# Increased Urinary Albumin Excretion, Endothelial Dysfunction, and Chronic Low-Grade Inflammation in Type 2 Diabetes

## Progressive, Interrelated, and Independently Associated With Risk of Death

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**In 328 type 2 diabetic patients followed for 9.0 years (mean), we investigated whether endothelial dysfunction and chronic inflammation (estimated from plasma markers) can explain the association between (micro)albuminuria and mortality. Of the patients, 113 died. Mortality was increased in patients with baseline microalbuminuria or macroalbuminuria (odds ratios as compared with normoalbuminuria, 1.78 [$P < 0.05$] and 2.86 [$P < 0.01$]) and in patients with soluble vascular cell adhesion molecule 1 in the third tertile and C-reactive protein in the second and third tertiles (odds ratios as compared with the first tertile, 2.05 [$P < 0.01$], and 1.80 [$P < 0.05$] and 2.92 [$P < 0.01$]). These associations were mutually independent. The mean yearly change in urinary albumin excretion was 9.4%; in von Willebrand factor, 8.1%; in tissue-type plasminogen activator, 2.8%; in soluble vascular cell adhesion molecule 1, 5.2%; in soluble E-selectin, −2.3%; in C-reactive protein, 3.8%; and in fibrinogen, 2.3%. The longitudinal development of urinary albumin excretion was significantly and independently determined by baseline levels of and the longitudinal development of BMI, systolic blood pressure, serum creatinine, glycated hemoglobin and plasma von Willebrand factor (baseline only), soluble E-selectin (baseline only), tissue-type plasminogen activator, C-reactive protein, and fibrinogen. The longitudinal developments of markers of endothelial function and inflammation were interrelated. In type 2 diabetes, increased urinary albumin excretion, endothelial dysfunction, and chronic inflammation are interrelated processes that develop in parallel, progress with time, and are strongly and independently associated with risk of death. *Diabetes* 51:1157–1165, 2002**

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CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; GEE, generalized estimating equation.

In type 2 diabetes, there is a strong association between microalbuminuria and risk of cardiovascular disease, which is independent of conventional risk factors such as hypertension and smoking (1). The nature of this association is poorly understood. It is unlikely to be causal because there is no plausible mechanism directly linking atherothrombotic disease to the quantitatively trivial albumin loss characteristic of microalbuminuria. A conceptually attractive explanation is that microalbuminuria is a marker of a pathophysiological process that causes both increased renal albumin loss and atherothrombosis. For example, it has been suggested that microalbuminuria reflects the severity of atherosclerosis (2). However, recent evidence indicates that atherosclerosis per se cannot explain the association between microalbuminuria and atherothrombosis in type 2 diabetes (3,4).

Dysfunction of the vascular endothelium and chronic low-grade inflammation are key features of the pathophysiology of atherothrombosis (5). Microalbuminuria in type 1 and type 2 diabetic patients, as well as in nondiabetic individuals, is associated with endothelial dysfunction and low-grade inflammation (6–13). We therefore hypothesized that endothelial dysfunction and/or chronic low-grade inflammation can explain the association between (micro)albuminuria and atherothrombotic disease in type 2 diabetes.

To investigate this hypothesis, we followed a cohort of patients with type 2 diabetes prospectively for 10 years. We repeatedly measured urinary albumin excretion and markers of endothelial dysfunction and chronic low-grade inflammation. We assessed their associations with mortality as well as their mutual relationships.

### RESEARCH DESIGN AND METHODS

**Patients.** The study population was based on all subjects ($n = 363$) with type 2 diabetes who were <66 years of age and attending the Hvidöre Hospital during the period of 1 January to 31 December 1987 (baseline examination [14]). Type 2 diabetes was defined as diabetes treated by diet alone or by diet combined with oral hypoglycemic agents; as treatment with insulin plus diabetes onset after the age of 40 years and a BMI above normal (≥25 kg/m$^2$ in women, ≥27 kg/m$^2$ in men) at the time of diagnosis; or as treatment with

insulin, normal BMI, and a glucagon-stimulated C-peptide value ≥0.60 pmol/ml. We excluded 31 non-Caucasian patients and 4 who did not hand in baseline urine collections. The cohort thus consisted of 328 patients.

**General procedures.** Annual follow-up examinations were performed from 1990 until and including 1997. Prior cardiovascular disease was defined as previous myocardial infarction or stroke (World Health Organization cardiovascular questionnaire). Current smokers were defined as subjects smoking one or more cigarettes, cigars, or pipes a day. Former smokers were defined as subjects who reported having quit smoking. Never-smokers were patients who described themselves as never having smoked. A positive smoking history included current and former smokers. Arterial blood pressure was measured after a 10-min rest with the patient supine. Two readings were made for each evaluation and averaged. At the baseline examination, we used a Hawksley random zero sphygmomanometer (Hawksley & Sons, Lancing, U.K.) recording phase I (systolic) and phase V (diastolic). From 1990 onward, an automatic oscillometric manometer (Takeda Medical UA-751, Tokyo) was used. Arterial hypertension was defined according to the World Health Organization criteria in use at the start of the study: systolic blood pressure ≥160 mmHg, diastolic blood pressure ≥95 mmHg, and/or antihypertensive treatment currently prescribed. Retinopathy was assessed by direct ophthalmoscopy at baseline and, from 1990, by fundus photography after pupillary dilatation. The degree of retinopathy was classified as nil, background, or proliferative. Body weight was measured, and the BMI was calculated as body weight (kg)/height $(m)^2$.

Blood was taken in the nonfasting state. $HbA_{1c}$ level was determined by an isoelectric focusing method (15) at baseline and by high-performance liquid chromatography thereafter (DIAMAT Analyzer; Biorad, Richmond, CA). The normal range was 4.1–6.1% in both assays. Serum concentrations of creatinine, total cholesterol, HDL cholesterol, and triglycerides were measured with standard methods.

The 24-h urine collection was carried out during unrestricted daily life activity. If bacterial growth was found, urine collection was repeated after treatment. The urinary albumin concentration was determined by radioimmunoassay (sensitivity, 0.5 mg/l; coefficient of variation [CV], 9% [16]) until 1992 and by enzyme-linked immunosorbent assay (ELISA) thereafter (sensitivity, 0.001 mg/l; CV, 8.3% [17]). The correlation between the two methods was $r = 0.99$ (17). At the baseline examination, patients were classified as having normoalbuminuria (albumin excretion <30 mg/24 h), microalbuminuria (30–299 mg/24 h), or macroalbuminuria (≥300 mg/24 h). At the follow-up examinations, we used the same criteria, except that patients with micro- or macroalbuminuria at a particular follow-up examination who had normo- or microalbuminuria (according to the above criteria) at subsequent examinations were classified as having micro- and macroalbuminuria, respectively. The number of 24-h urine collections performed by each patient during follow-up depended on the degree of albuminuria, reflecting the clinical routine at our clinic. Patients with normoalbuminuria collected at least one sample before each examination, and patients with micro- or macroalbuminuria usually collected three. Some patients did more than three collections during a year, and, for these patients, all available data have been used in the analyses.

Markers of endothelial function (von Willebrand factor, tissue-type plasminogen activator, soluble E-selectin, and soluble vascular cell adhesion molecule 1) and inflammatory activity (C-reactive protein and fibrinogen) were determined in duplicate in EDTA (1987) or citrated (after 1987) plasma that had been stored at −70°C. von Willebrand factor was determined using a homemade ELISA (18). Data are given as the percentage of pooled human plasma (set at 100%). The interassay CV was 9.1%, and the maximal (cutoff) value was 400%. Tissue-type plasminogen activator was measured using the Thrombonostika tPA ELISA according to the manufacturer's instructions (Organon-Teknika, Turnhout, Belgium). The interassay CV was 9.7%, and the cutoff value was 50 ng/ml. Soluble E-selectin was determined by ELISA according to the manufacturer's instructions (Bender MedSystems, Vienna, Austria). The interassay CV was 8.2%, and the cutoff value was 250 ng/ml. Soluble vascular cell adhesion molecule 1 was measured using an ELISA system from BioSource Europe SA (Nivelles, Belgium). The interassay CV was 27.4%, and the cutoff value was 3,750 ng/ml. C-reactive protein was measured by a homemade ELISA (19). The interassay CV was 13.9%, and the cutoff value was 20 mg/l. Fibrinogen was measured by immunoturbidimetry from 1990 onward (20).

All 328 patients were traced through the national register at the beginning of 1998. The observation period was defined as the number of days from the date of examination in 1987 to the date of death or 1 January 1998. Approval for the study was obtained from the Ethical Committee of Copenhagen County.

**Statistical analysis.** Cox regression was used to analyze the relationships between baseline normo-, micro-, or macroalbuminuria and markers of endothelial function and inflammatory activity (divided into tertiles) on the one hand and mortality on the other. The relationships were first analyzed without adjustment and then after adjustment for potential confounders. Adjustment for blood pressure level (shown in RESULTS) and for presence of hypertension gave comparable results.

We used generalized estimating equations (GEEs) to analyze the longitudinal relationships among urinary albumin excretion, markers of endothelial function, and markers of inflammatory activity (21). We first assessed baseline values of the markers of endothelial function and inflammatory activity as time-independent determinants of the longitudinal development of urinary albumin excretion. Next, we assessed the longitudinal associations between the markers and urinary albumin excretion by adding the marker concentrations as time-dependent determinants. The same two analyses were carried out to assess the longitudinal relationship between markers of endothelial function and markers of inflammatory activity. Finally, we assessed the longitudinal relationships between cardiovascular risk factors and markers of endothelial function and inflammatory activity. Outcome variables that were not normally distributed were log-transformed before analysis. Two-tailed $P$ values <0.05 were considered statistically significant. We used SPSS (SPSS, Chicago) for Cox analyses and SPIDA (Statistical Package for Interactive Data Analysis; Statistical Computing Laboratory, Macquarie, Australia) for GEE analyses.

## RESULTS

After a mean duration of follow-up of 9.0 years (SD 2.9; range 0.2–10.9), 113 (34%) patients had died (58 [51%] of cardiovascular disease and 9 [3%] of end-stage renal disease). Patients who died, compared with those who survived, were older, were more often men, had a longer duration of diabetes, had higher systolic blood pressure and more often had hypertension, had higher serum cholesterol and triglyceride concentrations, more often had prior cardiovascular disease, had a higher $HbA_{1c}$, more often had retinopathy, had a higher serum creatinine concentration and urinary albumin excretion, and had higher plasma concentrations of von Willebrand factor, tissue-type plasminogen activator, soluble vascular cell adhesion molecule 1, and C-reactive protein (Table 1).

**Baseline characteristics as determinants of mortality.** Table 2 shows that all conventional risk factors, except serum HDL cholesterol, were significantly associated with mortality after adjustment for age, sex, and diabetes duration. Micro- and macroalbuminuria were strongly associated with mortality (Table 3). Adjustment for markers of endothelial function and inflammatory activity did not importantly affect the associations (Table 3).

In univariate analyses, von Willebrand factor, soluble vascular cell adhesion molecule 1, and C-reactive protein were significantly associated with mortality (Table 3, model 1). Adjustment for conventional cardiovascular risk factors diminished the relative risk for von Willebrand factor but not those for soluble vascular cell adhesion molecule 1 or C-reactive protein (Table 3, models 2 and 4). Adjustment for urinary albumin excretion did not importantly affect the risk estimates (Table 3, model 3) nor did mutual adjustment of soluble vascular cell adhesion molecule 1 and C-reactive protein.

**Cardiovascular risk factors and plasma markers of endothelial function and inflammatory activity as determinants of the longitudinal development of urinary albumin excretion.** At baseline, 92 patients (28%) had microalbuminuria, and 45 (14%) had macroalbuminuria. Among the 191 patients with normoalbuminuria at baseline, microalbuminuria developed in 70 (37%) and macroalbuminuria in 60 (31%), including 21 who had first

TABLE 1
Baseline characteristics of 328 patients with type 2 diabetes

| | Survived | Died |
|---|---|---|
| *n* | 215 | 113 |
| Age (years) | 51.7 ± 9.1 | 57.9 ± 6.2 |
| Sex (% men) | 58.6 | 67.3 |
| BMI (kg/m$^2$) | 28.5 ± 5.0 | 29.0 ± 5.2 |
| Smoking (never/former/current %) | 29.8/25.1/45.1 | 24.8/24.8/50.4 |
| Diabetes duration (years) | 5.0 (2.0–10.0) | 9.0 (5.0–14.0) |
| Diabetes treatment (diet/insulin/other %) | 41.9/16.7/41.5 | 23.0/39.9/37.2 |
| Blood pressure (mmHg) | | |
| Systolic | 146.1 ± 21.4 | 159.7 ± 22.5 |
| Diastolic | 84.9 ± 11.2 | 87.3 ± 12.4 |
| Hypertension (%) | 45.1 | 67.3 |
| Antihypertensive drug treatment (%) | 31.6 | 48.7 |
| Serum total cholesterol (mmol/l) | 6.0 (5.1–6.9) | 6.7 (5.4–7.9) |
| Serum HDL cholesterol (mmol/l) | 1.0 (0.9–1.2) | 1.0 (0.8–1.3) |
| Serum triglycerides (mmol/l) | 1.7 (1.2–2.8) | 2.2 (1.4–3.5) |
| $HbA_{1c}$ (%) | 7.6 (6.4–9.1) | 8.6 (7.1–9.8) |
| Prior cardiovascular disease (%) | 3.3 | 22.1 |
| Retinopathy (background/proliferative %) | 22.8/2.3 | 44.2/7.1 |
| Serum creatinine (μmol/l) | 73.0 (63.0–85.0) | 81.0 (69.5–98.0) |
| Urinary albumin excretion (mg/24 h) | 13.0 (6.0–44.0) | 61.0 (14.0–245.0) |
| Normo-/micro-/macroalbuminuria (%) | 70.7/20.5/8.8 | 34.5/41.6/23.9 |
| Plasma von Willebrand factor (%) | 83.6 (54.9–112.2) | 112.8 (69.2–139.9) |
| Plasma tissue-type plasminogen activator (μg/l) | 6.3 (4.2–9.7) | 7.2 (4.8–11.0) |
| Plasma soluble vascular cell adhesion molecule 1 (μg/l) | 640 (443–946) | 881 (588–1,295) |
| Plasma soluble E-selectin (μg/l) | 64.1 (42.9–88.4) | 65.6 (47.6–97.0) |
| Plasma C-reactive protein (mg/l) | 2.9 (0.9–5.9) | 4.8 (2.2–10.5) |

Data are means ± SD or medians (25th–75th percentile) unless otherwise indicated.

TABLE 2
Associations between conventional risk factors at baseline and mortality during a 10-year follow-up in 328 patients with type 2 diabetes

| | Model 1 | Model 2 |
|---|---|---|
| Smoking habits | 1.11 (0.71–1.72) | — |
| BMI | 1.46 (0.92–2.32) | 1.53 (0.95–2.48) |
| | 2.36 (1.42–3.91)* | 1.98 (1.17–3.37)† |
| Systolic blood pressure | 2.15 (1.21–3.80)* | 2.10 (1.23–3.95)* |
| | 2.84 (1.63–4.95)* | 3.51 (1.96–6.31)* |
| Diastolic blood pressure | 0.91 (0.55–1.51) | — |
| | 1.79 (1.13–2.82)† | — |
| Serum total cholesterol | 1.04 (0.63–1.73) | 0.92 (0.55–1.54) |
| | 2.21 (1.39–3.54)* | 1.55 (0.95–2.53) |
| Serum HDL cholesterol | 1.38 (0.88–2.17) | — |
| | 0.76 (0.47–1.24) | — |
| Serum triglycerides | 1.41 (0.87–2.29) | — |
| | 1.91 (1.20–3.05)* | — |
| $HbA_{1c}$ | 1.31 (0.78–2.19) | 1.29 (0.76–2.19) |
| | 2.39 (1.47–3.87)* | 2.02 (1.22–3.35)* |
| Serum creatinine | 1.10 (0.64–1.90) | — |
| | 1.83 (1.05–3.20)† | — |
| Prior cardiovascular disease | 2.89 (1.81–4.62)* | 3.64 (2.20–6.03)* |

Model 1: relative risks (95% CI) adjusted for age, sex, and diabetes duration for the second and third tertile of the distribution of the variable under consideration, with the first tertile as the reference category. The cutoff values for the second and third tertile were as follows: BMI, 25.9 and 30.4 kg/m$^2$; systolic blood pressure, 138 and 158 mmHg; diastolic blood pressure, 81 and 90 mmHg; serum total cholesterol, 5.5 and 6.8 mmol/l; serum HDL cholesterol, 0.9 and 1.2 mmol/l; serum triglycerides, 1.5 and 2.4 mmol/l; $HbA_{1c}$, 7.0 and 8.9%; and serum creatinine, 68.0 and 83.1 μmol/l. For smoking habits, the table shows the relative risks associated with ever-smoking versus never having smoked. Model 2: relative risks (95% CI) after backward selection of all risk factors shown in the table, adjusted for age, sex, and diabetes duration. *$P < 0.01$; †$P < 0.05$.

developed microalbuminuria. The mean overall increase in urinary albumin excretion was 9.4% per year. After adjustment, the longitudinal development of urinary albumin excretion was significantly determined by baseline levels of and the longitudinal development of BMI, systolic blood pressure, serum creatinine, $HbA_{1c}$ and plasma von Willebrand factor (baseline only), soluble E-selectin (baseline only), tissue-type plasminogen activator, C-reactive protein, and fibrinogen (Table 4).

**Associations between the longitudinal developments of markers of endothelial function and inflammatory activity.** The mean yearly change in von Willebrand factor was 8.1%; in tissue-type plasminogen activator, 2.8%; in soluble vascular cell adhesion molecule 1, 5.2%; in soluble E-selectin, −2.3%; in C-reactive protein, 3.8%; and in fibrinogen, 2.3%. Table 5 shows that the longitudinal development of C-reactive protein was positively and significantly associated with the longitudinal development of all markers of endothelial function. For fibrinogen, the associations were significant only for von Willebrand factor. Conversely, the longitudinal development of markers of endothelial function was positively and, in general, significantly associated with the longitudinal development of C-reactive protein; the longitudinal development of von Willebrand factor was also associated with the longitudinal development of fibrinogen. The longitudinal developments of C-reactive protein and fibrinogen were mutually positively associated. The longitudinal developments of all markers of endothelial function were positively interrelated, except for the association between tissue-type plasminogen activator and soluble vascular cell adhesion molecule 1.

Inflammatory activity might be linked to the longitudinal development of urinary albumin excretion through endothelial dysfunction. However, adjustment for markers of endothelial function did not strongly affect the associations between markers of inflammatory activity and the longitudinal development of urinary albumin excretion (results not shown).

**Conventional cardiovascular risk factors as determinants of the longitudinal development of markers of endothelial function and inflammatory activity.** In both univariate and adjusted analyses, the longitudinal development of BMI and $HbA_{1c}$ was significantly positively

TABLE 3
Associations between mortality and urinary albumin excretion and plasma concentrations of markers of endothelial function and inflammatory activity at baseline during a 10-year follow-up in 328 patients with type 2 diabetes

| | Model 1 | Model 2 | Model 3 | Model 4 | |
|---|---|---|---|---|---|
| | | | | | + C-reactive protein‡ |
| Urinary albumin excretion | 3.01 (1.96–4.60)* | 2.36 (1.54–3.63)* | — | 1.78 (1.12–2.83)† | 1.86 (1.17–2.96)† |
| | 4.16 (2.54–6.81)* | 4.74 (2.82–7.96)* | — | 2.86 (1.61–5.09)* | 2.76 (1.52–4.85)* |
| von Willebrand factor | 1.08 (0.64–1.85) | 1.02 (0.59–1.76) | 0.96 (0.55–1.65) | 0.80 (0.46–1.40) | — |
| | 2.66 (1.67–4.24)* | 1.89 (1.17–3.08)* | 1.41 (0.85–2.32) | 1.16 (0.70–1.43) | — |
| Tissue-type plasminogen activator | 0.99 (0.61–1.59) | 1.06 (0.65–1.71) | 1.01 (0.62–1.64) | 0.78 (0.46–1.30) | — |
| | 1.31 (0.83–2.06) | 1.48 (0.94–2.34) | 1.42 (0.90–2.25) | 1.10 (0.68–1.79) | — |
| | | | | | + C-reactive protein |
| Soluble vascular cell adhesion molecule 1 | 1.95 (1.16–3.29)* | 1.52 (0.89–2.58) | 1.49 (0.87–2.56) | 1.86 (1.05–3.28)† | 1.74 (0.99–3.08) |
| | 2.54 (1.53–4.21)* | 2.05 (1.22–3.42)* | 1.84 (1.08–3.12)† | 2.26 (1.29–3.94)* | 2.11 (1.20–3.70)* |
| Soluble E-selectin | 1.28 (0.79–2.05) | 1.28 (0.79–2.08) | 1.08 (0.66–1.77) | 1.00 (0.60–1.66) | — |
| | 1.35 (0.85–2.16) | 2.03 (1.24–3.32)* | 1.54 (0.93–2.55) | 1.04 (0.60–1.82) | — |
| | | | | | + Soluble vascular cell adhesion molecule 1 |
| C-reactive protein | 1.76 (1.04–3.00)† | 1.80 (1.06–3.08)† | 1.47 (0.86–2.53) | 1.28 (0.73–2.22) | 1.26 (0.72–2.21) |
| | 2.86 (1.73–4.71)* | 2.92 (1.76–4.85)* | 2.23 (1.33–3.75)* | 1.86 (1.17–2.96)† | 1.66 (0.95–2.92) |

Data are relative risks (95% CI) for micro- and macroalbuminuria, with normoalbuminuria as the reference category, and for the second and third tertile of distribution of the variable under consideration, with the first tertile as the reference category. The cutoff values for the second and third tertile were as follows: von Willebrand factor, 67.9 and 111.9%; tissue-type plasminogen activator, 5.2 and 8.1 μg/l; soluble E-selectin, 52.8 and 79.2 μg/l; soluble vascular cell adhesion molecule 1, 546.4 and 908.2 μg/l; and C-reactive protein, 1.9 and 5.5 mg/l. Model 1: unadjusted; model 2: adjusted for age, sex, diabetes duration, and prior cardiovascular disease; model 3: same as model 2, plus adjusted for urinary albumin excretion; and model 4: same as model 3, plus adjusted for BMI, systolic blood pressure, serum total cholesterol, and $HbA_{1c}$. *$P < 0.01$; †$P < 0.05$; ‡additional adjustment for soluble vascular cell adhesion molecule-1 or von Willebrand factor showed comparable results.

associated with the longitudinal development of all markers, except soluble vascular cell adhesion molecule 1 (Table 6). To test whether BMI and $HbA_{1c}$ were related to mortality through endothelial dysfunction and inflammatory activity, we reanalyzed the mortality data (Table 2) with adjustment for markers of endothelial function or inflammatory activity. As compared with model 1 in Table 2, the association between BMI and mortality was weakened after adjustment for C-reactive protein (relative risks for the second and third tertile, 1.06 [0.64–1.75] and 1.70 [0.99–2.91]). In addition, the association between $HbA_{1c}$ and mortality (Table 2) was weakened after adjustment for C-reactive protein (relative risks, 1.06 [0.62–1.81] and 1.97 [1.19–2.23]), von Willebrand factor (relative risks, 1.18 [0.70–1.99] and 1.99 [1.20–3.28]), and soluble E-selectin (relative risks, 1.25 [0.74–2.12] and 2.11 [1.28–3.49]). The associations between both BMI and $HbA_{1c}$ and urinary albumin excretion (Table 4) were not importantly different after adjustment for markers of endothelial function or inflammatory activity. The associations between systolic blood pressure and serum cholesterol on the one hand and mortality (Table 2) and the development of urinary albumin excretion (Table 4) on the other were also not importantly different after adjustment for markers of endothelial function or inflammatory activity.

The above analyses show that conventional cardiovascular risk factors, markers of endothelial dysfunction and inflammatory activity, and increased urinary albumin excretion are interrelated. We therefore applied several time-lag GEE models to investigate whether these processes occurred in a certain sequence in time (22). However, because of the high relative stability over time of the factors under consideration, these time-lag models did not reveal strong evidence for specific sequences in time (results not shown).

## DISCUSSION

There are three major findings from this prospective investigation in individuals with type 2 diabetes. First, patients with (micro)albuminuria and high plasma concentrations of soluble vascular cell adhesion molecule 1 (a putative marker of endothelial dysfunction) and C-reactive protein (a marker of inflammation) had an increased risk of death. Importantly, these associations were not only independent of conventional cardiovascular risk factors, but also of each other, which suggests that the pathway linking (micro)albuminuria to mortality does not involve endothelial dysfunction or increased inflammatory activity to the extent reflected by these markers. Second, however, markers of endothelial dysfunction and of inflammatory activity were strongly associated with increases in urinary albumin excretion during the 10-year follow-up. These associations were independent of major risk factors for developing (micro)albuminuria, such as high blood pressure and poor glycemic control. These findings thus show that both endothelial dysfunction and inflammation are involved in the pathogenesis of (micro)albuminuria but that these phenomena cannot explain the latter's association with risk of death. Third, conventional cardiovascular risk factors, in particular obesity and poor glycemic control, were associated with increases in markers of endothelial dysfunction and inflammatory activity during follow-up. Taken together, these findings suggest that in patients with type 2 diabetes, conventional risk factors increase the risk of death in part through causing endo-

TABLE 4
Cardiovascular risk factors and plasma concentrations of markers of endothelial function and inflammatory activity as determinants of the longitudinal development of urinary albumin excretion (natural log-transformed) over a 10-year period in 328 patients with type 2 diabetes

| | Baseline (single) measurement of determinants | | Longitudinal (multiple) measurements of determinants | |
|---|---|---|---|---|
| | Model 1 | Model 2 | Model 1 | Model 2 |
| BMI (per 1 kg/m$^2$) | 0.067 (0.024 to 0.110)* | 0.070 (0.031 to 0.109)* | 0.043 (0.016 to 0.070)* | 0.035 (0.004 to 0.066)† |
| Smoking (ever vs. never) | 0.222 (−0.286 to 0.730) | 0.245 (−0.208 to 0.698) | 0.070 (−0.357 to 0.497) | 0.131 (−0.226 to 0.488) |
| Systolic blood pressure (per 10 mmHg) | 0.026 (0.018 to 0.034)* | 0.014 (0.003 to 0.024)† | 0.123 (0.100 to 0.155)* | 0.141 (0.100 to 0.183)* |
| Diastolic blood pressure (per 10 mmHg) | 0.421 (0.222 to 0.611)* | 0.102 (−0.110 to 0.314) | 0.152 (0.099 to 0.205)* | 0.003 (−0.077 to 0.083) |
| Total cholesterol (per 1.0 mmol/l) | 0.183 (0.020 to 0.346)† | 0.001 (−0.154 to 0.164) | 0.105 (0.046 to 0.164)* | 0.040 (−0.040 to 0.120) |
| HDL cholesterol (per 0.1 mmol/l) | −0.052 (−0.104 to 0.000)† | 0.023 (−0.023 to 0.080) | −0.007 (−0.028 to 0.014) | 0.003 (−0.020 to 0.026) |
| Triglycerides (per 1.0 mmol/l) | 0.147 (0.051 to 0.243)* | 0.092 (0.000 to 0.182)† | 0.040 (0.016 to 0.064)* | 0.021 (−0.010 to 0.052) |
| Creatinine (per 10 μmol/l) | 0.144 (0.061 to 0.228)* | 0.071 (0.017 to 0.126)* | 0.057 (0.027 to 0.087)* | 0.063 (0.033 to 0.093)* |
| $HbA_{1c}$ (per 1.0%) | 0.289 (0.191 to 0.387)* | 0.175 (0.067 to 0.283)* | 0.027 (−0.006 to 0.060) | 0.058 (0.017 to 0.099)* |
| Prior cardiovascular disease (yes vs. no) | 0.719 (−0.110 to 1.548) | 0.261 (−0.519 to 1.041) | 0.526 (−0.142 to 1.194) | 0.092 (−0.533 to 0.717) |
| von Willebrand factor (per 10% point) | 0.079 (0.028 to 0.131)* | 0.047 (0.000 to 0.094)† | 0.014 (0.000 to 0.028)† | 0.009 (−0.008 to 0.026) |
| Tissue-type plasminogen activator (per 1.0 μg/l) | 0.015 (−0.005 to 0.035) | 0.019 (0.001 to 0.037)† | 0.011 (0.000 to 0.023)† | 0.011 (−0.001 to 0.023)† |
| Soluble vascular cell adhesion molecule 1 (per 100 μg/l) | 0.038 (−0.005 to 0.081) | 0.017 (−0.016 to 0.050) | 0.018 (−0.002 to 0.037) | 0.014 (−0.004 to 0.032) |
| Soluble E-selectin (per 10 μg/l) | 0.076 (0.019 to 0.133)* | 0.076 (0.029 to 0.123)* | 0.035 (0.004 to 0.066)† | 0.004 (−0.028 to 0.035)§ |
| C-reactive protein (per 1.0 mg/l) | 0.083 (0.039 to 0.125)* | 0.061 (0.022 to 0.100)* | 0.017 (0.005 to 0.288)* | 0.017 (0.005 to 0.288)† |
| Fibrinogen (per 1.0 μmol/l)‡ | 0.243 (0.137 to 0.349)* | 0.132 (0.034 to 0.230)* | 0.070 (0.041 to 0.099)* | 0.061 (0.032 to 0.090)† |

Data are regression coefficients (95% CI) calculated with GEE analysis, which reflects averaged associations. For example, a regression coefficient of 0.067 for the association between BMI and urinary albumin excretion (top left) means that for each increase in BMI of 1 kg/m$^2$, urinary albumin excretion increases by $e^{0.067} = 1.07$ mg/24h. Model 1: univariate; model 2: adjusted for sex, age diabetes duration, prior cardiovascular disease, and classic cardiovascular risk factors (BMI, smoking, systolic and diastolic blood pressure, total cholesterol, HDL cholesterol, triglycerides, creatinine, and $HbA_{1c}$). *$P < 0.01$; †$P < 0.05$; ‡because fibrinogen was measured from 1990 onward, the coefficients for fibrinogen reflect the association with the longitudinal development of urinary albumin excretion over a 7-year period; §the point estimate of the regression coefficient of soluble E-selectin decreased after adjustment for BMI, total cholesterol, and systolic and diastolic blood pressure.

thelial dysfunction, increased inflammatory activity, and (micro)albuminuria; that these phenomena progress with time and are interrelated; and that their associations with risk of death are mutually independent and thus may reflect distinct pathophysiological pathways (Fig. 1).

We chose to investigate individuals with type 2 diabetes who were at high risk of cardiovascular disease, as illustrated by the fact that at a mean age of 54 years and a mean diabetes duration of 6 years, the baseline prevalence of (micro)albuminuria was 42% and that of prior cardiovascular disease was 10%; after 10 years of follow-up, 34% had died. Such characteristics increase the chance of finding associations between endothelial dysfunction and inflammatory activity on the one hand and mortality and the development of urinary albumin excretion on the other if such associations do indeed exist. We cannot be certain that our findings can be generalized to patients with a less adverse risk profile. Nevertheless, the association between (micro)albuminuria and cardiovascular risk holds both in investigations that are population-based (i.e., include subjects at lower risk) and in those that are hospital based (i.e., include subjects at higher risk) (1), suggesting that our findings may also be valid in type 2 diabetic patients with a less adverse prognosis than those we studied.

The nature of the association between (micro)albuminuria and risk of death has remained enigmatic. Our findings clearly support the hypothesis that endothelial dysfunction and inflammatory activity are involved in the pathogenesis of (micro)albuminuria (6–11). At the same time, our findings establish that endothelial dysfunction and inflammatory activity can, at most, explain a minor part of the association between (micro)albuminuria and risk of death. Because the sensitivity and the specificity of individual marker proteins are limited (6), we used a panel of markers. Importantly, all were positively associated with the development of urinary albumin excretion (Table 4). The most important limitation of this approach is that it does not provide precise mechanistic insights into how endothelial dysfunction and inflammatory activity cause (micro)albuminuria. Thus, we do not know whether the association between, for example, tissue-type plasminogen activator and urinary albumin excretion reflects endo-

TABLE 5
Relationships among the longitudinal developments of plasma markers of inflammatory activity and of plasma markers of endothelial function over a 10-year period in 328 patients with type 2 diabetes

| Outcome variable of interest | Determinants | | | | | |
|---|---|---|---|---|---|---|
| | von Willebrand factor | Tissue-type plasminogen activator | Soluble vascular cell adhesion molecule | Soluble E-selectin | Fibrinogen‡ | C-reactive protein |
| C-reactive protein | | | | | | |
| Model 1 | 0.046 (0.034 to 0.058)* | 0.016 (0.008 to 0.024)* | 0.019 (0.009 to 0.029)* | 0.068 (0.046 to 0.090)* | 0.142 (0.113 to 0.171)* | — |
| Model 2 | 0.032 (0.022 to 0.041)* | 0.011 (0.003 to 0.019)* | 0.017 (0.005 to 0.029)* | 0.045 (0.023 to 0.067)* | 0.148 (0.119 to 0.177)* | — |
| Fibrinogen‡ | | | | | | |
| Model 1 | 0.085 (0.040 to 0.130)* | 0.009 (−0.013 to 0.031) | 0.025 (−0.006 to 0.056) | 0.019 (−0.040 to 0.078) | — | 0.155 (0.126 to 0.184)* |
| Model 2 | 0.067 (0.029 to 0.105)* | 0.005 (−0.017 to 0.027) | −0.004 (−0.035 to 0.027) | 0.024 (−0.039 to 0.087) | — | 0.154 (0.123 to 0.185)* |
| von Willebrand factor | | | | | | |
| Model 1 | — | 0.010 (0.006 to 0.014)* | 0.026 (0.022 to 0.030)* | 0.026 (0.018 to 0.034)* | 0.022 (0.012 to 0.032)* | 0.013 (0.009 to 0.017)* |
| Model 2 | — | 0.009 (0.005 to 0.013)* | 0.024 (0.020 to 0.028)* | 0.020 (0.010 to 0.030)* | 0.020 (0.010 to 0.030)* | 0.011 (0.007 to 0.015)* |
| Tissue-type plasminogen activator | | | | | | |
| Model 1 | 0.024 (0.016 to 0.032)* | — | 0.003 (−0.003 to 0.009) | 0.053 (0.041 to 0.065)* | 0.006 (–0.008 to 0.020) | 0.010 (0.006 to 0.014)* |
| Model 2 | 0.019 (0.013 to 0.025)* | — | 0.007 (0.001 to 0.013)† | 0.044 (0.032 to 0.056)† | 0.003 (–0.013 to 0.019) | 0.006 (0.002 to 0.010)* |
| Soluble vascular cell adhesion molecule 1 | | | | | | |
| Model 1 | 0.033 (0.027 to 0.039)* | 0.002 (−0.002 to 0.006) | — | 0.008 (−0.004 to 0.020) | 0.007 (−0.003 to 0.017) | 0.004 (0.000 to 0.008) |
| Model 2 | 0.024 (0.019 to 0.028)* | 0.003 (−0.001 to 0.007) | — | 0.015 (0.001 to 0.029)† | 0.001 (−0.011 to 0.013) | 0.004 (0.000 to 0.004) |
| Soluble E-selectin | | | | | | |
| Model 1 | 0.012 (0.008 to 0.016)* | 0.008 (0.004 to 0.012)* | 0.004 (0.000 to 0.008) | — | 0.001 (−0.007 to 0.009) | 0.008 (0.004 to 0.012)* |
| Model 2 | 0.006 (0.003 to 0.009)* | 0.006 (0.004 to 0.008)* | 0.007 (0.003 to 0.011)* | — | 0.002 (−0.006 to 0.010) | 0.006 (0.002 to 0.010)* |

Data are regression coefficients (95% CI) calculated with GEE analysis, which reflects averaged associations. For example, a regression coefficient of 0.046 for the association between von Willebrand factor and C-reactive protein means that for each increase in von Willebrand factor of 10% point, C-reactive protein increases by $e^{0.046} = 1.05$ mg/l. For all outcome variables (except for fibrinogen), the natural log was used in the analyses. For von Willebrand factor, coefficients are expressed per 10% point; for tissue-type plasminogen activator, per 1.0 μg/l; for soluble vascular cell adhesion molecule 1, per 100 μg/l; for soluble E-selectin, per 10 μg/l; for fibrinogen, per 1.0 μmol/l; and for C-reactive protein, per 1.0 mg/l. Model 1: univariate; model 2: adjusted for sex, age, diabetes duration, prior cardiovascular disease, and classic cardiovascular risk factors (BMI, smoking, systolic and diastolic blood pressure, total cholesterol, HDL cholesterol, triglycerides, creatinine, and $HbA_{1c}$). *$P < 0.01$; †$P < 0.05$; ‡because fibrinogen was measured from 1990 onward, the coefficients for fibrinogen reflect the association with the longitudinal development of markers of endothelial function over a 7-year period.

TABLE 6
Relationships among the longitudinal developments of cardiovascular risk factors (determinants) and plasma concentrations of markers of endothelial function and inflammatory activity (outcome variables) over a 10-year period in 328 patients with type 2 diabetes

| | von Willebrand factor | Tissue-type plasminogen activator | Soluble vascular cell adhesion molecule 1 | Soluble E-selectin | C-reactive protein | Fibrinogen‡ |
|---|---|---|---|---|---|---|
| BMI | | | | | | |
| Model 1 | 0.012 (0.004 to 0.020)* | 0.036 (0.024 to 0.048)* | −0.009 (−0.021 to 0.003) | 0.030 (0.020 to 0.039)* | 0.083 (0.065 to 0.101)* | 0.057 (0.006 to 0.108)† |
| Model 2 | 0.011 (0.003 to 0.032)* | 0.031 (0.019 to 0.043)* | −0.009 (−0.021 to 0.003) | 0.022 (0.012 to 0.032)* | 0.077 (0.059 to 0.095)* | 0.055 (0.006 to 0.104)† |
| Smoking | | | | | | |
| Model 1 | 0.074 (−0.024 to 0.172) | 0.108 (−0.097 to 0.247) | 0.016 (−0.111 to 0.143) | −0.032 (−0.136 to 0.072) | 0.323 (0.064 to 0.582)† | 0.379 (−0.236 to 0.994) |
| Model 2 | 0.092 (0.000 to 0.186) | 0.121 (−0.018 to 0.260) | 0.029 (−0.091 to 0.149) | −0.033 (−0.139 to 0.073) | 0.400 (0.179 to 0.621)* | 0.309 (−0.242 to 0.860) |
| Systolic blood pressure | | | | | | |
| Model 1 | 0.006 (−0.006 to 0.017) | −0.004 (−0.019 to 0.010) | 0.009 (−0.005 to 0.022) | 0.013 (0.004 to 0.022)* | 0.003 (−0.022 to 0.029) | 0.109 (0.029 to 0.189)* |
| Model 2 | −0.007 (−0.021 to 0.007) | −0.018 (−0.036 to 0.001) | 0.012 (−0.008 to 0.031) | 0.000 (−0.013 to 0.006) | −0.009 (−0.043 to 0.025) | 0.091 (−0.016 to 0.198) |
| Diastolic blood pressure | | | | | | |
| Model 1 | 0.021 (−0.001 to 0.043) | 0.021 (−0.008 to 0.050) | −0.012 (−0.036 to 0.012) | 0.051 (0.033 to 0.069)* | 0.024 (−0.027 to 0.075) | 0.071 (−0.090 to 0.232) |
| Model 2 | 0.033 (0.006 to 0.060)† | 0.033 (−0.004 to 0.070) | −0.013 (−0.050 to 0.024) | 0.040 (0.018 to 0.062)* | 0.028 (−0.039 to 0.095) | −0.006 (−0.258 to 0.138) |
| Total cholesterol | | | | | | |
| Model 1 | 0.015 (−0.005 to 0.035) | 0.062 (0.039 to 0.086)* | −0.022 (−0.044 to 0.000) | 0.066 (0.046 to 0.086)* | 0.044 (0.003 to 0.085)† | 0.029 (−0.116 to 0.174) |
| Model 2 | 0.001 (−0.026 to 0.028) | 0.026 (−0.001 to 0.053) | −0.031 (−0.062 to 0.000) | 0.041 (0.023 to 0.059)* | 0.000 (−0.053 to 0.053) | 0.083 (−0.103 to 0.269) |
| HDL cholesterol | | | | | | |
| Model 1 | −0.004 (−0.012 to 0.004) | −0.012 (−0.021 to −0.003)* | −0.004 (−0.016 to 0.007) | −0.009 (−0.015 to −0.002)* | −0.045 (−0.062 to −0.028)* | −0.117 (−0.161 to −0.072)* |
| Model 2 | 0.004 (−0.003 to 0.011) | −0.003 (−0.013 to 0.007) | −0.002 (−0.014 to 0.010) | −0.002 (−0.008 to 0.004) | −0.030 (−0.046 to −0.013)* | −0.123 (−0.174 to −0.073)* |
| Triglycerides | | | | | | |
| Model 1 | 0.011 (0.001 to 0.021)† | 0.032 (0.014 to 0.050)* | 0.004 (−0.006 to 0.014) | 0.025 (0.009 to 0.041)* | 0.042 (0.024 to 0.060)* | −0.043 (−0.092 to 0.006) |
| Model 2 | 0.006 (−0.004 to 0.016) | 0.018 (0.002 to 0.038)† | 0.015 (0.003 to 0.027)† | 0.006 (−0.006 to 0.178) | 0.017 (−0.005 to 0.039) | −0.096 (−0.190 to −0.002)† |
| Creatinine | | | | | | |
| Model 1 | 0.012 (0.006 to 0.018)* | −0.016 (−0.028 to −0.005)* | 0.027 (0.019 to 0.036)* | −0.011 (−0.019 to −0.002)† | 0.000 (−0.013 to 0.013) | 0.121 (0.069 to 0.172)* |
| Model 2 | 0.015 (0.008 to 0.021)* | −0.012 (−0.024 to 0.001) | 0.027 (0.018 to 0.036)* | −0.005 (−0.014 to 0.004) | 0.013 (0.002 to 0.025)† | 0.127 (0.077 to 0.177)* |
| $HbA_{1c}$ | | | | | | |
| Model 1 | 0.030 (0.018 to 0.041)* | 0.040 (0.022 to 0.058)* | −0.011 (−0.029 to 0.007) | 0.049 (0.039 to 0.059)* | 0.064 (0.039 to 0.090)* | 0.051 (−0.039 to 0.141) |
| Model 2 | 0.030 (0.016 to 0.044)* | 0.022 (0.000 to 0.044)* | −0.003 (−0.021 to 0.015) | 0.040 (0.028 to 0.052)** | 0.049 (0.020 to 0.078)* | 0.071 (−0.029 to 0.171) |
| Prior cardiovascular disease | | | | | | |
| Model 1 | 0.045 (−0.114 to 0.204) | 0.024 (−0.148 to 0.196) | 0.179 (0.020 to 0.338)† | −0.039 (−0.190 to 0.112) | 0.400 (0.043 to 0.757)† | 1.813 (0.521 to 3.105)* |
| Model 2 | −0.023 (−0.184 to 0.138) | −0.022 (−0.189 to 0.145) | 0.111 (−0.044 to 0.266) | −0.070 (−0.215 to 0.075) | 0.296 (−0.037 to 0.629) | 1.497 (0.101 to 2.893)† |

Data are regression coefficients (95% CI) from GEE analysis, which reflects averaged associations. For example, a regression coefficient of 0.012 for the association between BMI and von Willebrand factor means that for each increase in BMI of 1.0 $kg/m^2$, von Willebrand factor increases by $e^{0.012} = 1.01\%$ point. All outcome variables except fibrinogen were natural log-transformed. For BMI, coefficients are expressed per 1.0 $kg/m^2$; for smoking, ever versus never; for blood pressures, per 10 mmHg; for total cholesterol, per 1.0 mmol/l; for HDL cholesterol, per 0.1 mmol/l; for triglycerides, per 1.0 mmol/l; for creatinine, per 10 μmol/l; and for $HbA_{1c}$, per 1.0%. Model 1: univariate; model 2: adjusted for sex, age, diabetes duration, prior cardiovascular disease, and classic cardiovascular risk factors (BMI, smoking, systolic and diastolic blood pressure, total cholesterol, HDL cholesterol, triglycerides, creatinine, and $HbA_{1c}$). *$P < 0.01$; †$P < 0.05$; ‡because fibrinogen was measured from 1990 onward, the coefficients for fibrinogen reflect associations over a 7-year period.

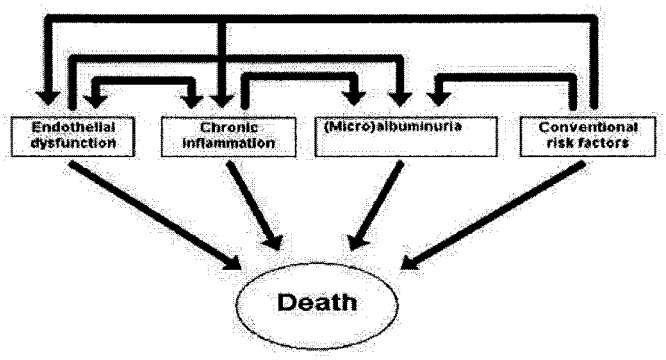


**FIG. 1. Postulated pathways leading to death in type 2 diabetes.**

thelial dysfunction in general or whether it specifically means that impairment of the regulation of fibrinolysis is involved. Similarly, we cannot exclude that the association between fibrinogen and urinary albumin excretion in part reflects fibrinogen's procoagulant and viscous properties. These issues require further investigation.

Inflammatory activity and endothelial dysfunction increased over time; these increases were strongly interrelated (Table 5). C-reactive protein and fibrinogen may reflect the presence of cytokines such as tumor necrosis factor-α and interleukin-6, which can cause endothelial dysfunction either directly or indirectly (5–7). C-reactive protein may in addition have biological properties that cause endothelial dysfunction (23). Our data also raise the possibility that endothelial dysfunction causes an increase in inflammatory activity, possibly through the elaboration of proinflammatory cytokines, thus potentially creating a vicious cycle of inflammatory activity and endothelial dysfunction.

Even though endothelial dysfunction and inflammatory activity were interrelated, we found no evidence that the association between inflammatory markers and mortality or the development of urinary albumin excretion could be explained by markers of endothelial dysfunction. Therefore, there may be distinct pathways through which inflammatory activity and endothelial dysfunction contribute to these adverse outcomes, which suggests that both may be targets for intervention.

BMI and $HbA_{1c}$ were consistently positively associated with the longitudinal development of markers of inflammatory activity and endothelial dysfunction (Table 6). Adipocytes synthesize mediators such as cytokines, nonesterified fatty acids, and leptin, which may induce inflammation and alter endothelial function (5–7,24,25). The associations of $HbA_{1c}$ with inflammation and endothelial function may reflect the combined biological effects of hyperglycemia, Amadori products, and advanced glycated end products (6,26,27).

Strengths of our study include its prospective design; the repeated assessment of risk factors, marker proteins, and urinary albumin excretion, which allowed an assessment of their interrelationships that had important advantages over conventional regression analyses (22); and the completeness of the follow-up. Limitations include its relatively small size, which resulted in insufficient power to assess determinants of cardiovascular mortality and morbidity and of end-stage renal disease, and changes in guidelines for intervention during the follow-up, which may have diluted the influence of, for example, blood pressure.

We conclude that in individuals with type 2 diabetes, conventional risk factors, especially obesity and poor glycemic control, may increase the risk of death in part through causing endothelial dysfunction, increased inflammatory activity, and (micro)albuminuria; that these phenomena are interrelated and progressive; and that their associations with risk of death are mutually independent. Our data provide a basis for investigating the effects of specific interventions to decrease inflammatory activity and improve endothelial dysfunction in type 2 diabetes.

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