Hemostasis Variables in Type I Diabetic Patients Without Demonstrable Vascular Complications

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OBJECTIVE — To determine hemostasis variables in type I diabetic patients without clinically demonstrable micro- and macroangiopathy and to relate them to glycemic control.

RESEARCH DESIGN AND METHODS — Fifty patients and 50 comparable control subjects were enrolled in this study. The patients were subdivided in two groups, according to their level of HbA_{1c} (group 1, $n = 30$, $HbA_{1c} \leq 8\%$; group 2, $n = 20$, $HbA_{1c} > 8\%$). We determined the platelet count, the platelet aggregation in the spontaneous state and in the presence of ADP or collagen, β-thromboglobulin, platelet factor 4, fibrinogen, von Willebrand factor (factors VIII:C, VIIIR:Ag, and VIIIR:VW), plasma and urinary fibrinopeptide A, euglobulin lysis time, anticoagulant proteins C and S, and plasma viscosity.

RESULTS — All coagulation variables were significantly higher in diabetic patients compared with control subjects. Moreover, when the patients were subdivided according to their levels of HbA_{1c}, the hemostatic disturbances appeared significantly more pronounced in the poorly controlled than in the well-controlled subjects.

CONCLUSIONS — This study confirms the existence of a state of hypercoagulability in type I diabetes. This hypercoagulability may be related to poor glycemic control. Our study suggests that the hemostasis disturbances precede demonstrable vascular complications.

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RECEIVED FOR PUBLICATION 30 NOVEMBER 1992 AND ACCEPTED IN REVISED FORM 8 APRIL 1993.

TYPE I DIABETES, INSULIN-DEPENDENT DIABETES MELLITUS; TYPE II DIABETES, NON-INSULIN-DEPENDENT DIABETES MELLITUS; WHO, WORLD HEALTH ORGANIZATION; BP, BLOOD PRESSURE; sBP, SYSTOLIC BLOOD PRESSURE; dBP, DIASTOLIC BLOOD PRESSURE; RIA, RADIOIMMUNOASSAY; HPLC, HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY; CV, COEFFICIENT OF VARIATION; HDL, HIGH-DENSITY LIPOPROTEIN; LDL, LOW-DENSITY LIPOPROTEIN; *V*, VELOCITY AT TANGENT OF STEEPEST PART OF AGGREGATION CURVE; MAX, MAXIMAL AMPLITUDE OF AGGREGATION CURVE; PF 4, PLATELET FACTOR 4; FPA, FIBRINOPEPTIDE A; ELISA, ENZYME-LINKED IMMUNOSORBENT ASSAY; ANOVA, ANALYSIS OF VARIANCE; BMI, BODY MASS INDEX.

Abnormalities of the hemostatic system have been widely described in diabetes and related, at least in part, to the quality of glycemic control. Thus, numerous clinical studies have shown in type I or II diabetic patients or both an hypercoagulable state, manifested by increased platelet aggregation, augmented plasma β-thromboglobulin and PF 4 as a result of platelet activation, elevated von Willebrand factor, raised coagulation factors, and impaired fibrinolysis (1–6). Increased blood viscosity has also been observed (7).

This hypercoagulable state could play a role in the genesis of diabetic vascular disease (1,4,8–11). However, conflicting data have been published (12–19). Moreover, most studies were conducted in patients with established micro- or macroangiopathy or both. Because of different methodologies and variations in patient groups, it is not yet clear whether these coagulation abnormalities precede and are causally related to or are the consequence of diabetic angiopathy. Therefore, this study aimed to determine the coagulation state in 50 type I diabetic patients without clinically demonstrable micro- and macroangiopathy and to relate it to their chronic glycemic control.

RESEARCH DESIGN AND METHODS

— We studied 50 (25 male and 25 female) Belgian type I diabetic individuals and 50 (24 male and 26 female) comparable nondiabetic control subjects. All were Caucasian. Type I diabetes was diagnosed according to WHO criteria (20). The patients, whose clinical and biological characteristics are shown in Tables 1 and 2, were selected from our diabetes clinic on the basis of no demonstrable vascular complications. Thus, none of them had evidence of diabetic retinopathy by fundoscopy through dilated pupils ($n = 50$), fluorescein angiography ($n = 5$), or both, or of incipient nephropathy, defined as a urinary albumin excretion rate >30 mg/24 h. In

TABLE 1—*Clinical characteristics of study subjects*

	Nondiabetic control subjects	Diabetic patients		
		Both groups	Group 1 ($HbA_{1c} \leq 8\%$)	Group 2 ($HbA_{1c} > 8\%$)
n		50	30	20
Sex (M/F)	24/26	25/25	16/14	9/11
Age (yr)*	36 ± 10	36 ± 10	37 ± 9	35 ± 11
Duration of diabetes (yr)	—	11 ± 7	11 ± 8	11 ± 7
BMI (kg/m^2)	23 ± 2	24 ± 2	24 ± 2	23 ± 2
sBP (mmHg)	124 ± 8	126 ± 10	127 ± 11	125 ± 9
dBP (mmHg)	79 ± 2	78 ± 4	77 ± 5	80 ± 2
Daily insulin dose (U/kg body wt)	—	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.2
Complications				
Retinopathy	—	0	0	0
Nephropathy	—	0	0	0
Neuropathy	—	16	5	11
Contraceptive hormones	9/26	5/25	1/14	4/11
Cigarette smokers	20	17	7	10

*Data are means ± SD.

32 patients, albuminuria was assessed in one 24-h collection. In 18 patients, albuminuria was measured in two collections. On the basis of history and physical examination, no signs existed of past or current atherosclerotic coronary, cerebral, or peripheral vascular disease. sBP and dBP levels (Korotkoff phase I and V, respectively) were measured by a physician on the right arm with a sphygmomanometer after at least 5-min rest in the supine position and were normal in all the patients. Similarly, resting electrocardiographic records were also normal. If there was any doubt about the existence of a vascular complication and/or if hypertension defined according to modified WHO criteria was present (BP >160–95 mmHg), the patient was not included in the study. A peripheral neuropathy, established by electromyography, was present in 16 diabetic subjects. No patient had evidence of liver and renal disease. Five female diabetic patients

Table 2—*Biological characteristics of study subjects*

	Nondiabetic control subjects	Diabetic patients		
		Both groups	Group 1 ($HbA_{1c} \leq 8\%$)	Group 2 ($HbA_{1c} > 8\%$)
n		50	30	20
HbA_{1c} (%)	4.1 ± 0.7	8.2 ± 1.2	7.4 ± 0.7	9.4 ± 0.7
C-peptide (pM)				
Fasting	1.56 ± 0.33	0.05 ± 0.10	0.07 ± 0.12	0.02 ± 0.07
Postprandial	4.26 ± 0.46	0.13 ± 0.25	0.16 ± 0.30	0.08 ± 0.14
Cholesterol (mg/dl)	205 ± 38	200 ± 38	199 ± 30	202 ± 47
Triglycerides (mg/dl)	109 ± 74	119 ± 64	116 ± 60	124 ± 69
LDL cholesterol (mg/dl)	125 ± 34	123 ± 34	119 ± 32	130 ± 35
HDL cholesterol (mg/dl)	57 ± 16	56 ± 17	56 ± 18	55 ± 15
Plasma creatinine (mg/dl)	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Albuminuria (mg/24 h)*	—	17 ± 8	15 ± 7	21 ± 9†

Data are means ± SD.
*Normal, <30 mg/24 h.
†$P = 0.035$.

and 9 female control subjects used contraceptives. No subject received any other drug, i.e., antiaggregating agents or anticoagulants, that might affect the hemostatic mechanisms. The number of cigarette smokers was comparable in diabetic patients (n = 17) and control subjects (n = 20).

Subsequently, the patients were divided into two groups, according to their level of HbA_{1c} (Tables 1 and 2). Thus, patients in group 1 (n = 30; HbA_{1c} ≤8%) were in better glycemic control than those in group 2 (n = 20; HbA_{1c} >8%). Tables 1 and 2 show that both groups were comparable with regard to several clinical and biological characteristics. However, albuminuria, although within the normal range, was higher in group 2 than in group 1: 21 ± 9 vs. 15 ± 7 mg/24 h (means ± SD; P = 0.035).

The control subjects were healthy volunteers randomly recruited from the personnel and the students of the University Clinic of Mont-Godinne. All diabetic and normal subjects were aware of the experimental nature of the study and gave informed consent for the blood donations.

Laboratory studies

C-peptide levels were measured by RIA with a commercially available kit (C-PEP/RIA/CT, Medgenix, Fleurus, Belgium). HbA_{1c} was assayed by HPLC using an ion-exchange chromatographic column (Kyoto Daiichi, HA–8121, Menarini, Brussels, Belgium). At HbA_{1c} values of 5.1 and 17.5%, the intra-assay CVs were 1 (n = 20) and 0.9% (n = 11), respectively. At HbA_{1c} values of 4.2 and 7.5%, the interassay CVs were 1.9 (n = 20) and 1.4% (n = 20), respectively. Microalbuminuria was assayed by RIA (Albumine-RIA, Pharmacia, Uppsala, Sweden).

Enzymatic techniques were used to measure cholesterol and triglycerides. HDL cholesterol was determined after precipitation of other lipoproteins with phosphotungstic acid. LDL cholesterol was calculated using Friedewald's formula.

Platelet counts were performed on K3 EDTA anticoagulated blood samples with a Coulter STKS calibrated on Coulter S cal (Coulter, Hialean, FL). For platelet aggregation tests, fibrinogen, factor VIII:C, factor VIIIR:Ag, factor VIIIR:VW, euglobulin clot lysis time, and proteins C and S, blood was collected by venipuncture and diluted (9:1, vol:vol) in 109 mM trisodium citrate. Platelet aggregation results are expressed as V and Max. These data are obtained on PAP–4 Bio/Data aggregometer (Bio/Data, Hatboro, PA) by mixing ADP or collagen with platelet-rich plasma obtained from centrifugation of citrated blood (10 min at 120 g). The final concentrations of ADP and collagen were 5 μM and 190 μg/ml, respectively. Spontaneous aggregation was established after a 30-min incubation in the aggregometer. β-Thromboglobulin and PF 4 were measured by enzyme immunoassay Asserachrom BTG and PF 4 (Stago, Asnieres, France) on plasma collected on Diatube-H (Stago) without tourniquet and with a 20-gauge needle. Platelet depletion was obtained by a two-stage centrifugation (2500 g at 4°C). Fibrinogen was determined according to Clauss with Fibrinomat (BIO Merieux, Lyon, France) on KC10 (Amelung, Lembo, Germany). A one-stage coagulometric assay was used for factor VIII:C determination according to Hardisty on KC10 (Amelung). Enzyme immunoassay Asserachrom (Stago) was used for factor VIIIR:Ag (vWF:Ag). Factor VIIIR:VW (ristocetin cofactor activity) was measured on PAP–4 Bio/Data aggregometer using Bio/Data lyophilized platelets, Aggrecetin Bio/Data, according to the factory recommendations (Bio/Data). Calibration of factor VIII:C, factor VIIIR:Ag, and factor VIIIR:VW was similarly performed on plasma pooled from 30 healthy volunteer subjects (20–40 yr of age).

Plasma FPA was measured by the competitive technique of enzyme immunoassay Asserachrom FPA (Stago) on samples collected on the medium provided in the kit. Urinary fibrinopeptide was measured using an ELISA-based kit (Byk Belga, Brussels, Belgium). Euglobulin clot lysis time was performed before and after ischemia achieved by a sphyngomanometer for 10 min at a pressure intermediate between dBP and sBP. The measurement was performed according to Van Kaulla. Plasma viscosity of heparinized plasma (15 U/ml) was measured at 37°C with an LVT viscosimeter (Wells Brookfield, Stoughton, MA). Results were expressed in relative units with a calibration on distilled water (relative viscosity = 1). The levels of proteins C and S were estimated by an ELISA Asserachrom (Stago). Calibration of proteins C and S was similarly performed on plasma pooled from 30 healthy volunteer subjects (20–40 yr of age).

Blood drawing was performed in the fasting state except for C-peptide, which was determined in both fasting and postprandial states.

Statistical analysis

All numerical variables are expressed as means ± SD. Wilcoxon rank sum test was used to compare variables between diabetic patients and control subjects and to compare the three groups (diabetic groups 1 and 2, control subjects) two by two, after a Kruskal-Wallis ANOVA by ranks has revealed a global heterogeneity between them. The χ^2 square test was used for comparing proportions. Correlations were assessed by Spearman rank correlation coefficient. The simultaneous influence of various factors on hemostatic parameters was assessed by multiple linear regression. All tests performed were two-tailed. Differences were considered significant at $P < 0.05$.

RESULTS

Coagulation variables in all diabetic versus nondiabetic subjects

As shown in Table 3, the diabetic patients had a higher platelet count than

Table 3—*Hemostasis parameters in nondiabetic subjects and diabetic patients*

	Nondiabetic subjects	Diabetic patients
Platelet count (10^3/mm^3)	232 ± 38	263 ± 49*
Platelet aggregation (%)		
In response to ADP†		
V	35 ± 12	41 ± 10‡
Max	68 ± 11	74 ± 13§
In response to collagen		
V	33 ± 8	39 ± 10‡
Max	75 ± 9	81 ± 11‡
Spontaneous		
V	2 ± 2	5 ± 7†
Max	1 ± 2	5 ± 6†
β-Thromboglobulin (ng/ml)	27 ± 7	43 ± 19†
PF 4 (ng/ml)	3.4 ± 1.2	5.5 ± 2.6†
Fibrinogen (mg/dl)	285 ± 54	304 ± 67
Factor VIII:C (%)	96 ± 14	107 ± 31
Factor VIIIR:Ag (%)	91 ± 13	114 ± 37*
Factor VIIIR:VW (%)	91 ± 13	117 ± 33*
Factor VIII:C/factor VIIIR:Ag	1.06 ± 0.14	0.96 ± 0.18*
Plasma FPA (ng/ml)	1.5 ± 0.5	2.1 ± 0.6*
Urinary FPA (ng/mg creatinine)	1.75 ± 0.48	2.37 ± 0.78*
Euglobulin lysis time (min)		
Before ischemia	221 ± 167	224 ± 187
After ischemia	92 ± 98	122 ± 110
Plasma viscosity (cp)	1.7 ± 0.1	1.9 ± 0.1*
Protein C (%)	90 ± 13	99 ± 19‡
Protein S (%)	86 ± 15	95 ± 21‡

Data are means ± SD.
*$P < 0.001$ vs. nondiabetic patients.
†V, velocity (% aggregation/min); max, maximal aggregation.
‡$P < 0.01$.
§$P < 0.05$.

the nondiabetic subjects. They demonstrated an increased platelet aggregation in the spontaneous state and in the presence of ADP or collagen. When compared with control subjects, plasma β-thromboglobulin and PF 4 were also significantly enhanced in the diabetic group. Factor VIII:C was slightly, but not significantly higher in diabetic patients than in the control subjects ($P > 0.1$). In contrast, factor VIIIR:Ag and factor VIIIR:VW were significantly more elevated in the diabetic patients than in the control subjects. We also observed significantly higher plasma and urinary FPA in the diabetic group than in the control group. No significant difference in the euglobulin lysis time was observed between diabetic and nondiabetic subjects. Plasma viscosity levels were higher in diabetic patients than in the control subjects. Table 3 shows that the anticoagulant proteins C and S were also significantly higher in the diabetic than in the control group.

Coagulation variables in poorly versus well-controlled diabetic patients

When the patients were subdivided according to their levels of HbA_{1c}, the hemostatic disturbances appeared significantly more pronounced in the poorly than in the well-controlled subjects. Indeed, as shown in Table 4, the platelet count was higher in group 2 (HbA_{1c} >8%) than in group 1 (HbA_{1c} ≤8%). The basal and activated platelet aggregation were also significantly increased in group 2 versus group 1 (Table 4). Figure 1 shows that β-thromboglobulin and PF 4 levels were significantly higher in group 2 than in group 1: 54 ± 18 and 6.6 ± 2.3 vs. 35 ± 15 and 4.8 ± 2.5 ng/ml, respectively. Figure 2 shows that the plasma fibrinogen levels were 341 ± 51 and 280 ± 66 mg/dl in the two groups ($P = 0.003$). Figure 2 also shows that factor VIII:C was 124 ± 36 and 95 ± 21% in poorly and well-controlled patients, respectively ($P = 0.003$). Factor VIIIR:Ag was 137 ± 45 and 99 ± 2% in groups 2 and 1 ($P = 0.003$). Similarly, factor VIIIR:VW was 137 ± 35 and 103 ± 24% ($P = 0.001$). Factor VIII:C-to-factor VIIIR:Ag ratios were 0.94 ± 0.14 and 0.98 ± 0.20 in groups 2 and 1, respectively (NS). As indicated in Fig. 3, plasma FPA levels were slightly but not significantly higher in group 2 than in group 1: 2.2 ± 0.7 vs. 2.0 ± 0.5 ng/ml, respectively ($P > 0.1$). In contrast, the difference in urinary FPA reached the level of significance: 2.69 ± 0.90 vs. 2.15 ± 0.60 ng/mg creatinine ($P = 0.013$). The euglobulin lysis time was not significantly different between groups but, after ischemia, it was increased ($P = 0.025$) in group 2 versus control subjects (Fig. 4). Plasma viscosity was 1.9 ± 0.1 and 1.8 ± 0.1 cp in poorly and well-controlled patients, respectively ($P = 0.005$). No statistical difference in protein C (group 1: 96 ± 19%; group 2: 105 ± 18%) or in protein S (group 1: 94 ± 23%; group 2: 97 ± 18%) was observed. However, their levels were significantly increased in group 2 when compared with the control subjects ($P = 0.013$; Fig. 5).

Table 5 illustrates the correlation analysis between the hemostasis variables and HbA_{1c} in the total group of diabetic patients. A correlation was found between most of the hemostasis parameters and HbA_{1c}. Furthermore, a

Table 4—*Platelet count and aggregation in the two groups of diabetic patients and in control subjects*

	Nondiabetic control subjects	Diabetic patients	
		Group 1	Group 2
Platelet count (10^3/mm^3)	232 ± 38	250 ± 48	283 ± 43*†
Platelet aggregation (%)			
In response to ADP			
V	35 ± 12	40 ± 11	43 ± 9‡
Max	68 ± 11	70 ± 13	79 ± 11§†
In response to collagen			
V	33 ± 8	36 ± 10	43 ± 9§†
Max	75 ± 9	78 ± 11	85 ± 9§†
Spontaneous			
V	2 ± 2	4 ± 6	7 ± 6§†
Max	1 ± 2	4 ± 6‖	7 ± 7*†

Data are means ± SD.
*P < 0.05 vs. group 1.
†P < 0.001 vs. nondiabetic control subjects.
‡P < 0.05 vs. nondiabetic control subjects.
§P < 0.02 vs. group 1.
‖P < 0.05 vs. nondiabetic control subjects.

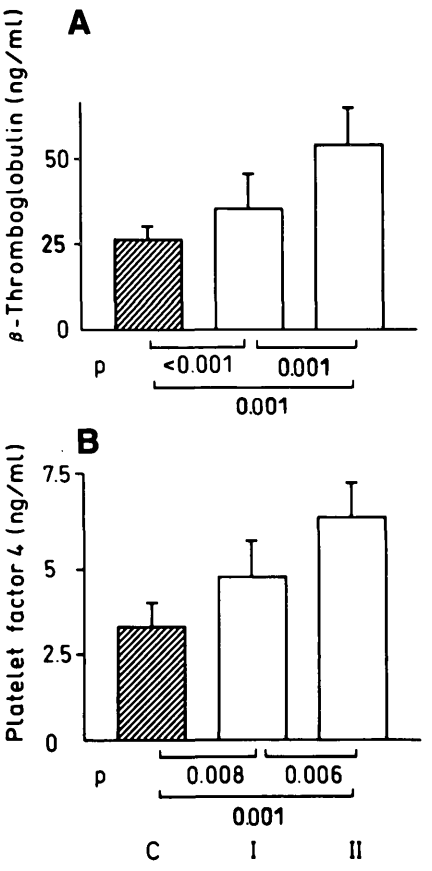

Figure 1—*Plasma β-thromboglobulin (A) and PF 4 (B) in control subjects (C) and in diabetic patients with an HbA_{1c} level <8% (I) or >8% (II).*

relation was noted between albuminuria and HbA_{1c} (r = 0.325; P = 0.021). A significant relationship persisted between HbA_{1c} and most of the hemostasis parameters after adjustment for albuminuria, duration of diabetes, and smoking habits.

Coagulation variables in well-controlled diabetic patients versus nondiabetic subjects

Several hemostasis parameters were comparable in well-controlled patients (HbA_1 ≤8%) and nondiabetic subjects. Thus, the platelet count and the activated aggregation in the presence of ADP or collagen were not significantly higher in diabetic patients than in control subjects (Fig. 2; Table 4). As shown in Fig. 2, fibrinogen was 250 ± 48 (group 1) and 232 ± 38 mg/dl (control subjects; NS). Similarly, factors VIII:C and VIIIR:Ag levels were comparable: 95 ± 21 and 99 ± 21% in diabetic patients, 96 ± 14 and 91 ± 13% in control subjects. As shown in Fig. 4, the euglobulin lysis time was also comparable in the well-controlled diabetic subjects (before ischemia, 197 ± 168 min; after ischemia, 96 ± 84 min) and in the control group (before ischemia, 221 ± 167 min; after ischemia, 92 ± 98 min).

However, several other parameters remained significantly different in diabetic patients and nondiabetic subjects, despite the good glycemic control. Thus, β-thromboglobulin and PF 4 were 35 ± 15 and 4.8 ± 2.5 ng/ml in well-controlled diabetic patients vs. 27 ± 7 and 3.4 ± 1.2 ng/ml in control subjects (P <0.001 and P = 0.008, respectively; Fig. 1). Factor VIIIR:VW also remained different: 103 ± 24 vs. 91 ± 13% in diabetic patients and control subjects, respectively (P = 0.019; Fig. 2). Plasma and urinary FPA levels were significantly higher in diabetic patients than in control subjects: 2.0 ± 0.5 vs. 1.5 ± 0.5 ng/ml (P = 0.001) and 2.15 ± 0.60 vs. 1.75 ± 0.48 (P = 0.002), respectively (Fig. 3). Finally, plasma viscosity was 1.8 ± 0.1 (group 1) and 1.7 ± 0.1 cp (control subjects) (P < 0.001).

As far as anticoagulant proteins were concerned, we observed protein C levels of 96 ± 19% in diabetic patients with HbA_1 <8% and 90 ± 13% in control subjects (NS). Protein S levels were 94 ± 23 and 86 ± 15% in diabetic patients of group 1 and control subjects (P = 0.039; Fig. 5).

CONCLUSIONS— This study evidenced a state of hypercoagulability in type I diabetic patients, when compared with nondiabetic subjects. Indeed, we observed an abnormal platelet behavior that was characterized by an increased platelet count, a spontaneous hyperaggregability, a platelet hypersensitivity to different aggregating agents and a pathological platelet activation, as assessed by high levels of β-thromboglobulin and PF 4. We also reported elevated von Wille-

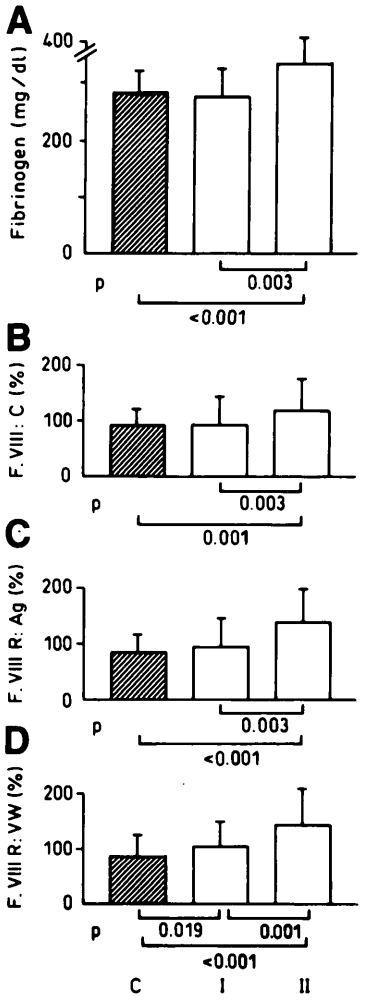

Figure 2—*Plasma fibrinogen (A) and factor VIII (factors VIII:C [B], VIIIR:Ag [C], and VIIIR:VW [D]) in control subjects (C) and in diabetic patients with an HbA_{1c} level <8% (I) or >8% (II).*

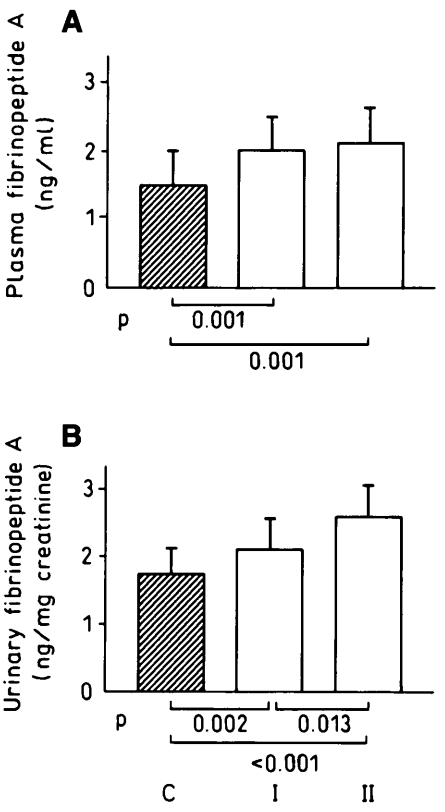

Figure 3—*Plasma (A) and urinary FPA (B) in control subjects (C) and in diabetic patients with an HbA_{1c} level <8% (I) or >8% (II).*

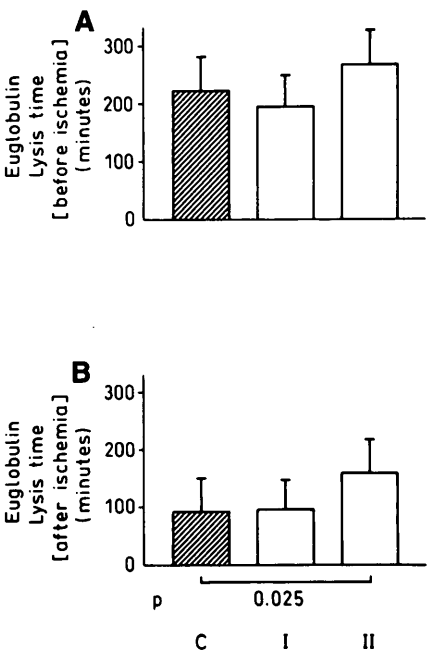

Figure 4—*Euglobulin lysis time before (A) and after ischemia (B) in control subjects (C) and in diabetic patients with an HbA_{1c} level<8% (I) or >8% (II).*

brand factor, which was determined by measuring the antigen (factor VIIIR:Ag) and its activity (factor VIIIR:VW). This clearly expresses endothelium damage. Diabetic patients also demonstrated high plasma and urine FPA values, which reflect a coagulation activation and, consequently, the formation of fibrin. Proteins C and S, two physiological inhibitors of coagulation, previously reported as decreased (21,22) or increased (23) in diabetic patients, were found elevated in this study. Antithrombin III was not measured in our study. Many investigations have previously indicated that it may be decreased in diabetes (24). The high levels of FPA in this study indirectly support such a view, because decreased antithrombin III results in an increased thrombin formation and activation of the coagulation cascade. An increased plasma viscosity was also evidenced in our study. Overall, these results confirmed and extended data obtained by others.

We also demonstrated an influence of chronic glycemic control, as assessed by HbA_{1c} values, on these hemostasis disturbances. The latter were indeed systematically more pronounced in poorly than in well-controlled patients. Interestingly, diabetic patients with an HbA_{1c} level <8% nearly normalized several hemostasis parameters: e.g., platelet count, aggregation, factor VIII:C, factor VIIIR:Ag, and euglobulin lysis time tended toward normal values. These data agree with those of Vukovich et al. (25), who showed that hypercoagulability and endothelial cell dysfunction, as assessed by factor VIII measurements, could be corrected in diabetic patients by 2 wk of intensified insulin therapy. However, despite good metabolic control, platelet specific proteins, factor VIIIR:VW, FPA levels, and plasma viscosity remained abnormal in our diabetic patients, when compared with nondiabetic subjects. These data suggest that strict chronic normoglycemia is required to normalize the hypercoagulable state characterizing type I diabetes. This concept is reinforced by the correlations found in this study between HbA_{1c} and most of the hemostasis parameters. Data of the literature also support our observations: Mayfield et al. (26) were able to reduce, but not to normalize, thromboxane A_2, a platelet activation marker, during a brief

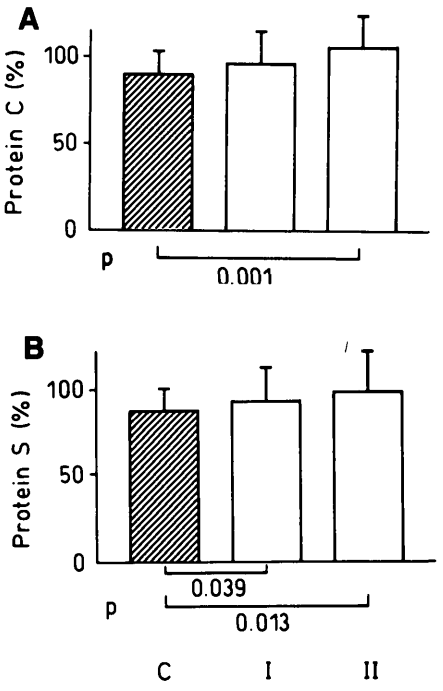

Figure 5—*Plasma protein C (A) and protein S (B) in control subjects (C) and in diabetic patients with an HbA_{1c} level <8% (I) or >8% (II).*

period of continuous insulin infusion. Ceriello et al. (27) demonstrated, in nondiabetic volunteer subjects, that a short period of moderate hyperglycemia (10 mM) was able to produce a sustained increase in FPA values. Moreover, a significant relationship between FPA and glycemic control was also found (28). Thus, hemostatic disturbances in diabetes appear as a continuum, with abnormalities already present in the case of mild hyperglycemia. The higher levels of anticoagulant proteins, in particular protein S, found in our well-controlled diabetic patients, might represent a protective response to the other hemostatic disturbances that favored hypercoagulability (23).

In this study, a correlation was found between HbA_{1c} and albuminuria. Few studies, if any, have considered the influence of confounders such as albuminuria levels still in the normal range on the relationship between HbA_{1c} and hemostasis variables. Our data indicated that most of the hemostatic disturbances remained significantly related to glycemic control, even when albuminuria was taken into account. Possibly, more pronounced endothelium dysfunction, as a consequence of poorer glycemic control, would be responsible for the higher levels of albuminuria in group 2 than in group 1.

Hypercoagulability has been claimed to be a result rather than a cause of diabetic vascular disease. Indeed, more marked abnormalities were reported in patients with diabetic micro- or macroangiopathy or both than in those without (1,4,8,10,29,30). It was even suggested that elevated levels of β-thromboglobulin, PF 4, and factor VIIIR:Ag were found only in diabetic patients with an abnormal albumin excretion rate, when compared with normoalbuminuric diabetic subjects (31, 32). Stehouwer et al. (33,34) also reported that higher levels of factor VIIIR:Ag were observed in diabetic patients who developed nephropathy than in those who did not. Our results are not entirely in accordance with these data. We observed that abnormal hemostasis parameters were present in young type I diabetic patients without clinically detectable microvascular complication or

Table 5—*Correlations between hemostasis parameters and HbA_{1c}*

	HbA_{1c}		Multiple linear regression (*P* values)			
	r_s	*P* value	HbA_{1c}	Albuminuria	Duration of diabetes	Smoking habits
Platelet count	0.418	0.002	<0.001	NS	0.020	NS
Platelet aggregation						
ADP						
V	0.089	NS	NS	NS	NS	NS
Max	0.426	0.002	0.015	NS	NS	NS
Collagen						
V	0.385	0.006	0.016	NS	NS	NS
Max	0.343	0.015	0.034	NS	NS	NS
Spontaneous						
V	0.366	0.009	NS	NS	NS	NS
Max	0.296	0.037	NS	NS	NS	NS
β-thromboglobulin	0.641	<0.001	<0.001	NS	NS	NS
PF 4	0.332	0.018	0.022	NS	NS	NS
Fibrinogen	0.334	0.018	NS	0.031	NS	NS
Factor VIII						
:C	0.492	<0.001	<0.001	0.049	NS	NS
R:Ag	0.513	<0.001	<0.001	0.049	NS	NS
R:VW	0.566	<0.001	<0.001	NS*	NS	NS
FpA						
Plasma	0.267	NS*	NS	NS	NS	NS
Urine	0.382	0.006	0.035	0.038	NS	NS
Euglobulin lysis time						
Before ischemia	0.129	NS	NS	NS	NS	0.042
After ischemia	0.245	NS*	NS	NS	NS	NS*
Plasma viscosity	0.508	<0.001	0.005	NS*	NS	NS
Protein C	0.376	0.007	NS*	NS	NS*	NS
Protein S	0.131	NS	NS	NS	NS	NS
Albuminuria	0.325	0.021				
Duration of diabetes	−0.063	NS				

*$0.05 < P < 0.1$.

macrovascular disease. Colwell et al. (11) previously demonstrated alterations in platelet and endothelial function occurring early in the diabetic state that may contribute to vascular disease. Our data are also supported by Lamberton et al. (15) and Ford et al. (35), who observed in type I and II diabetic patients an activation of the coagulation system that preceded the appearance of clinically detectable complications. Borkenstein and Muntean (36) reported the presence of alterations in factor VIIIR:Ag in type I diabetic children without vascular disease. Moreover, in 15 type I diabetic adults, Vukovich et al. (25) also reported factor VIII:C and factor VIIIR:Ag alterations, despite the absence of micro- and macroangiopathy.

The latter data and our own results, although we cannot exclude that our patients may not have been completely free from a preclinical microvascular disease, are consistent with disturbances of hemostasis being an early event in the course of diabetes. The observation in our study of elevated factor VIIIR:Ag before microalbuminuria supports this concept. It is not clear whether these hemostasis abnormalities are a cause of angiopathy or only the consequence of silent, chronic endothelium damage.

In conclusion, our study confirms the existence of hypercoagulability in type I diabetes and shows that the latter may be related to a poor chronic glycemic control. Our study further suggests that hypercoagulability precedes demonstrable vascular complications.

References

1. Bensoussan D, Lzvy-Toledano S, Passa P, Caen J, Canivet J: Platelet hyperaggregation and increased plasma level of von Willebrand factor in diabetics with retinopathy. *Diabetologia* 11:307–12, 1975
2. Fuller J, Keen H, Jarrett R, Omer T, Meade T, Chakrabarth R, North W, Stirling Y: Haemostatic variables associated with diabetes and its complications. *Br Med J* 2:964–69, 1979
3. Borsey D, Dawes J, Fraser D, Prowse C, Elton R, Clarke B: Plasma beta-thromboglobulin in diabetes mellitus. *Diabetologia* 18:353–57, 1980
4. Christe M, Fritschi J, Lammle B, Tran T, Marbet G, Berger W, Duckert F: Fifteen coagulation and fibrinolysis parameters in diabetes mellitus and in patients with vasculopathy. *Thromb Haemost* 52:138–48, 1984
5. Davi G, Catalano I, Averna M, Notarbartolo A, Strano A, Ciabattoni G, Patrono C: Thromboxane biosynthesis and platelet function in type II diabetes mellitus. *N Engl J Med* 322:1769–74, 1990
6. Schernthaner G, Vukovich T, Gisinger CH: Anomalies de l'hémostase au cours du diabéte sucré. *J Diabétologie de l'Hôtel Dieu*. Flammarion, Paris 1991, p. 91–108
7. Paisey R, Harkness J, Hartog M, Chadwick T: The effect of improvement in diabetic control on plasma and whole blood viscosity. *Diabetologia* 18:345–49, 1980
8. Preston F, Ward J, Marcola B, Porter N, Timperley W, O'Malley B: Elevated β-thromboglobulin levels and circulating platelet aggregates in diabetic microangiopathy. *Lancet* 1:238–40, 1978
9. Ishibashi T, Tanaka K, Taniguchi Y: Platelet aggregation and coagulation in the pathogenesis of diabetic retinopathy in rats. *Diabetes* 30:601–606, 1981
10. Dornan T, Rhymes I, Cederholm-Williams S, Rizza C, Pepys M, Bron A, Turner R: Plasma haemostatic factors and diabetic retinopathy. *Eur J Clin Invest* 13:231–35, 1983
11. Colwell J, Winocour P, Halushka P: Do platelets have anything to do with diabetic microvascular disease? *Diabetes* 32 (Suppl. 2):14–19, 1983
12. Elving L, Casparie A, Miedema K, Russchen C: Plasma β-thromboglobulin in diabetics with and without microangiopathic complications (Letter). *Diabetologia* 21:160–61, 1981
13. Rak K, Beck P, Udvardy M, Pflieger G, Misz M, Boda Z: Plasma levels of beta-thromboglobulin and factor VIII-related antigen in diabetic children and adults. *Thromb Res* 29:155–62, 1983
14. Fritschi J, Christe M, Lammle B, Marbet G, Berger W, Duckert F: Platelet aggregation, β-thromboglobulin and platelet factor 4 in diabetes and in patients with vasculopathy. *Thromb Haemost* 52:236–39, 1984
15. Lamberton RP, Goodman A, Kassoff A, Rubin C, Treble D, Saba T, Merimee T, Dodds W : Von Willebrand Factor (VIII R:Ag), fibronectin, and insulin-like growth factors I and II in diabetic retinopathy and nephropathy. *Diabetes* 33: 125–29, 1984
16. Pasi K, Enayat MS, Horrocks P, Wright A, Hill F: Qualitative and quantitative abnormalities of von Willebrand antigen in patients with diabetes mellitus. *Thromb Res* 59:581–91, 1990
17. Cho N, Becker D, Ellis D, Kuller L, Drash A, Orchard T: Spontaneous whole blood platelet aggregation, hematological variables and complications in insulin-dependent diabetes mellitus: the Pittsburgh Epidemiology of Diabetes Complications Study. *J Diabetic Complications* 6:12–18, 1992
18. Stehouwer C, Zellenrath P, Polak B, Baarsma G, Nauta J, Donker A, Den Ottolander G: Von Willebrand factor and early diabetic retinopathy: no evidence for a relationship in patients with type 1 (insulin-dependent) diabetes mellitus and normal urinary albumin excretion. *Diabetologia* 35:555–59, 1992
19. Silveira A, Sjoberg S, Blomback M, Ostman J: Von Willebrand factor antigen in plasma and urine in patients with type I (insulin-dependent) diabetes mellitus with and without nephropathy. *J Diabetic Complications* 6:89–95, 1992
20. World Health Organization: *WHO Expert Committee on Diabetes Mellitus*. Geneva, World Health Org., 1980 (Tech. Rep. Ser., no. 646)
21. Vukovich T, Schernthaner G: Decreased protein C levels in patients with insulin-dependent type I diabetes mellitus. *Diabetes* 35:617–19, 1986
22. Schernthaner G, Vukovich T, Knobl P, Hay U, Muller M: The effect of near-normoglycemic control on plasma levels of coagulation factor VII and the anticoagulant proteins C and S in insulin-dependent diabetic patients. *Br J Haema-*

tol 73:356–59, 1989
23. Vagano S, Mannucci P, D'Angelo A, Gelfi C, Gensini G, Rostagno C, Neri Serneri G: Protein C antigen is not an acute phase reactant and is often high in ischemic heart disease and diabetes. *Thromb Haemost* 52:263–66, 1984
24. Ceriello A, Dello Russo P, Zuccotti C, Florio A, Nazarro S, Pietrantuono C, Rosato GB: Decreased antithrombin III activity in diabetes may be due to nonenzymatic glycosylation. A preliminary report. *Thromb Haemost* 50:633–34, 1983
25. Vukovich T, Schernthaner G, Knobl P, Hay U: The effect of near-normoglycemic control on plasma factor VIII/von Willebrand factor and fibrin degradation products in insulin dependent diabetic patients. *J Clin Endocrinol Metab* 69:84–89, 1989
26. Mayfield R, Halushka P, Wohltmann H, Lopes-Virella M, Chambers J, Loadholt C, Colwell J: Platelet function during continuous insulin infusion treatment in insulin-dependent diabetic patients. *Diabetes* 34:1127–33, 1985
27. Ceriello A, Giugliano D, Quatraro A, Dello Russo P, Marchi E, Torella R: Hyperglycemia may determine fibrinopeptide A plasma level increase in humans. *Metabolism* 38:1162–63, 1989
28. Jones RL: Fibrinopeptide-A in diabetes mellitus: relation to levels of blood glucose, fibrinogen disappearance and hemodynamic changes. *Diabetes* 34:836–43, 1985
29. Coller B, Frank R, Milton R, Gralnick H: Plasma cofactors of platelet function: correlation with diabetic retinopathy and hemoglobins A_{1a-c}. *Ann Int Med* 88:311–16, 1978
30. Porta M, Townsend C, Clover G, Nanson M, Alderson A, Mc Craw A, Kohner E: Evidence for functional endothelial cell damage in early diabetic retinopathy. *Diabetologia* 20:597–601, 1981
31. Jensen T, Bjerre-Knudsen J, Feldt-Rasmussen B, Deckert T: Features of endothelial dysfunction in early diabetic nephropathy. *Lancet* 1:461–63, 1989
32. O'Donnell MJ, Le Guen C, Lawson N, Gyde O, Barnett A: Platelet behaviour and haemostatic variables in type 1 (insulin-dependent) diabetic patients with and without albuminuria. *Diabetic Med* 8:624–28, 1991
33. Stehouwer C, Stroes E, Hackeng W, Mulder P, Ottolander G: Von Willebrand factor and development of diabetic nephropathy in IDDM. *Diabetes* 40:971–76, 1991
34. Stehouwer C, Nauta J, Zeldenrust G, Hackeng W, Donker A, Ottolander G: Urinary albumin excretion, cardiovascular disease and endothelial dysfunction in non-insulin-dependent diabetes mellitus. *Lancet* 340:319–23, 1992
35. Ford I, Singh T, Kitchen S, Makris M, Ward J, Preston F: Activation of coagulation in diabetes mellitus in relation to the presence of vascular complications. *Diabetic Med* 8:322–29, 1991
36. Borkenstein M, Muntean W: Elevated factor VIII activity and factor VIII related antigen in diabetic children without vascular disease. *Diabetes* 31:1006–09, 1982