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Plasminogen Activator Inhibitor-1 and Tissue-Plasminogen Activator in Minority Adolescents with Type 2 Diabetes and Obesity

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ABSTRACT

Increased plasminogen activator inhibitor-1 (PAI-1) and decreased tissue-plasminogen activator (t-PA) activities lead to impaired fibrinolysis, which is critical for cardiovascular disease. We studied these hemostatic factors at fasting state and after an oral fat load in 12 type 2 diabetic and 17 nondiabetic obese adolescents, matched for age, sex, body mass index, and sexual maturation. Plasma PAI-1, t-PA, and glucose as well as serum C-peptide, insulin, total cholesterol, triglyceride, and HDL and LDL cholesterol levels were measured at 0, 2, 4, and 6 h after the fat load. Metabolic responses were expressed as the area under the curve (AUC). PAI-1 activities were significantly greater in patients than in control subjects [fasting, 23.4 ± 2.6 *versus* 12.9 ± 2.0 U/mL ($p < 0.004$); AUC, 101.7 ± 12.1 *versus* 57.6 ± 6.5 $U \cdot h^{-1} \cdot mL^{-1}$ ($p < 0.003$)]. Fasting t-PA activities were significantly lower in the patients than in the control subjects (0.8 ± 0.3 *versus* 6.5 ± 2.7 U/mL; $p < 0.001$). Triglyceride was the only lipid parameter that was significantly different in the patients than in the control subjects [fasting, 1.5 ± 0.2 *versus* 0.9 ± 0.1 mM ($p < 0.05$); AUC, 15.7 ± 2.9 *versus* 7.9 ± 0.6 $mmol \cdot h^{-1} \cdot L^{-1}$ ($p < 0.02$)]. The PAI-1 activities decreased significantly during the loading tests ($p < 0.0001$), whereas the t-PA activities did not change. Insulin resistance estimated by the homeostasis model assessment was greater in the patients than in the control subjects (14.4 ± 2.8 *versus* 4.6 ± 0.7; $p < 0.0001$). We conclude that elevated PAI-1 and diminished t-PA activities, suggestive of suppressed fibrinolysis, are present in our adolescents with type 2 diabetes; adding another risk factor for cardiovascular disease and acute high fat load does not further negatively affect this suppressed fibrinolysis. **(*Pediatr Res* 58: 483–487, 2005)**

Abbreviations

AUC, area under the curve
BMI, body mass index
CVD, cardiovascular disease
HOMA, homeostasis model assessment
PAI-1 plasminogen activator inhibitor-1
t-PA, tissue-plasminogen activator

Insulin resistance followed by absolute or relative insulin deficiency is a known course of type 2 diabetes development (1,2). Type 2 diabetes, obesity, dyslipidemia, and abnormal factors related to blood clotting are risk factors of cardiovascular disease (CVD) and are often seen in patients with insulin resistance (2–7). Tissue-plasminogen activator (t-PA) initiates fibrinolysis. Plasminogen activator inhibitor-1 (PAI-1) binds rapidly to t-PA and has an inhibitory activity against t-PA (6). As a result, abnormal PAI-1 and/or t-PA activities contribute to a thrombotic tendency (6). Elevated plasma PAI-1 activities and antigens have been reported in adults with type 1 diabetes, type 2 diabetes, and obesity as well as in children with type 1 diabetes and obesity (8–20). Elevated plasma t-PA antigens have been reported in adults with type 1 diabetes, type 2 diabetes, and obesity as well as in children with type 1 diabetes and obesity (8,13,19,21). Decreased plasma t-PA activities and a decreased capacity of endothelial cells to secret t-PA in response to a fibrinolytic stimulus were also reported in adults with diabetes (7,14,22). Normal PAI-1 and t-PA antigens, however, have been shown in children and adults with type 1 diabetes (17,19). Because there are few data about PAI-1 and t-PA in children and adolescents with type 2 diabetes, there is a continuing increase in type 2 diabetes and obesity in this population, which is thought to be due to high caloric intake and sedentary life style (especially in minority groups) (23–25), and CVD begins in childhood (26,27), we studied the PAI-1 and t-PA activities at fasting state as well as an impact of a fat load assigned to simulate the fat content of a high-fat, fast-food meal (28) to these critical factors in minority adolescents with type 2 diabetes and obesity.

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METHODS

Study groups. Twelve type 2 diabetic obese and 17 nondiabetic obese adolescents (predominantly blacks) participated in our study. All type 2 diabetic patients had *1*) negative antibodies to glutamic acid decarboxylase, islet cell, and insulin and *2*) fasting serum C-peptide levels >0.2 nmol/L (29). Mean duration of diabetes was 7.1 ± 2.5 mo. All nondiabetic subjects had fasting glucose levels <6.1 mM (1) and normal HbA_{1c}. Nine patients with diabetes were taking insulin, and three were taking insulin and metformin. Metformin was stopped ~6 wk before the fat-loading test. Both diabetes and control groups were age, sex, body mass index (BMI), and sexual maturation matched. Clinical characteristics are shown in Table 1. The study was approved by the Institutional Review Board of the State University of New York Health Science Center (Brooklyn, NY). Each subject and legal guardian gave consent to participate.

Fat-loading tests and measurements. After overnight fasting and withholding of insulin injection (at least 12 h), the subjects ingested a fat load over a period of <5 min. The fat load was a mixture of 350 mL of pasteurized heavy cream (G.A.F. Seelig, Inc., Woodside, NY), 15 mL of fat-free milk (G.A.F. Seelig), 15 mL of chocolate syrup (Hershey chocolate syrup, Hershey, PA), and 1 tablespoon of granulated sugar. It contained 117 g of fat (70 g of saturated fat, 467 mg of cholesterol), 41.5 g of carbohydrate, and 0.5 g of protein. It provided 1242 cal: 86.4% from fat, 13.4% from carbohydrate, and 0.2% from protein. The fat load was modified from what was used by Patsch *et al.* (30) and was assigned to simulate the fat content of a high-fat, fast-food meal (28). Venous blood samples were obtained at 0, 2, 4, and 6 h after the ingestion of the fat loads. The venous blood is processed to plasma, serum, and acidified plasma for glucose; lipid profile, C-peptide, and insulin; and PAI-1 and t-PA, respectively. The lipid profile included total cholesterol, triglyceride and HDL and LDL cholesterol determinations. The acidified plasma for t-PA was obtained by collecting 9 vol of blood in an ice-cold test tube that contained 1 vol of 0.13 M sodium citrate, mixing, adding acetate (1 vol acetate: 2 vol citrated blood), placing on ice, and centrifuging within 30 min at 1500 × *g* for 5 min. Plasma was transferred to a new tube, and 20% acetic acid was added (0.05 vol of 20% acetic acid:1 vol plasma) to acidify the plasma. All samples for PAI-1 and t-PA were frozen and stored at −70°C until assayed. PAI-1 and t-PA activity measurements were performed using chromogenic assay (Spectrolyse; American Diagnostica Inc., Greenwich, CT). Total cholesterol, triglyceride, and HDL cholesterol concentrations were measured by standard colorimetric methods (Vitros; Ortho-Clinical Diagnostics, Inc., Rochester, NY) (31–33). LDL cholesterol concentrations were calculated using the Friedewald formula [LDL cholesterol = total cholesterol − (HDL cholesterol + triglycerides/5)] (34). All samples for C-peptide and insulin were centrifuged at 4°C. The sera were frozen and stored at −20°C until assayed. C-peptide determinations were performed in duplicate by double-antibody RIA (C-peptide ^{125}I RIA kits; DiaSorin, Inc., Stillwater, MN), with a lower limit of detection of 0.2 nmol/L. Insulin determinations were performed in duplicate by RIA (Human Insulin Specific RIA kits; Linco Research, Inc., St. Charles, MO), with a lower limit of detection of 14 pmol/L. Glucose concentrations were analyzed by a glucose oxidase method (Glucose Analyzer; Beckman Coulter, Inc., Brea, CA). Immunologic marker determinations (glutamic acid decarboxylase, islet cell autoantibodies, and insulin autoantibodies) were determined by RIA in the patients with diabetes and were all negative. HbA_{1c} was measured by HPLC.

Statistics. Metabolic responses to the fat load were expressed as the area under the curve (AUC) calculated by trapezoidal estimation. Insulin resistance was estimated by the homeostasis model assessment (HOMA = [fasting glucose × fasting insulin]/22.5) (35). Differences in age, BMI, HbA^{1c}, fasting metabolic parameters, and AUC between the two groups were examined by Mann-Whitney tests because these parameters were not normally distributed and our sample size was small. Differences in the metabolic responses between the two groups and across the four time points were also examined by repeated measures ANOVA. Both Mann-Whitney tests and repeated measures ANOVA yielded similar results for the differences of the metabolic parameters during the loading tests between the two groups. Only *p* values from the Mann-Whitney tests were reported. Differences in sex and sexual maturation were examined by Fisher's exact tests. Correlations were evaluated, and multiple linear regression analyses were used when indicated. The statistical package for social sciences for Windows, version 10.5 (SPSS, Inc., Chicago, IL) was used for the statistical analyses. Significance was accepted at the level $p < 0.05$. Data are presented as mean ± SEM.

Table 1. *Clinical characteristics of study groups*

	Diabetes (n = 12)	Control (n = 17)
Age (y)	14.0 ± 0.7	13.2 ± 0.5
Sex (n; female/male)	7/5	9/8
BMI (kg/m^2)	32.7 ± 1.1	32.0 ± 2.6
Tanner staging		
2	1	3
3	2	5
4	4	7
5	5	2
Race	92% black	77% black
	8% Asian	23% Hispanic

Values are means ± SEM.

RESULTS

Fasting state. Fasting PAI-1 activities were significantly greater in the diabetes group than in the control group. Fasting t-PA activities in the diabetes group were significantly lower than in the control group. Triglyceride was the only lipid parameter that was significantly different in the patients than in the control subjects. As expected, the fasting glucose and HbA_{1c} levels were significantly higher in the diabetes group when compared with the control group. The fasting C-peptide and insulin levels were also significantly greater in the diabetes group than in the control group. HOMA was greater in the patients with diabetes than in the control subjects (Table 2).

Postprandial state. PAI-1 AUC was significantly greater in the diabetes group than in the control group (Fig. 1*A*). t-PA AUC in the diabetes group was relatively lower than in the control group (Fig. 1*B*). Triglyceride responses in the diabetes group were significantly higher than in the control groups (Fig. 1*C*). Total cholesterol, HDL cholesterol, and LDL cholesterol AUCs did not differ between the two groups [28.5 ± 2.1 *versus* 25.9 ± 1.2 mmol · h^{-1} · L^{-1} (p = NS); 6.0 ± 0.3 *versus* 7.1 ± 0.4 mmol · h^{-1} · L^{-1} (p = NS); 16.9 ± 2.0 *versus* 14.8 ± 1.4 mmol · h^{-1} · L^{-1} (p = NS), respectively). As expected, glucose AUC was significantly higher in the diabetes group when compared with the control group (Fig. 1*D*). The C-peptide and insulin AUCs were also significantly greater in the diabetes group than in the control group (Fig. 1*E* and *F*, respectively).

When we compared changes of the metabolic parameters across the four time points, PAI-1 activities decreased significantly ($p < 0.0001$). t-PA activities did not change. Triglycerides increased significantly ($p < 0.0001$). Total cholesterol levels increased significantly ($p < 0.005$), whereas HDL cho-

Table 2. *Results of metabolic parameters at fasting state in study groups*

	Diabetes (n = 12)	Control (n = 17)	p Values
PAI-1 activity (U/mL)	23.4 ± 2.6	12.9 ± 2.0	0.004
t-PA activity (U/mL)	0.8 ± 0.3	6.5 ± 2.7	0.001
Triglyceride (mmol/L)	1.5 ± 0.2	0.9 ± 0.1	0.05
Total cholesterol (mmol/L)	4.7 ± 0.3	4.2 ± 0.2	NS
HDL cholesterol (mmol/L)	1.1 ± 0.1	1.2 ± 0.1	NS
LDL cholesterol (mmol/L)	2.9 ± 0.3	2.6 ± 0.2	NS
Glucose (mmol/L)	9.2 ± 1.3	4.6 ± 0.1	0.007
C-peptide (nmol/L)	0.9 ± 0.1	0.7 ± 0.04	0.03
Insulin (pmol/L)	244.1 ± 27.6	151.8 ± 19.8	0.008
HOMA	14.4 ± 2.8	4.6 ± 0.7	0.0001
HbA_{1c} (%)	9.2 ± 1.0	5.4 ± 0.2	0.001

Values are expressed as means ± SEM.

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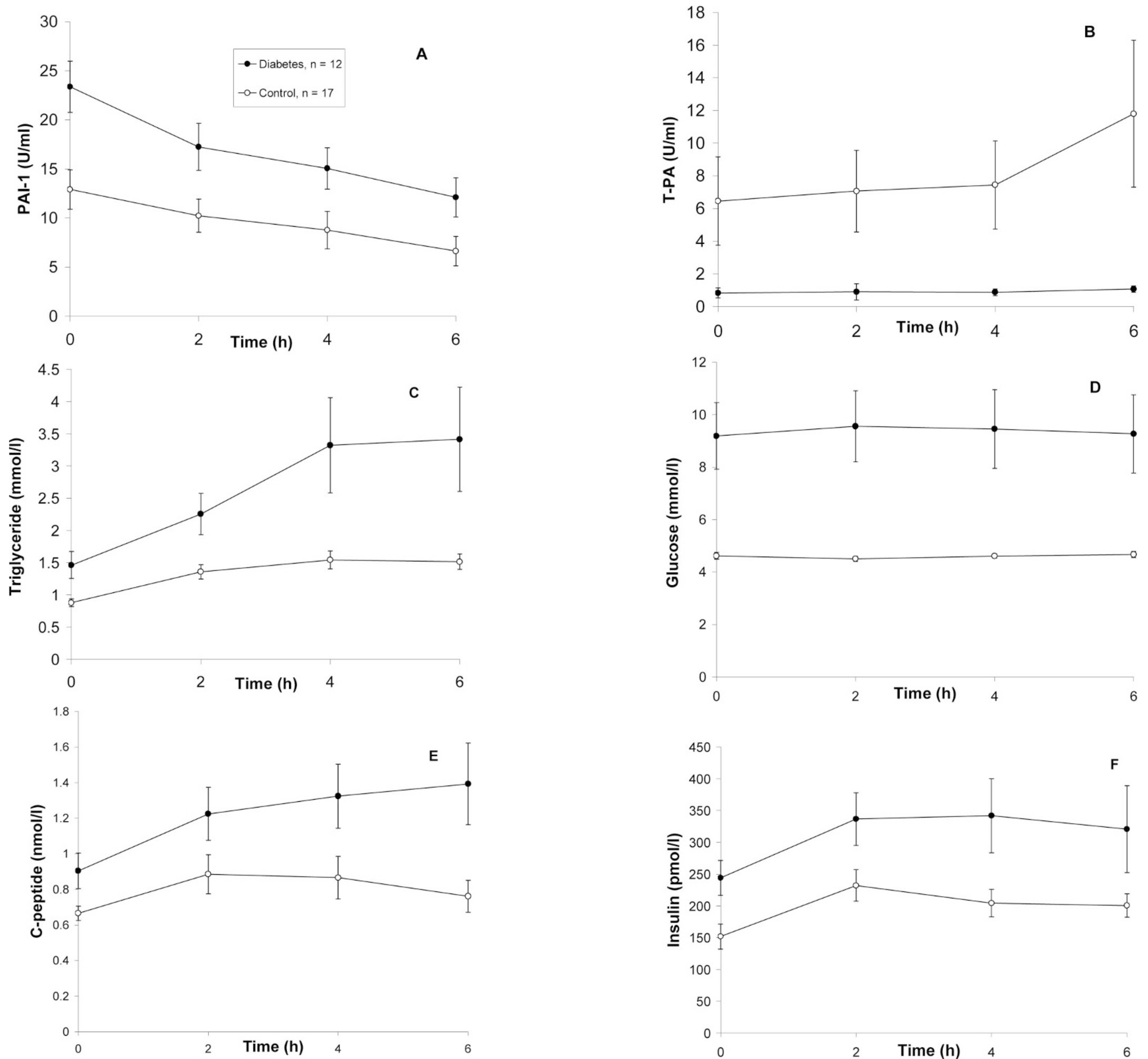

Figure 1. PAI-1, t-PA, triglyceride, glucose, C-peptide, and insulin responses during the fat-loading tests in diabetes ($n = 12$) and control ($n = 17$) groups; mean ± SEM. (*A*) AUC of PAI-1 (101.7 ± 12.1 *vs* 57.6 ± 6.5 U · h^{-1} · mL^{-1}; $p < 0.003$). (*B*) AUC of t-PA (7.8 ± 2.7 *vs* 58.3 ± 19.3 U · h^{-1} · mL^{-1}; p = NS). (*C*) AUC of triglyceride (15.7 ± 2.9 *vs* 7.9 ± 0.6 mmol · h^{-1} · L^{-1}; $p < 0.02$). (*D*) AUC of glucose (56.2 ± 8.1 *vs* 27.6 ± 0.5 mmol · h^{-1} · L^{-1}; $p < 0.01$). (*E*) AUC of C-peptide (7.3 ± 0.8 *vs* 4.8 ± 0.5 nmol · h^{-1} · L^{-1}; $p < 0.009$). (*F*) AUC of insulin (1865.0 ± 263.3 *vs* 1167.2 ± 74.0 pmol · h^{-1} · L^{-1}; $p < 0.01$).

lesterol levels decreased significantly ($p < 0.04$) and LDL cholesterol levels did not change. As expected, glucose levels did not change significantly. C-peptide and insulin levels increased significantly ($p < 0.001$ and 0.006, respectively).

Correlations. As a whole group, fasting PAI-1 activities were significantly correlated with fasting triglycerides ($r = 0.54$, $p < 0.004$), fasting insulin ($r = 0.50$, $p < 0.009$), and HOMA ($r = 0.70$, $p < 0.001$). Fasting t-PA activities were significantly correlated with fasting cholesterol levels ($r = -0.51$, $p < 0.009$), fasting LDL cholesterol levels ($r = -0.52$, $p < 0.008$), and BMI ($r = -0.60$, $p < 0.002$). After multiple linear regression analyses, only correlation between fasting PAI-1 levels and HOMA remained significant. Postprandially, significant correlation was also found between PAI-1 AUCs and HOMA ($r = 0.78$, $p < 0.0001$) after multiple linear regression analyses (Fig. 2).

DISCUSSION

This study shows for the first time that adolescents with type 2 diabetes have both fasting and postprandial unfavorable PAI-1 and t-PA activities when compared with nondiabetic

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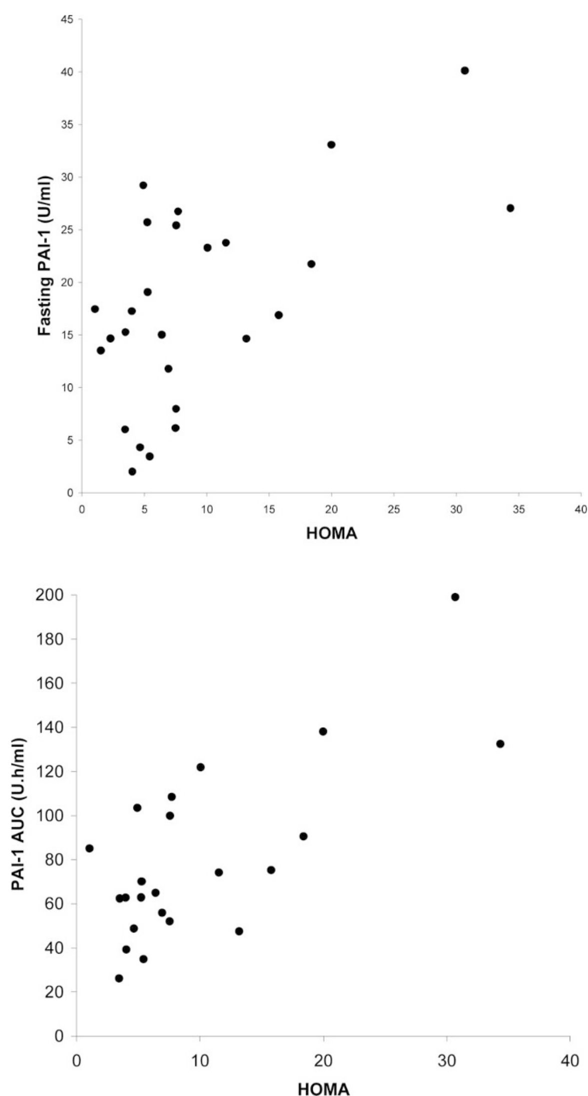

Figure 2. HOMA was significantly correlated with fasting PAI-1 ($r = 0.70$, $p < 0.001$; $n = 26$) and PAI-1 AUC ($r = 0.78$, $p < 0.0001$; $n = 23$).

adolescents who are equally obese and that these profiles are not negatively affected by an acute oral high-fat load. Because PAI-1 binds rapidly to t-PA and forms an inactive t-PA–PAI-1 complex, we measured PAI-1 and t-PA activities, rather than antigens. Increased PAI-1 and decreased t-PA activities are suggestive of reduced fibrinolytic activities and are unfavorable for CVD (6,8,16,19,36). Patients who have diabetes and developed peripheral artery disease or late complications of diabetes showed a shift toward an antifibrinolytic pathway with diminished t-PA and increased PAI-1 antigen and activity (12,17). Our adolescents with type 2 diabetes had mean duration of the disease of 7.1 ± 2.5 mo; therefore, interference of any coexistent atherosclerosis as overt late complication of diabetes is very unlikely to affect the results of PAI-1 and t-PA measurements.

At fasting and during postprandial states, PAI-1, triglyceride, glucose, C-peptide, and insulin levels were significantly higher in patients than in control subjects. Fasting t-PA activities were significantly lower in the patients than in the control subjects. Insulin resistance, represented by HOMA, was greater in the patients than that in the control subjects. A clustering of these cardiovascular risk factors, including increased PAI-1/decreased t-PA activities, hypertriglyceridemia, diabetes, and hyperinsulinemia, increases the risk for CVD.

The increased triglyceride levels during the fat-loading tests observed in our study are consistent with findings reported by others (37–39) and have been discussed previously (40). The relationship between elevated PAI-1, insulin resistance, and hypertriglyceridemia is established (2,6,17,18,36,41) and is shown in our study. Therefore, elevated PAI-1 activities were anticipated during the fat-loading tests. On the contrary, the PAI-1 activities decreased significantly. Both increased and decreased PAI-1 activities after fat loads have been reported (39,42–47). The difference in the postprandial PAI-1 responses has been explained by different genotypes of PAI-1, different fat types and content, and that PAI-1 has a circadian rhythm (PAI-1 concentrations decrease during the day) (39,44,48,49). Whereas increase in triglycerides alone, as here in our study, or insulin alone (50) seems insufficient to increase PAI-1 concentrations, a combination of hypertriglyceridemia, hyperinsulinemia, and hyperglycemia can increase PAI-1 in blood in normal humans (51). Therefore, our results also support the hypothesis that several abnormal metabolic parameters are essential for the elevation of PAI-1 levels. In addition, chronic exposure to high fat loads may result differently. t-PA activities seemed unrelated to insulin resistance and were not affected by the fat-loading tests as reported previously (45). Nevertheless, the patients with type 2 diabetes had relatively lower t-PA activities than the control subjects throughout the fat-loading tests.

The differences in the PAI-1 levels in our diabetic and nondiabetic obese subjects, who had comparable BMIs, may be due to the difference in fat distribution (52–55). However, further investigation (*e.g.* computed tomography or magnetic resonance imaging to measure fat distribution) is needed to determine whether indeed our diabetic subjects have central fat distribution and our obese subjects have peripheral fat distribution. Furthermore, glycation, the result of hyperglycemia in the diabetic patients, may enhance the production of PAI-1 and attenuate that of t-PA (22,56,57).

We conclude that elevated PAI-1 and diminished t-PA activities, suggestive of suppressed fibrinolysis, are present in our adolescents with type 2 diabetes, adding another risk factor for CVD, and high fat intake does not further negatively affect this suppressed fibrinolysis.

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