Fibrinogen Is a Marker for Nephropathy and Peripheral Vascular Disease in Type 1 Diabetes

Studies of plasma fibrinogen and fibrinogen gene polymorphism in the DCCT/EDIC cohort

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ABSTRACT — We examined whether plasma fibrinogen levels and the β-fibrinogen gene G^{-455}→A polymorphism were related to microvascular or macrovascular disease in patients (n = 909) with type 1 diabetes enrolled in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC). Univariate regression showed that fibrinogen levels were correlated with BMI (r = 0.15; $P < 0.0001$), HbA_{1c} (r = 0.11; P = 0.0014), total cholesterol (r = 0.17; $P < 0.0001$), and LDL cholesterol (r = 0.16; $P < 0.0001$) in all patients. In men, but not women, waist-to-hip ratio (r = 0.20; $P < 0.0001$) and triglycerides (r = 0.13; P = 0.0047) also became powerful predictors of fibrinogen level; in women, but not men, fibrinogen was correlated with both diastolic (r = 0.16; P = 0.0011) and systolic (r = 0.11; P = 0.0241) blood pressure. Fibrinogen was correlated with urinary albumin excretion rates in men (r = 0.13; P = 0.0033), but not in women. In both sexes, however, the development of proteinuria (albumin excretion >300 mg/24 h) was accompanied by 1.5-fold increment in plasma fibrinogen compared with patients with normal excretion or microalbuminuria. In addition, high fibrinogen levels were associated with a lower average ankle-brachial index in women (r = −0.13; P = 0.0075), but not men. Multiple regression analyses demonstrated that plasma fibrinogen was independently correlated with high albumin excretion rate in men, and with low average ankle-brachial index in women. Fibrinogen was not correlated with the severity of retinopathy. Carotid artery intima-medial thickness was not correlated with fibrinogen, and the G^{-455}→A polymorphism in the 5′ promoter region of the β-fibrinogen gene did not influence circulating fibrinogen levels. However, the presence of the more common G^{-455} allele was associated with greater intima-medial thickness in the internal carotid artery (ANCOVA P = 0.045). Last, hyperfibrinogenemia in type 1 diabetes is associated with components of the insulin resistance syndrome trait cluster, and the association is influenced by sex.

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Abbreviations: ABI, ankle-brachial index; CVD, cardiovascular disease; DCCT, Diabetes Control and Complication Trial; EDIC, Epidemiology of Diabetes Interventions and Complications; ETDRS, Early Treatment Diabetic Retinopathy Study; IMT, intimal-medial thickness; MUSC, Medical University of South Carolina; UTR, untranslated region.

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

Epidemiological studies in the general population indicate an association between fibrinogen levels and the subsequent development of all the major atherosclerotic cardiovascular events, including myocardial infarction, stroke, and peripheral arterial disease (1–4). Whereas cardiovascular disease (CVD) is common in type 1 diabetes, the role of fibrinogen is unclear. In one study, no significant relationship was found between fibrinogen and symptomatic or electrocardiographic evidence of CVD (5). It is possible that this was due to the relatively imprecise methods for assessment of vascular disease. In contrast, several studies have shown an association between hyperfibrinogenemia and microalbuminuria/albuminuria in type 1 diabetes (6–11). It is not clear whether hyperfibrinogenemia is a primary abnormality and precedes the development of diabetic nephropathy. Alternatively, or in addition, elevated fibrinogen levels may occur secondary to nephropathy but could contribute to further decline in renal function and the nephropathy associated with increased CVD risk.

A number of mechanisms may explain the association between hyperfibrinogenemia and vascular disease. Fibrinogen may be indirectly associated with vascular disease as a marker of unstable lesions that are undergoing subintimal hemorrhage or with potent risk factors such as smoking (3). In addition, hyperfibrinogenemia may be an indicator of inflammatory vascular changes and endothelial dysfunction (12). Alternatively, fibrinogen may be directly involved in atherosclerosis and thrombosis (13–15). Hyperfibrinogen levels lead to enhanced coagulant activity and are associated with increased blood viscosity. Fibrinogen is also a cofactor in platelet activation and may directly contribute to plaque formation,

where it is converted to fibrin and fibrinogen degradation products (13,16).

Multiple factors influence plasma fibrinogen levels, including age, sex, smoking status, and lipid levels, and fibrinogen is associated with markers of insulin resistance (2,17). Evidence also suggests a genetic influence, with estimates of heritability ranging between 30 and 50% (18,19). The interaction between genetic and environmental determinants is unclear.

Fibrinogen is composed of three pairs of nonidentical polypeptide chains denoted Aα, Bβ, and γ. Regulation of fibrinogen gene expression is not fully understood; however, synthesis of the Bβ chain has been shown to be the rate-limiting step in fibrinogen synthesis. Genetic studies in nondiabetic subjects have shown an association between β-fibrinogen gene polymorphisms and fibrinogen levels (18–23). β-Fibrinogen gene variation may also influence the association between smoking and hyperfibrinogenemia (19,21). Polymorphisms of the β-fibrinogen gene have also been shown to be associated with myocardial infarction, stroke, and peripheral vascular disease in nondiabetic subjects (22–25).

The aims of this study, therefore, were to investigate the association between fibrinogen levels and micro- and macrovascular complications in well-characterized type 1 diabetic subjects from the Diabetes Control and Complication Trial/Early Treatment Diabetic Retinopathy Study (DCCT/EDIC) cohort. We also wished to determine the impact of β-fibrinogen gene variation on fibrinogen levels and to investigate if genotype predicts development of vascular complications.

Table 1—*Clinical characteristics of 909 patients with type 1 diabetes in the DCCT/EDIC cohort*

Parameter	Men	Women	*P*
n	504	405	
Age (years)	39.9 ± 6.7	39.2 ± 7.2	0.12
Duration of diabetes (years)	17.1 ± 4.6	17.8 ± 4.9	**0.03**
BMI (kg/m^2)	27.2 ± 3.9	26.3 ± 4.3	**0.002**
Waist-to-hip circumference ratio	0.9 ± 0.1	0.8 ± 0.7	**<0.0001**
Systolic blood pressure (mmHg)	123 ± 13	116 ± 14	**<0.0001**
Diastolic blood pressure (mmHg)	77 ± 9	73 ± 9	**<0.0001**
Total cholesterol (mg/dl)	189 ± 37	188 ± 34	0.43
Triglycerides (mg/dl)	98 ± 72	77 ± 49	**<0.0001**
LDL cholesterol (mg/dl)	118 ± 32	110 ± 30	**<0.0001**
HDL cholesterol (mg/dl)	52 ± 13	63 ± 15	**<0.0001**
HbA$_{1c}$ (%)	8.2 ± 1.3	8.2 ± 1.4	0.95
Mean HbA$_{1c}$ during DCCT (%)	8.1 ± 1.3	8.1 ± 1.5	0.60
Albumin excretion rate (mg/24 h)	133 ± 749	91 ± 757	0.40
Log albumin excretion rate (mg/24 h)	2.7 ± 1.4	2.5 ± 1.2	**0.01**
ETDRS retinopathy score	4.6 ± 3.3	4.4 ± 3.4	0.29
Average ankle-brachial index	1.12 ± 0.12	1.09 ± 0.10	**<0.0009**
Common carotid IMT (mm)	0.69 ± 0.09	0.66 ± 0.08	**<0.0001**
Internal carotid IMT (mm)	0.71 ± 0.17	0.64 ± 0.16	**<0.0001**
Intensive treatment subgroup during DCCT (*n*)	50	52	0.49
Smoking (*n*)	19	18	0.76
Hypertension (*n*)*	43	27	**<0.0001**
Albumin excretion rate ≥40 mg/24 h (*n*)	15	12	0.11
Fibrinogen genotype (GG/GA/AA) (*n*)	58/37/5	63/31/6	0.17

Data are as means ± SD. *Hypertension is defined as previously documented hypertension or blood pressure ≥140/90 mmHg at the time the blood sample was obtained for analysis of fibrinogen. *P* values in **bold type** are statistically significant.

RESEARCH DESIGN AND METHODS

Patients and blood samples. Study subjects were recruited from the DCCT/EDIC cohort of patients with type 1 diabetes (26,27) as part of a collaborative project between investigators at the Medical University of South Carolina (MUSC) and the DCCT/EDIC research group. The primary goal of the project is to identify risk factors and mechanisms for macrovascular disease in type 1 diabetes.

Sample collection. A 105.5-ml blood sample was collected after an overnight fast of at least 8 h from 957 patients consecutively appearing for their biennial exam according to the DCCT/EDIC research protocol (27). The initial 63-ml aliquot was collected and used for nonrelated research protocols. The subsequent 11-ml aliquot (nine volumes) of blood was collected using 0.13 mol/l trisodium citrate, pH 4 (one volume), as an anticoagulant. The blood sample and the anticoagulant were mixed by inverting the collection tube, which was maintained at 4°C for a period not exceeding 2 h before further processing. Erythrocytes were sedimented by centrifugation (3,000*g*) for 15 min. The plasma was transferred into polypropylene cryovials (Sarstedt, Newton, NC) and immediately stored at −20°C for a period not exceeding 30 days. Samples were then shipped on dry ice to the study site via overnight courier and were stored at −70°C for up to 2 months until fibrinogen levels were determined. Samples were thawed one time and the assays run immediately. Results are reported for the analysis of 909 samples. Results from 48 analyses were unavailable due to instrument failure, insufficient sample quantity, or severe sample hemolysis. Samples taken at the same venipuncture were shipped on dry ice to the DCCT/EDIC trial's Central Biochemistry Laboratory, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, for analysis of lipid profile, serum creatinine, and HbA$_{1c}$ (26). Plasma for lipoprotein-related analyses and blood cells for DNA analyses were sent to MUSC. The studies were approved by the Institutional Review Boards at all DCCT/EDIC Clinical Study Centers and at MUSC, and all patients signed an informed consent.

Clinical procedures conducted by the DCCT/EDIC Research Group. Each EDIC subject undergoes a standardized annual history and physical examination, resting electrocardiograms, and blood pressure determinations. In the DCCT/EDIC cohort, HbA$_{1c}$ is measured annually using high-performance liquid chromatography (26), and the fasting lipid profile (total cholesterol, total triglyceride, HDL cholesterol, calculated LDL cholesterol levels) is determined in alternate years.

Table 2—*Relationships between plasma fibrinogen level and clinical parameters: correlation coefficients*

	All patients (n = 909)		Men (n = 504)		Women (n = 405)	
Parameter	r*	P	r*	P	r*	P
Age (years)	**0.13**	**0.0002**	**0.17**	**0.0001**	0.08	0.09
Duration of type 1 diabetes (years)	0.02	0.54	0.01	0.81	0.02	0.74
BMI (kg/m^2)	**0.15**	**<0.0001**	**0.14**	**0.0019**	**0.19**	**0.0002**
Waist-to-hip circumference ratio	0.04	0.27	**0.20**	**<0.0001**	0.04	0.39
Triglycerides (mg/dl)	0.07	0.05	**0.13**	**0.0047**	0.00	0.97
Total cholesterol (mg/dl)	**0.17**	**<0.0001**	**0.18**	**<0.0001**	**0.15**	**0.0025**
LDL cholesterol (mg/dl)	**0.16**	**<0.0001**	**0.19**	**<0.0001**	**0.15**	**0.0028**
HDL cholesterol (mg/dl)	0.01	0.74	−0.08	0.08	0.05	0.39
HbA_{1c} (%)	**0.11**	**0.0014**	**0.12**	**0.0101**	**0.10**	**0.0480**
Systolic blood pressure (mmHg)	0.05	0.16	0.04	0.40	**0.11**	**0.0241**
Diastolic blood pressure (mmHg)	**0.07**	**0.0367**	0.05	0.31	**0.16**	**0.0011**
Log albumin excretion rate (mg/24 h)	**0.10**	**0.0039**	**0.13**	**0.0033**	0.06	0.21
ETDRS retinopathy score	0.04	0.26	0.02	0.68	0.07	0.16
Common carotid IMT (mm)	0.03	0.32	0.08	0.09	0.02	0.74
Internal carotid IMT (mm)	0.03	0.39	0.05	0.33	0.06	0.25
Average ankle-brachial index	**−0.07**	**0.0305**	−0.02	0.66	**−0.13**	**0.008**

*Pearson correlation coefficient with plasma fibrinogen level. Values in **bold type** are statistically significant.

Assessment of renal function. Renal function was assessed in alternate years by measurement of urine albumin excretion rates from 4 h urine collections (27) as described previously (28). Ninety-eight percent of assessments were conducted within 1 year of the fibrinogen sample collection.

Assessment of retinopathy status. Seven-field stereoscopic fundus photographs were taken by certified photographers and were centrally assessed by graders unaware of the original DCCT treatment-group assignments or current clinical status. The graders used the protocol of the Early Treatment Diabetic Retinopathy Study (ETDRS) (29). The overall level of severity of retinopathy in both eyes was determined for each patient according to the ETDRS interim scale (30). Sixty percent of ETDRS assessments were done within 1 year, and 39% within 2 years, of sample collection for fibrinogen determination.

Table 3—*Effects of discontinuous variables on the serum fibrinogen level in type 1 diabetes*

Discontinuous variable	Number studied	Fibrinogen level (mg/dl)	P (ANOVA)	P (ANCOVA)*
Sex			0.0045	0.0006
Men	504	300 ± 5		
Women	405	321 ± 5		
Smoking status			0.3742	0.1187
Yes	166	316 ± 9		
No	731	308 ± 4		
Hypertension†			0.0208	0.6608
Yes	323	321 ± 6		
No	576	303 ± 4		
HbA_{1c} (by quartile)			0.0001	0.0085
<7.3%	219	288 ± 7		
7.3–7.9%	224	312 ± 7		
8.0–8.9%	229	310 ± 7		
≥9.0%	232	328 ± 7		

Data are means ± SE. *ANCOVA P value adjusts for effects of age, duration of diabetes, DCCT treatment group, BMI, waist-to-hip ratio, serum triglycerides, LDL cholesterol, and HDL cholesterol in all analyses, in addition to HbA_{1c}, sex, hypertension, and smoking status when not considered to be the dependent variable. †Individuals are classified as hypertensive if taking antihypertensive medications with a history of hypertension plus individuals with a blood pressure of ≥140/90 at the clinic visit.

Assessment of macrovascular disease. Ankle-brachial index (ABI), a measure of peripheral vascular status, is measured annually in EDIC subjects using both posterior tibial and dorsalis pedis arteries (26). A normal ABI is taken as 0.8–1.4, with high (>1.4) levels reflecting vascular rigidity and low (<0.8) levels reflecting vascular occlusion (26).

Carotid intimal-medial thickness (IMT) was measured a maximum of 3 years before this study using B-mode ultrasonography as previously described (31).

Fibrinogen analyses. Quantitative determinations of fibrinogen level in plasma were made using a commercially available assay kit (Diagnostica Stago) based on the clotting method of Von Clauss (32) in an ST4 agglutinometer (Diagnostica Stago). Assay reagents and plasma samples were prepared according to the manufacturer's protocol. Fibrinogen control samples (Fibrinogen Control N, Fibrinogen Control P; Diagnostica Stago) were included with each batch of samples analyzed. Interassay coefficient of variation averaged 6.4%; intra-assay coefficient averaged 3.9%.

Genetic analyses. Genomic DNA was isolated from peripheral blood using a DNA isolation kit (Gentra Systems, Minneapolis, MN). Genotyping of the $G^{-455}\rightarrow A$ polymorphism (numbered from the transcriptional start site) in the 5′ untranslated region (UTR) was carried out by amplification of the polymorphic

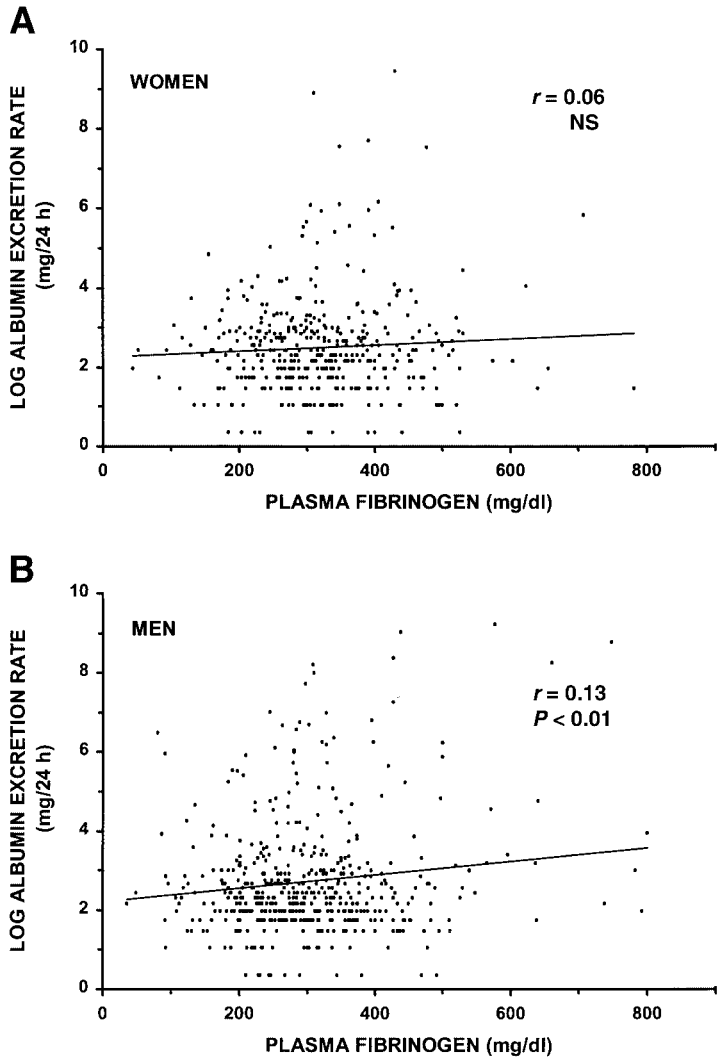

Figure 1—*The relationship between plasma fibrinogen and the log albumin excretion rate in type 1 diabetes. The study group consisted of 405 women (*A*) and 504 men (*B*) with type 1 diabetes in the DCCT/EDIC cohort. The urinary albumin excretion rate was measured in a 4-h timed collection. Simple Pearson correlation was performed between fibrinogen and albuminuria as a continuous variable.*

region by PCR followed by digestion with the restriction enzyme *Hae*III. A 757-bp amplification product was generated by PCR of genomic DNA using the following primers: Fibrinogen5′UTR*f*: 5′-TTTGTT GAACATTTTACCTTATGTGA-3′ and Fibrinogen5′UTR*r*:5′-CATTGTCGTTGA CACCTTCC-3′. *Taq* DNA polymerase was purchased from Gibco-BRL (Gaithersburg, MD). Reaction samples (20 μl) were denatured (3 min, 95°C), and PCR was carried out for 40 cycles (9600 thermal cycler; Perkin-Elmer, Foster City, CA). Each cycle consisted of three segments; denaturation (95°C, 30 s), primer annealing (60°C, 60 s), and primer extension (72°C, 60 s, 1.5 mmol/l $MgCl_2$); an additional extension period (5 min, 72°C) was applied upon completion of the 40 cycles. This PCR product was directly digested with *Hae*III according to the manufacturer's instructions (New England Biolabs, Beverly, MA), in 25-μl volumes for 6 h at 37°C. Digested products were resolved on 2% agarose gels and visualized with ethidium bromide under ultraviolet light. Restriction digestion of the 757-bp PCR product with *Hae*III results in two DNA fragments of 570 and 187 bp in GG homozygotes and a single fragment of 757 bp in AA homozygotes, reflecting lack of the restriction site.

Statistical methods. Urinary albumin excretion rates were log transformed because they were not normally distributed. Simple Pearson correlation was performed between fibrinogen and other continuous variables. Single and multiple linear regression models were used to assess the association between fibrinogen and other continuous, clinically relevant variables, where the clinical variables were considered as dependent and fibrinogen as independent. Multiple linear regression analyses tested whether fibrinogen was significantly associated with the clinical variables after adjusting for covariates such as age, sex, duration of diabetes, DCCT treatment group, waist-to-hip ratio, HbA_{1c}, BMI, triglycerides, and total and LDL cholesterol. Deviation from Hardy-Weinberg equilibrium was assessed by χ^2 (2 degrees of freedom [df]). The hypothesis that clinical variables, fibrinogen level, and vascular complications differed among β-fibrinogen genotypes was tested using ANOVA and by ANCOVA after considering the confounding effects of concomitant variables while main effects were tested. ANCOVA *F* test was used to determine if two or more adjusted least-square means were significantly different between or across genotypes. Bonferroni adjustment was applied for multiple comparisons. SAS software (version 8; SAS Institute, Cary, NC) was used in all statistical analyses. All significance tests for the comparisons were two sided, and results were considered significant at $P < 0.05$.

RESULTS — Clinical characteristics of the 909 patients are delineated in Table 1. These characteristics do not differ significantly from those in the 1,301 patients who comprise the overall DCCT/EDIC cohort (data not shown). The study group comprised nearly equal numbers of men and women of similar age who were randomized equally to the intensive and conventional treatment subgroups during the DCCT. Proportionately more men than women were hypertensive, and men exhibited significantly higher systolic and diastolic blood pressure levels ($P < 0.0001$). Plasma levels of triglycerides and LDL cholesterol were significantly higher in men than in women; HDL cholesterol levels were significantly lower (all $P < 0.0001$). The severity of retinopathy did not differ between men and women; however, nephropathy, measured as the log albumin excretion rate, was marginally increased in men ($P = 0.01$). Periph-

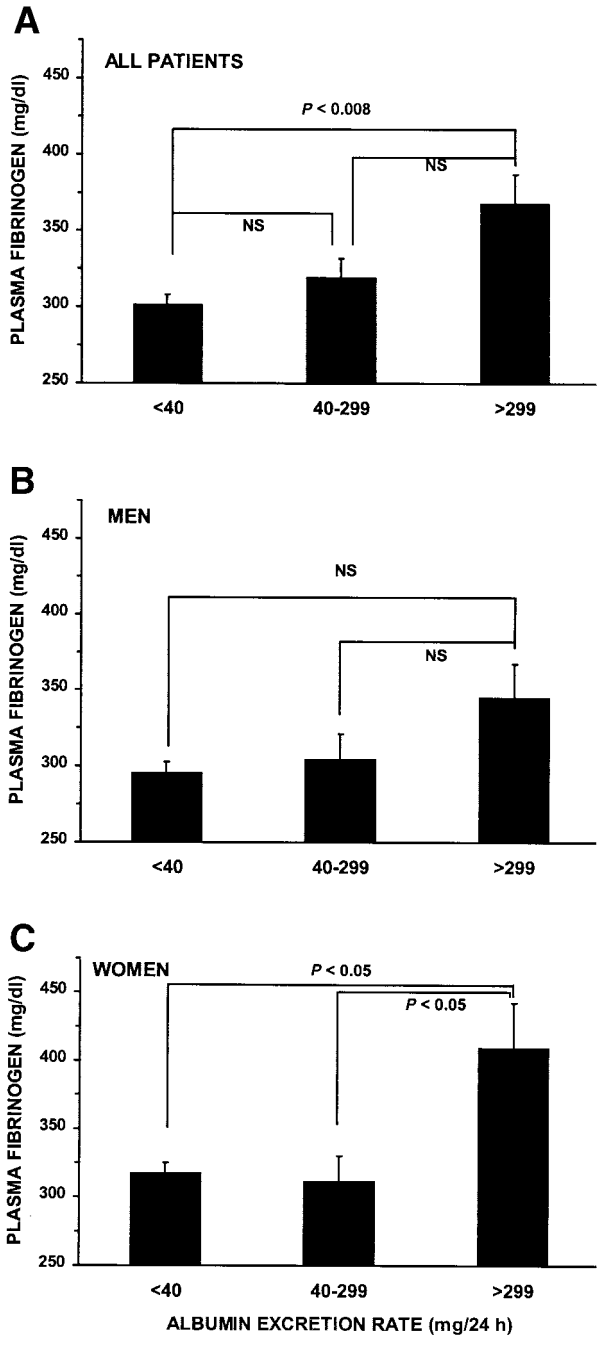

Figure 2—*The effects of albuminuria status on plasma fibrinogen level in type 1 diabetes. Mean ± SE plasma fibrinogen levels are shown in type 1 diabetic patients with normal urinary albumin excretion (0–39 mg/24 h), microalbuminuria (40–299 mg/24 h), and proteinuria (albumin excretion >299 mg/24 h). All 909 patients (women and men) are shown in* A*, 405 women in* B*, and 504 men in* C*.*

eral vascular disease was more advanced in men than in women as demonstrated by significantly increased IMT in both the common carotid and internal carotid arteries, and by a significantly elevated ABI ($P < 0.001$). The percentage distribution of fibrinogen genotypes was similar for both men and women.

Table 2 shows the relationship between plasma fibrinogen and other parameters using univariate regression analysis. When all patients were considered, statistically significant correlations between fibrinogen and age, BMI, triglycerides, total and LDL cholesterol, current HbA_{1c}, diastolic blood pressure, log albumin excretion rate, and the average ABI were observed. However, close examination of the univariate regressions revealed marked differences between men and women. In men, all of these correlations remained statistically significant except for blood pressure. In addition, the relationship with waist-to-hip ratio was statistically significant, and, in fact, was the strongest predictor of plasma fibrinogen level ($r = 0.20$; $P < 0.001$). In women, fibrinogen was correlated with BMI, total and LDL cholesterol, HbA_{1c}, and both diastolic and systolic blood pressure, but not with waist-to-hip ratio, age, or triglycerides.

Table 3 shows discontinuous analyses for several variables. Female patients had higher mean plasma fibrinogen level than males (321 ± 5 vs. 300 ± 5 mg/dl; $P = 0.0045$). In both sexes, the relationship between fibrinogen and HbA_{1c} was nonlinear, with lower fibrinogen levels in patients in the lower quartile with HbA_{1c} values <7.3%, and a higher mean value in the highest quartile with HbA_{1c} ≥9.0% (Table 3). There was no significant effect of smoking status on plasma fibrinogen in these patients.

Univariate regression analyses also demonstrated that fibrinogen was related to markers of nephropathy and peripheral vascular disease and that the relationship was dependent on sex. Fibrinogen was positively correlated with urinary albumin excretion rate in the group as a whole (Table 2); however, this relationship was statistically significant only in men ($r = 0.13$; $P = 0.003$) and not in women (Fig. 1). It was also instructive to examine this relationship with albuminuria as a discontinuous variable in Fig. 2. Whereas there was a tendency for fibrinogen to increase with the development of microalbuminuria (albumin excretion rate 40–299 mg/24 h) compared with normal individuals (<40 mg/24 h), this difference did not achieve statistical significance. In contrast, there was a marked 1.5-fold elevation in plasma fibrinogen noted in patients with proteinuria (albumin excretion rate ≥300 mg/24 h). These differences in mean fibrinogen level as a function of albuminuria category were statistically significant in women and in the group as a whole, but did not quite achieve significance in men. Thus, there were indications in both men (Fig. 1) and women (Fig. 2) that fibrinogen was associated with nephropathy. The clear and pronounced elevation in patients with proteinuria (and not with microalbuminuria) suggests that high fibrinogen levels were acquired with more severe degrees of albuminuria and did not precede the development of microalbuminuria. Thus, elevated fibrinogen could contribute to the acceleration of atherogenesis that ac-

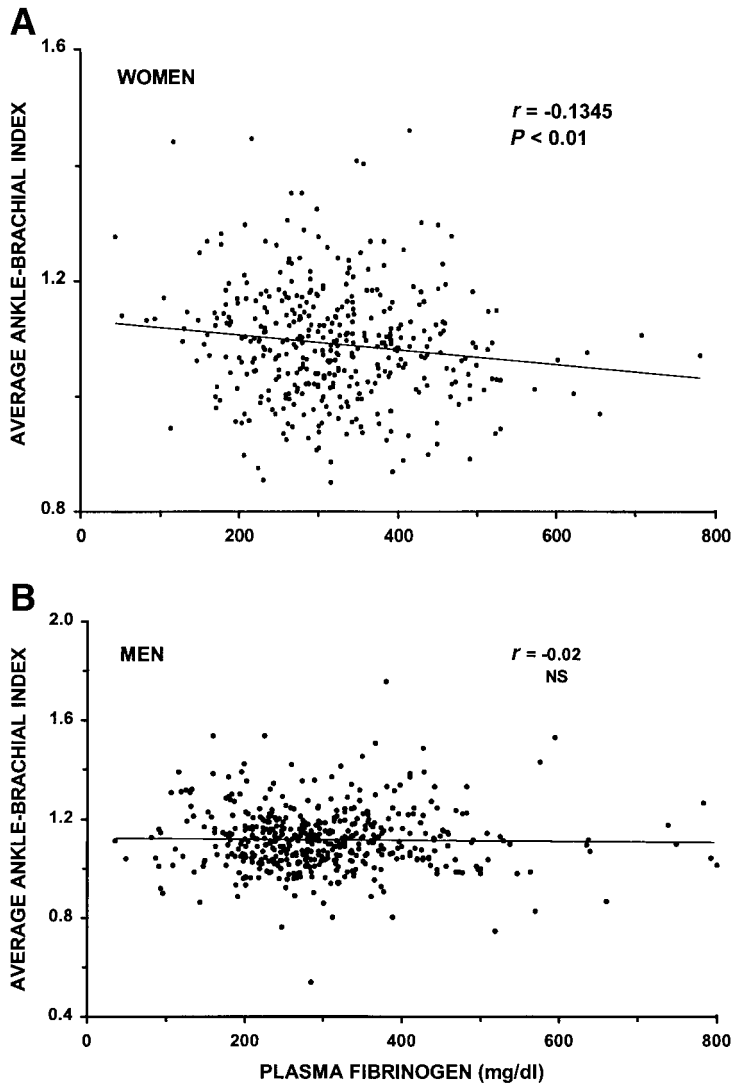

Figure 3—*The relationship between plasma fibrinogen and the average ABI in type 1 diabetes. The study group consisted of 405 women (*A*) and 504 men (*B*) with type 1 diabetes in the DCCT/EDIC cohort. The average ABI is the mean of four measurements taken from the right and left dorsalis pedis artery and posterior tibial artery in individual patients. Simple Pearson correlation was performed between fibrinogen and ankle-brachial index as a continuous variable.*

companies proteinuria. There was no relationship between fibrinogen level and the severity of another microvascular complication, retinopathy, as assessed by ETDRS retinopathy score in Table 2.

Plasma fibrinogen was also correlated with the ABI, a measure of peripheral vascular disease, and this relationship also exhibited sex dimorphism (Table 2). In women, the relationship between fibrinogen and the average value (Fig. 3) of the four ABI measurements in each individual (i.e., right and left dorsalis pedis and posterior tibial arteries) was highly significant. High fibrinogen levels were associated with lower ABI measurements (i.e., more severe peripheral vascular disease) with respect to both the dorsalis pedis ($r = -0.12$; $P = 0.0160$) and posterior tibial ($r = -0.15$; $P = 0.0038$) arteries (data not shown). However, in men, plasma fibrinogen was not significantly correlated with ABI measurements (Fig. 3, Table 2).

We determined whether plasma fibrinogen concentration was independent of various clinical parameters and of vascular disease complications. The higher fibrinogen level in women and the relationship between fibrinogen and HbA_{1c} remained highly significant after adjusting for multiple covariables (Table 3).

Multiple regression analyses were conducted to determine whether plasma fibrinogen was independently correlated with various clinical parameters and diabetes complications. Multiple regression equations for albumin excretion rate and the average ABI are depicted in Table 4. Plasma fibrinogen was found to be an independent predictor of the albumin excretion rate in men ($P = 0.016$) but not in women. Other factors that were statistically significant in the multiple regression equation for albumin excretion were age, DCCT treatment group, HbA_{1c}, systolic blood pressure, triglycerides, and LDL cholesterol. As regards peripheral vascular disease, plasma fibrinogen was independently associated with the average ABI measurement in women ($P = 0.009$), but not in men. Other risk factors that achieved statistical significance in the regression equation for ABI included BMI, waist-to-hip ratio, and systolic blood pressure in men, but only BMI in women. Age, DCCT treatment group, current HbA_{1c}, diastolic blood pressure, triglycerides, LDL and HDL cholesterol, and smoking status did not otherwise significantly contribute to the multiple regression equation.

The relationship between fibrinogen and carotid wall thickness, another quantitative measure of macrovascular disease, was also investigated. Unlike the marker for peripheral vascular disease, there was no association between plasma fibrinogen and either common carotid IMT or internal carotid IMT determined by duplex ultrasonography in men or women (Table 2). Similarly, fibrinogen was not significantly related to common or internal carotid IMT after adjusting for multiple covariables (including age, DCCT treatment group, HbA_{1c}, BMI, waist-to-hip ratio, blood pressures, and lipid panel) in multiple regression analyses (data not shown).

The distribution of β-fibrinogen $G^{-455}\rightarrow A$ genotypes was 60% for GG, 35% for GA, and 6% for AA% for all subjects and did not differ significantly between sexes (Table 1). These frequencies conformed to Hardy-Weinberg equilibrium and were similar to those previously reported in nondiabetic subjects. This polymorphism in the 5′ promoter region of the β-fibrinogen gene did not influence fibrinogen levels. There were no significant differences in mean plasma fibrino-

Table 4—*Multiple regression analyses for factors related to albumin excretion rate and average ABI: independent effects of plasma fibrinogen*

Covariate in multiple regression equation	Log albumin excretion rate (mg/24 h)		Average ABI	
	β	*P*	β	*P*
All patients				
Plasma fibrinogen	**0.001**	**0.024**	0.000	0.17
Age (years)	**−0.022**	**0.001**	0.001	0.12
Sex	−0.038	0.76	**−0.046**	**<.0001**
DCCT treatment group	**−0.385**	**<.0001**	−0.006	0.26
HbA_{1c}	**0.175**	**<.0001**	−0.005	0.15
BMI	−0.021	0.10	**0.003**	**0.002**
Waist-to-hip ratio	0.255	0.74	**−0.166**	**0.021**
Systolic blood pressure	**0.029**	**<.0001**	−0.001	0.08
Diastolic blood pressure	−0.007	0.24	−0.000	0.38
Triglycerides	**0.004**	**<.0001**	0.000	0.52
LDL cholesterol	**0.004**	**0.009**	0.000	0.41
HDL cholesterol	0.005	0.14	0.000	0.22
Smoking (Y/N)	0.211	0.07	−0.012	0.27
Men				
Plasma fibrinogen	**0.002**	**0.016**	0.000	0.96
Age (years)	−0.019	0.059	0.001	0.07
DCCT treatment group	**−0.405**	**0.001**	−0.003	0.76
HbA_{1c}	**0.149**	**0.005**	−0.008	0.24
BMI	−0.001	0.94	**0.004**	**0.045**
Waist-to-hip ratio	−0.704	0.55	**−0.254**	**0.024**
Systolic blood pressure	**0.030**	**<.0001**	**−0.001**	**0.032**
Diastolic blood pressure	−0.006	0.48	0.000	0.73
Triglycerides	**0.004**	**<.001**	0.000	0.21
LDL cholesterol	0.004	0.07	0.000	0.90
HDL cholesterol	**0.013**	**0.009**	0.001	0.21
Smoking (Y/N)	**0.357**	**0.033**	−0.014	0.39
Women				
Plasma fibrinogen	0.001	0.44	**0.001**	**0.009**
Age (years)	**−0.024**	**0.005**	0.000	0.63
DCCT treatment group	**−0.338**	**0.005**	−0.017	0.11
HbA_{1c}	**0.200**	**<.0001**	−0.002	0.66
BMI	**0.036**	**0.023**	**0.004**	**0.007**
Waist-to-hip ratio	0.573	0.58	−0.125	0.17
Systolic blood pressure	**0.028**	**<.0001**	0.000	0.83
Diastolic blood pressure	−0.007	0.41	0.000	0.51
Triglycerides	**0.005**	**0.003**	0.000	0.47
LDL cholesterol	0.003	0.19	0.000	0.22
HDL cholesterol	−0.003	0.49	0.000	0.60
Smoking (Y/N)	0.001	0.99	−0.008	0.58

Values in **bold type** are statistically significant.

gen levels according to genotype, either before or after adjusting for covariables including age, sex, BMI, current HbA_{1c}, and smoking status (Table 5). Furthermore, in measured genotype analyses (Table 5), the fibrinogen gene polymorphism was not associated with urinary albumin excretion rate, retinopathy score, ABI, or common carotid IMT. However, the association with IMT of the internal carotid artery did just reach statistical significance (ANOVA $P = 0.050$; ANCOVA $P = 0.045$). Homozygosity for the more common G allele was associated with increased IMT (0.687 ± 0.008 mm) compared with homozygosity for the less common A allele (0.642 ± 0.026 mm), with heterozygotes having an intermediary mean value (0.659±.010 mm) (post hoc pairwise comparisons GG versus GA, $P = 0.037$; GG versus AA, $P = 0.1046$; GA versus AA, $P = 0.544$).

CONCLUSIONS — Our cross-sectional analyses indicated that fibrinogen levels are related to microvascular and macrovascular disease in type 1 diabetes. Fibrinogen levels were associated with nephropathy status in type 1 diabetic subjects (Table 2; Figs. 1 and 2). In men but not women, there was a significant correlation between fibrinogen levels and albumin excretion rate across the entire range of albumin loss ($r = 0.10$; $P = 0.0039$), with no evidence of a threshold for increased fibrinogen. In women, discontinuous analysis demonstrated that, whereas fibrinogen levels were similar in normoalbuminuric and microalbuminuric subgroups, there was a significant increase in plasma fibrinogen with macroalbuminuria. Therefore, in both men and women, fibrinogen was related to nephropathy as manifested by the severity of albuminuria. The results of previous studies in diabetic subjects have not been consistent and have shown varying associations between the presence of micro- and/or macroalbuminuria and elevated fibrinogen concentrations (5–11,33–35), most probably as a result of the varying influence of multiple additional factors and drug treatments.

In the present study, analysis of covariance was used to control for effects of multiple other cardiovascular risk factors (including HbA_{1c}) and showed that hyperfibrinogenemia is independently associated with nephropathy status and albumin excretion rate. It is not clear, however, whether hyperfibrinogenemia occurs secondary to the onset of nephropathy or is a primary factor that antedates microalbuminuria. Further prospective evidence is required to confirm hyperfibrinogenemia as an independent predictor of future diabetic nephropathy. Our longitudinal follow-up in the EDIC cohort (in progress) will address this issue. However, even if hyperfibrinogenemia is a secondary abnormality, it could still contribute to a worsening of existing nephropathy and to the associated increase in vascular disease risk associated with albuminuria.

Regarding other covariables, smoking status did not affect plasma fibrinogen ($P = 0.37$) in our patients, contrary to previous studies in both the general pop-

Table 5—*Effects of $G^{-455}\rightarrow A$ fibrinogen gene polymorphism on plasma fibrinogen level and vascular complications in type 1 diabetes*

Parameter and genotype	Number of patients	Mean	SE	*P* (ANOVA)	*P* (ANCOVA)*
Plasma fibrinogen level (mg/dl)				0.651	0.416*
GG	444	307	5		
GA	256	314	7		
AA	41	302	17		
Log albumin excretion rate (mg/24h)				0.580	0.548*
GG	438	2.6	0.1		
GA	256	2.6	0.1		
AA	40	2.8	0.2		
ETDRS retinopathy score (grade 1–23)				0.527	0.537*
GG	442	4.6	0.2		
GA	252	4.3	0.2		
AA	40	4.8	0.5		
Average ABI				0.278	0.299
GG	438	1.10	0.005		
GA	354	1.11	0.007		
AA	40	1.12	0.018		
Common carotid IMT (mm)				0.987	0.928*
GG	425	0.677	0.004		
GA	247	0.678	0.006		
AA	39	0.676	0.014		
Internal carotid IMT (mm)				**0.050**	**0.045***
GG	425	0.687	0.008		
GA	247	0.659	0.010		
AA	39	0.642	0.026		

*ANCOVA for fibrinogen level adjusted for age, sex, BMI, HbA_{1c}, and smoking status; ANCOVAs for log albumin excretion rate, ETDRS score, common carotid IMT, and internal carotid IMT adjusted for age, sex, DCCT treatment group, BMI, and HbA_{1c} level. Values in **bold type** are statistically significant.

ulation and type 1 diabetic subjects (2,5,14,15). It is possible that this represents a type 2 error due to the limited number of current smokers in the EDIC study. Sex did affect fibrinogen levels, with higher values observed in women, as reported in other studies. Multiple reports (36–38), but not all (39,40), have demonstrated that fibrinogen levels are increased in diabetes, although the mechanism has not been clarified. The results of the present study show that one factor contributing to this relationship may be hyperglycemia, since fibrinogen levels are elevated in patients as a function of poor glycemic control (Tables 2 and 3).

In contrast to the association with diabetic nephropathy, fibrinogen levels were not related to another microvascular complication, retinopathy (Table 2). Although retinopathy and nephropathy are often concomitant, this is not always the case, and the data indicate that different risk factors may influence the development of microvascular complications at different sites.

With respect to macrovascular disease, the association between high plasma fibrinogen and peripheral vascular disease in type 1 diabetes is a novel observation. We found an inverse relationship between fibrinogen and the ABI, which is a measure of subclinical peripheral vascular disease (Table 2). This relationship was statistically significant in the group as a whole, but this was due to a significant correlation in women only. Furthermore, hyperfibrinogenemia was independently associated with ABI in women after controlling for conventional vascular risk factors and other covariables (Table 4). Although the magnitude of the independent association is not large, it is possible that this may be an underestimate of the true impact of fibrinogen. This is because the range of ABI values was quite narrow, with few values <0.8, indicating that, as a whole, the DCCT/EDIC cohort had not yet developed clinically overt peripheral vascular disease. Additional measurements of fibrinogen as the incidence of more severe and clinically overt peripheral arterial disease increases may help refine the degree of risk conferred by fibrinogen. Furthermore, while it is statistically valid to determine the independent association with ABI, biologically, fibrinogen may interact with other risk factors to contribute to the overall vascular disease risk. These results in type 1 diabetes are consistent with previous reports in nondiabetic patients with peripheral vascular disease, where an inverse correlation between fibrinogen and ABI values has also been demonstrated (41–46).

We did not observe an association between fibrinogen levels and carotid artery IMT determined by ultrasound. Carotid IMT has previously been shown to be associated with conventional vascular risk factors in the EDIC cohort, including total cholesterol, LDL cholesterol, BMI, and waist-to-hip ratio in men, systolic blood pressure, and smoking (31). It should be pointed out that the measurement of carotid IMT was performed up to 3 years before measurement of fibrinogen and was also in the relatively normal range, which are potential sources of error. However, carotid IMT has been shown to be associated with the presence of coronary artery disease, and it is of interest that no association between fibrinogen and electocardiographic or symptomatic evidence of coronary artery disease was demonstrated in one previous study in type 1 diabetes (5). Further measurements of plasma fibrinogen over time, combined with ongoing assessment of events and measurement of coronary artery disease by the DCCT/EDIC study, will more directly examine whether there is a relationship between fibrinogen and coronary artery disease in type 1 diabetes.

The results of the present study show that the β-fibrinogen $G^{-455}\rightarrow A$ polymorphism does not influence fibrinogen levels in type 1 diabetes (Table 5). We chose to examine the $G^{-455}\rightarrow A$ polymorphism because of recent in vitro evidence suggesting that this polymorphism is associated with altered transcriptional activity in β-fibrinogen promoter/luciferase reporter constructs and influences the binding of a specific, but as yet unidentified, nuclear protein that regulates transcription (47). Our results are in contrast with those of previous studies

(22,23,25,48–50) that have demonstrated an association between this polymorphism and fibrinogen levels. The explanation for the difference in the results of these studies may involve heterogeneity in the genetic factors regulating fibrinogen levels in diabetic and nondiabetic subjects. Moreover, multiple other variables in diabetes could influence the interaction between genotype and circulating fibrinogen levels. Along these same lines, fibrinogen gene polymorphisms have been shown to influence the effect of smoking on fibrinogen levels in nondiabetic subjects (21,49); however, we found no evidence of an association between the β-fibrinogen $G^{-455}\rightarrow A$ polymorphism and fibrinogen levels in either current smokers or nonsmokers.

Evidence indicates a role for genetic factors in the pathogenesis of nephropathy in type 1 diabetes (51,52). The results of this study show that the β-fibrinogen gene polymorphism does not contribute to this genetic susceptibility (Table 5). Similarly, there was no association between the $G^{-455}\rightarrow A$ polymorphism and ABI values in type 1 diabetes. The β-fibrinogen gene polymorphism was, however, independently associated with internal carotid artery IMT. This observation, together with the finding that circulating fibrinogen was not affected by this promoter polymorphism, suggests that the relationship with internal carotid IMT is the result of linkage disequilibrium with some other adjacent causal gene.

Last, an insulin resistance syndrome score has been developed based on clinical risk factors in patients with type 1 diabetes and was validated using euglycemic-hyperinsulinemic clamp studies (53). Fibrinogen levels were significantly associated ($P < 0.0001$) with this insulin resistance syndrome score (data not shown). Waist-to-hip ratio, triglycerides, hypertension status, and glycemic control are factors used to define this score and predict glucose disposal rates in type 1 diabetic patients. In the present study, fibrinogen levels were correlated with BMI, waist-to-hip ratio, HbA_{1c} level, and triglycerides in men, and with BMI, HbA_{1c} level, and both systolic and diastolic blood pressure in women. Thus, while there is considerable overlap of the traits, sex seems to influence which are presented. The present study demonstrates conclusively, however, that elevated plasma fibrinogen correlates with several traits that comprise the insulin resistance syndrome in patients with type 1 diabetes and suggests that elevated fibrinogen may be a component of the trait cluster.

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