Increased Incorporation of Antiplasmin Into the Fibrin Network in Patients With Type 1 Diabetes

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CARDIOVASCULAR AND METABOLIC RISK

OBJECTIVE

Diabetes is associated with various vascular complications and is suggested to induce a prothrombotic state. In the current study, we characterized antiplasmin incorporation into fibrin in relation to other fibrinolytic compounds in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

A total of 236 patients with type 1 diabetes and 78 control subjects were investigated. The incorporation of antiplasmin into the fibrin network and the plasma levels of plasminogen activator inhibitor type 1 (PAI-1) activity, tissue plasminogen activator (tPA) activity, tPA/PAI-1 complex, plasmin-antiplasmin complex, antiplasmin, factor XIII, and D-dimer were measured. In addition, we used global assays to study fibrinolysis.

RESULTS

The incorporation of antiplasmin into the fibrin network was significantly higher in patients with type 1 diabetes than in control subjects without diabetes (1.65 ± 0.25 vs. 1.35 ± 0.18 mg/L, respectively; $P < 0.0001$). The patients also had lower PAI-1 activity (2.19 units/mL [interquartile range 0.96–5.42] vs. 4.25 units/mL [1.95–9.0]; $P = 0.0012$) and antiplasmin level in plasma (78.5 ± 13.3 vs. 83.2 ± 15.4 mg/L; $P < 0.05$), resulting in a higher fibrinolytic capacity (shorter clot lysis time; $P = 0.0090$). We did not find any important sex differences regarding fibrinolysis in the patients or in the control subjects.

CONCLUSIONS

Patients with type 1 diabetes incorporate more antiplasmin into the fibrin network than control subjects without diabetes do and have a reduced PAI-1 activity and a shorter clot lysis time. These results suggest that patients with type 1 diabetes produce a fibrin clot that is more resistant to fibrinolysis, which, however, may be counteracted by an increased fibrinolytic potential in plasma.

Diabetes is linked to microvascular disturbances causing neuropathy, retinopathy, and nephropathy, as well as macrovascular complications such as stroke, coronary heart disease, and nonhealing foot ulcers (1). Hypercoagulability and impaired fibrinolysis are pathophysiologically important processes of vascular complications (2). Diabetes has been considered a prothrombotic state (3), partly due to impaired fibrinolysis (4), but has also been associated with increased risk of bleeding when

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antithrombotic therapies are instituted (5,6). It is not uncommon that studies of hemostasis and vascular disease do not distinguish between type 1 and type 2 diabetes despite their different etiologies and pathophysiologies (7). Most studies on hemostasis in diabetic patients have been performed in the context of type 2 diabetes or in diabetic populations where the diabetes disease was poorly defined or described. Since patients with type 1 diabetes constitute a minority of the diabetic population, patients with type 2 diabetes are most likely overrepresented in studies of diabetes and cardiovascular disease (8). However, type 1 diabetes afflicts 15–20% of the diabetic population, which is a considerable number of individuals (9). Moreover, like in type 2 diabetes, cardiovascular morbidity and mortality in type 1 diabetes patients are significantly increased in both sexes, at all ages, and, interestingly, with the highest standardized mortality rates found in young women (10). Disturbances of the fibrinolytic function is associated with cardiovascular disease, mainly due to elevated plasminogen activator inhibitor-1 (PAI-1) (11), but relationships have also been observed with global assays that reflect fibrinolytic capacity of plasma (12,13). The aim of the current study was to assess the fibrinolytic function specifically in type 1 diabetes and whether fibrinolysis was influenced by sex.

RESEARCH DESIGN AND METHODS

Study Population and Setting

A total of 236 age- and sex-matched patients (130 men, 106 women) with type 1 diabetes were recruited from the outpatient clinic at the Department of Endocrinology and Diabetology, Danderyd University Hospital, Stockholm, Sweden, between January and December 2009. All patients were from the Stockholm area. Patients eligible for the study were between the ages of 20 and 70 years with type 1 diabetes. Patients treated with anticoagulants, nonsteroid anti-inflammatory drugs, or platelet inhibitors, including aspirin, were not included.

Seventy-eight control subjects without diabetes, also from the Stockholm area, were recruited during 2009–2010 from the Swedish Population Registry. Control subjects did not have a history of diabetes, and normal plasma glucose levels were required for inclusion.

Investigation Procedures

All patients arrived at the Clinical Research Laboratory between 8:00 and 9:00 A.M. after a 10-h fast. Arm blood pressure (mmHg) was measured in the supine position after 20 min of rest. Venous blood sampling was performed as described below. Measurements of length, weight, and waist-to-hip ratio were performed, and the prevalence of albuminuria was assessed with urinary dipstick tests (Clinitek; Bayer HealthCare). Retinopathy was based on fundoscopic findings performed within the last 6 months.

Laboratory Analyses

Venous blood sampling was performed after 20 min of supine rest. Blood for coagulation and fibrinolytic assays were collected in 5-mL tubes containing 0.129 mol/L sodium citrate (BD; Becton, Dickinson and Company, Franklin Lakes, NJ), while samples for analyses of tissue plasminogen activator (tPA) activity were collected in TriniLIZEStabilyte tubes. The tubes were centrifuged within 30 min at 2,000*g* for 20 min, after which plasma was separated, immediately frozen, and kept at −70°C until analysis.

Fibrinogen

Fibrinogen concentration was determined by a turbidimetric method (14).

Prothrombinfragment 1+2

Prothrombinfragment 1+2 was analyzed using a commercially available method (Enzygnost F1+2 [monoclonal]; Siemens Healthcare Diagnostics Products, Marburg, Germany).

PAI-1 Activity

A commercially available kit (TriniLIZE PAI-1 activity; Trinity Biotech Plc., Bray, Ireland) was used. The manufacturer states that the analysis has a detection range of 2.0–50 units/mL. For improvement of the precision at lower PAI-1 levels, extra measuring points were added at the lower end of the calibration curve. In addition, a point-to-point approach was used instead of linear regression as described earlier (15), with the intra-assay coefficient of variation (CV) 11.5% for the low control (PAI-1 0.9 units/mL) and 5.7% for the high control (PAI-1 19.4 units/ mL) and an interassay CV 4.5% and 3.6%, respectively.

tPA

TriniLIZEtPA activity, a commercially available kit, was used. For healthy humans, the basal level is between 0.2 and 2 units/mL according to the manufacturer.

tPA/PAI-1 Complex

The complex between tPA and PAI-1 was analyzed using TintElize tPA–PAI-1 (Biopool, Umeå, Sweden). The reference range of this analyte is 0.6–6.7 μg/L according to the manufacturer.

Antiplasmin

Antiplasmin antigen in plasma was determined using a standard ELISA, using the same polyclonal antiplasmin goat IgG fraction as both catch and detection antibodies. For detection, the antibodies were conjugated with horseradish peroxidase (16). The CV of the method was $<$5% both within and between series.

Incorporation of Antiplasmin Into Fibrin Network

A fibrin clot (i.e., a fibrin network) was formed after addition of thrombin to recalcified plasma. The clot was washed by saline and solubilized with urea, and then fibrin-bound antiplasmin was measured by the ELISA technique.

In brief, 100 μL platelet-poor plasma $CaCl_2$ was added to a final concentration of 20 mmol/L, and thereafter thrombin was added to a final concentration of 0.2 units/mL. The mixture was incubated at 37°C for 30 min and then stored overnight in a moist chamber at room temperature. The next day, the clot was transferred from the tube to a silk cloth, placed above a filter paper. The clot was washed with saline, which was added slowly on top of the cloth repeatedly.

The fibrin mesh was removed by a pipette tip and transferred into a new tube and 200 μL of 6 mol/L urea was added. Then the tube was incubated in a water bath at 37°C for 120 min. The clot was completely resolved during this process. The tubes were then kept frozen at −70°C until the analysis of antiplasmin was performed. The intra-assay CV was 3.8%, and the interassay CV was 4.2%.

Plasmin-Antiplasmin Complex

Determination of plasmin-antiplasmin complex (PAP) was performed by a classical two-site ELISA. The microtiter wells were coated with a goat antibody raised against PAP and suitably adsorbed by immobilized plasminogen. The antibodies were reacting toward a major neoantigen

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epitope in the antiplasmin moiety of the complex, and the affinity was ~200 times higher for the complex compared with free antiplasmin. The measuring antibody was a horseradish peroxidase–conjugated goat antiplasminogen IgG. Purified PAP was used as a standard (0–12 mg/L). By dilution of the plasma samples 1:200 in phosphate-EDTA-Tween buffer (0.04 mol/L sodium phosphate buffer, pH 7.3, containing 0.1 mol/L NaCl, 0.005 mol/L EDTA, and 0.05% Tween 20), the influence in the assay of free antiplasmin present in plasma was negligible. The minimal detectable concentration using this procedure is ~0.1 mg/L. The accuracy measured as the recovery of pure PAP added to several different plasma samples was close to 100%. CV measured at 1.5 mg/L was <10%. The reference range, based on a large control sample from another study, is ~1–3 mg/L. This method has previously been described (17).

Factor XIII Antigen

An ELISA method was developed using commercially available antibodies: capture Ab, sheep anti-human factor XIII (FXIII) purified IgG (cat. no. SAFXIII-IG; Enzyme Research Laboratories, South Bend, IN); detecting Ab, sheep anti-human FXIII peroxidase conjugate (cat. no. SAFXIII-HRP; Enzyme Research Laboratories). Intra-assay CV was 3.2% and interassay CV 9.5%

D-dimer

D-dimer was analyzed using a commercially available method (Technozym D-Dimer ELISA; Technoclone, Vienna, Austria). The reference range stated by the manufacturer is 0–250 μg/L.

Turbidimetric Assays Measuring Clot Formation and Clot Lysis

This method was performed according to a method described by Carter et al. (18). The turbidimetric lysis assay was performed as follows: 75 μL assay buffer (0.05 mol/L TRIS-HCl, 0.15 mol/L NaCl, pH 7.4) with or without the addition of tPA (final concentration 83 ng/mL) was added to 25 μL citrated plasma (in duplicate) in a microtiter plate, and 50 μL of a mixture of thrombin (final concentration 0.03 units/mL) and $CaCl_2$ (final concentration 7.5 mmol/L) prepared in assay buffer was added with a multichannel pipette.

A single-chain tissue type plasminogen activator (human recombinant) was used (Technoclone) and dissolved in 0.1 mol/L potassium phosphate buffer containing 3.5 mg/mL L-arginine and 0.001% polysorbate Tween 80. The specific activity of tPA is at least 400,000 units/mg. Plates were shaken and the absorbance read at 340 nm every 18 s for 240 cycles. Maximum clot absorbance (max abs) was defined as the absorbance at 40 min of the curve obtained in the absence of tPA. Clot lysis time was the time in seconds from max abs of the curve obtained in the presence of tPA to a 50% fall in absorbance. Max abs had an intra-assay CV of 5.3% and an interassay CV of 4.7%. For clot lysis time, the intra-assay CV was 2.0% and the interassay CV was 9.8%.

Glycated Hemoglobin

Glycated hemoglobin (HbA_{1c}) levels were analyzed by the Mono S method using high-performance liquid chromatography (Variant II; Bio-Rad Laboratories, Hercules, CA) and with a reference level <5.2%. The HbA_{1c} values (Mono S [%]) have been converted to HbA_{1c} National Glycohemoglobin Standardization Program (%) and International Federation of Clinical Chemistry (mmol/mol).

Statistical Analyses

Data are presented as mean ± SD for normally distributed data and median with lower- to upper-quartile values for nonnormally distributed data. Nonnormal and skew distributions were converted and checked to be normally distributed after log transformation. Further analysis was performed by an overall factorial ANOVA including the factors sex and patient/control. The predefined aim to study a putative influence of sex was analyzed by orthogonal contrasts if sex or interaction with sex resulted in a P value <0.10 in the overall ANOVA. The P values concerning the univariate comparison of hemostasic and fibrinolytic variables in patients with type 1 diabetes and control subjects were adjusted for the influence of age, statin use, and systolic and diastolic blood pressure by ANCOVA. The statistical software Statistica 12 (StatSoft, Inc., Tulsa, OK) was used for all these analyses.

Multivariate regression was performed by projection to latent structures regression using the nonlinear iterative partial least squares algorithm. Variables of importance for the projection (VIPs) were listed. VIPs with a value >0.8 are considered important (19). With multivariate methods, it is possible to investigate the relations between all variables in a single context. When fitting a projection to latent structures (PLS) model, this model finds the linear (or polynomial) relationship between a matrix Y (dependent variables) and a matrix X (predictor variables). The statistical software SIMCA P+ (version 12.0.1.0; Umetrics, Ltd., Umeå, Sweden) was used. SIMCA-P+ does also compute the influence on Y of every term in the model, called VIP. VIP is the sum over all model dimensions of the contributions (variable influence).

Ethical Considerations

The protocol of this trial was approved by the local ethics committee of Karolinska Hospital. Written informed consent was obtained from all patients and control subjects.

RESULTS

The baseline characteristics of patients and control subjects (including separation for sex) are presented in Table 1. As shown, 236 patients (130 men, 106 women) and 78 control subjects (34 men, 44 women) were included. Patients were a few years younger than control subjects. The systolic blood pressure was slightly higher in the patients than in the control subjects (P = 0.04), while levels of total cholesterol and LDL cholesterol were lower in patients (most likely due to the fact that a third of the patients received treatment with statins).

Sixteen percent of the patients were treated with continuous subcutaneous insulin infusion, while the others received intermittent doses of short-acting insulin with meals and long-acting insulin analogs once or twice daily.

Demographic data of interest for this study are presented in Table 2. The patients had type 1 diabetes since 22.5 ± 14.2 years with a range from 1 to 67 years. Female patients had higher HbA_{1c} than male patients (8.1 ± 1.4 vs. 7.7 ± 1.1%, P = 0.02, or 65 ± 16.4 vs. 61 ± 13.1 mmol/mol, P = 0.02). Retinopathy was present in 62% of the patients, while 25% had microalbuminuria.

The coagulation and fibrinolytic variables are presented in Table 3. We found a greater incorporation of antiplasmin into the fibrin network in the patients compared with the control

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Table 1—Baseline characteristics in patients with type 1 diabetes (n = 236) and control subjects (n = 78)

	Type 1 diabetic subjects	Control subjects	P
Total population	236 (100)	78 (100)	
Female	106 (45)	44 (56)	
Male	130 (55)	34 (44)	
Age (years)	44 ± 13	49 ± 10	0.008
Female	44 ± 13	48 ± 10	0.052
Male	44 ± 13	49 ± 10	0.071
BMI (kg/m^2)	24.8 ± 4.1	24.6 ± 3.2	0.60
Female	24.1 ± 4.5	24.2 ± 3.4	0.51
Male	25.5 ± 3.6	25.2 ± 2.9	0.91
Waist-to-hip ratio	0.88 ± 0.1	0.85 ± 0.07	0.009
Female	0.82 ± 0.08	0.81 ± 0.07	0.42
Male	0.93 ± 0.08	0.90 ± 0.05	0.05
Smokers	35 (15)	15 (19)	0.48
Systolic BP (mmHg)	128 ± 18	124 ± 12	0.04
Diastolic BP (mmHg)	73 ± 9	77 ± 8	$<$0.001
S-creatinine (mmol/L)	77 ± 20	75 ± 12	0.44
P-glucose (mmol/L)	10.2 ± 4.5	5.1 ± 0.5	$<$0.001
Total cholesterol (mmol/L)	4.5 ± 0.8	5.2 ± 0.7	$<$0.001
Triglycerides (mmol/L)	0.81 ± 0.7	0.84 ± 0.4	0.74
LDL cholesterol (mmol/L)	2.6 ± 0.7	3.4 ± 0.7	$<$0.001
HDL cholesterol (mmol/L)	1.7 ± 0.5	1.6 ± 0.4	0.07
ACE inhibitors	63 (27)	1 (1)	
Statins	76 (32)	1 (1)	
Estrogens, n/N total females in each group (%)	15/106 (14)	4/44 (9)	

Data are means ± SD or n (%) unless otherwise indicated. No patients were on aspirin or anticoagulants. BP, blood pressure; P-glucose, plasma glucose.

subjects (1.65 ± 0.25 vs. 1.35 ± 0.18 mg/L, $P < 0.0001$), whereas the antiplasmin concentrations in plasma were lower in patients than in control subjects (P = 0.032). Furthermore, PAI-1 activity in plasma was lower in patients than in control subjects: 2.19 units/mL (interquartile range 0.96–5.42) vs. 4.25 units/mL (1.95–9.0); P = 0.0012). tPA activity could only be analyzed in patients, since blood samples had not been stored in TriniLIZEStabilyte tubes in the controls. These tubes contain citrated anticoagulant at low pH, which is necessary for the analysis.

tPA activity was correlated to PAI-1 activity (Fig. 1). The data were skewed and therefore log transformed before analysis.

There was no influence of sex on antiplasmin incorporation, tPA activity, or PAI-1 activity. However, we found an influence of sex concerning concentrations of antiplasmin in plasma, which were higher in females (81 ± 11.4 vs. 76.6 ± 14.4 mg/L in female vs. male patients and 87 ± 11.4 vs. 76.1 ± 11.4 mg/L in female vs. male control subjects; $P <$ 0.0001, ANOVA), as was D-dimer in plasma (median concentrations 42.3 vs. 33.6 μg/L in female vs. male patients and 48.5 vs. 21.0 in female vs. male control subjects; P = 0.00016, ANOVA).

In order to get an idea of possible mechanisms behind the increased incorporation of antiplasmin into the fibrin network in diabetes, we performed a multivariate PLS regression analysis. The PLS regression was highly significant ($P <$ 0.0001). The most important variables that were positively correlated with the incorporation of antiplasmin into fibrin among patients were in decreasing order tPA activity, plasma fibrinogen, PAP, and clot lysis time. The most important variables that were negatively correlated in patients were, in decreasing order, PAI-1, FXIII, plasma glucose, and tPA/PAI-1 complex. The only variable explaining incorporation of antiplasmin into fibrin in control subjects was FXIII.

These most important variables (VIPs) explaining the increase in incorporation of antiplasmin into fibrin in type 1 diabetic patients are shown in Fig. 2.

Since fibrinolysis can be influenced by different medications (20), a comparison of the results with regard to drug treatment was performed and is summarized below. Patients on ACE inhibitors had no significant differences in PAI-1 activity or antiplasmin incorporation into fibrin clots compared with patients who were not on ACE inhibitors. However, there was a small difference, with a longer clot lysis time (926 ± 227 s) in patients on ACE inhibitors versus patients not on ACE inhibitors (838 ± 213 s) ($P <$ 0.01). In addition, patients treated with ACE inhibitors were older (53 ± 12 vs. 43 ± 12 years, $P <$ 0.01) and had a longer duration of diabetes (29 ± 15 vs. 20 ± 13 years, $P <$ 0.01).

Patients on statin treatment had a prolonged clot lysis time (906 ± 251 vs. 837 ± 202 s; P = 0.02), had higher PAI-1 activity (3.2 units/mL [interquartile range 1.2–8.0] vs. 1.9 units/mL [0.8–4.1]; $P <$ 0.01), were older (52 ± 11 vs. 43 ± 13 years; $P <$ 0.01), had a higher BMI (26 ± 4 vs. 24 ± 3.8 kg/m^2; $P <$ 0.01), and had longer diabetes duration (26 ± 14 vs. 21 ± 14 years; $P <$ 0.01) than patients not receiving statin treatment.

Table 2—Demographic characteristics of patients with type 1 diabetes

	All patients (n = 236)	Males (n = 130)	Females (n = 106)	P
Diabetes duration (years)	22.5 ± 14.2	22.8 ± 14.3	22.1 ± 14.1	0.72
HbA_{1c} (%)	7.9 ± 1.3	7.7 ± 1.1	8.1 ± 1.4	0.02
HbA_{1c} (mmol/mol)	63 ± 14.2	61 ± 13.1	65 ± 16.4	
Retinopathy[a]	146 (62)	81 (62)	65 (61)	0.98
Microalbuminuria	59 (25)	28 (22)	31 (29)	0.16

Data are means ± SD or n (%). HbA_{1c} according to National Glycohemoglobin Standardization Program (%) and International Federation of Clinical Chemistry and Laboratory Medicine (mmol/mol). [a]Nonproliferative and proliferative.

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Table 3—Hemostatic and fibrinolytic variables in patients with type 1 diabetes and control subjects

	Type 1 diabetic subjects (*n* = 236)	Control subjects (*n* = 78)	*P*#	*P**
Fibrinogen (g/L)	2.98 ± 0.80	3.19 ± 0.60	0.04	<0.0001
Fragment 1+2 (pmol/L)	220 (164–294)	235 (178–332)	0.047	0.35
PAI-1 activity (U/mL)	2.19 (0.96–5.42)	4.25 (1.95–9.00)	<0.0001	0.0012
tPA activity (U/mL)	0.86 (0.61–1.07)	M.D.		
tPA/PAI-1 complex (μg/L)	2.25 (1.7–3.0)	2.6 (1.8–3.4)	0.24	0.98
Antiplasmin, plasma (mg/L)	78.5 ± 13.3	83.2 ± 15.4	0.038	0.032
Antiplasmin, fibrin (mg/L)	1.65 ± 0.25	1.35 ± 0.18	<0.000001	<0.000001
PAP complex (mg/L)	0.25 (0.21–0.32)	0.24 (0.20–0.30)	0.36	0.33
FXIII (AU/mL)	0.83 ± 0.19	0.88 ± 0.20	0.077	0.30
D-dimer (μg/L)	35.8 (22.8–58.2)	36.5 (19–62.3)	0.51	0.64
Clot lysis time (s)	858 ± 228	927 ± 208	0.014	0.0090

Data are means ± SD or median (lower to upper quartiles). M.D., missing data. #Univariate analysis. **P* values adjusted for age, use of statins, and systolic and diastolic blood pressure by ANCOVA.

There were no significant differences in antiplasmin incorporation into the fibrin clot with regard to drug use. No significant differences were seen in the fibrinolytic variables between women using estrogen compared with nonusers. There were only weak correlations between the different fibrinolytic parameters and HbA_{1c} ($r^2 < 0.1$).

C-reactive protein (CRP) was analyzed with two different methods in patients and control subjects (CRP and high-sensitivity CRP, respectively). The values were generally low, and there were no signs of increased inflammation in any of the groups (data not shown).

CONCLUSIONS

The novel finding of the current study is that patients with type 1 diabetes have a statistically highly significant increased incorporation of antiplasmin into the fibrin network, and the findings are very similar regardless of sex. These data were demonstrated by a new robust method for determination of antiplasmin incorporation into the fibrin network. An increased incorporation of antiplasmin into fibrin will cause a more stable fibrin network, and it is indeed possible that this can contribute to diabetes complications. On the other hand, the patients had reduced plasma levels of antiplasmin and fibrinogen and shorter clot lysis time, and, notably, the important fibrinolysis inhibitor PAI-1 was at only half the value in the control subjects; the latter findings indicate an increased fibrinolysis potential.

Antiplasmin is one of the most important endogenous regulators of fibrinolysis, and its concentration in plasma seems strictly regulated (21). Indeed, reduced plasma levels may result in bleeding (22), and elevated antiplasmin levels have been associated with an increased risk for acute myocardial infarction (23). Circulating antiplasmin will bind and inhibit any free plasmin, but it also binds to fibrin and is accumulated in fibrin-rich clots. Thus, antiplasmin will attenuate fibrin degradation through binding and inhibition of plasmin accumulated in the fibrin network of the clot or the thrombus. Despite reduced plasma antiplasmin levels, the patients in the current study had increased incorporation of antiplasmin into their fibrin. Using a different method, Grant and colleagues demonstrated that patients with type 2 diabetes also seem to have increased incorporation of antiplasmin into the fibrin

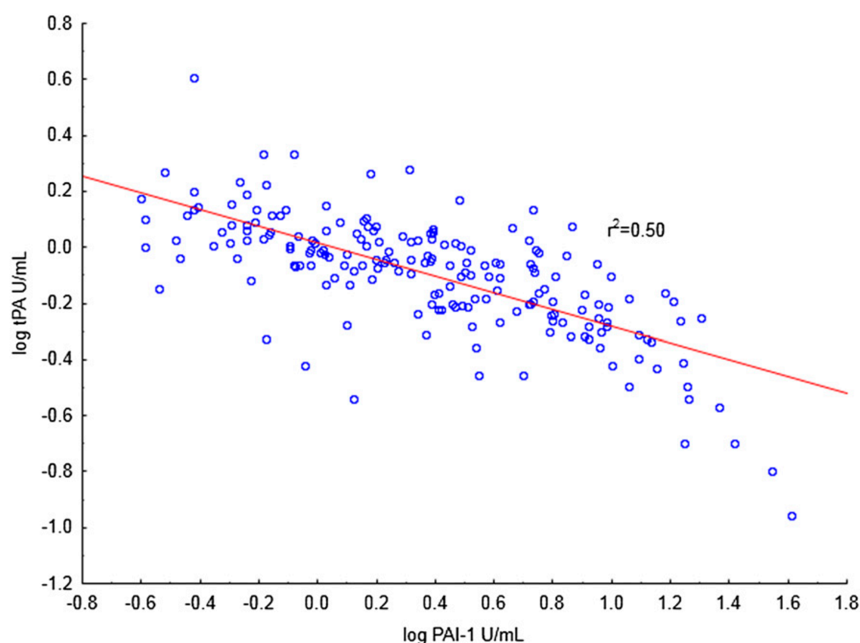

Figure 1—Correlation between active tPA (units per milliliter) and active PAI-1 (units per milliliter) in patients with type 1 diabetes. Data are log transformed owing to skewed distributions.

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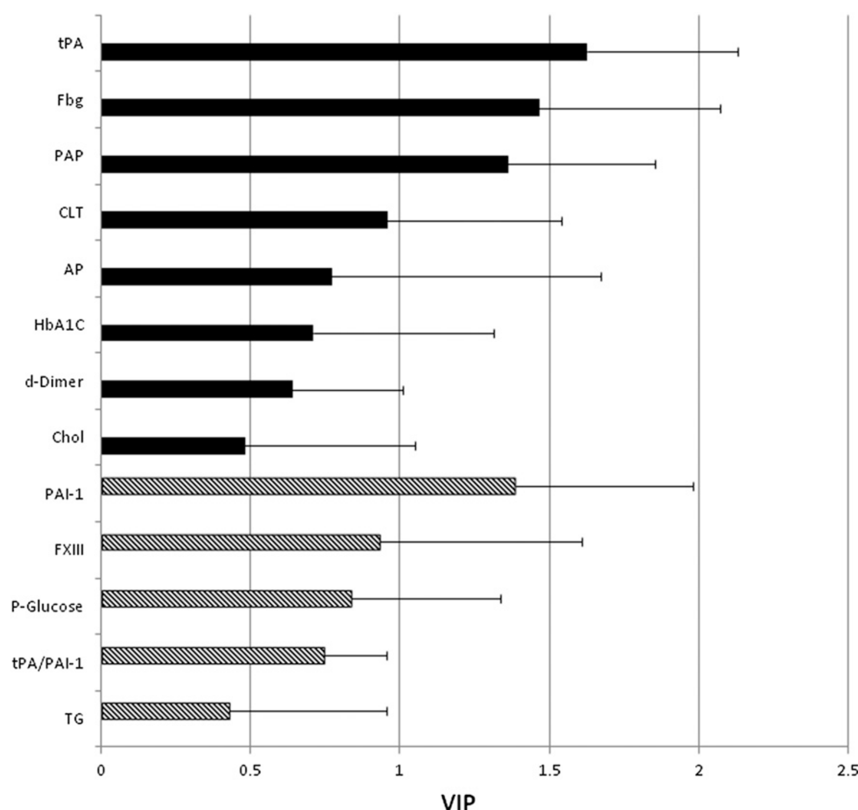

Figure 2—Multivariate PLS regression analysis showing the VIP that explain the increase in incorporation of antiplasmin into fibrin network in patients with type 1 diabetes. The variables that were positively correlated with the incorporation of antiplasmin into fibrin are shown as black bars, while the most important negatively correlated variables are shown as striped bars. AP, antiplasmin in plasma; Chol, cholesterol; CLT, clot lysis time; Fbg, fibrinogen; P-Glucose, plasma glucose; TG, triglycerides.

network (24). As mentioned earlier, increased antiplasmin incorporation into fibrin would thus be expected to cause a more stable fibrin network. However, in our patients with type 1 diabetes, the increased incorporation of antiplasmin into fibrin seemed counteracted by reduced plasma levels of PAI-1 activity and fibrinogen. We also found a shorter clot lysis time in patients than in control subjects. In order to try to understand these somewhat surprising findings and find out more about the mechanism for the increased incorporation of antiplasmin into the fibrin network, we used multivariate PLS regression analysis. The factors that had a significant influence on the antiplasmin incorporation could thus be identified. Interestingly, the factors that had the highest influence pointed in the direction of an increased fibrinolytic capacity. These factors were (in statistical order) higher tPA activity, lower PAI-1 activity, and elevated PAP. These findings were surprising to us, and it would be very interesting to repeat our study in patients with type 2 diabetes, especially viewed against the above-mentioned findings by Grant and colleagues (24).

The main difference in the fibrinolytic function between patients with type 1 and type 2 diabetes is higher PAI-1 activity in type 2 diabetes (25,26), while the incorporation of antiplasmin in fibrin seems to be increased in both these patient groups (24) compared with control subjects.

Another puzzling finding in our study was that incorporation of antiplasmin into the fibrin network is associated with a decreased FXIII concentration, as found in both the patient group and the control group. One possible way to explain this is that FXIII might be consumed in those individuals, but this has to be confirmed.

Women with type 1 diabetes seem to be more afflicted by cardiovascular disease and stroke than men when comparing standardized mortality rates (10,27). We did not, however, find any distinct pattern concerning sex-related differences in fibrinolysis that mechanistically could shed light on these variations in cardiovascular complications between sexes in patients with type 1 diabetes (28). Only two hemostatic variables differed, and these were circulating antiplasmin levels and D-dimer levels, which both were increased in women.

A sex-related difference is the observation that women have generally somewhat higher HbA_{1c} than men (29,30), a finding that was reproduced in our study (8.1 $\pm$ 1.4 vs. 7.7 $\pm$ 1.1%, P = 0.02, or 65 $\pm$ 16.4 and 61 $\pm$ 13.1 mmol/mol, P = 0.02). Glycemic control influences the risk of cardiovascular complications (31,32), and improved glycemic control reduces the risk of complications (33). However, in the current study we found no strong relationship between long-term glycemic control as assessed by levels of HbA_{1c} and fibrinolytic variables.

We found that patients with type 1 diabetes had lower PAI-1 activity, in agreement with another study (34), but this is not widely recognized in the literature. The dominating concept is, rather, that diabetes is a condition associated with impaired fibrinolysis. However, this is mainly due to the fact that type 2 diabetes is more common than type 1 diabetes. It should be recognized that plasma PAI-1 levels are influenced by a lot of different factors, like body weight, waist-to-hip ratio, inflammation, and estrogen treatment, but none of these factors could explain the results obtained in the current study of type 1 diabetes. Hypothetically, there might be a connection between the lack of endogenous insulin production and low PAI-1 activity in type 1 diabetes (35). Type 1 and type 2 diabetes are two different diseases: one in which there is a complete lack of production of insulin (type 1 diabetes) and one in which insulin resistance with high levels of insulin is the dominating feature (type 2 diabetes). Further studies are needed to investigate the possible role of insulin influencing plasma PAI-1 concentration.

Notably, low PAI-1 has been associated with increased bleeding tendency (15,36). As there are differences in PAI-1 activity between patients with type 1 and type 2 diabetes, we suggest that attention should be paid to the type of

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diabetes when performing and analyzing large clinical studies on antithrombotic drugs, especially in the era of new antithrombotic drugs where intensified prophylactic antithrombotic treatment is increasingly common.

In this study, we did not include patients on treatment with aspirin or anticoagulation because these drugs may have affected some of the laboratory analyses performed. It could be argued that the study group therefore is not representative for patients with type 1 diabetes and cardiovascular disease, since this pharmacological treatment is often used in this category of patients. This is in line with the observation that none of the patients included had a previous macrovascular event in their history. Furthermore, the lower levels of fibrinogen in the diabetic patients than in the control subjects may have been due to this exclusion criterion. Notably, the shorter clot lysis time might in part be due to the lower fibrinogen levels in the patients, although PAI-1 activity concentration seems to be the major predictor of clot lysis time (18,37). It should also be emphasized that the patients that were on treatment with ACE inhibitors or statins (more risk factors and/or more advanced disease) had signs of impaired fibrinolysis with a prolonged clot lysis time and elevated PAI-1 activity (statin-treated patients). Together, this would fit with the idea that vascular disease progression and increased risk factor burden are also associated with impaired fibrinolysis in type 1 diabetes. Progressive impairment of fibrinolytic capacity, which seems to go along with increased risk factor burden, would result in an augmented thrombotic risk if there is a concomitant propensity of increased incorporation of antiplasmin in the fibrin network. Since recent evidence suggests that fibrinogen in diabetic patients becomes glycated (38), which has been shown by our own group, an investigation of the mechanisms should be a follow-up of the reported findings

The role for high fibrinolytic capacity in patients with type 1 diabetes is not easy to understand, but it might be involved in pathophysiological mechanisms. This has to be studied further. It is known that the fibrinolytic process is also involved in tissue remodeling and neovascularization, where PAI-1 appears to modulate cellular responses linked to vascular remodeling (39). Proteases of the fibrinolytic system may also play a role in angiogenesis (40), and in patients with type 1 diabetes there is an increased risk for proliferation of retinal vessels and a high fibrinolytic capacity could be a contributing factor.

In conclusion, the current study demonstrates that patients with type 1 diabetes incorporate more antiplasmin into their fibrin network. A high incorporation of antiplasmin into fibrin may result in a clot, which is more resistant to fibrinolysis. However, the increased antiplasmin incorporation could be counteracted by a decreased PAI-1 activity in plasma, decreased antiplasmin levels, and a reduced clot lysis time, together reflecting an increased fibrinolytic capacity. We cannot demonstrate any important influence of sex on fibrinolysis. Thus, these findings indicate a complex hemostatic situation in patients with type 1 diabetes, i.e., an increased risk of thrombotic complications simultaneously with an enhanced fibrinolytic potential. Since enhanced fibrinolysis may be associated with an increased bleeding risk, we suggest that antithrombotic therapy be used with caution in this patient group until further research can provide better guidance on this issue.

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Author Contributions. A.Å. assembled and analyzed data, performed some of the basic statistical analyses, and wrote the manuscript. G.J. and H.W. designed the study, handled the funding, and edited the manuscript. G.E. performed the coagulation and fibrinolysis laboratory assays. P.H. acquired data, performed the advanced statistical analyses, and edited the manuscript. B.W. designed the laboratory work, developed the analysis of incorporation of antiplasmin into the fibrin network, and edited the manuscript. A.Å. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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