Open access • Journal Article • DOI:10.2337/DIAB.44.5.561

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Published on: 01 May 1995 - Diabetes (American Diabetes Association)

Topics: Microalbuminuria, Glycated hemoglobin, Microangiopathy and Endothelial dysfunction

Endothelial Dysfunction Precedes Development of Microalbuminuria in IDDM

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In patients with insulin-dependent diabetes mellitus (IDDM), microalbuminuria is a predictor of widespread severe microangiopathy and macroangiopathy. Patients with microalbuminuria show generalized dysfunction of the vascular endothelium, but it is unknown whether endothelial dysfunction precedes the development of microalbuminuria. We examined a cohort of 17 IDDM patients at baseline and on three occasions during a follow-up of (median) 64 months (range 51–89). All had normal (<15 μg/min) urinary albumin excretion (UAE) at the first three examinations. At the fourth examination, 11 patients had normal UAE and 6 had microalbuminuria (median 25.7 μg/min [range 15.3–42.8]). Compared with patients with normal UAE, microalbuminuric patients had significantly higher plasma levels of von Willebrand factor (vWF), a marker of endothelial dysfunction, at the second (200% [168–274] vs. 131% [69–186]), third (208% [188–270] vs. 125% [82–190]), and fourth examinations (231% [202–269] vs. 132% [88–208], $P < 0.0001$), but not at baseline (128% [98–161] vs. 122% [87–210]). An increase in vWF preceded the occurrence of microalbuminuria by ~3 years. The groups did not differ with regard to age, diabetes duration, blood pressure, mean glycated hemoglobin and cholesterol, smoking habits, or extent of retinopathy. Endothelial dysfunction, as estimated by plasma vWF concentration, precedes and may predict the development of microalbuminuria in IDDM. *Diabetes* 44:561–564, 1995

In insulin-dependent diabetes mellitus (IDDM), microalbuminuria predicts the development not only of diabetic nephropathy, but also of severe retinopathy, neuropathy, hypertension, and macrovascular disease (1,2). Microalbuminuria is thought to be a marker of widespread vascular damage (1,3), which may underlie the propensity of microalbuminuric patients to develop severe extrarenal vascular disease.

The endothelium is an important locus of control of vascular functions. It actively regulates vascular tone and permeability, the balance between coagulation and fibrinolysis, the composition of the subendothelial matrix, and the mitogenesis of vascular smooth muscle and renal mesangial cells (4–6). In cross-sectional studies in IDDM, there is a close association between microalbuminuria and endothelial dysfunction. Thus, in patients with microalbuminuria, the vascular endothelium tends to increase, rather than decrease, vascular resistance (7,8); fails to restrict the passage of macromolecules (1,3); and loses its anticoagulant and profibrinolytic properties (9,10). In addition, there is an increase in the plasma concentration of markers of endothelial injury and dysfunction, such as von Willebrand factor (vWF) (9,11), a glycoprotein involved in primary hemostasis and secreted mainly by endothelial cells.

Some aspects of endothelial function have been reported to be disturbed in IDDM patients with normal urinary albumin excretion (UAE) (12–15), but whether these are of prognostic significance is not known. Answering that question is important because it would increase our knowledge about the pathogenesis of microalbuminuria and allow early recognition of high-risk patients. Therefore, we wished to investigate whether endothelial dysfunction precedes the occurrence of microalbuminuria. To do this, we followed a group of IDDM patients and measured UAE and plasma vWF concentration at regular intervals (as an estimate of endothelial function).

RESEARCH DESIGN AND METHODS

Cohort study. Between June 1985 and August 1989, 65 IDDM patients (fasting C-peptide levels < 0.01 nmol/l) were recruited and gave informed consent, as previously reported (11,12). Clinical and laboratory data, as detailed below, were obtained at baseline (first examination) and after a median follow-up of 3 years (second examination). This study was completed in 59 patients and showed that the development of microalbuminuria was paralleled by increases in the plasma vWF concentration, but we could not determine whether such increases preceded the occurrence of microalbuminuria (11). To further investigate the time course of the development of complications of diabetes in relation to changes in vWF, patients were invited for further follow-up examinations when, at the second examination, they had normal UAE, no diabetic retinopathy on ophthalmoscopy, and fair glycemic control (HbA_{1c} < 8.5%) and used no medication other than insulin (12). Patients with poorly controlled diabetes were excluded to avoid, as much as possible, fluctuations of plasma vWF concentrations associated with short-term changes in glycemic control (16). Seventeen patients fulfilled these criteria and underwent third and fourth examinations, which are the subjects of this report. The median follow-up in these patients was 24 months (range 11–47), 40 months (27–64), and 64 months (51–89) between the first and the second, the first and the third, and the first and the fourth examinations, respectively.

Clinical and laboratory studies. Detailed descriptions are given elsewhere (11,12,17). At each examination, we recorded age, diabetes duration, body mass index (BMI), blood pressure (BP; diastolic phase V, to the nearest 5 mmHg; twice, after 15 min of supine rest, using a

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Received for publication 10 November 1994 and accepted in revised form 19 January 1995.

ANOVA, analysis of variance; BP, blood pressure; BMI, body mass index; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; UAE, urinary albumin excretion; vWF, von Willebrand factor.

standard sphygmomanometer with an appropriately sized cuff), insulin dose, current smoking habits, and current medication. After an overnight fast, blood was drawn from an antecubital vein for measurement of glucose (glucose oxidase method), vWF antigen (see below), glycated hemoglobin (spectrophotometric assay; Bio-Rad, Richmond, VA; normal range 3.5–6.5%), serum creatinine concentration (modified Jaffé reaction), and serum total cholesterol (enzymatically; Boehringer Mannheim, Mannheim, Germany). Within 2 months of each examination, data on UAE and retinal status had been collected for all patients (see below).

Main outcome measures

UAE. On the basis of the median UAE in at least three consecutive 4-h (0800–1200) urine collections, patients were classified as having normal (<15 μg/min) or increased UAE. Note that median values are reported, which minimizes the influence of occasional outliers. Microalbuminuria was defined (in advance [11,12]) as UAE of 15–200 μg/min. (We chose 15 μg/min as cutoff because the median UAE in healthy volunteers in our laboratory is 5.7 μg/min, with >95% having an UAE <15 μg/min [11].) Patients were instructed to avoid strenuous exercise and to empty their bladders completely on arising and at noon. Completeness of urine collections was checked by comparing creatinine excretion among samples, whereas absence of infection was ensured by examining urinary sediments; new urine was collected when necessary.

Retinopathy. At the first and second examinations, the ocular fundus was examined by an ophthalmologist after dilatation of the pupils. At the third and fourth examinations, ophthalmoscopy was repeated on each patient by two ophthalmologists. In addition, fluorescein angiograms were obtained after intravenous injection of 5 ml 10% sodium fluorescein.

Endothelial function. Plasma vWF antigen concentration was measured by immunoelectrophoresis (11). The intra-assay and interassay variations were 4.1 and 8.7%, respectively; the same assay was used throughout the study. Levels of and changes in vWF were expressed as percentages of normal pooled plasma, the antigen level of which is defined as 100% (normal range 50–150; 100% = 1.00 IU/ml). We were careful to avoid increases in vWF associated with hypoglycemia, exercise, smoking, prolonged (>2 min) venous occlusion, and intercurrent illness. In our experience, the within-person day-to-day variability of plasma vWF was thus reduced to ~10%.

The clinical and laboratory data, including UAE and retinal status, were collected without knowledge of the results of the vWF measurements.

Statistical analysis. Data are presented as means ± SD or as medians and ranges, unless otherwise indicated. Patients were divided into two groups according to the absence or presence of increased UAE at the fourth examination. These groups were then compared with regard to clinical and laboratory characteristics and changes therein at examinations 1–4, using standard parametric and nonparametric testing as indicated. A similar procedure was followed with the absence or presence of retinopathy as the outcome of interest. Finally, correlation analysis was used to study the relationship between (changes in) vWF levels and potential determinants of endothelial injury. All testing was two-sided. P values <0.05 were considered statistically significant.

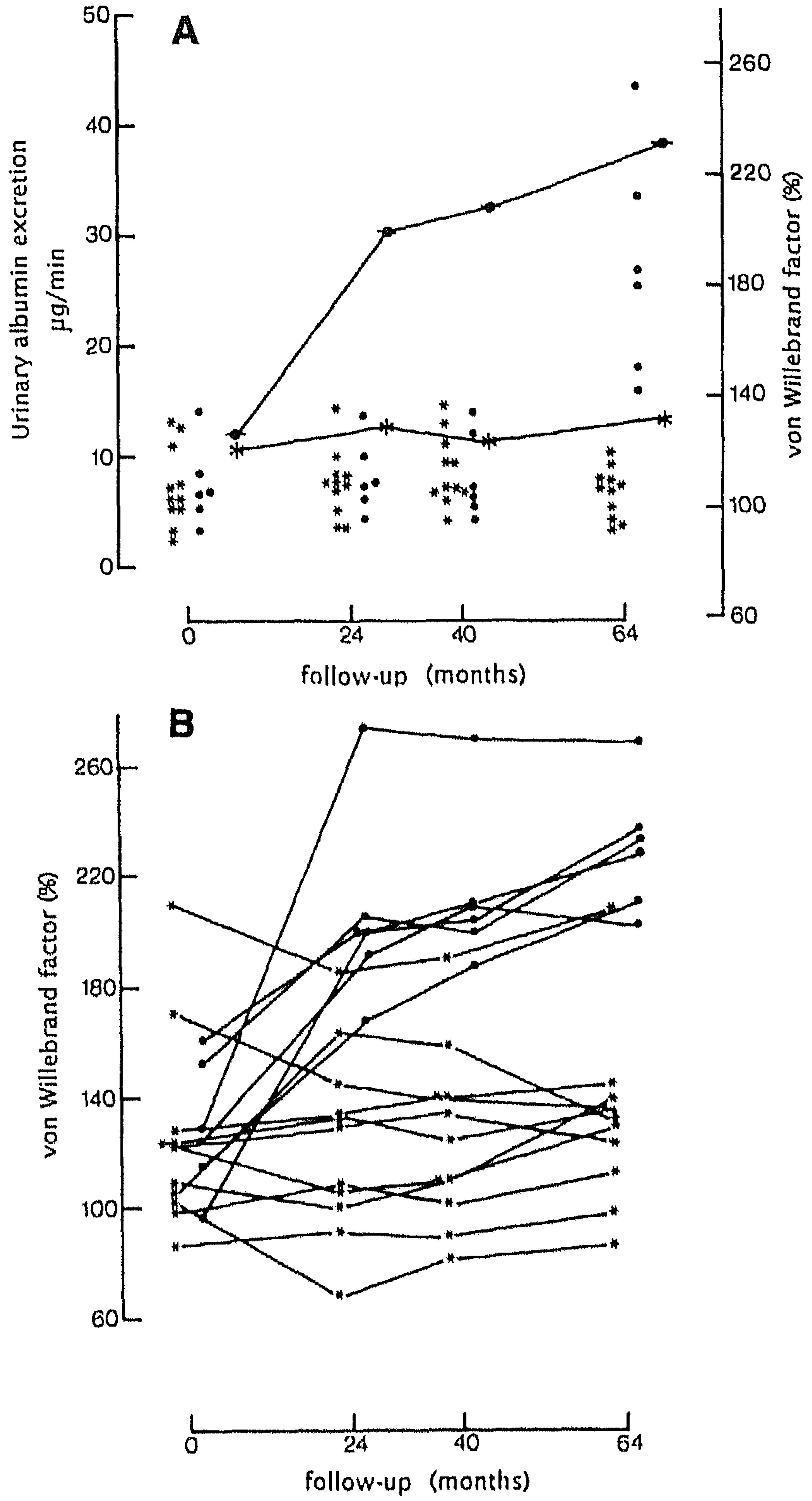

FIG. 1. Time course of UAE and plasma vWF in patients with normal UAE (*) and microalbuminuria (●) at the final follow-up. *A*: scattergram of UAE (* and ●) and medians of vWF (*——* and ●——●). *B*: vWF (normal range 50–150% [0.50–1.50 IU/ml]).

RESULTS

All 17 patients completed the study, except 1 refused fluorescein angiography at the third examination and another declined both ophthalmoscopy and angiography at the fourth. There was no clinical evidence of macrovascular complications in any patient at the third examination, but at the fourth, one patient (with normal UAE) had had a stroke, as confirmed by chart review. There were only two smokers among the patients; this factor was therefore omitted from further analyses. In one patient with persistently normal UAE, antihypertensive medication (a β-receptor antagonist) was started between the third and fourth examinations.

UAE. At the fourth examination, 11 patients had normal UAE (7.2 [3.1–9.9] μg/min) and 6 had microalbuminuria (25.7 [15.3–42.8] μg/min) (Fig. 1). Thus, the cumulative incidence of microalbuminuria was 35%, yielding an incidence density (cases per 100 person-years of follow-up) of 6.3. The main difference between the groups with normal versus increased UAE was the plasma vWF concentration (Table 1 and Fig. 1), which was higher in the microalbuminuric patients at the second (median 200% [range 168–274] vs. 131% [69–186]), third (208% [188–270] vs. 125% [82–190]), and fourth examinations (231% [202–269] vs. 132% [88–208], $P < 0.0001$ by analysis of variance [ANOVA] and by Student's t tests), but not at baseline (128% [98–161] vs. 122% [87–210]). UAE at the fourth examination was related to both the plasma vWF level ($r = 0.70$, $P = 0.002$) and the change in plasma vWF between examinations 1 and 4 ($r = 0.80$, $P < 0.0001$). A plasma vWF level above the upper limit of normal (i.e., 150%) at two or more of the first three examinations predicted the presence of microalbuminuria at the fourth examination with 100% sensitivity (6 of 6) and 82% specificity (9 of 11). These results were not materially altered by exclusion of the patient who received antihypertensive treatment or by exclusion of the two smokers.

TABLE 1
Clinical and laboratory data for patients with IDDM

	Normal UAE	Microalbuminuria
Sex (M/W)	5/6	3/3
Age (years)	49 ± 13	47 ± 19
Diabetes duration (years)	16 ± 3	14 ± 9
Follow-up duration (months)	64 (51–85)	67 (54–89)
BMI (kg/m²)	24.2 ± 3.2	23.9 ± 2.4
Smoker (yes/no)	1/10	1/5
Blood pressure (mmHg)		
First examination	139/84 ± 24/6	140/83 ± 28/15
Second examination	132/81 ± 19/6	142/85 ± 21/10
Third examination	135/83 ± 15/4	145/85 ± 15/9
Fourth examination	146/83 ± 25/7	140/83 ± 16/5
Mean	138/83 ± 19/4	140/84 ± 19/8
Serum total cholesterol (mmol/l)		
First/second examination	6.2 ± 0.8/6.3 ± 0.7	6.0 ± 0.8/6.1 ± 0.7
Third/fourth examination	6.1 ± 0.7/5.6 ± 1.3	5.9 ± 0.7/5.8 ± 0.8
Glycated hemoglobin (%)		
First/second examination	8.0 ± 1.0/6.9 ± 0.8	8.4 ± 1.3/6.8 ± 1.3
Third/fourth examination	6.7 ± 0.8/7.5 ± 1.9	6.7 ± 1.0/6.6 ± 0.7
Mean	7.2 ± 0.9	7.0 ± 0.9
Serum creatinine (μmol/l)	80 ± 7	77 ± 8
Diabetic retinopathy (yes/no)		
Fundoscopy	4/7	3/2
Fluorescein angiography	8/3	3/2

Data are number of patients, means ± SD, or medians (ranges) at the fourth examination.

Retinopathy. At the fourth examination, retinopathy was absent on fundoscopy in nine patients and present in seven. When angiography, a more sensitive method, was used as the criterion, retinopathy was absent in 5 patients and present in 11. Retinopathy was mild and consisted of microaneurysms (in nine patients), small hemorrhages (in five patients), and dilated capillaries (in seven patients; angiographic data). No patient had proliferative changes. Patients in whom retinopathy was absent on fundoscopy did not differ from those in whom it was present with regard to follow-up duration, sex distribution, age, diabetes duration, BMI, or serum creatinine at the fourth examination. In addition, ANOVA indicated no differences, at examinations 1–4, in UAE, glycated hemoglobin, serum cholesterol, or systolic and diastolic BP. Plasma vWF was also similar (124% [98–210] vs. 122% [87–170], 135% [101–200] vs. 145% [69–274], 140% [102–210] vs. 140% [82–270], and 139% [112–237] vs. 136% [88–269], respectively). Analysis of the angiographic data yielded similar results.

vWF. vWF and the change in vWF were related to UAE (see above) but not to potential determinants of endothelial injury, such as age, diabetes duration, glycated hemoglobin, BP, and serum cholesterol. Similarly, vWF was not related to the blood glucose concentration at the time of blood sampling, which varied between 3.6 and 16.9 mmol/l.

DISCUSSION

This study supports the hypothesis that the vascular endothelium is an early and relevant target in the pathogenesis of diabetic microangiopathy. Endothelial dysfunction, as estimated by plasma vWF concentration, preceded the development of microalbuminuria in IDDM by as much as 3 years. In accordance with an earlier report (12), vWF levels were not related to the development of early diabetic retinopathy, which suggests that retinal endothelial injury is not accompanied by widespread endothelial dysfunction resulting in increases in vWF.

Endothelial dysfunction in microalbuminuric IDDM appears to be generalized in that it affects many aspects of endothelial function (1,3,7,9–11,13). In IDDM patients with normal UAE, however, endothelial dysfunction is more variable, restricted in nature, and less severe (13). As a working hypothesis, we suggest that endothelial dysfunction in IDDM develops gradually, with sustained increases in plasma vWF and UAE as relatively advanced features.

The type of endothelial dysfunction reflected by increased vWF levels is particularly closely related to microalbuminuria not only in IDDM (11), but also in non-insulin-dependent diabetes mellitus (NIDDM) (13,17) and essential hypertension (18). Microalbuminuria reflects an increased transcapillary passage of macromolecules (1), a phase that, in IDDM, is preceded by a clear and persistent increase in vWF levels, as shown by our study. Such data are not available for NIDDM or hypertension, although high vWF levels in NIDDM may similarly increase the risk of development of microalbuminuria and the risk of clinical cardiovascular disease once microalbuminuria is present (17). It is not clear, however, whether the prognostic value of vWF is related to its specific functions, i.e., enhancement of platelet adhesion and factor VIII availability, or whether it is simply a marker of endothelial injury and dysfunction. Nevertheless, both UAE and vWF deserve consideration as clinically useful markers of vascular status.

Determinants of increases in vWF, i.e., of endothelial injury, were not identified. Increases in vWF are nonspecific with respect to the type of injury and can be induced by hypertension, smoking, hypercholesterolemia, hyperglycemia, activation of coagulation, and cytokines (13,18,19). The pathogenesis of microalbuminuria is likely to involve hyperglycemia and an early rise in BP (1,3,20–22). Our study was small and excluded patients with poor glycemic control, thereby limiting the ability to find a relation between UAE or vWF and glycated hemoglobin (11) or BP (20–22). In addition, susceptibility to the development of diabetic nephropathy (and presumably microalbuminuria) is thought to vary among individuals (23). Variability among individuals in the susceptibility to injury of the endothelium might play a role, and this would hamper the identification of determinants of endothelial injury in cohort studies. Finally, other factors not assessed in this study may be important, such as insulin resistance, hyperinsulinemia, dyslipidemia, and activation of coagulation (19,24–26).

Are our findings generalizable? Clearly, selection bias cannot be completely excluded in a small study such as this. On the other hand, the patients were not selected on the basis of plasma vWF; therefore, the relationship between UAE and vWF is unlikely to have been affected by selection bias. In addition, the incidence density of microalbuminuria observed (6.3 or 4.2 [4 of 17; Fig. 1] with 20 μg/min [1,3,22] instead of 15 μg/min as cutoff) is within the range reported by others (1.1–8.2 [20–22,27–31]). Finally, the exclusion of patients with poor glycemic control may have increased the within-person stability of plasma vWF levels. Thus, the applicability of our results to patients with poorly controlled diabetes is somewhat uncertain.

Nevertheless, a sustained increase in plasma vWF in patients with IDDM is associated with a high risk of development of microalbuminuria. We suggest that such patients are candidates for intensified treatment of hyperglycemia and of rises in BP (even small ones).

ACKNOWLEDGMENTS

C.D.A.S. is supported by a fellowship from the Diabetes Fonds Nederland (Diabetes Research Fund) and the Netherlands Organisation for Scientific Research (NWO).

We thank W. Devillé, MD, for help with the statistical analysis and G. J. van Kamp, PhD, for measurement of vWF levels.

REFERENCES

1. Jensen T: Albuminuria: a marker of renal and generalized vascular disease in insulin-dependent diabetes mellitus. *Dan Med Bull* 38:134–144, 1991
2. Messent JWC, Elliott TG, Hill RD, Jarrett RJ, Keen H, Viberti GC: Prognostic significance of microalbuminuria in insulin-dependent diabetes mellitus: a twenty-three year follow-up study. *Kidney Int* 41:836–839, 1992
3. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A: Albuminuria reflects widespread vascular damage: the Steno hypothesis. *Diabetologia* 32:219–226, 1989
4. Vane JR, Änggård EE, Botting RM: Mechanisms of disease: regulatory functions of the vascular endothelium. *N Engl J Med* 323:27–36, 1990
5. Ross R: The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362:801–809, 1993
6. Remuzzi G, Benigni A: Endothelins in the control of cardiovascular and renal function. *Lancet* 342:589–593, 1993
7. Elliott TG, Cockcroft JR, Groop PH, Viberti GC, Ritter JM: Inhibition of nitric oxide synthesis in forearm vasculature of insulin-dependent diabetic patients: blunted vasoconstriction in patients with microalbuminuria. *Clin Sci* 83:687–693, 1993
8. Collier A, Leach JP, McLellan A, Jardine A, Morton JJ, Small M: Plasma endothelinlike immunoreactivity levels in insulin-dependent diabetes mellitus patients with microalbuminuria. *Diabetes Care* 15:1038–1040, 1992
9. Jensen T, Bjerre-Knudsen J, Feldt-Rasmussen B, Deckert T: Features of endothelial dysfunction in early diabetic nephropathy. *Lancet* i:461–463, 1989
10. Gruden G, Cavallo-Perin P, Bazzab M, Stella S, Vuolo A, Pagano G: PAI-1 and factor VII activity are higher in insulin-dependent diabetes mellitus patients with microalbuminuria. *Diabetes* 43:426–429, 1994
11. Stehouwer CDA, Stroes ESG, Hackeng WHL, Mulder PGH, den Ottolander GJH: von Willebrand factor and development of diabetic nephropathy in insulin-dependent diabetes mellitus. *Diabetes* 40:971–976, 1991 (Erratum, *Diabetes* 40:1746, 1991)
12. Stehouwer CDA, Zellenrath P, Polak BCP, Baarsma GS, Nauta JJP, Donker AJM, den Ottolander GJH: von Willebrand factor and early diabetic retinopathy: no evidence for a relationship in patients with type I (insulin-dependent) diabetes mellitus and normal urinary albumin excretion. *Diabetologia* 35:555–559, 1992
13. Stehouwer CDA, Donker AJM: Urinary albumin excretion and cardiovascular disease risk in diabetes mellitus: is endothelial dysfunction the missing link? *J Nephrol* 6:72–92, 1993
14. Porta M, La Selva M, Molinatti P, Molinatti GM: Endothelial cell function in diabetic microangiopathy. *Diabetologia* 30:601–609, 1987
15. Calver A, Collier J, Vallance P: Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. *J Clin Invest* 90:2548–2554, 1992
16. Vukovich TC, Schernthaner G, Knöbl PN, 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
17. Stehouwer CDA, Nauta JJP, Zeldenrust GC, Hackeng WHL, Donker AJM, den Ottolander GJH: Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus. *Lancet* 340:319–323, 1992
18. Pedrinelli R, Giampietro O, Carnassi F, Mellilo E, Dell'Omo G, Catapano G, Matteuci E, Talarico L, Morale M, de Negri F, di Bello V: Microalbuminuria and endothelial dysfunction in essential hypertension. *Lancet* 344:14–18, 1994
19. Conlan MG, Folsom AR, Finch A, Davis CE, Sorlie P, Marcucci G, Wu KK: Associations of factor VIII and von Willebrand factor with age, race, sex, and risk factors for atherosclerosis: the Atherosclerosis Risk in Communities (ARIC) Study. *Thromb Haemost* 70:380–385, 1993
20. Mathiesen ER, Ronn B, Jensen T, Storm B, Deckert T: Relationship between blood pressure and urinary albumin excretion in the development of microalbuminuria. *Diabetes* 39:245–250, 1990
21. Microalbuminuria Collaborative Study Group, United Kingdom: Risk factors for development of microalbuminuria in insulin-dependent diabetic patients: a cohort study. *Br Med J* 306:1235–1239, 1993
22. Poulsen PL, Hansen KW, Mogensen CE: Ambulatory blood pressure in the transition from normo- to microalbuminuria: a longitudinal study in insulin-dependent diabetes mellitus patients. *Diabetes* 43:1248–1253, 1994
23. Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease: evidence for genetic susceptibility for diabetic nephropathy. *N Engl J Med* 320:1161–1165, 1989
24. Yip J, Mattock MB, Morocutti A, Sethi M, Trevisan R, Viberti GC: Insulin resistance in insulin-dependent diabetic patients with microalbuminuria. *Lancet* 342:883–887, 1993
25. Groop L, Ekstrand A, Forsblom C, Widen E, Groop PH, Teppo AM, Eriksson J: Insulin resistance, hypertension and microalbuminuria in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 36:642–647, 1993
26. Ceriello A: Coagulation activation in diabetes mellitus: the role of hyperglycaemia and therapeutic prospects. *Diabetologia* 36:1119–1125, 1993
27. Jerums G, Cooper ME, Seeman E, Murray RML, McNeil JJ: Spectrum of proteinuria in type I and type II diabetes. *Diabetes Care* 10:419–427, 1987
28. Rudberg S, Persson B, Dahlquist G: Increased glomerular filtration rate as a predictor of diabetic nephropathy: an 8-year prospective study. *Kidney Int* 41:822–828, 1992
29. Raal FJ, Kalk WJ, Taylor DR, Osler CE, Panz VR: The relationship between the development and progression of microalbuminuria and arterial blood pressure in type I (insulin-dependent) diabetes mellitus. *Diabetes Res Clin Pract* 16:221–227, 1992
30. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
31. Coonrod BA, Ellis D, Becker DJ, Bunker CH, Kelsey SF, Lloyd CE, Drash AL, Kuller LH, Orchard TJ: Predictors of microalbuminuria in individuals with insulin-dependent diabetes mellitus: Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes Care* 16:1376–1383, 1993