Impaired Fibrinolytic Compensation for Hypercoagulability in Obese Patients With Type 2 Diabetes: Association With Increased Plasminogen Activator Inhibitor-1

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In patients with type 2 diabetes, fibrinolysis is considered impaired by increased plasma concentrations of plasminogen activator inhibitor (PAI)-1. However, several investigators found both coagulation and fibrinolysis to be activated in these patients. We further characterized the balance between coagulation and fibrinolysis in lean and obese patients with type 2 diabetes. We studied 112 type 2 diabetic patients (66 lean, 46 obese) and 69 age-matched healthy subjects (46 lean, 23 obese). We measured plasma concentrations of fibrinogen and prothrombin F1+2 (F1+2) as indicating coagulation activity and plasmin-antiplasmin complex (PAP) and D dimer as indicating fibrinolytic activity. Plasma PAI-1 concentrations also were determined. Plasma concentrations of F1+2, PAP, D dimer, and PAI-1 were higher in diabetic patients than in control subjects. Plasma fibrinogen and F1+2 were similar between lean and obese diabetic patients, but plasma PAP and D dimer were significantly lower in obese than lean diabetic patients ($P < .0001$, $P = .0194$, respectively). By multivariate analysis, plasma PAI-1 and body mass index (BMI) were independent factors in diabetic patients predicting PAP, while BMI and glycosylated hemoglobin (HbA_{1c}) independently predicted D dimer. Plasma PAI-1 concentrations were significantly higher in obese than lean diabetic patients ($P < .0001$). In conclusions, both coagulation and fibrinolytic systems are enhanced in lean and obese type 2 diabetic patients compared with healthy subjects. Although the degree of activation of coagulation was similar between lean and obese diabetic patients, the fibrinolytic activity was lower in obese than lean patients. Fibrinolytic compensation for hypercoagulation is incomplete in obese patients with type 2 diabetes, partly because of elevated PAI-1 in the blood.
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TYPE 2 DIABETIC patients have a high incidence of atherosclerosis and related thrombosis, leading to increased morbidity and mortality from coronary artery disease, cerebrovascular disease, and peripheral vascular disease.[1-3] The superimposition of thrombosis on atherosclerosis is associated with hypercoagulation, hypofibrinolysis, and/or platelet alternations,[4,5] which is caused by several factors, including endothelial dysfunction, disturbed lipid metabolism, monocyte invasion, and smooth muscle cell proliferation.[6] Type 2 diabetic patients have a well-established tendency toward hypercoagulability,[7-9] but fibrinolysis has been conflictingly reported to be either attenuated or enhanced.[4,10-13] The fibrinolytic system involves conversion of plasminogen to plasmin by tissue plasminogen activator (t-PA). Blood concentrations of plasminogen activator inhibitor-1 (PAI-1), which negatively regulate fibrinolysis in the blood by inhibiting t-PA,[14] are elevated in type 2 diabetic patients.[15,16] Thus, type 2 diabetic patients are believed to be hypofibrinolytic due to an increase in PAI-1.[17] However, our investigational group, as well as others, has reported that both coagulation and fibrinolysis are enhanced concomitantly in type 2 diabetic patients.[9,12]

Recent studies have suggested that adipocytes may be important sources of elevated blood concentrations of PAI-1 in type 2 diabetes and obesity.[18,19] We hypothesize that a fibrinolysis defect is seen in obese diabetics, but not in lean diabetics. Therefore, the present study was undertaken to both further characterize the balance between coagulation and fibrinolysis in patients with type 2 diabetes and to investigate the influence of body weight on coagulation and fibrinolytic systems in diabetic patients. Here we demonstrate that fibrinolytic compensation for hypercoagulability is incomplete only in obese patients with type 2 diabetes.

MATERIALS AND METHODS

We studied 112 type 2 diabetic patients and 69 healthy control subjects. The diagnosis of type 2 diabetes was made according to the criteria of the World Health Organization. All patients who fulfilled the following inclusion criteria were considered for the study: (1) no episodes of ketoacidosis, (2) diagnosis of diabetes after the age of 30 years, and 3) if any, insulin therapy started after at least 5 years of known disease. Based on body mass index (BMI), the diabetic patients were divided into 2 groups: lean diabetic patients with a BMI less than 25.0 kg/m^2 (n = 66) and obese diabetic patients with a BMI greater than 25 kg/m^2 (n = 46).[20] BMI in lean diabetic patients was 21.2 ± 2.4 kg/m^2 and in obese patients 27.6 ± 2.1 kg/m^2. Among the control subjects, 23 subjects had a BMI greater than 25.0. None of the subjects received any medication affecting coagulation or fibrinolysis. Excluded from the study were patients with any liver disease and subjects with either a creatinine clearance less than 50 mL/min (an index of glomerular filtration rate [GFR]) or with a serum creatinine concentration exceeding 1.3 mg/dL. Patients with type 2 diabetes were treated with diet alone (n = 15), sulfonylureas (n = 62), or insulin (n = 35). None of the patients were taking biguanides or thiazolinediones that could have reduced or altered plasma PAI-1 levels.[21,22] Thirty-nine of the diabetic patients had hypertension, defined as systolic blood pressure exceeding 140 and/or diastolic blood pressure exceeding 90 mm Hg, or alternatively as use of antihypertensive agents, and were treated with an angiotensin-converting enzyme (ACE) inhibitor (n = 12: lean, n = 6; obese, n = 6) and /or a calcium blocker (n = 32).

Venous blood was obtained between 6:00 and 7:00 AM after an overnight fast and collected in a tube containing 3.8% sodium citrate. Plasma concentrations of fibrinogen, a surrogate marker of thrombin activation,[23] were measured by the von Claus method. Plasma concen-

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Table 1. Characteristics of Control Subjects and Type 2 Diabetic Patients

Variables	Control Subjects	Diabetic Patients
No. (M/F)	69 (36/33)	112 (57/55)
Age (yr)	54.5 ± 12.1	57.9 ± 10.9
BMI (kg/m^2)	24.3 ± 2.9	23.7 ± 3.9
Diabetes duration (yr)	—	9.2 ± 7.2
FPG (mmol/L)	5.0 ± 0.5	9.5 ± 0.4*
HbA_{1c} (%)	—	9.8 ± 2.3
F1+2 (nmol/L)	0.82 ± 0.18	1.07 ± 0.47*
PAP (μg/mL)	0.40 ± 0.14	0.93 ± 0.50*
D dimer (μg/mL)	0.27 ± 0.14	1.00 ± 1.47*
PAI-1 (ng/mL)	9.0 ± 7.3	12.3 ± 11.7†
Treatment (D/OHA/insulin)	—	15/62/35

NOTE. Data are mean ± SD.

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; PAP, plasmin-α2-antiplasmin complex; PAI, plasminogen activator inhibitor; D, diet alone; OHA, oral hypoglycemic agents.

*$P < .001$, †$P < .05$ *v* control.

trations of prothrombin fragments 1+2 (F1+2), a direct marker of thrombin generation, and plasmin-α2-antiplasmin (PAP), a measure of fibrinolytic activity, were determined by sandwich enzyme immunoassay (EIA) (Enzygnost F1+2 micro and Enzygnost PAP micro, obtained from Dade Behring, Marburg, Germany). Plasma concentrations of D dimer, a degraded product of fibrin cleaved by plasmin, were measured by a commercial kit (LPIA-5500; Iatron Laboratories, Tokyo, Japan). Intra- and interassay coefficient of variation (CV) were 1.16% and 1.99%, respectively. Plasma PAI-1 concentration was measured with an enzyme-linked immunosorbent assay (ELISA) (Biopool IMULYSE PAI-1, Umea, Sweden). This method detects active and latent and PAI-1, as well as PAI-1 bound to t-PA. Intra- and interassay CV of the PAI-1 method were 2.26% to 3.77% and 3.57% to 4.76%, respectively. Plasma insulin concentration was determined by a radioimmunoassay.

Statistical Analysis

Data are presented as mean ± SD unless otherwise indicated. Differences between groups were analyzed by unpaired *t* tests or Mann-Whitney test. If data were distributed not to be normal, nonparametric tests were used. Correlation was determined by Pearson's correlation analysis or Spearman Rank test. Multivariate analysis was performed to clarify the relationships between markers of fibrinolysis and the clinical variables. A *P* value less than .05 was accepted as statistically significant. Statistical analyses were performed using SPSS programs (SPSS, Chicago, IL).

RESULTS

Clinical characteristics of subjects are summarized in Table 1. There were no significant differences in age or BMI between the control subjects and diabetic patients. Plasma F1+2 concentration was higher in diabetic patients than in control subjects ($P < .001$; Table 1). Plasma PAP and D dimer concentrations also were higher in diabetic patients than in control subjects ($P < .001$; $P < .001$, respectively; Table 1). PAI-1 concentration was significantly higher in diabetic patients than in control subjects (9.0 ± 7.3 *v* 12.3 ± 11.7 ng/mL, $P < .05$).

We found no differences in fasting plasma glucose or glycosylated hemoglobin (HbA_{1c}) between lean and obese type 2 diabetic patients (9.8 ± 4.4 *v* 8.6 ± 2.6 mmol/L, $P = .0877$; 10.2% ± 2.5% *v* 9.4% ± 2.0%, $P = .0681$, respectively). As for blood lipid concentrations, triglyceride (TG) was higher in obese than lean diabetic patients (193 ± 94 *v* 166 ± 160 mg/dL, $P = .0094$), while no differences in total cholesterol or high-density lipoprotein (HDL)-cholesterol were found between the 2 diabetic groups (data not shown). Plasma concentrations of fibrinogen were comparable between lean and obese diabetic patients (364 ± 88 *v* 361 ± 69 mg/dL, $P = .8564$). F1+2 concentrations also did not significantly differ between the 2 diabetic groups (1.08 ± 0.55 *v* 1.06 ± 0.33 nmol/L, $P = .8503$). However, plasma PAP concentrations were significantly lower in obese than in the lean diabetic subjects (0.70 ± 0.30 *v* 1.08 ± 0.54 μg/mL, $P < .0001$; Fig 1A). In addition, plasma D dimer was lower in obese than lean diabetic patients (0.59 ± 0.45 *v* 1.28 ± 1.83 μg/mL, $P = .0194$; Fig 1B). In lean diabetic patients, plasma PAP concentration correlated positively with plasma fibrinogen ($r = .46$, $P < .0001$; (Fig 2A). In obese patients, however, we found no significant correlation between plasma PAP and fibrinogen concentration ($r = .23$, $P = .1506$; Fig 2B).

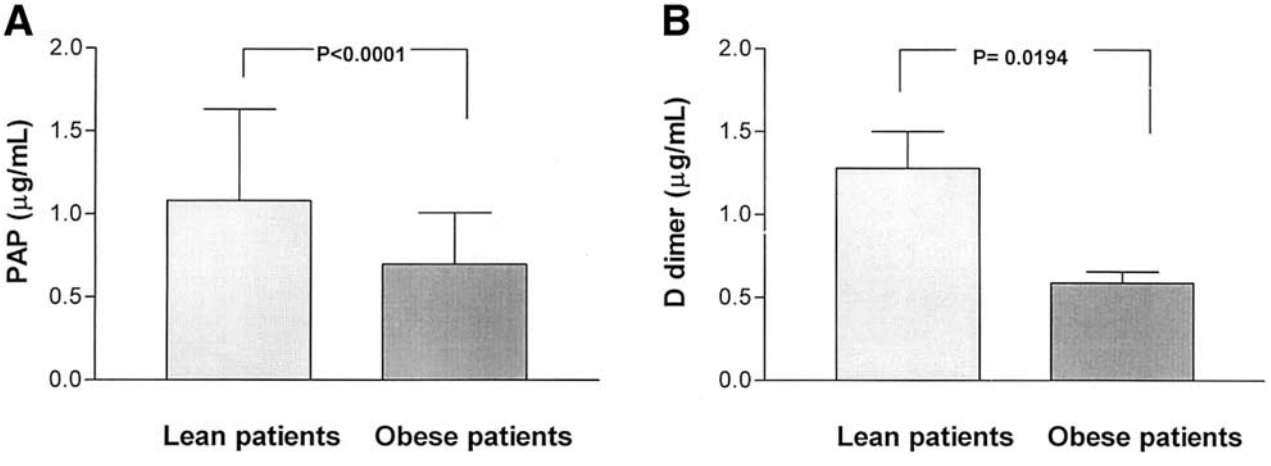

Fig 1. Plasma concentrations of PAP complex (A) and D dimer (B) in the lean and obese patients with type 2 diabetes. Data for PAP are mean ± SD; data for D dimer are mean ± SEM.

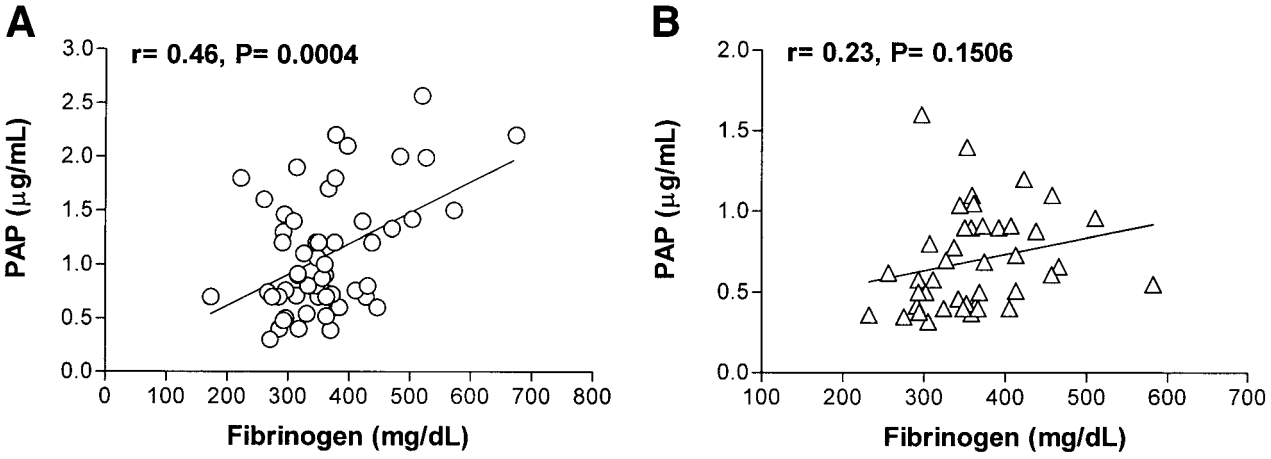

Fig 2. Correlation between plasma concentrations of PAP complex and fibrinogen in lean (A) and obese (B) patients with type 2 diabetes.

Considering all type 2 diabetic subjects together, plasma PAP concentration showed a strong negative correlation with plasma PAI-1 concentration (r = -.54, $P < .0001$; Fig 3A). Curvilinear analysis showed a significant inverse correlation between plasma PAP and PAI-1 (R^2 = .15; Fig 3B). We found no correlation between plasma PAI-1 and fasting insulin concentrations (r = .01, P = .966).

Multivariate analysis demonstrated that BMI and PAI-1 were independent factors for predicting the plasma concentrations of PAP (P = .009, P = .001, respectively). BMI, HbA_{1c}, and fibrinogen predicted plasma concentrations of the D dimer (P = .003, P = .038, and P = .028, respectively) in the type 2 diabetic patients (Table 2). PAP, but not insulin, independently predicted PAI-1 (P = .028; data not shown).

Plasma concentrations of PAI-1 were higher in the obese than lean diabetic patients (17.6 ± 13.6 *v* 8.1 ± 7.9 ng/mL, $P <$ .0001; Fig 4). We then compared plasma concentrations of PAI-1 in patients treated and those not treated with ACE inhibitors, because some studies have suggested that treatments with ACE inhibitor has a lowering effect on plasma PAI-1 antigen in humans.[24,25] We found no significant differences in plasma concentrations of PAI-1 between patients treated with ACE inhibitors and those not treated with ACE inhibitors (9.7 ± 6.6 *v* 12.6 ± 12.1 ng/mL, $P > .5$).

DISCUSSION

An arterial thrombus includes both platelet aggregates and a fibrin meshwork.[7] Formation of fibrin deposits in arteries induced by hypercoagulation plays a crucial role in the development of atherothrombosis.[26] Spontaneous dissolution of thrombus is regulated mainly by fibrinolytic activity, which ultimately is dependent on generation of plasmin.[27] Thus, impaired fibrinolysis may contribute to the pathogenesis of athrothrombosis, leading to coronary artery disease and cerebrovascular disease. Several studies have demonstrated that plasma concentrations of PAI-1, a main inhibitor of the fibrinolytic system, are elevated in obese subjects and in patients with type 2 diabetes.[15,16]

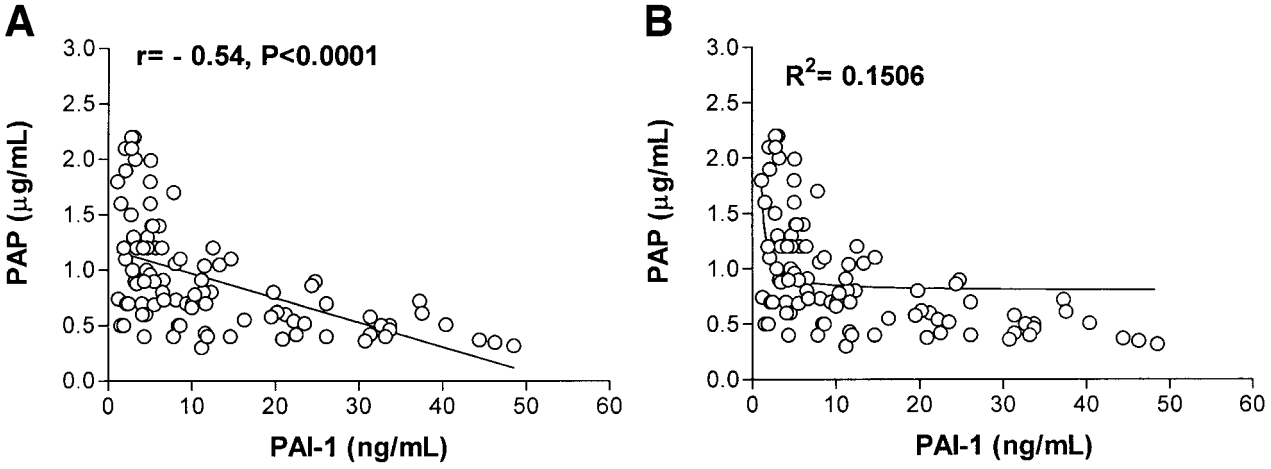

Fig 3. Correlation between plasma concentrations of PAP complex and PAI-1 in type 2 diabetic patients. (A) Linear regression analysis. (B) Curvilinear regression.

Table 2. Multivariate Analysis of the Relationship Between Fibrinolytic Activity Indicated by PAP and D Dimer and Clinical Variables in Type 2 Diabetic Patients

Variables	Plasma PAP		Plasma D Dimer	
	β	*P* Value	β	*P* Value
Age (yr)	−0.070	.957	0.1744	.186
Diabetes duration (yr)	0.1692	.185	0.0370	.781
BMI (kg/m^2)	−0.3250	.009	−0.3826	.003
FPG (mmol/L)	−0.2419	.056	−0.0319	.811
HbA_{1c} (%)	0.0435	.735	−0.2707	.038
TC (mg/dL)	−0.0427	.740	0.0822	.536
TG (mg/dL)	0.0374	.771	−0.0321	.810
HDL-cholesterol (mg/dL)	−0.0507	.693	−0.2041	.121
Insulin (μU/mL)	0.0089	.945	0.1101	.406
Fibrinogen (g/L)	0.1839	.149	0.2865	.028
PAI-1 (ng/mL)	−0.4709	.001	0.0664	.618

NOTE. The partial coefficient is indicated as β.

Abbreviations: PAP, plasmin-antiplasmin complex; FPG, fasting plasma glucose; BMI, body mass index; TC, total cholesterol; TG, triglyceride; PAI, plasminogen activator inhibitor.

Increased PAI-1 concentrations in blood may induce hypofibrinolysis, contributing to the increased risk of cardiovascular and cerebrovascular disease in type 2 diabetic patients. However, several investigators have reported that in patients with type 2 diabetes, fibrinolytic activity in response to hypercoagulation is enhanced.[9,12]

The present study demonstrated that both coagulation and fibrinolysis are activated simultaneously in type 2 diabetic patients, because plasma concentrations of fibrinogen and F1+2 (coagulation markers), as well as those of PAP and D dimer (fibrinolytic markers), were higher in diabetic patients than in control subjects. Diabetic patients are recognized to exhibit a hypercoagulable state.[7-9] Furthermore, because activation of fibrinolysis is a response secondary to a preceding occurrence of hypercoagulation in blood, the coexistence of activated coagulation and fibrinolysis is a reasonable finding in patients with type 2 diabetes.[9,12]

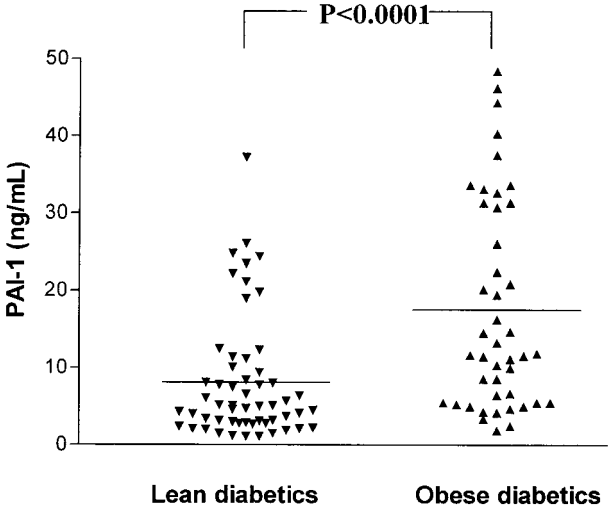

Fig 4. Comparison of plasma concentrations of PAI-1 in lean and obese patients with type 2 diabetes. Vertical bars indicate values of the mean.

We found that fibrinolytic activity was significantly lower in obese than lean patients with type 2 diabetes. However, activation of coagulation was comparable between the diabetic groups, because no differences in plasma concentrations of fibrinogen or F1+2 were seen between groups. The most striking finding in the present study was that despite a similar degree of hypercoagulation between lean and obese diabetic patients, the endogenous fibrinolytic activity was lower in obese than lean diabetic patients. In addition, the present study showed that markers of fibrinolytic activity increased in parallel with activity of coagulation in lean, but not obese, diabetic patients. On the basis of these results, we speculate that fibrinolytic compensation for hypercoagulation is incomplete in obese patients with type 2 diabetes. Thus, relative hypofibrinolysis in a hypercoagulable state may contribute to a high risk of coronary artery disease in obese diabetic patients. Imbalance between coagulation and fibrinolysis may play a role in the development of atherothrombosis in obese patients with type 2 diabetes.

Considering all type 2 diabetic subjects, our simple regression analysis showed plasma concentrations of PAP to have a strong inverse correlation with PAI-1 concentrations. Multivariate analysis showed that BMI and PAI-1 concentrations were independent factors determining plasma concentrations of PAP in diabetic patients. Thus, fibrinolytic activity is regulated largely by plasma PAI-1 concentration in patients with type 2 diabetes. The present study showed that plasma concentrations of PAI-1 were comparable between healthy subjects and lean patients with type 2 diabetes, while obese patients with type 2 diabetes had higher concentrations of PAI-1 than healthy subjects or lean diabetic patients. PAI-1 is produced in multiple sites including the liver,[28] endothelial cells,[29,30] megakaryocytes,[31] and adipose tissue.[18,19] However, the present observation that obese diabetic patients had much higher plasma PAI-1 concentrations than lean diabetic patients suggests that PAI-1 release from adipose tissue contributes particularly to elevated plasma PAI-1 in obese patients with type 2 diabetes. Impairment of fibrinolytic compensation for hypercoagulation in obese patients with type 2 diabetes apparently may result partly

from high plasma concentrations of PAI-1. The present study extends this observation to Japanese patients with type 2 diabetes.

Although the precise cause of elevated plasma concentrations of PAI-1 in patients with type 2 diabetes remains unclear, PAI-1 concentration is known to correlate well with fasting plasma insulin. Hyperinsulinemia secondary to insulin resistance, therefore, may be the primary determinant of elevation of plasma PAI-1 in type 2 diabetic patients.[15,32] In vitro study[33] also demonstrated that either insulin or its precursor can augment the expression of PAI-1 in hepatocytes. However, in the present study, we found no significant correlation between plasma concentration of PAI-1 and fasting insulin concentration in type 2 diabetic patients. A possible explanation for this discrepancy is that 35 of the diabetic patients were treated with insulin injections; therefore, exogenous insulin may have spuriously influenced fasting plasma insulin concentration. Furthermore, insulin therapy reportedly suppressed PAI-1 in type 2 diabetic subjects.[34] Thus, administration of insulin may help to account for the lack of correlation between plasma PAI-1 and insulin concentrations in the present study. Alternatively, it is possible that chronic hyperglycemia itself can affect plasma PAI-levels. An in vitro study demonstrated that glucose, in concentrations seen in the plasma of hyperglycemic patients, induces the synthesis of PAI-1 in aortic endothelial cells.[30] A previous study reported that glucose-responsive elements in the human PAI-1 promoter can be localized to 2 Sp1 sites just upstream from the transcription start site.[35] The present study also shows that plasma concentrations of PAI-1 are higher in the diabetic patients than healthy controls, despite the similar BMI between the 2 groups. Thus, it is more likely that the synergistic action of hyperglycemia and obesity causes elevation of plasma PAI-1 concentrations in patients with type 2 diabetes.

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