Increased Plasminogen Activator Inhibitor-1 Activity in Offspring of Type 2 Diabetic Patients

Lack of association with plasma insulin levels

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OBJECTIVE — To determine whether a dysregulation of the fibrinolytic system exists in normal glucose tolerant offspring of type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — In this cross-sectional study, 32 offspring of type 2 diabetic patients and 26 subjects with no family history of diabetes were studied. With respect to the metabolic parameters, plasma fasting and 2-h postload (75 g glucose) glucose and insulin levels, total cholesterol, triglycerides, and HDL cholesterol concentrations were determined. To evaluate the status of hemostatic factors, fibrinogen, tissue plasminogen activator (tPA) antigen level, plasminogen activator inhibitor-1 (PAI-1) antigen level, and PAI-1 activity were assessed. The statistical analyses included the Mann-Whitney *U* test to check the significance of differences between variables in the two groups and Spearman's rank correlation tests to check the interrelationships between the hemostatic and metabolic parameters in the offspring group.

RESULTS — All subjects had normal glucose tolerance according to the American Diabetes Association criteria. Plasma fasting and postload insulin concentrations were significantly higher in offspring compared with control group ($P < 0.00001$ and $P < 0.01$, respectively). Plasma fasting and postload glucose, fibrinogen, tPA antigen, total cholesterol, and BMI were comparable between the groups. The offspring had significantly higher waist-to-hip ratio (WHR) ($P = 0.03$), higher triglycerides ($P = 0.01$), and lower HDL cholesterol ($P < 0.01$) compared with the control group. PAI-1 antigen level and PAI-1 activity were higher in the offspring ($P = 0.05$ and $P = 0.04$, respectively). In the offspring group, PAI-1 activity was correlated with plasma PAI-1 antigen level ($r = 0.40$, $P = 0.02$), fibrinogen ($r = 0.45$, $P = 0.01$), and HDL cholesterol ($r = -0.36$, $P = 0.04$). However, tPA antigen level, fasting and postload plasma glucose and insulin, total cholesterol, triglycerides, WHR, and BMI did not correlate with PAI-1 activity.

CONCLUSIONS — These data suggest that normal glucose tolerant offspring of type 2 diabetic subjects have elevated PAI-1 activity indicating to hypofibrinolysis in this group. The elevated PAI-1 activity has no association with plasma insulin concentration.

Diabetes Care 23:88–92, 2000

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Received for publication 24 July 1999 and accepted in revised form 7 October 1999.

Abbreviations: CV, coefficient of variation; PAI-1, plasminogen activator inhibitor-1; tPA, tissue plasminogen activator; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

In prospective population studies, hyperinsulinemia has been shown to be associated with increased incidence of cardiovascular diseases in men (1,2). Cardiovascular diseases are major causes of mortality and morbidity in diabetic populations and are frequently present even at the time of diagnosis. The insulin resistance syndrome that precedes the onset of overt diabetes is associated with metabolic alterations and abnormalities in hemostasis (3,4). The cluster of cardiovascular risk factors in the prediabetic state may explain the high prevalence of cardiovascular diseases present at the diagnosis of overt disease.

Impaired fibrinolysis increases the risk of cardiovascular diseases, particularly the risk of myocardial infarction (5,6). Hypofibrinolysis favors the intravascular deposition of fibrin. Fibrinolysis is regulated through plasminogen activators, and especially inhibitors, primarily the plasminogen activator inhibitor-1 (PAI-1) (7). Several studies have found high PAI-1 levels in conditions associated with insulin resistance, such as hypertriglyceridemia (8,9), obesity (10,11), type 2 diabetes (12), and coronary artery disease (13). In all of these conditions, a significant correlation has been found between PAI-1 and plasma insulin levels.

First-degree relatives of type 2 diabetic subjects are supposed to be genetically prone to the development of clinical disease. They have been shown to exhibit a high prevalence of glucose intolerance, hyperinsulinemia, and insulin resistance that may precede the diagnosis of diabetes by decades (14–16). It might be possible that hemostatic dysregulation may be present even in normal glucose tolerant first-degree relatives. To our knowledge, PAI-1 activity and its relationship with plasma insulin levels have not previously been studied in offspring of type 2 diabetic subjects. In the present study, we aimed to investigate the hemostatic parameters, including tissue plasminogen activator (tPA), fibrinogen, PAI-1 antigen, and PAI-1 activity, in a group of diabetic patients' offspring in comparison with healthy control subjects who have no family

Table 1—*Comparison of clinical and metabolic parameters between offspring and control group*

Variable	Offspring	Control group	*P* value*
n	32	26	—
Age (years)	37 (20–49)	35 (22–51)	NS
BMI (kg/m^2)	24.8 (22.2–28.9)	24.6 (21.4–28.0)	NS
WHR	0.79 (0.69–0.98)	0.75 (0.69–0.93)	0.03
sBP (mmHg)	107.5 (90–140)	112.5 (90–140)	NS
dBP (mmHg)	70 (50–90)	70 (50–90)	NS
Fasting glucose (mmol/l)	5.2 (3.5–6.1)	4.9 (4.0–5.9)	NS
Postload glucose (mmol/l)†	5.7 (3.8–7.5)	5.3 (4.2–7.2)	NS
Fasting insulin (pmol/l)	170.8 (106.2–707.5)	101.9 (33.0–319.3)	<0.00001
Postload insulin (pmol/l)†	441.2 (126.3–1,288)	285.5 (86.1–1,021)	<0.01
Total cholesterol (mmol/l)	4.5 (2.7–6.4)	4.1 (3.1–5.5)	NS
Triglycerides (mmol/l)	1.3 (0.5–4.46)	0.8 (0.27–4.39)	0.01
HDL cholesterol (mmol/l)	0.98 (0.65–1.57)	1.25 (0.45–1.72)	<0.01

Data are medians (range). dBP, diastolic blood pressure; sBP, systolic blood pressure. *By Mann-Whitney *U* test; †2 h after 75-g oral glucose.

history of diabetes. We also investigated the interrelationships between these parameters and plasma insulin levels.

RESEARCH DESIGN AND METHODS

Subjects

We studied 32 (22 women, 10 men) nondiabetic offspring recruited from the type 2 diabetic population attending the hospital. Of these subjects, 20 (62.5%) had diabetic mothers, 10 (31.2%) had diabetic fathers, and 2 (6.3%) had diabetic fathers and mothers both. There were 26 (17 women, 9 men) normoglycemic subjects without a family history of diabetes who served as the control group. None of the participants had clinical antecedents of stroke, coronary heart disease, or peripheral vascular disease. Subjects with a disease or treatment known to affect hemostatic variables, glucose tolerance, or insulin secretion or action were excluded. Blood samples for tPA antigen, PAI-1 antigen, PAI-1 activity, and fibrinogen were drawn in the morning after an overnight fast, avoiding venous stasis. At the same time, samples for blood glucose, total cholesterol, plasma triglycerides, HDL cholesterol, and insulin were obtained. The status of glucose tolerance was determined by a standard 2-h oral glucose tolerance test (75 g). Blood was drawn for glucose and insulin before and 2 h after glucose ingestion, and the results were interpreted according to the 2-h glucose levels as recently recommended by American Diabetes Association (ADA) (17). Height and weight were measured by following a standardized protocol. BMI (weight/height^2[kg/m^2]) was used as an estimate of overall adiposity. Waist circumference was measured at the level of the umbilicus in the standing position, and hip circumference was measured at the level of greater trochanters. The waist-to-hip ratio (WHR) was used as a measure of upper-body adiposity. Smoking history was obtained from all participants. There were 11 (34.3%) subjects in the offspring group and 7 (26.9%) subjects in the control group who were current smokers. Other subjects had never smoked in their lives.

The study was approved by the ethical committee of Hacettepe University, and informed written consent was obtained from all participants.

Methods

Glucose was measured by a glucose-oxidase method. Insulin was measured by a radioimmunoassay kit (Insulin-CT; CIS Biointernational, Gif-Sur-Yvette, France) that shows a cross-reactivity with porcine proinsulin of 14.1%. The interassay coefficient of variation (CV) was 8.8% at 137 pmol/l, and the intra-assay CV was 8.2% at 130 pmol/l. The lower limit of detectibility was 14.35 pmol/l. For tPA antigen, PAI-1 antigen, and PAI-1 activity, vacutainers containing sodium citrate 0.109 mol/l were used to collect the blood directly. Plasma was prepared by centrifugation of the blood within 10 min after the venipuncture for 15 min at 3,000*g* at 4°C and stored at −20°C in small aliquots in plastic tubes until assay. After thawing at 37°C, samples were used within 2 h.

Quantitative determination of tPA and PAI-1 antigen levels were performed using commercial enzyme-linked immunosorbent assay kits (Asserachrom TPA/PAI; Diagnostica Stago, Asnières-Sur-Seine, France). Plasma PAI-1 activity was determined by the synthetic chromogenic method (18) using a colorimetric assay (Stachrom PAI; Diagnostica Stago). Fibrinogen was measured by a clot-rate assay using the Diagnostica Stago ST4 instrument. Serum total cholesterol, triglycerides, and HDL cholesterol were measured by automated methods.

Statistics

Because of the non-normal distribution of the data, nonparametric Mann-Whitney *U* test was used to check the significance of differences between the study groups. Spearman's rank correlation coefficients were calculated to assess the correlation between the variables in the offspring group. The statistical level of significance was set at 0.05. The program SPSS for Windows 5.0 was used (Chicago, IL).

RESULTS — Table 1 shows the comparison of the clinical and metabolic characteristics of the groups studied. All subjects were considered to have normal glucose tolerance according to ADA criteria (17), because none of them had 2-h postload plasma glucose values >7.8 mmol/l. Offspring were well matched to the control subjects with regard to age and BMI. All subjects were considered to be nonobese because they had BMIs <30 kg/m^2. No significant differences were found between offspring and control subjects with respect to fasting and postload plasma glucose, serum total cholesterol, and systolic and diastolic blood pressures. The offspring had significantly higher WHRs (P = 0.03), higher triglycerides (P = 0.01), and lower HDL cholesterol ($P < 0.01$) compared with those of the control subjects. Fasting and postload insulin concentrations were significantly higher in the offspring group compared with those in the control group (median fasting insulin: 170.8 vs. 101.9 pmol/l, respectively, $P < 0.00001$; median postload insulin: 441.2 vs. 285.5 pmol/l, respectively, $P < 0.01$).

The comparison of the hemostatic parameters between the study groups is shown in Table 2. Plasma fibrinogen and tPA antigen concentrations were comparable between offspring and control subjects. Plasma PAI-1 antigen concentration was higher in offspring (median 232.2 ng/ml) compared with control subjects (median 150.0 ng/ml), the difference between the

two groups being at the borderline level of statistical significance ($P = 0.05$). Similarly, plasma PAI-1 activity was significantly higher in offspring compared with control subjects (median 28.5 vs. 22.0 IU/ml, respectively, $P = 0.04$).

Spearman's correlation analyses

Plasma PAI-1 activity was significantly and positively correlated with plasma PAI-1 antigen ($r = 0.40$, $P = 0.02$) and fibrinogen ($r = 0.45$, $P = 0.01$) concentrations. PAI-1 activity also had a significant inverse correlation with HDL cholesterol concentration ($r = -0.36$, $P = 0.04$). However, plasma tPA antigen level, fasting and postload plasma glucose, fasting and postload plasma insulin, total cholesterol, triglycerides, WHR, and BMI had no correlation with PAI-1 activity (details not shown).

The plasma PAI-1 antigen concentration was significantly correlated with the WHR ($r = 0.36$, $P = 0.04$), plasma fibrinogen ($r = 0.37$, $P = 0.03$), plasma tPA antigen ($r = 0.39$, $P = 0.02$), and HDL cholesterol ($r = -0.34$, $P = 0.05$). No correlation was found between plasma PAI-1 antigen concentrations and fasting/postload glucose, fasting and postload insulin, total cholesterol, triglycerides, and BMI (details not shown).

Plasma fasting and postload insulin concentration had no correlation with WHR, BMI, triglycerides, total cholesterol, and HDL cholesterol concentrations (details not shown).

Table 2—*Comparison of the hemostatic parameters between offspring and control group*

Variable	Offspring	Control group	*P* value*
n	32	26	—
Fibrinogen (g/l)	2.9 (1.2–4.9)	2.7 (1.4–4.0)	NS
tPA-antigen (ng/ml)	44.8 (17.6–84.0)	37.6 (17.2–73.6)	NS
PAI-1 antigen (ng/ml)	232.2 (36.3–554.0)	150.0 (15.8–416.0)	0.05
PAI-1 activity (IU/ml)	28.5 (8.8–69.1)	22.0 (0–51.2)	0.04

Data are medians (range). NS, not significant. *By Mann-Whitney *U* test.

CONCLUSIONS

In the present study, we have demonstrated for the first time that nonobese normal glucose tolerant offspring of type 2 diabetic patients have increased PAI-1 activity, which may indicate to hypofibrinolysis. Our results also indicate a lack of association between plasma insulin (fasting and postload) and PAI-1 activity. Hypofibrinolysis and hyperinsulinemia found in these individuals are separate risk factors that may lead to early development of cardiovascular diseases.

To the best of our knowledge, the only study concerning the status of the fibrinolytic system in nondiabetic offspring of type 2 patients was conducted by Fernandez-Castaner et al. (19). The authors studied hemostatic variables in 46 nondiabetic offspring (15 glucose-intolerant) in comparison with 21 healthy control subjects who had no family history of diabetes. They found increased prothrombin fragment 1+2 and D-dimer in the offspring indicating to activated coagulation. In that study, although plasma tPA and PAI-1 antigen levels were higher in offspring compared with those in the control group, the difference was not statistically significant. However, the authors have not studied the plasma PAI-1 activity in the offspring, and correlation analyses between plasma insulin and tPA/PAI-1 levels were also lacking.

In subjects with hyperinsulinemia, PAI-1 activity has been shown to be high and the activity of tPA low (20). We found elevated basal and postload insulin concentrations in the offspring compared with control subjects. Although plasma tPA concentrations were comparable, plasma PAI-1 activity was significantly higher in the offspring than control subjects. However, we have not measured the plasma tPA activity in our study.

It has previously been stated that correlations of insulin resistance (whole-body glucose uptake in hyperinsulinemic-euglycemic clamp studies) with fasting or postload insulin levels were remarkably consistent, ranging from −0.58 to −0.74 (21). Thus, it may be speculated that the offspring in our study are relatively insulin resistant compared with our control subjects. We found significantly higher triglycerides, basal and postload insulin levels, and WHR, and significantly lower HDL cholesterol in diabetic patients' offspring. Taken together, these data may indicate the presence of some of the components of "insulin resistance syndrome" in these subjects (3,22). Recently, hypercoagulability and hypofibrinolysis have been related to the insulin resistance syndrome (23,24). Consistent with this finding, our data also indicate to hypofibrinolysis in the offspring that accompanies a cluster of cardiovascular risk factors. Hypofibrinolysis associated with aforementioned cardiovascular risk factors may lead to accelerated atherothrombosis and, thereby, the high prevalence of cardiovascular diseases in diabetic subjects at the time of diagnosis. However, whether this issue is true can be resolved by further prospective controlled studies that should be performed in normal and impaired glucose tolerant offspring of type 2 diabetic patients who have these risk factors.

Although the offspring in our study were hyperinsulinemic compared with the normal control subjects, we could not demonstrate any correlation between PAI-1 activity and plasma insulin concentration. Indeed, controversy exists with respect to the relationship between plasma insulin and PAI-1 activity in vitro and in vivo. In vitro data have shown that both insulin (25) and its precursors (26) are able to stimulate PAI-1 synthesis from hepatocytes. Accordingly, a relationship between insulin and its precursors and PAI-1 activity has been shown in a large, healthy normal glucose tolerant population from northern Sweden (27). In that study, however, neither proinsulin nor insulin concentrations predicted the serum PAI-1 activity in a multivariate linear regression model. Similar to our observation, a significant positive correlation ($r = 0.36$) was present between fibrinogen and PAI-1 activity in this study (27). Given that we found comparable fibrinogen levels between offspring and control subjects, the relatively elevated PAI-1 activity in the offspring cannot be explained solely by the fibrinogen status.

In contrast with our observations, in the Insulin Resistance Atherosclerosis Study by Festa et al. (28), a strong and independent relationship between PAI-1 antigen and insulin has been found consistently across varying degrees of glucose tolerance. The correlation coefficient for normal glucose tolerant subjects was 0.38.

In vivo in normal subjects, insulin infusion was shown to decrease plasma PAI-1 activity within hours (29). Similar data were also reported by Grant et al. (30) and Potter van Loon et al. (31). Lack of effect of insulin on short-term regulation of PAI-1 activity in normoglycemic subjects has also been confirmed by Vuorinen-Markkola et al. (32). It has been suggested

that the in vitro stimulatory effect of insulin on PAI-1 synthesis in hepatocytes may be counteracted by an inhibitory effect of insulin on PAI-1 synthesis and release in extrahepatic tissues, or by stimulation of PAI-1 degradation by insulin (32). These complicated interactions between insulin and PAI-1 may explain why we have not found any association between insulin and PAI-1 activity in our study subjects. It may be speculated also that long-term mild hyperinsulinemia might have led to an upregulation in plasma PAI-1 activity in these subjects, even though immediate insulin and PAI-1 levels are not correlated.

In conclusion, our data suggest that nonobese normal glucose tolerant offspring of type 2 diabetic subjects have elevated PAI-1 activity indicating to hypofibrinolysis in this group. Despite the presence of hyperinsulinemia and, possibly, insulin resistance, there is no association between insulin levels and PAI-1 activity in these subjects. Hypofibrinolysis associated with enhanced PAI-1 activity may be a risk factor for early development of atherosclerosis in these subjects who are genetically prone to the development of diabetes in the future. Large-scale controlled prospective studies including normal and impaired glucose tolerant offspring are required to elucidate whether the increased prevalence of cardiovascular diseases present even at the diagnosis of overt diabetes is related to the clustering of cardiovascular risk factors, including hypofibrinolysis, in the prediabetic state.

Acknowledgments — This work was supported in part by funds from Hacettepe University and Scientific and Technical Research Council of the Turkish Republic (TUBITAK).

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