis. Baseline characteristics were compared using χ^2 tests or ANOVA. Subjects were followed from baseline to the sixth (1995–1998) and seventh (1998–2001) offspring exams. We used the exam visit date that a new case of diabetes was identified as the date of diagnosis. We calculated the diabetes incidence rate by dividing the number of diabetes cases by the number of person-years of follow-up from baseline to diagnosis or censoring at exam 6 or 7. We used hazard ratios from proportional hazards regression models to estimate relative risks and 95% CIs for incident diabetes conditioned on baseline clinical covariates. We constructed a series of nested models predicting risk of diabetes, using separate models for each biomarker. Continuous covariates including triglycerides, HDL cholesterol, obesity and insulin resistance measures, and levels of PAI-1, vWF, and CRP were modeled per interquartile range (IQR) increase to allow comparisons of their relative effect sizes. Models were sequentially adjusted for age and sex, potential diabetes risk factors, measures of obesity and insulin resistance, and levels of CRP. First-order interaction terms for sex–by–PAI-1 or vWF levels on risk of diabetes were not significant (P value >0.2), so we present sex-combined analyses. Area under the receiver-operator characteristic curve values were estimated using c-statistics generated from proportional hazards regression models. We performed all analyses using SAS (SAS Institute, Cary, NC) and considered a two-sided value of $P < 0.05$ to be statistically significant.

TABLE 1
Baseline characteristics of 2,924 nondiabetic men and women

	Men	Women
n	1,333	1,591
Age (years)	54 ± 9.8	54 ± 9.8
Age range (years)	30 ± 83	26 ± 82
Physical activity index	36.0 ± 7.0	33.6 ± 4.9
Current ERT	—	9.7
Current smoker	18.5	19.3
Current alcohol use	76.7	66.0
Current aspirin/NSAID use	64.0	56.2
Blood pressure >130/85 or treatment	51.0	38.6
HDL cholesterol <50 mg/dl (women) or <40 (men)	39.5	34.5
Parental history of diabetes	15.0	18.8
IFG/IGT	39.5	26.8
Triglycerides (mg/dl)	125 (98)	109 (76)
Waist circumference (cm)	97.8 (13.3)	83.8 (17.8)
BMI (kg/m^2)	27.5 (4.9)	25.3 (6.2)
HOMA-IR	6.7 (3.1)	5.8 (2.4)
PAI-1 antigen (ng/ml)	20.0 (16.8)	15.5 (14.8)
vWF antigen (% control)	120.8 (66.8)	117.8 (63.4)
CRP (mg/l)	1.36 (3.72)	1.56 (4.70)
Number of diabetes events	86	67
Developed diabetes over follow-up	6.45	4.21
Person-years of follow-up	8,901	10,763
Crude incidence rate (per 1,000 per year)	9.66	6.23

Data are means ± SD, median (IQR), or percent, unless otherwise indicated. ERT, estrogen replacement therapy; NSAID, nonsteroidal anti-inflammatory drug.

RESULTS

Baseline characteristics, PAI-1 and vWF levels, and risk of type 2 diabetes over follow-up. The baseline characteristics of study subjects are shown in Table 1. Twenty-seven percent of women and 40% of men had IFG/IGT at baseline. Mean PAI-1 levels were higher ($P < 0.0001$) in men compared with women, and vWF levels were similar ($P = 0.2$). During 19,664 person-years of follow-up, 153 new cases of diabetes occurred for a cumulative incidence rate of 5.23% and an average incidence rate of 7.78 cases per 1,000 person-years. Plasma levels of PAI-1, vWF, and CRP were all correlated (Spearman correlation coefficients: PAI-1 vs. CRP, 0.33; vWF vs. CRP, 0.16; and PAI-1 vs. vWF, 0.11; all $P < 0.0001$). Levels of PAI-1 and CRP were higher comparing smokers with

TABLE 2
Age- and sex-adjusted levels of PAI-1 antigen, vWF antigen, and CRP by levels of major diabetes risk factors

	PAL-1 (ng/ml)	vWF (% control)	CRP (mg/l)
Parental history of diabetes	21.4 ± 6.8	127.7 ± 2.0	3.91 ± 0.39
No parental history of diabetes	21.2 ± 3.1	124.9 ± 0.9	3.95 ± 0.18
P value for trend	0.8	0.20	0.9
NGT	18.8 ± 0.3	122.4 ± 1.0	3.38 ± 0.20
IFG/IGT	26.3 ± 0.5	131.4 ± 1.5	5.10 ± 0.28
P value	<0.0001	<0.0001	<0.0001
Triglycerides			
Quartile 1	14.5 ± 0.5	119.9 ± 1.7	2.44 ± 0.33
Quartile 2	17.8 ± 0.5	126.2 ± 1.7	3.66 ± 0.32
Quartile 3	23.4 ± 0.5	127.8 ± 1.7	4.48 ± 0.32
Quartile 4	29.3 ± 0.5	127.5 ± 1.7	5.16 ± 0.33
P value for trend	<0.0001	0.0025	<0.0001
Waist circumference			
Quartile 1	13.8 ± 0.5	122.6 ± 1.6	2.48 ± 0.32
Quartile 2	18.1 ± 0.5	120.8 ± 1.7	3.51 ± 0.33
Quartile 3	22.7 ± 0.5	126.4 ± 1.7	3.92 ± 0.33
Quartile 4	30.7 ± 0.5	131.6 ± 1.7	5.85 ± 0.32
P value for trend	<0.0001	<0.0001	<0.0001
BMI			
Quartile 1	13.4 ± 0.5	121.1 ± 1.7	2.16 ± 0.32
Quartile 2	18.1 ± 0.5	123.7 ± 1.7	3.33 ± 0.32
Quartile 3	22.7 ± 0.5	125.6 ± 1.7	3.84 ± 0.32
Quartile 4	30.7 ± 0.5	131.2 ± 1.7	6.36 ± 0.32
P value for trend	<0.0001	<0.0001	<0.0002
HOMA-IR			
Quartile 1	14.0 ± 0.5	117.3 ± 1.7	2.47 ± 0.34
Quartile 2	17.2 ± 0.5	121.2 ± 1.7	3.73 ± 0.33
Quartile 3	22.2 ± 0.5	125.2 ± 1.7	4.12 ± 0.33
Quartile 4	31.6 ± 0.5	135.4 ± 1.7	5.42 ± 0.33
P value for trend	<0.0001	<0.0001	<0.0001

Data are least squares means ± SE.

nonsmokers: median (interquartile range) levels for PAI-1 were 19.3 (17.3) vs. 17.1 (15.6), respectively, $P = 0.003$, and for CRP were 2.3 (5.6) vs. 1.3 (3.8), respectively, $P < 0.0001$; vWF levels were similar: 119 (66) vs. 119 (65), $P = 0.9$. Biomarker levels were correlated with other major type 2 diabetes risk factors (Table 2) and with the incidence of type 2 diabetes (Fig. 1). Figure 1 shows a positive, graded relationship for the incidence of diabetes across increasing quartiles of PAI-1, vWF, and CRP. Figure 1 also shows these associations stratified by normal glucose tolerance (NGT) or IFG/IGT at baseline; the gradient of diabetes incidence across increasing quartiles of PAI-1, vWF, and CRP appeared to be steeper for subjects with IFG/IGT than for those with NGT, although the significance of glucose tolerance status-by-biomarker level first-order interactions were not significant (*P* values all >0.1), consistent with a statistically similar gradient of diabetes incidence for both NGT and IFG/IGT subgroups. In age- and sex-adjusted regression models, major diabetes risk factors and increasing levels of PAI-1, vWF, and CRP were all significantly associated with increased risk for diabetes (Table 3). The area under the receiver-operator characteristic curve for age- and sex-adjusted levels of PAI-1 were 0.72 and for levels of vWF and CRP were 0.63.

Effect of adjustment for major diabetes risk factors including obesity, insulin resistance, and inflammation. Results of the regression modeling strategy are displayed in Table 4. Higher levels of PAI-1 and vWF significantly increased risk for diabetes after multivariable adjustment for age, sex, physical activity, HDL cholesterol and triglyceride level, smoking, parental history of diabetes, blood pressure level, IFG/IGT, and use of exogenous estrogen, alcohol, aspirin or nonsteroidal anti-inflammatory drugs, and blood pressure therapy (Table 4, *model 1*). After adjustment for these risk factors and measures of obesity, HOMA-IR, and inflammation, alone or together, PAI-1 levels increased the relative risk of incident diabetes by 18% per IQR increase ($P = 0.03$; Table 4, *model 5*) and vWF levels by 39% per IQR increase ($P = 0.009$). In these models, adjusted CRP levels were not associated with risk of diabetes (Table 4, *model* 5: relative risk 1.01 mg/l per IQR [4.2] increase, $P \geq 0.7$), while waist circumference ($P = 0.001$) and HOMA-IR ($P = 0.0004$) were also independent risk factors for incident diabetes. Area under the receiver-operator characteristic curve values in models 1–4 ranged from 0.85 to 0.86 for PAI-1 and from 0.84 to 0.86 for vWF. Results were similar when BMI instead of waist circumference was modeled as the measure of obesity or when a term for change in BMI or waist circumference over follow-up was included in regression models. Finally, in a model with simultaneous adjustment for major diabetes risk factors and levels of PAI-1, vWF, and CRP, vWF remained a strong, independent risk factor for new cases of diabetes (relative risk 1.37 [95% CI 1.07–1.75], $P = 0.01$), levels of PAI-1 remained significant (1.17 [1.003–1.36], $P = 0.046$), and levels of CRP were not associated with risk of diabetes ($P = 0.8$).

We assessed effect modification by inflammation, obesity, insulin resistance, parental history of diabetes, and glucose tolerance on risk of diabetes by testing the significance of their first-order interactions with PAI-1 or vWF levels, none of which were significant (*P* values all >0.3).

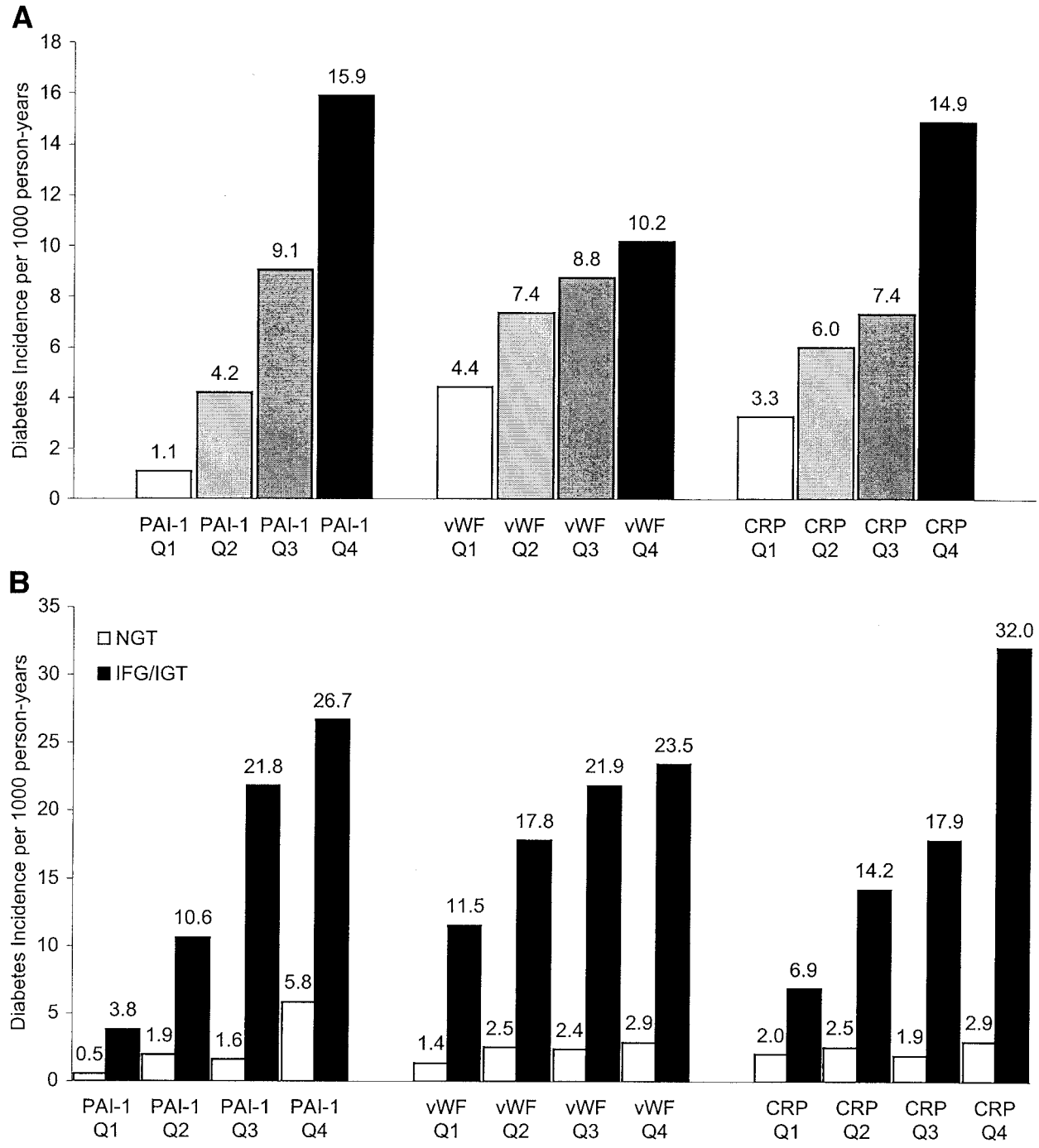

FIG. 1. Age- and sex-adjusted incidence rates for type 2 diabetes according to quartile of plasma levels of PAI-1, vWF, or CRP. *A*: Overall sample. *B*: Sample stratified by NGT (n = 1,970) or IFG/IGT (n = 954).

In particular, multivariate-adjusted (Table 4) relative risks of diabetes associated with elevated levels of PAI-1 or vWF were similar among subjects with NGT or IFG/IGT at baseline: for PAI-1, the relative risk (95% CI) for incident diabetes in NGT was 1.63 (1.27–2.09) per IQR increase and in IFG/IGT was 1.27 (1.09–1.49, P value for interaction = 0.1); for vWF in NGT, the relative risk was 1.33 (0.80–2.20) and in IFG/IGT was 1.40 (1.07–1.82, P value for interaction >0.99). Last, in a subsidiary analysis we excluded 209 cases of prevalent CVD at baseline. In the remaining sample, multivariable-adjusted (Table 4, *model 5*) relative risks for incident diabetes were 1.17 (0.99–1.38) per IQR increase for PAI-1 and 1.33 (1.03–1.72) per IQR increase for vWF.

DISCUSSION

In this community-based population sample, PAI-1 and vWF levels had positive, graded relationships with the 7-year incidence of type 2 diabetes. This association was independent of effects of other risk factors, including obesity, HOMA-IR, and IFG/IGT (three well-established diabetes risk factors); levels of triglycerides (a known mediator of PAI-1 levels [26]); and inflammation (a novel

TABLE 3
Age- and sex-adjusted relative risks for incident type 2 diabetes among 2,924 initially nondiabetic men and women

	Relative risk (95% CI)	*P* value
Parental history of diabetes	1.90 (1.32–2.75)	0.0006
IFG/IGT	8.67 (5.75–13.1)	<0.0001
Triglycerides (per 84-mg/dl increase)	1.21 (1.13–1.29)	<0.0001
Waist circumference (per 19.7-cm increase)	3.16 (2.56–3.90)	<0.0001
BMI (per 5.8-kg/m^2 increase)	2.17 (1.88–2.51)	<0.0001
HOMA-IR (per 2.81-unit increase)	1.74 (1.60–1.89)	<0.0001
PAI-1 antigen (per 15.8-ng/ml increase)	1.55 (1.41–1.70)	<0.0001
vWF antigen (per 65% control increase)	1.49 (1.21–1.85)	0.0002
CRP (per 4.2-mg/l increase)	1.06 (1.02–1.09)	0.001

Data are relative risks (95% CI) for incident diabetes adjusted for age and sex. All covariates were modeled as relative risk for diabetes per IQR increase, except for parental history of diabetes and IFG/IGT, where risk was modeled relative to those without the condition.

diabetes risk factor [13,27]). After adjustment for all these potentially confounding factors, vWF increased the relative risk for new cases of diabetes by 39% per IQR increase, and PAI-1 increased the relative risk by 18%. Effects of PAI-1 and vWF were not modified by variation in levels of other major diabetes risk factors, including glucose tolerance status. Although many prior studies suggest an association of endothelial dysfunction with risk of type 2 diabetes, the data are limited by cross-sectional study designs or limited ability to fully control for confounding factors (8–18). The present data build on prior data to demonstrate that elevated levels of these biomarkers of endothelial dysfunction are significant, independent precursors of type 2 diabetes in the community

Endothelial dysfunction reflects an imbalance in the regulatory function of vascular endothelial cells and is characterized by impaired endothelium-dependent nitric oxide–mediated vasodilatation (28), elevated plasma levels of cellular adhesion molecules (11,29), microalbuminuria (30), and impaired fibrinolysis, marked by elevated plasma levels of PAI-1 and vWF (31). The primary physiologic function of PAI-1 and vWF is to maintain hemostatic balance in the vasculature (32,33), but because the endothelium is a primary source of PAI-1 and vWF, elevated levels also reflect stimulation or injury of endothelial cells (32,34). Elevated plasma levels of biomarkers of endothelial dysfunction are modestly correlated with impaired endothelium-dependent vasodilatation in forearm skin or the brachial artery (11,29) and are highly correlated with insulin resistance (12,35,36), providing one mechanism by which endothelial dysfunction might confer risk for type 2 diabetes. In the arteriolar microcirculation, impaired endothelium-dependent vasomotion may limit insulin-mediated capillary recruitment and redistribution of skeletal

TABLE 4
Multivariable relative risks for incident type 2 diabetes associated with increasing PAI-1 antigen or vWF antigen, after adjustment for diabetes risk factors obesity, insulin resistance, and inflammation

Variable	Model 1	Model 2	Model 3	Model 4	Model 5
PAI-1 (per 15.8-ng/ml increase)					
Relative risk (95% CI)	1.35 (1.19–1.54)	1.24 (1.08–1.43)	1.24 (1.07–1.43)	1.35 (1.19–1.53)	1.18 (1.01–1.37)
P value	<.0001	0.003	0.004	<.0001	0.03
Waist circumference (per 19.7-cm increase)					
Relative risk (95% CI)	—	2.05 (1.57–2.67)	—	—	1.65 (1.23–2.21)
P value	—	<0.000	—	—	0.009
HOMA-IR (per 2.81-unit increase)					
Relative risk (95% CI)	—	—	1.36 (1.21–1.53)	—	1.28 (1.13–1.45)
P value	—	—	<0.001	—	0.002
CRP (per 4.2-mg/l increase)					
Relative risk (95% CI)	—	—	—	1.02 (0.98–1.07)	1.01 (0.95–1.08)
P value	—	—	—	0.3	0.7
vWF (per 65% control increase)					
Relative risk (95% CI)	1.40 (1.12–1.77)	1.37 (1.08–1.73)	1.41 (1.11–1.79)	1.41 (1.11–1.78)	1.39 (1.09–1.77)
P value	0.004	0.009	0.005	0.004	0.009
Waist circumference (per 19.7-cm increase)					
Relative risk (95% CI)	—	2.21 (1.71–2.85)	—	—	1.71 (1.28–2.29)
P value	—	<.0001	—	—	0.0003
HOMA-IR (per 2.81-unit increase)					
Relative risk (95% CI)	—	—	1.42 (1.27–1.58)	—	1.30 (1.15–1.48)
P value	—	—	<.0001	—	<.0001
CRP (per 4.2-mg/l increase)					
Relative risk (95% CI)	—	—	—	1.02 (0.98–1.07)	1.01 (0.95–1.07)
P value	—	—	—	0.3	0.8

The *top* and *bottom* halves of the table show relative risks and 95% CIs for incident diabetes per IQR increase in PAI-1 antigen or vWF antigen, respectively. All models have been adjusted for sex, physical activity, HDL cholesterol and triglyceride levels, smoking, parental history of diabetes, blood pressure level, IFG/IGT, use of exogenous estrogen, alcohol, aspirin or NSAIDs, and blood pressure therapy. Models were sequentially adjusted for these risk factors and waist circumference, HOMA-IR, and levels of CRP as shown.

muscle blood flow from nonnutritive to nutritive flow routes, diminishing insulin delivery to insulin-sensitive muscle tissue (6,37–39). Altered endothelial permeability also may impair insulin delivery to the interstitium, where insulin levels appear to be a rate-limiting step determining insulin effectiveness (40). Endothelial dysfunction is also associated with many features of the pre-diabetic "metabolic syndrome," including obesity and elevated triglycerides, blood pressure, and levels of CRP (5,35,41–43). We found that risk for diabetes associated with elevated levels of vWF and PAI-1 was attenuated, but not eliminated, by adjustment for metabolic syndrome–related factors. To the extent that elevated levels of vWF and PAI-1 reflect endothelial dysfunction, our analysis provides strong evidence that it is an independent precursor to type 2 diabetes. However, obesity and insulin resistance were also independent risk factors, suggesting that these and endothelial dysfunction may not increase risk via completely overlapping pathways.

In this analysis we considered elevated plasma levels of both PAI-1 and vWF to reflect underlying endothelial dysfunction. However, the weak correlations ($r = 0.11$) that we observed between levels of these biomarkers support the view that they are under substantially different regulatory control. PAI-1 is secreted by hepatocytes, adipose tissue, and vascular smooth muscle cells as well as endothelium (33) and may predict diabetes in part because elevated levels also reflect visceral obesity and insulin resistance. This would explain why the association of PAI-1 with incident diabetes was so strongly attenuated after adjustment for waist circumference and HOMA-IR. vWF, on the other hand, is thought to be more specific for endothelial dysfunction, as it secreted almost exclusively by endothelial cells activated by proinflammatory cytokines (32). Diabetes risk associated with vWF levels was minimally attenuated by adjustment for BMI or waist circumference, HOMA-IR, or CRP, supporting our contention that the relationship of endothelial dysfunction with diabetes is unlikely to be completely explained by visceral fat, insulin resistance, or low-grade inflammation. However, our analysis does not exclude the intriguing possibility that perivascular fat, which is associated with central fat but does not necessarily produce a low-grade acute-phase response, may play a role in influencing endothelial dysfunction and insulin action through "vasocrine" signaling (44).

Several other lines of evidence support the hypothesis that endothelial dysfunction is a precursor of type 2 diabetes. In the Insulin Resistance Atherosclerosis Study (13), levels of log(PAI-1) increased the risk of incident type 2 diabetes by 61% per SD difference, even after adjustment for major diabetes risk factors and directly measured insulin resistance. This analysis did not further adjust for effects of inflammation. In the Nurses' Health Study (14), adjusted relative risks for incident diabetes in the top versus the bottom quintile were 5.4 for the cellular adhesion molecule E-selectin and 3.6 for intracellular adhesion molecule-1. However, while this analysis controlled for inflammation, it had limited ability to control for baseline glucose intolerance or levels of insulin resistance, dyslipidemia, or blood pressure. In a recent analysis of postmenopausal women (17), each one-unit decrease in flow-mediated vasodilation of the brachial artery was associated with a significant 32% increase in the 4-year relative risk for incident diabetes after adjustment for most major diabetes risk factors except insulin resistance or inflammation. However, two other studies (45,46) did not find an association of vWF levels with risk of diabetes after accounting for diabetes risk factors. Treatment with drugs having beneficial effects on endothelial function (including thiazolidinediones, metformin, renin-angiotensin system–acting agents, and HMG CoA reductase inhibitors) improve insulin sensitivity and can reduce risk of diabetes (47–53). Finally, mice with endothelial dysfunction by virtue of targeted knockout mutations in the endothelium-dependent nitric oxide synthase (eNOS) gene are insulin resistant and display features of the metabolic syndrome (54,55), and in humans, variation in the eNOS gene is associated with increased risk of type 2 diabetes (56).

Strengths of our analysis include the examination of a large community-based sample of nondiabetic men and women across a broad age spectrum, standardized assessment of diabetes outcomes, comprehensive measurement of a wide array of factors known to influence both diabetes risk and endothelial dysfunction, and biomarkers measured with reasonable and comparable assay variability. However, the analysis has several limitations. We did not perform oral glucose tolerance tests to diagnose incident diabetes, leaving some people with postchallenge diabetes in the nondiabetic sample. This misclassification would lead us to underestimate the risk of diabetes associated with PAI-1 and vWF levels. We used a surrogate index of insulin resistance and may have incompletely controlled for its effects. We did not account for the potential effects of subclinical atherosclerosis at baseline, but removal of clinical CVD did not affect the main conclusions. We did not account for levels of free fatty acids or adiponectin, which are diabetes risk factors and associated with endothelial function (57,58). Finally, subjects included in this analysis were at slightly lower risk of diabetes compared with those excluded, and because Framingham is in general a lower diabetes–risk population than populations including greater numbers of minority subjects, whether the same effects operate in other studies including a more diverse mix of race/ethnic groups requires further study.

In conclusion, elevated plasma levels of hemostatic markers of endothelial dysfunction preceded new cases of type 2 diabetes, independent of other major diabetes risk factors. Our findings have several important implications. Arterial endothelium may join fat, muscle, liver, and pancreas as a tissue fundamentally involved in the pathogenesis of type 2 diabetes. Other studies have shown endothelial dysfunction to be key to the pathogenesis of CVD, supporting the hypothesis of common antecedents for type 2 diabetes and CVD. Interventions that improve endothelial function could have beneficial effects on diabetes risk and help to slow the accelerating worldwide epidemic of type 2 diabetes and CVD.

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