Plasma Levels of Cellular Fibronectin in Diabetes

SUZAN D.J.M. KANTERS, MD, PHD
JAN-DIRK BANGA, MD, PHD
ALE ALGRA, MD, PHD
RINI C.J.M. FRIJNS, MD
JAAP J. BEUTLER, MD, PHD
ROB FIJNHEER, MD, PHD

OBJECTIVE — Cellular fibronectin is an endothelium-derived protein involved in subendothelial matrix assembly. Elevated plasma levels of cellular fibronectin therefore reflect loss of endothelial cell polarization or injury to blood vessels. Consequently, elevated plasma levels of circulating cellular fibronectin have been described in clinical syndromes with vascular damage, although not in diabetes or atherosclerosis.

RESEARCH DESIGN AND METHODS — We determined fibronectin levels in 52 patients with type 1 diabetes, 50 patients with type 2 diabetes, 54 patients with a history of ischemic stroke, 23 patients with renal artery stenosis, and 64 healthy subjects.

RESULTS — Circulating cellular fibronectin was significantly elevated in patients with diabetes (4.3 ± 2.8 μg/ml) compared with patients with ischemic stroke (2.0 ± 0.9 μg/ml), patients with renovascular hypertension (1.7 ± 1.1 μg/ml), and healthy subjects (1.4 ± 0.6 μg/ml). Patients with diabetes and at least one cardiovascular risk factor had an almost 2.5-fold increase in cellular fibronectin compared with diabetic subjects without such a risk factor. In multivariate regression analysis, higher triglycerides, current or past cigarette smoking, and higher urinary albumin excretion were independently associated with an increase in circulating cellular fibronectin in diabetes.

CONCLUSIONS — These results suggest that circulating cellular fibronectin may be a marker protein for endothelial cell activation, especially in diabetes. Prospective studies are needed to explore this possibility.

Diabetes Care 24:323–327, 2001

Change in endothelial function is considered an early pivotal step in the development of arterial disease. Endothelial function can be assessed either by vascular function tests using invasive or noninvasive techniques or by determination of plasma concentrations of specific endothelial proteins, such as von Willebrand factor (vWF). Circulating cellular fibronectin may be another useful marker of endothelial activation.

Fibronectins are large glycoproteins found in plasma, in extracellular matrix, and on cell surfaces. They promote cell-cell and cell-matrix interactions and thus play a role in tissue construction and reconstruction (1). A single gene encodes for various distinct fibronectin moieties, with differences in primary structure resulting from alternative splicing of the primary mRNA transcript (2). The predominant fibronectin in plasma is secreted by hepatocytes and lacks extra domain (ED) segments. The fibronectins found in subendothelial and connective tissue matrices are produced locally by endothelial cells and fibroblasts and often contain an extra type III structural domain called ED-A (3,4). Usually, <1–2% of total fibronectins in plasma consists of this cellular fibronectin (5).

Elevated plasma levels of cellular fibronectin have been reported in patients with rheumatoid vasculitis, in preeclamptic women, in patients with collagen vascular disorders, in acute trauma, in sepsis syndrome, and in thrombotic thrombocytopenic purpura (5–9). These observations suggest that the intravascular accumulation of cellular fibronectin reflects injury to blood vessels. Vessel wall damage with characteristic endothelial extracellular matrix changes is also found in subjects with diabetes. The accumulation of proteins in the subendothelial matrix in patients with diabetes is visible by light microscopy as periodic acid-Schiff–positive material, consisting of laminin, type IV collagen, and fibronectin (10). Plasma levels of circulating cellular fibronectin could therefore reflect matrix changes and hence vessel wall damage in patients with diabetes.

In the present study, we investigated whether the plasma level of cellular fibronectin reflects the vascular changes in patients with diabetes, compared with healthy nondiabetic individuals or nondiabetic patients with a history of ischemic stroke and nondiabetic patients with renal artery stenosis. Furthermore, we studied relations between various cardiovascular risk factors and circulating cellular fibronectin in diabetic individuals.

RESEARCH DESIGN AND METHODS

Patient characteristics

Diabetic patients, renovascular hypertensive patients, and control subjects were recruited from the outpatient clinic, and ischemic stroke patients were recruited from the clinic of the University Medical Center Utrecht. The study protocol was approved by the ethics committee of the hospital and all patients gave informed consent. We investigated the following groups of patients:

Group 1. We studied 102 patients with diabetes: 52 with type 1 and 50 with type 2 diabetes, aged 19–73 years, with a duration

From the Department of Internal Medicine (S.D.J.M.K., J.-D.B.), the Julius Center for Patient-Oriented Research (A.A.), the Department of Neurology (A.A., R.C.J.M.F.), the Department of Nephrology and Hypertension (J.J.B.), and the Department of Hematology (R.F.), University Medical Center, Utrecht, the Netherlands.

Address correspondence and reprint requests to Jan-Dirk Banga, MD, PhD, Department of Internal Medicine, G02.228, University Medical Center Utrecht, P.O. Box 85500, 3508 GA, Utrecht, the Netherlands. E-mail: j.d.banga@digd.azu.nl.

Received for publication 10 July 2000 and accepted in revised form 27 October 2000.

Abbreviations: BSA, bovine serum albumin; ED, extra domain; MoAb, monoclonal antibody; PBS, phosphate-buffered saline; vWF, von Willebrand factor.

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

Table 1—*Demographic, clinical, and biochemical characteristics of 102 patients with diabetes*

Age (years)	48.8 ± 14.6
Men	62
Type 1 diabetes	51
Diabetes duration (years)	13.5 ± 9.8
HbA_{1c} (%)	8.3 ± 1.3
Total cholesterol (mmol/l)	5.3 ± 0.9
HDL cholesterol (mmol/l)	1.2 ± 0.4
Triglycerides (mmol/l)	1.7 ± 1.0
LDL cholesterol (mmol/l)	3.3 ± 0.8
BMI (kg/m^2)	26.4 ± 4.2
Treatment for hypertension	25
Current or past cigarette smoking	64
History of vascular disease	
Angina pectoris	11
Myocardial infarction	10
Stroke	2
Intermittent claudication	4
Micro- or macroalbuminuria*	26

Data are means ± SD or %. *Urinary albumin excretion >30 mg/24 h.

of diabetes of 0.4–47 years, and 62% were men. Demographic, clinical, and biochemical characteristics of these patients are shown in Table 1. Of these patients, 73 were included in a lipid-lowering study, and 29 participated in a study on the effect of long-term physical exercise on glycemic control (11,12). Their baseline data were used for the current study. Inclusion criteria for the lipid-lowering study were as follows: type 1 or type 2 diabetes, LDL cholesterol >2.6 mmol/l or triglycerides >1.7 mmol/l or HDL cholesterol <0.9 mmol/l for men and <1.1 mmol/l for women. Exclusion criteria were as follows: premenopausal women without adequate birth control; liver or renal impairment (creatinine >200 μmol/l); other contraindication to the study medication; hyperlipidemia caused by hypothyroidism; or excessive alcohol consumption (>5 servings/day). The inclusion criterion for the study on physical exercise was type 1 diabetes for at least 2 years. Exclusion criteria were as follows: micro- or macroalbuminuria (>30 mg/24 h), proliferative retinopathy, disabling neuropathy, clinical manifestations of micro- or macrovasular disease, a BMI >30 kg/m^2, or any disease interfering with regular intensive physical activity.

Group 2. Plasma was sampled from 24 nondiabetic patients within 2 days of acute ischemic stroke and from 30 nondiabetic patients with a previous (>1 week) transient ischemic attack or minor ischemic stroke associated with a moderate-to-severe stenosis of the internal carotid artery (13). These 54 ischemic stroke patients were 32–88 years of age, and 70% were men. The diagnosis of acute ischemic stroke was established by history, clinical examination, and cerebral computed tomography or magnetic resonance imaging. Some 38% of the patients with an acute ischemic stroke and 47% of the patients with a symptomatic stenosis of the internal carotid artery had a history of hypertension.

Group 3. We included 23 nondiabetic hypertensive patients with angiographic evidence of >50% atherosclerotic stenosis of a renal artery (renovascular and atherosclerotic hypertensive patients) in our study (14). They were 45–71 years of age, had a mean arterial pressure in the range of 97–153 mmHg (mean 125), and 57% were men. Their duration of hypertension was in the range of 0.5–36 years (mean 9). Of these patients, 10 also had evidence of coronary artery disease, 4 had proven atherosclerosis of the carotid arteries, and 11 had evidence of atherosclerosis in the aorta, or in femoral or iliac arteries.

Group 4. Healthy control subjects numbered 64 individuals, were 19–79 years of age, and 42% were men. They were asymptomatic for vascular disease and were not being administered cardiovascular medication.

Blood sampling and laboratory investigations

Blood was sampled by an evacuated tube system and collected in citrate anticoagulant (1:10 in 3.1% citrate). It was immediately centrifuged at 2,000*g* for 15 min at 4°C. The supernatant was removed and centrifuged in the same way a second time. Plasma samples were stored at −70°C. Circulating cellular fibronectin was measured with an enzyme-linked immunosorbent assay developed at the University Medical Center Utrecht. Microtiter plates were coated overnight with IgM monoclonal antibody (MoAb) 3E2 (1.4 μg/ml) against cellular (ED-A) fibronectin antibodies (Sigma, St. Louis, MO) in carbonate buffer (50 mmol/l Na_2CO_3/$NaHCO_3$; pH 9.6) at 4°C and then blocked with phosphate-buffered saline (PBS)/3% bovine serum albumin (BSA). Wells were washed with PBS/0.05% Tween. Samples were diluted to an appropriate concentration with PBS/0.05% Tween/1% BSA. Ligand capture was detected by addition of peroxidase-conjugated anti-human fibronectin antibodies (0.6 μg/ml) (Dako, Glostrup, Denmark). Binding of the antibody was detected by adding 0.25 μg/ml *O*-phenylene-diamine in 0.1 mol/l citrate-phosphate buffer (pH 5) with 2.5 mmol/l H_2O_2. The reaction was stopped by adding 3 mol/l sulfuric acid. Absorbance at 490 nm was read on a microplate reader. As a standard, cellular fibronectin was purified from cultured human fetal lung fibroblasts (5,9). ED-A fibronectin was isolated from the medium of GM-1380 human fetal lung fibroblast grown in Dulbecco's modified Eagle's medium containing 10% fibronectin-depleted fetal calf serum by gelatin-affinity chromatography (5). MoAb 3E2 was raised using fibronectin antigen released by cultures of human breast cancer cell lines (15,16). The interassay coefficient of variation of a standard (1.0 μg cellular fibronectin per milliliter) measured in 12 microtiter plates was 8.1%, and the intra-assay coefficient of variation was 4.0% (12 samples). An amount of 0.4 μg/ml in plasma could be detected in the linear range of the standard curve. MoAb 3E2 is specific for the ED-A domain, and the critical residues were recently identified (17).

Statistical analysis

Data are presented as means ± SD. Differences between groups of patients and control subjects were described by 95% CIs based on the *t* test for independent samples. These differences were adjusted for age by linear regression analysis.

Linear regression analysis was used to evaluate the determinants of circulating cellular fibronectin in diabetes. Age, duration of diabetes, HbA_{1c}, total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol, BMI, and urinary albumin excretion were entered as continuous variables into the regression analyses. Sex, type of diabetes, treatment for hypertension, current or past cigarette smoking, history of vascular disease, and at least one cardiovascular risk factor were entered as dichotomous variables. Variables selected from univariate analysis (selected by significance level of ≤0.10 or less) were sequentially entered into the multivariate model until no remaining candidate variable had a significance level of ≤0.10. $P < 0.05$ was considered statistically significant.

RESULTS — Mean age (± SD) of the patients with diabetes (n = 102) was 49 ± 15 years, and 62% were men. These character-

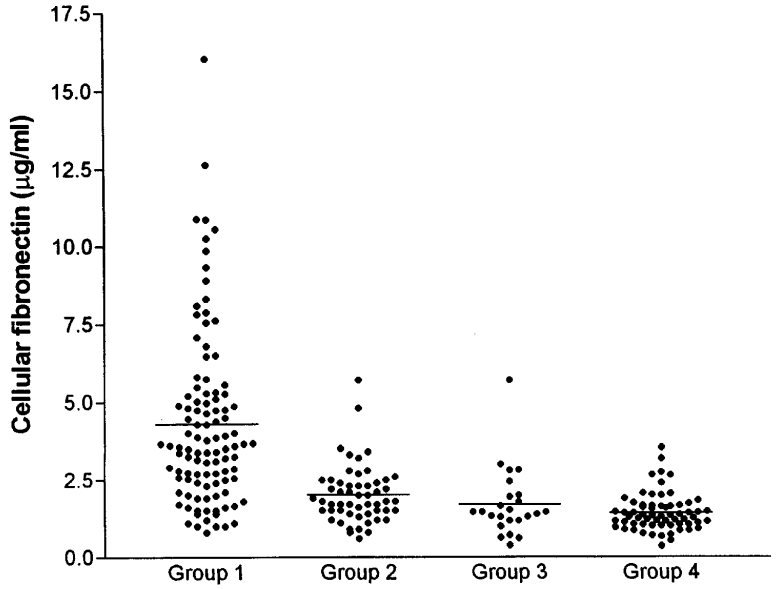

Figure 1—*Plasma levels of cellular fibronectin in patients with diabetes (Group 1; 4.3 ± 2.8 µg/ml [mean ± SD]), ischemic stroke patients (Group 2; 2.0 ± 0.9 µg/ml), renovascular hypertensive patients (Group 3; 1.7 ± 1.1 µg/ml), and healthy control subjects (Group 4; 1.4 ± 0.6 µg/ml).*

istics were 62 ± 13 years and 70% for the ischemic stroke patients (n = 54), 59 ± 8 years and 57% for the renovascular hypertensive patients (n = 23), and 49 ± 18 years and 42% for the healthy control subjects, respectively. Levels of circulating cellular fibronectin are summarized in Fig. 1. Table 2 shows the unadjusted and the age-adjusted differences in circulating cellular fibronectin between groups. Levels were significantly elevated in diabetic patients compared with ischemic stroke patients, renovascular hypertensive patients, and healthy control subjects. Circulating cellular fibronectin was significantly higher in ischemic stroke patients than in healthy control subjects. There was no difference in plasma levels between renovascular hypertensive patients and healthy control subjects.

In the healthy control group, circulating cellular fibronectin levels were similar in men and women: 1.5 ± 0.6 µg/ml (n = 26) and 1.4 ± 0.6 µg/ml (n = 38), respectively. Circulating cellular fibronectin increased with age in the healthy control group. The regression coefficient was 0.014 (95% CI 0.006–0.022), i.e., for each year increase in age, the mean increase in circulating tissue-specific fibronectin was 0.014 µg/ml.

Results of univariate regression analysis in the diabetic patients are shown in Table 3. Plasma levels of cellular fibronectin were higher for type 2 (n = 50) than for type 1 (n = 52) diabetes: 4.9 ± 2.3 and 3.8 ± 3.1 µg/ml, respectively (difference 1.1; 95% CI 0.06–2.2). The plasma level of cellular fibronectin increased by 0.04 µg/ml for each 10 mg/24 h increase in urinary albumin excretion (0.004 µg/ml for each mg/24 h). Mean levels were 3.7 ± 2.3, 4.4 ± 1.9, and 9.0 ± 3.9 µg/ml for diabetic patients with normoalbuminuria (<30 mg/24 h; n = 70), microalbuminuria (30–300 mg/24 h; n = 17), and macroalbuminuria (>300 mg/24 h; n = 7), respectively. When these differences were analyzed by the t test for independent samples, no statistically significant difference in plasma levels between patients with normoalbuminuria and microalbuminuria was found. However, circulating cellular fibronectin was elevated in patients with macroalbuminuria compared with subjects with normoalbuminuria and microalbuminuria. Circulating cellular fibronectin was 5.1 ± 2.6 and 4.2 ± 2.8 µg/ml for diabetic patients with (n = 15) or without (n = 87) a history of vascular disease, respectively (difference 0.9; 95% CI −0.6 to 2.5). Seventeen diabetic patients had no micro- or macroalbuminuria, no history of vascular disease, no treatment for hypertension, no current or past cigarette smoking, and no hyperlipidemia (HDL cholesterol >1.1 mmol/l, triglycerides <2.3 mmol/l, and LDL cholesterol <3.4 mmol/l) (18). Circulating cellular fibronectin was 2.0 ± 1.1 µg/ml in these subjects without cardiovascular risk factors, compared with 4.8 ± 2.8 µg/ml in the 85 diabetic patients with risk factors (difference 2.8; 95% CI 1.5–4.2). Furthermore, in univariate regression analysis, age, HDL cholesterol, triglycerides, BMI, treatment for hypertension, and current or past cigarette smoking were associated with plasma levels of cellular fibronectin. Type of diabetes, HDL cholesterol, BMI, and treatment for hypertension were no longer statistically significant predictors of circulating cellular fibronectin after adjustment for age. The age-adjusted regression coefficients of the other variables remained essentially the same.

Results of multivariate regression analysis are shown in Table 4. Higher triglycerides, current or past cigarette smoking, and higher urinary albumin excretion were independently associated with an increase in circulating cellular fibronectin in diabetes. The other variables, including type of diabetes, were not independently associated with plasma levels of cellular fibronectin.

The group of ischemic stroke patients consisted of patients with an acute ischemic stroke and subjects with more chronic cerebral ischemia. Circulating cellular fibronectin was 2.2 ± 1.0 µg/ml (n = 24) and 1.8 ± 0.9 µg/ml (n = 30) for both subgroups, respectively (difference 0.4; 95% CI −0.1 to 0.9). The plasma levels of each of these

Table 2—***Unadjusted and age-adjusted differences in plasma levels of cellular fibronectin between groups of patients and control subjects***

	Unadjusted difference (95% CI)	Age-adjusted difference (95% CI)
Group 1–Group 2	2.3 (1.6 to 3.1)	2.9 (2.1 to 3.7)
Group 1–Group 3	2.7 (1.5 to 3.9)	3.2 (2.1 to 4.4)
Group 1–Group 4	2.9 (2.2 to 3.6)	2.9 (2.2 to 3.6)
Group 2–Group 3	0.4 (−0.1 to 0.8)	0.3 (−0.2 to 0.8)
Group 2–Group 4	0.6 (0.3 to 0.9)	0.4 (0.1 to 0.7)
Group 3–Group 4	0.2 (−0.2 to 0.6)	0.1 (−0.3 to 0.4)

Group 1: patients with diabetes; Group 2: ischemic stroke patients; Group 3: renovascular hypertensive patients; and Group 4: healthy control subjects.

Table 3—*Univariate regression analysis of variables for the prediction of cellular fibronectin in the patients with diabetes* (n = *102*)

Age (years)	0.055 (0.020 to 0.090)
Sex (men = 0, women = 1)	−0.67 (−1.78 to 0.44)
Type of diabetes (type 1 = 0, type 2 = 1)	1.12 (0.06 to 2.18)
Diabetes duration (years)	0.030 (−0.025 to 0.085)
HbA_{1c} (%)	0.24 (−0.18 to 0.66)
Total cholesterol (mmol/l)	0.50 (−0.13 to 1.13)
HDL cholesterol (mmol/l)	−1.93 (−3.19 to −0.67)
Triglycerides (mmol/l)	1.05 (0.52 to 1.57)
LDL cholesterol (mmol/l)	0.37 (−0.28 to 1.02)
BMI (kg/m^2)	0.14 (0.01 to 0.27)
Treatment for hypertension (no = 0, yes = 1)	1.54 (0.31 to 2.77)
Current or past cigarette smoking (no = 0, yes = 1)	1.91 (0.85 to 2.98)
Urinary albumin excretion (10 mg/24 h)	0.040 (0.027 to 0.054)
History of vascular disease (no = 0, yes = 1)	0.93 (−0.59 to 2.45)
At least one cardiovascular disease risk factor (no = 0, yes = 1)	2.84 (1.49 to 4.18)

Data are regression coefficients (95% CI). For each unit increase of the predictor variable, the regression coefficient reflects the mean increase of circulating cellular fibronectin.

subgroups were significantly higher than those in healthy control subjects.

CONCLUSIONS — Plasma levels of cellular fibronectin were elevated in diabetic patients. Diabetic subjects with cardiovascular risk factors had higher plasma levels of cellular fibronectin than those without risk factors. Elevated triglycerides, current or past cigarette smoking, and higher urinary albumin excretion were independently associated with an increase in circulating cellular fibronectin in diabetes. Levels were comparable between renovascular hypertensive patients and healthy control subjects and increased in ischemic stroke patients compared with control subjects.

There is ample evidence of microangiopathy in diabetes: increased urinary albumin excretion, high levels of vWF and propeptide vWF, high levels of vascular cell adhesion molecule 1, and impaired endothelium-dependent vasodilatation (19–23). These signs are not specific for diabetes-associated vasculopathy and are also found in atherosclerosis without diabetes. The predictive value of these factors for cardiovascular morbidity is limited. We observed an increase of circulating cellular fibronectin in diabetic patients compared with nondiabetic patients with severe atherosclerotic vascular diseases. This increase in patients with diabetes may suggest a different type of endothelial cell activation. Elevated levels of cellular fibronectin can also be found in patients with severe nonatherosclerotic vascular diseases: vasculitis, preeclampsia, acute trauma, sepsis syndrome, and thrombotic thrombocytopenic purpura (5–9). Therefore, elevated plasma levels of cellular fibronectin may reflect a common pathway of endothelial cell activation in these circumstances, which are not found in atherosclerosis without diabetes. Prospective studies could establish the usefulness of cellular fibronectin as a marker of endothelial dysfunction in individuals with diabetes.

Fibronectin exists in a soluble protomeric form in micromolar concentrations in blood plasma and in an insoluble multimeric form in the extracellular matrix. Plasma fibronectin is synthesized in the liver by hepatocytes and contains neither ED-A nor ED-B, whereas cellular fibronectin contains variable amounts of either or both ED-A and ED-B (1). In this study, we focused on ED-A. Approximately 1% of the total fibronectin concentration in blood plasma consists of cellular fibronectin. Cellular fibronectin is synthesized by endothelial cells, fibroblasts, and smooth muscle cells. Under normal conditions, the main components of the matrix proteins are basement membrane proteins, collagen type IV, and laminin. Significant amounts of fibronectin are also found, but such fibronectin is strictly devoid of ED-A and ED-B. After experimental endothelial denudation, there is rapid (24–48 h) upregulation in the expression of fibronectin mRNA, including both ED-A–positive and ED-B–positive isoforms (24). This expression is sustained even after re-endothelialization is achieved. The changes in ED-A and ED-B fibronectin variants are regulated by alternative splicing, and it seems clear that inclusion of these segments increases under specific pathological circumstances (25). In diabetes, an increased concentration of different matrix proteins is found (10). The large vessels as well as the small arteries contain more laminin and fibronectin. The retinopathy in diabetes is associated with an increased concentration of ED-A–positive cellular fibronectin localized in the subendothelial matrix (26). There is little passive accumulation of fibronectin in preexisting extracellular matrix (27). The polymerization of fibronectin into the extracellular matrix and its insoluble state makes release into the circulation unlikely. Although apical and basal stimulation with transforming growth factor-β1 increased fibronectin synthesis, the secretory response differed depending on which surface was being stimulated (28). Apical secretion of fibronectin and expression of ED-A fibronectin mRNA increased only after apical stimulation. Changes in polarized secretion in diabetes could induce a higher concentration of cellular fibronectin in blood plasma.

To our knowledge, no studies have been published on plasma levels of cellular fibronectin in diabetes. Furthermore, it is unknown whether cellular fibronectin is a general marker for atherosclerosis. From our findings, we speculate that circulating cellular fibronectin may be a marker for diabetic angiopathy. Such a reflection of subendothelial changes in diabetic subjects

Table 4—*Multivariate regression analysis of variables for the prediction of cellular fibronectin in the patients with diabetes* (n = *102*)

Triglycerides (mmol/l)	0.67 (0.21–1.12)
Current or past cigarette smoking (no = 0, yes = 1)	1.52 (0.61–2.42)
Urinary albumin excretion (10 mg/24 h)	0.037 (0.025–0.049)

Data are regression coefficients (95% CI). For each unit increase of the predictor variable, the regression coefficient reflects the mean increase of circulating cellular fibronectin. R^2 = 0.406, i.e., 40.6% of the variation in cellular fibronectin is explained by the three variables in the model.

could be very useful for the follow-up of patients in epidemiological studies and clinical trials.

Circulating cellular fibronectin should be further explored as a potential useful marker protein for endothelial cell activation. The association with diabetes, vascular complications, and other risk factors especially merits further study.

Acknowledgments — This study was financially supported in part by the Dutch Prevention Fund (grant 28-2437). S.D.J.M.K. is the recipient of a grant from the Dutch Organization for Scientific Research (NWO).

The authors wish to thank Dr. P.C. Ligtenberg for providing patient data and plasma samples.

References

1. Hynes RO: Fibronectins. *Sci Am* 254:42–51, 1986
2. Hynes RO: Molecular biology of fibronectin. *Ann Rev Cell Biol* 1:67–90, 1985
3. Yamada K, Kennedy DW: Fibroblast cellular and plasma fibronectin are similar but not identical. *J Cell Biol* 80:492–498, 1979
4. Peters JH, Sporn LA, Ginsberg MH, Wagner DD: Human endothelial cells synthesize, process, and secrete fibronection molecules bearing an alternatively spliced type II homology (ED1). *Blood* 75:1801–1808, 1990
5. Peters JH, Maunder RJ, Woolf AD, Cochrane CG, Ginsberg MH: Elevated plasma levels of ED1+ ("cellular") fibronectin in patients with vascular injury. *J Lab Clin Med* 113:586–597, 1989
6. Friedman SA, de Groot C, Taylor R, Golditch B, Roberts JM: Plasma cellular fibronectin as a measure of endothelial involvement in preeclampsia and intrauterine growth retardation. *Am J Obstet Gynecol* 170:838–841, 1994
7. Van der Plas RM, Schiphorst ME, Huizinga EG, Hene RJ, Verdonck LF, Sixma JJ, Fijnheer R: Von Willebrand factor proteolysis is deficient in classis, but not in bone marrow transplantation-associated, thrombotic thrombocytopenic purpura. *Blood* 93: 3798–3802, 1999
8. Voskuyl AE, Emeis JJ, Hazes JMW, Van Hogezand RA, Biemond I, Breedveld FC: Levels of circulating cellular fibronectin are increased in patients with rheumatoid vasculitis. *Clin Exp Rheumatol* 16:429–434, 1998
9. Fijnheer R, Frijns CJ, Korteweg J, Rommes H, Peters JH, Sixma JJ, Nieuwenhuis HK: The origin of P-selectin as a circulating plasma protein. *Thromb Haemost* 77:1081–1085, 1997
10. Andresen JL, Rasmussen L, Ledet T: Diabetic macroangiopathy and atherosclerosis. *Diabetes* 45 (Suppl. 1):S91–S94, 1996
11. Kanters SDJM, Algra A, De Bruin TWA, Erkelens DW, Banga JD: Intensive lipid lowering strategy in patients with diabetes mellitus. *Diabet Med* 16:500–508, 1999
12. Ligtenberg PC, Blans M, Hoekstra JBL, Van der Tweel I, Erkelens DW: No effect of long-term physical activity on the glycemic control in type 1 diabetes patients: a cross-sectional study. *Neth J Med* 55:59–64, 1999
13. Frijns CJM, Kappelle LJ, Van Gijn J, Nieuwenhuis HK, Sixma JJ, Fijnheer R: Soluble adhesion molecules reflect endothelial cell activation in ischemic stroke and in carotid atherosclerosis. *Stroke* 28:2214–2218, 1997
14. Verhaar MC, Beutler JJ, Gaillard CA, Koomans HA, Fijnheer R, Rabelink TJ: Progressive vascular damage in hypertension is associated with increased levels of circulating P-selectin. *J Hypertens* 16:45–50, 1998
15. Garbasch C, Matthiessen ME, Olsen BE, Moe D, Kirkeby S: Immunohistochemistry of the intercellular matrix components and the epithelio-mesenchymal junction of the human tooth germ. *Histochem J* 26:110–118, 1994
16. Cattoretti G, Pileri S, Parravicini C, Becker MH, Poggi S, Bifulco, C, Key G, D'Amato L, Sabattini E, Feudale E: Antigen unmasking on formalin-fixed, paraffin-embedded tissue sections. *J Pathol* 171:83–98, 1993
17. Liao Y-F, Wieder KG, Classen JM, Van De Water L: Identification of two amino acids within the EIIIA (ED-A) segment of fibronectin constituting the epitope for two function-blocking monoclonal antibodies. *JBC* 274:17876–17884, 1999
18. Haffner SM: Management of dyslipidemia in adults with diabetes. *Diabetes Care* 21: 160–178, 1998
19. Dinneen SF, Gerstein HC: The association of microalbuminuria and mortality in non-insulin-dependent diabetes mellitus: a systematic overview of the literature. *Arch Intern Med* 157:1413–1418, 1997
20. Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA: Impaired nitric oxide–mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 27:567–574, 1996
21. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA: Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 88:2510–2516, 1993
22. Vischer UM, Emeis JJ, Bilo HJG, Stehouwer CDA, Thomsen C, Rasmussen O, Hermansen K, Wollheim CB, Ingerslev J: von-Willebrand factor (vWf) as a plasma marker of endothelial activation in diabetes: improved reliability with parallel determination of the vWf propeptide (vWf:AgII). *Thromb Haemost* 80:1002–1007, 1998
23. Van Mourik JA, Boertjes R, Voorberg J, Huisveld IA, Fijnvandraat K, Van Genderen PJJ, Fijnheer R: Von Willebrand factor propeptide in vascular disorders: a tool for discriminating between acute and chronic endothelial cell perturbation. *Blood* 94: 179–185, 1999
24. Bauters C, Marotte F, Hamon M, Oliviero P, Farhadian F, Robert V, Samuel JL, Rappaport L: Accumulation of fetal fibronectin mRNAs after balloon denudation of rabbit arteries. *Circulation* 92:904–911, 1995
25. Kornblihtt AR, Pesce CG, Alonso CR, Cramer P, Srebrow A, Werbajh S, Muro AF: The fibronectin gene as a model for splicing and transcription studies. *FASEB J* 10:248–257, 1996
26. Spirin KS, Saghizadeh M, Lewin SL, Zardi L, Kenney MC, Ljubimov AV: Basement membrane and growth factor gene expression in normal and diabetic human retinas. *Curr Eye Res* 18:490–499, 1999
27. Peters DMP, Mosher DF: Localization of cell surface sites involved in fibronectin fibrillogenesis. *J Cell Biol* 104:121–130, 1987
28. Wang A, Cohen DS, Palmer E, Sheppard D: Polarized regulation of fibronectin secretion and alternative splicing by transforming growth factor β. *J Biol Chem* 266:15598–15601, 1991