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ORIGINAL ARTICLE

Increased urinary orosomucoid excretion: a proposed marker for inflammation and endothelial dysfunction in patients with type 2 diabetes

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Objective. In a previous study, urinary orosomucoid excretion rate (UOER) independently predicted cardiovascular mortality in patients with type 2 diabetes. The aim of the present study was to determine whether increased UOER is associated with cardiovascular risk factors such as inflammation, impaired left ventricular function and endothelial dysfunction in patients with type 2 diabetes. ***Material and methods.*** We performed a cross-sectional study of 41 patients with type 2 diabetes (17 patients with normal UOER and 24 with increased UOER) with no history of cardiovascular disease and 21 healthy controls. Urinary orosomucoid was measured using a particle-enhanced immunoturbidimetric assay. Plasma interleukin-6 (IL-6), tissue plasminogen activator (tPA) and soluble intercellular adhesion molecule-1 (sICAM) were measured using ELISA. Endothelial function measured as vasodilatory capacity of the brachial artery and echocardiography were done in all participants. ***Results.*** Patients with diabetes and increased UOER had subclinically increased serum orosomucoid ($p<0.001$), C-reactive protein (CRP) ($p<0.001$), IL-6 ($p<0.001$), tPA ($p<0.003$) and sICAM ($p<0.003$) compared with healthy controls. In patients with type 2 diabetes, UOER was independently associated with increasing values of IL-6 (1.43 (1.06–1.93)) and tPA (1.82 (1.20–2.77)). Measurements by echocardiography showed no signs of cardiac dysfunction. ***Conclusions.*** Asymptomatic patients with type 2 diabetes and increased UOER displayed signs of chronic low-grade inflammation and endothelial dysfunction. UOER was independently related to markers of proinflammation and endothelial dysfunction in patients with type 2 diabetes. The previously shown relation between increased UOER and cardiovascular mortality is proposed to be caused by chronic low-grade inflammation and early endothelial dysfunction.

Keywords: Albuminuria; inflammation; intercellular adhesion molecule-1; orosomucoid; tissue plasminogen activator; vasodilation; ventricular function (left)

Introduction

Patients with type 2 diabetes have an increased risk of mortality compared to the background population, primarily due to cardiovascular diseases [1–3]. Orosomucoid is an acute phase reactant shown to exert a regulatory, dampening influence on the inflammatory cascade, thereby protecting against tissue damage from excessive inflammation [4,5]. Orosomucoid is a prominent component of the temporary proteinuria that occurs in association with exercise [6], acute inflammation [7,8] and acute coronary syndrome [9].

We have previously shown that urinary orosomucoid excretion rate (UOER) is an independent predictor of cardiovascular mortality in patients with type 2 diabetes [10,11], and that increased UOER is a powerful predictor of cardiovascular mortality, even in the subgroup of patients with normal urinary albumin excretion rate (UAER) [11]. The predictive value of UOER was independent of classic cardiovascular risk factors and UAER. The pathophysiologic mechanisms underlying the relation between UOER and increased risk of cardiovascular mortality are unknown.

Important factors involved in the development of atherosclerosis and the increased morbidity and mortality of type 2 diabetes are chronic low-grade inflammation [12–15], endothelial dysfunction [15,16] and left ventricular hypertrophy [17,18]. We suggest that an early and often pronounced increase in UOER associated with inflammation provides a pathophysiological link between UOER and the

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increased risk of cardiovascular mortality observed by us as previously documented [11]. In the present study, we address the hypothesis that UOER is an early marker of inflammation, endothelial dysfunction and impaired cardiovascular function in patients with type 2 diabetes.

Material and methods

Subjects

All diabetic patients attending the outpatient clinic at Amager Hospital, Copenhagen were screened by urinary analysis for orosomucoid and albumin. Patient files were screened from 344 Caucasian diabetic patients aged <70 years with dipstick-negative urine and UAER <200 μg/min at initial urinary screening. Inclusion of patients took place from May 2002 until June 2003. The exclusion and inclusion criteria are shown in Figure 1.

Type 2 diabetes was defined as fasting blood glucose $\geqslant$6.1 mmol/L or 2-h OGTT values of blood glucose $\geqslant$11.1 mmol/L and not being prone to keto-acidosis [19]. Based on reference values of healthy persons, patients were classified as having increased UOER if they had at least two out of three timed overnight urine samples with UOER >2.04 μg/min [20].

Healthy control persons were included by invitation. Exclusion criteria for healthy persons were: age $\geqslant$70 years; resting blood pressure $\geqslant$160/90 mmHg; fasting blood glucose $\geqslant$6.1 mmol/L; urine-stick positive for haemoglobin, leucocytes or nitrite; history of chronic diseases; pregnancy; regular intake of prescribed medicine, except for oral contraceptive pills or oral hormone replacement therapy.

Methods

History of cardiovascular diseases was assessed using Rose's questionnaire [21]. All participants were weighed wearing light clothing and height was measured. After 15 min with the patient resting in the supine position, blood pressure was measured using a standard mercury sphygmomanometer with an appropriately sized cut-off. The inclusion blood pressure was the mean value of the latter two of three measurements. Orthostatic hypotension was defined as a fall in blood pressure of $\geqslant$30/15 mmHg from

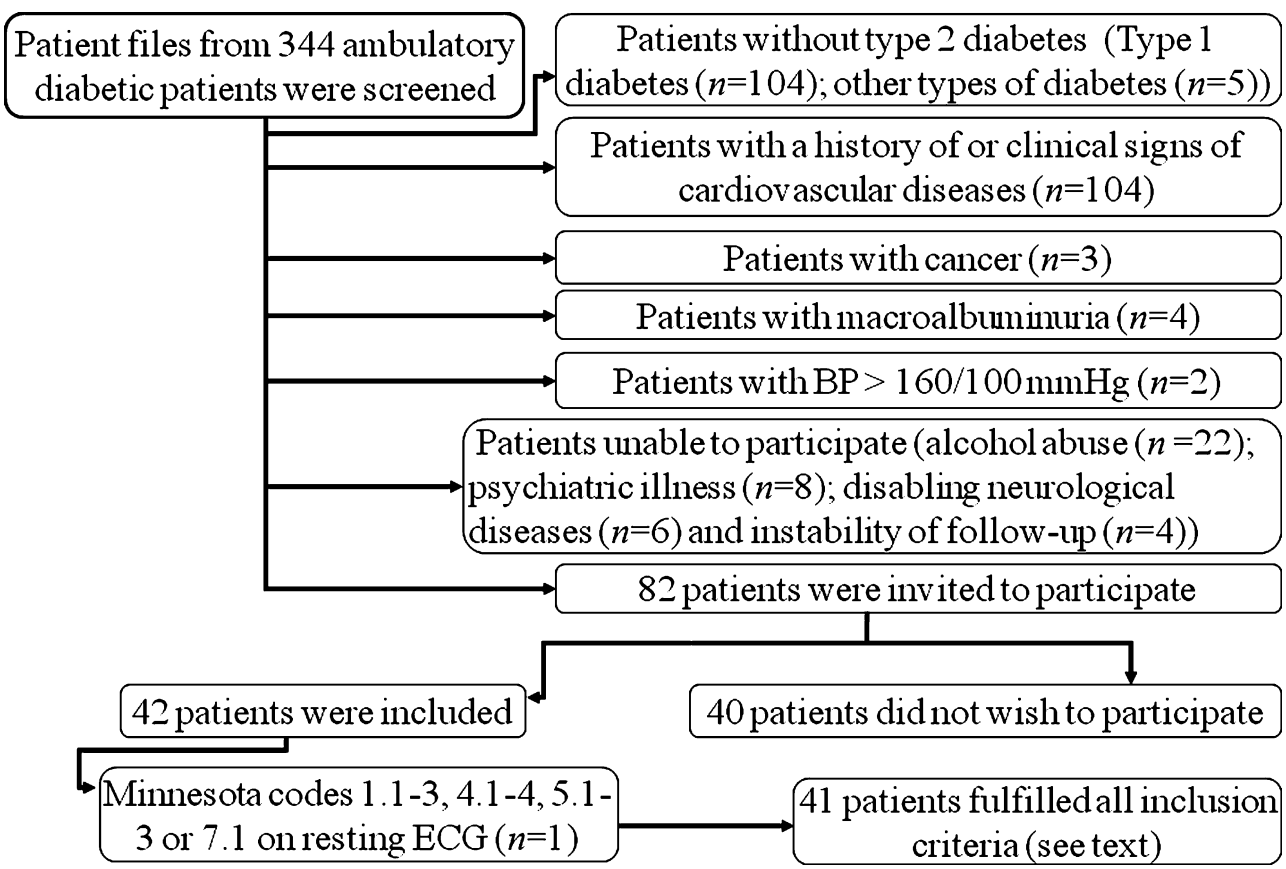

Figure 1. Diagram for exclusion and inclusion criteria of patients in the study.

initial values in the 10 min after participants rose to the standing position.

Vibration perception thresholds were measured using a handheld biothesiometer (Bio-Medical Instrument Company, Newbury, Ohio, USA) at the medial malleolus on both feet. The mean value of three registrations was used.

Standard 12-lead ECG was measured at rest and was Minnesota coded by two experienced investigators [22] blinded to the classification of the patients. Beat-to-beat variation was assessed as the difference between maximal and minimal heart rate during deep breathing. The mean value of five cycles was used [23].

Ophthalmoscopy in the diabetic patients was performed through dilated pupils by trained ophthalmologists, and classifications of retinal changes were: none, simplex or proliferative retinopathy.

The protocol of the artery dilatory capacity was: patients were fasting and having refrained from smoking in the 2 h before the investigation, and resting in a quiet room in the supine position for 10 min before the investigation. At baseline, the diameter of the brachial artery was measured in a longitudinal section using ultrasound (Acuson 128XP/10; Mountain View, Calif., USA) as the mean of measurements in four cardiac circles concurrently with the R-wave in a simultaneously recorded ECG. Blood flow was measured at a 70° angle to the artery using a pulsed-waved Doppler signal. The transducer position was kept in place throughout the study by a mechanical arm. A pneumatic tourniquet placed around the forearm distal to the segment of the artery being scanned was inflated to a pressure of 300 mmHg for 4.5 min. The arterial flow was measured as the maximal systolic flow within 15 s after deflation of the cuff. The diameter of the brachial artery (post-ischaemic diameter) was measured 50–60 s after deflation of the cuff. After a further 10 min rest, 400 μg nitroglycerine (Nitrolingual spray; Pohl-Boskamp, Germany) was given sublingually. Another measurement of the arterial diameter (post-nitroglycerine diameter) was taken 3–4 min afterwards. The post-ischaemic and post-nitroglycerine diameters were measured four times and the mean value was calculated. Measurements were stored digitally and results were calculated as mean values by two experienced investigators blinded to the classification of the patients. Flow-mediated dilatation as a percentage of change from baseline was calculated as: ((post-ischaemic diameter – baseline diameter)/baseline diameter).

Echocardiography was performed and stored digitally using an Agilent Sonos 4500 (Hewlett-Packard, Andover, Mass., USA) by one experienced investigator. Standard measurements of transthoracic two-dimensional echocardiography, M-mode and pulsed-wave Doppler were taken [24]. The recordings were measured by two experienced investigators blinded to the classification of the patients. The dimensions of the left ventricle were measured and the mass of the left ventricle was calculated [25] and corrected for body surface area [26] together with the diameter of the left atrium. As measures of the diastolic function, the mitral deceleration time, the peak velocity of early (E-wave) diastolic and late atrial (A-wave) transmitral flow were used, and the E/A wave velocity ratio was calculated. The AE interval was measured as the interval between the end of the A-wave and the start of the E-wave. All measurements were averaged over three cardiac cycles in expiration. Diastolic ventricular filling was categorized as normal or impaired relaxation [27]. The Tei index is defined as the sum of the isovolumic relaxation and contraction times divided by the ejection time (ET) [28] and equals the left ventricular inflow minus left ventricular outflow divided by the left ventricular outflow and was calculated as ((AE interval – ET)/ET).

Urine analyses

Urine samples were collected as timed overnight urine samples and stored without additives at 2–8℃ until analysis within 1 week of collection. To minimize variation, all participants were asked to refrain from vigorous exercise in the 24 h before sample collection. Each participant collected three samples, the first and second on two successive days; the third sample was collected 1 month after the first. All samples were analysed for leucocytes, nitrite and haemoglobin using Multistix (Bayer, Elkhart, USA). Urinary orosomucoid was analysed using a particle-enhanced immunoturbidimetric assay [20]. Urinary albumin was analysed using immunoturbidimetry.

Blood analyses

After at least 8 h of fasting, blood samples were collected from a cubital vein with the patient in the supine position. Four participants with signs of minor inflammatory conditions on the day of investigation returned when they were well for a new blood sample for inflammatory parameters. Plasma values of interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) are missing for these four patients. Serum albumin, orosomucoid (reference range: 0.45–1.17 g/L) and C-reactive protein (CRP) were determined using immunoturbidimetry.

Serum total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride and creatinine were measured using enzymatic, colorimetric methods. Blood haemoglobin and leucocytes were determined using photometry and flow cytometry, respectively. Blood glucose was determined using an enzymatic hexokinase method. Blood HbA_1c was determined as the HbA_1c (using immunoturbidimetry) in per cent of the total haemoglobin (using colorimetry). Plasma values of IL-6 (reference range <10 ng/L [29]), TNF-α, soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) were analysed from EDTA plasma using ELISA (R&D Systems, Minn., USA). Plasma tissue plasminogen activator (tPA) was analysed from citrate plasma using ELISA (Stago, Asnieres-sur-Seine, France). Plasma von Willebrand factor antigen (vWf) was analysed from citrate plasma using ELISA. Plasma N-terminal proBrain Natriuretic Peptide 1-21 (proBNP) was analysed in EDTA plasma using a modified RIA [30] (reference range <17 pmol/L for men and <30 pmol/L for women) [31]. For analyses of homocysteine, lithium-heparin plasma samples were placed on ice immediately after sample collection; analyses were done using a fluorescence-polarization immunoassay (reference range 4.5–12.4 μmol/L). All plasma samples were centrifuged at 2500*g* at 2–8°C within 30 min after sample collection and then stored at -20°C (-80°C for IL-6 and TNF-α) until analysis. Blood folate was analysed from EDTA plasma using an ion capture immunoassay. Serum thyroid stimulating hormone (TSH) was analysed using a microparticle enzyme immunoassay.

Ethics

All participants gave their written informed consent to taking part in the study. The protocol was approved by the local ethics committee in Copenhagen (KF 01- 238/01 and KF 11-071/02) and the study was carried out in accordance with the Helsinki Declaration 2000.

Statistical analyses

Statistical analyses were performed using Statistica for Windows version 6 (StatSoft Inc., Tulsa, Okla., USA). ANOVA was used for testing the differences among the three subgroups of participants. Log-transformed values of positive skewed variables were used for ANOVA; these variables are given as median and range. Two sample *t*-tests were used for the comparison of continuous variables, together with parametric correlation analysis of normally distributed continuous variables. The Mann-Whitney U-test was used for comparison of non-normally distributed parameters between two groups and the Pearson and Yates chi-square tests for comparison of dichotomous variables. Multiple linear regression analysis with backward stepwise selection was used for investigating the association between UOER and inflammatory and endothelial markers. Mutual values of urine and blood samples collected on the same day were used for these calculations.

Values of UAER and UOER from the first collected urine sample were used for comparison between groups. For CRP values below the detection limit of 1.0 mg/L, an assigned value of 0.9 mg/L was used for evaluation of the differences among the subgroups. Owing to technical difficulties, the data are incomplete for a few parameters; the number of investigated persons is stated in the tables of results. Bonferroni's correction was used at multiple comparison and a *p*-value of <0.05 was considered statistically significant.

Results

We included 17 Type 2 diabetic patients with normal UOER, 24 patients with increased UOER and 21 healthy control persons. Clinical and paraclinical data are given in Table I. The three subgroups were comparable in relation to age, distribution of sex, prevalence of smoking and beat-to-beat variation. Patients with diabetes had higher body mass index (BMI), triglyceride levels, systolic and diastolic blood pressure and lower levels of serum creatinine and serum LDL cholesterol than healthy control persons. There were no differences between the two subgroups of patients in duration of diabetes, prevalence of hypertension or retinopathy, treatment of diabetes, treatment with an angiotensin converting enzyme (ACE) inhibitor, BMI ($p=0.45$) systolic ($p=0.45$) or diastolic ($p=0.14$) blood pressure, vibration threshold ($p=0.11$), blood glucose ($p=0.21$), serum creatinine ($p=0.84$), serum LDL cholesterol ($p=0.96$) or serum triglyceride level ($p=0.23$). Patients with increased UOER had larger waist-to-hip ratio ($p<0.05$), higher levels of HbA_1c ($p<0.03$) and higher UAER in the first collected timed overnight urine sample ($p<0.0006$) than patients with normal UOER. Orthostatic hypotension was present in two control persons and in eight patients with increased UOER. Seven of these eight patients were treated with ACE inhibitors. Only two patients had symptoms of slight dizziness during the investigation.

Table I. Clinical and paraclinical parameters.

Variable	Control persons	Patients with normal UOER	Patients with increased UOER	*p*-value
Number (men/women)	21 (10/11)	17 (7/10)	24 (17/7)	0.12
Age (range) years	53±11 (30–67)	56±8 (42–67)	56±9 (33–68)	0.43
Duration of diabetes (years)	–	3.5 (0.5–35.8)	5.8 (0.6–14.1)	0.24
Hypertension (%)	0	65	67	0.84
Retinopathy, none/simplex/proliferative (%)	–	82/18/0	74/22/4[a]	0.63
Diet/tablet/insulin treatment (%)	0	18/41/41	0/63/38	0.08
ACE inhibitor treatment (%)	0	59	71	0.64
Smokers (%)	24	24	29	0.89
Beat-to-beat variation (min^{-1})	8.3±3.3	8.2±5.0	6.9±3.7	0.42
Body mass index (kg/m^2)	26±4	33±7	35±6	<0.0001
Waist/hip ratio	0.87±0.08	0.93±0.09[b]	0.99±0.08	<0.0001
Systolic blood pressure (mmHg)	128±14	140±16	143±11	<0.0009
Diastolic blood pressure (mmHg)	80±4	85±9	90±7	<0.0001
Vibration threshold left (mV)	14 (10–31)	20 (10–37)	28 (9−>50)	<0.005
Vibration threshold right (mV)	16 (9–33)	19 (9–39)	34 (4−>50)	<0.003
Blood glucose (mmol/L)	4.8±0.5	8.2±3.1	9.3±2.4	<0.0001
Blood HbA_1c (%)	5.2±0.2	7.6±1.5[c]	8.9±1.7	<0.0001
Serum creatinine (μmol/L)	76±14	66±18	67±11	<0.05
Serum cholesterol (mmol/L)	5.4±1.1	4.8±0.8	4.9±1.1	0.10
Serum HDL cholesterol (mmol/L)	1.3 (0.7–2.9)	1.2 (1.0–1.9)	1.2 (0.8–1.9)	0.65
Serum LDL cholesterol (mmol/L)	3.6±0.8	3.0±0.8	3.0±0.9	<0.05
Serum triglyceride (mmol/L)	1.0 (0.5–3.5)	1.3 (0.5–9.5)	1.8 (0.8–7.1)	<0.009
UOER (μg/min)	0.54 (0.18–2.04)	0.47 (0.17–5.62)	4.36 (1.30–41.16)	–
UAER (μg/min)	12 (6–46)	11 (8–58)[d]	23 (10–171)	<0.0001

Data are mean ± SD or median (range) unless otherwise stated; [a]$n=23$; [b]$p<0.05$, [c]$p<0.03$ and [d]$p<0.0006$ versus patients with increased UOER.

Markers of inflammation

Patients with increased UOER had higher levels of serum orosomucoid ($p<0.001$), CRP ($p<0.001$) and IL-6 ($p<0.001$) compared with healthy control persons (Table II). Patients with normal UOER had higher levels of serum CRP ($p<0.002$) and IL-6 ($p<0.006$) compared with healthy control persons (Table II). There were no differences in inflammatory markers between the two subgroups of patients with diabetes. Only one patient had a slightly increased value of IL-6 (10.2 ng/L). Two patients with increased UOER had a slightly elevated serum value of orosomucoid (1.23 and 1.29 g/L).

Correlation analysis of inflammatory parameters showed significant correlation to logUOER of the following variables: logCRP ($r=0.37$, $p<0.01$), serum orosomucoid ($r=0.47$, $p<0.01$) and logIL-6 ($r=0.39$, $p<0.01$). Some of the inflammatory markers were correlated to HbA_1c: logUOER ($r=0.54$, $p<0.01$); logIL-6 ($r=0.49$, $p<0.01$); logCRP ($r=0.44$, $p<0.01$) and serum orosomucoid ($r=0.37$, $p<0.01$). There was a significant correlation of logUOER and tPA ($r=0.42$, $p<0.01$), but not a significant correlation of logUAER and tPA ($r=0.23$, $p=0.07$).

In multiple regression analysis including the two groups of patients with type 2 diabetes, we found

Table II. Inflammatory variables.

Variable	Control persons ($n=21$)	Patients with normal UOER ($n=17$)	Patients with increased UOER ($n=24$)	ANOVA (*p*-value)
Blood white blood cells (10^9/L)	6.2±2.1	6.8±1.7	7.5±2.0	0.11
Serum C-reactive protein (mg/L)	1 (<1.0–3)[a, b]	3 (<1.0–15)	4 (1–16)	<0.0001
Serum orosomucoid (g/L)	0.75±0.12[a]	0.85±0.17	0.94±0.17	<0.001
Plasma IL-6 (ng/L)	0.85 (0.32–2.27)[a, b, c]	1.35 (0.41–6.77)[d]	2.18 (0.86–10.18)[e]	<0.0001
Plasma TNF-α (ng/L)	2.77 (1.16–8.97)[c]	3.32 (1.80–7.41)[d]	2.62 (1.53–9.39)[e]	0.62

Data are mean ± SD or median (range). [a]$p<0.0004$ versus patients with increased UOER; [b]$p<0.006$ versus patients with normal UOER; [c]$n=18$; [d]$n=16$; [e]$n=23$.

UOER to be independently associated with increasing values of IL-6 and tPA (Table III). UAER was associated with higher serum orosomucoid and negatively with tPA. The analyses were adjusted for sex, waist-to-hip ratio and HbA1c.

Markers of endothelial function and arterial dilatory capacity

Patients with increased UOER had higher levels of plasma sICAM ($p < 0.003$) and plasma tPA ($p < 0.001$) compared with healthy control persons (Table IV). There were no differences in sICAM ($p=0.11$) or tPA ($p=0.45$) between patients with increased and normal UOER. The relative increase in arterial diameter after flow-mediated and nitroglycerine-mediated dilation of the arterial diameter was similar in the three subgroups (Table IV). We found no differences in endothelial function or inflammatory markers between patients treated with ACE inhibitors versus persons without treatment (data not shown).

Markers of cardiac function and echocardiography

The plasma levels of homocysteine and proBNP were within the reference ranges or only slightly increased (Table V) and there were no differences among the three subgroups of investigated persons. Blood folate and TSH were similar in the three subgroups (data not given).

Findings of echocardiography are given in Table V. Two patients with increased UOER had an ejection fraction of the left ventricle below 55 %; the remaining participants had a normal left ventricular function with an ejection fraction above 55 %. None of the participants had valve disease. We found no differences in any of the parameters measured by echocardiography (Table V).

The prevalence of diastolic dysfunction in the three subgroups was equal. Six patients with normal UOER, seven with increased UOER and three control persons had impaired relaxation.

Discussion

The study showed increased inflammatory markers and signs of endothelial dysfunction in asymptomatic patients with type 2 diabetes and increased UOER. Furthermore, we found that UOER was associated with markers of inflammation and endothelial dysfunction independently of other risk markers.

We found increased inflammatory activity in the subgroup of patients with increased UOER

Table III. Multivariate regression analysis of UOER and UAER as independent predictors of inflammatory and endothelial markers (adjusted for sex, waist-to-hip ratio and HbA1c). Analysis of diabetic patients.

Dependent variable	Independent variable	Odds ratio (95 % CI)	*p*-value
Serum orosomucoid (g/L)	logUAER	1.58 (1.20–2.09)	0.003
Plasma IL-6 (ng/L)	logUOER	1.43 (1.06–1.93)	0.03
Plasma tissue plasminogen activator (ng/mL)	logUOER	1.82 (1.20–2.77)	0.008
	logUAER	0.61 (0.40–0.93)	0.03

Table IV. Endothelial function and arterial dilatory capacity.

Variable	Control persons ($n=21$)	Patients with normal UOER ($n=17$)	Patients with increased UOER ($n=24$)	ANOVA (*p*-value)
Plasma soluble intercellular adhesion molecule-1 (ng/mL)	257 (152–465)[a]	294 (224–405)	313 (219–903)	<0.003
Plasma vascular cell adhesion molecule-1 (ng/mL)	458 ± 59	480 ± 79	505 ± 137	0.30
Plasma tissue plasminogen activator (ng/mL)	35 ± 12[a,b]	52 ± 18	57 ± 19	<0.0002
Plasma von Willebrand factor antigen (kIU/L)	1.2 ± 0.4	1.2 ± 0.4	1.3 ± 0.3	0.62
Baseline diameter (mm)	3.6 ± 0.5	3.8 ± 0.7[c]	4.0 ± 0.6[d]	0.08
Post-ischaemic diameter (mm)	3.7 ± 0.5[e]	3.9 ± 0.7[c]	4.1 ± 0.5[f]	<0.05
Post-nitroglycerine diameter (mm)	4.0 ± 0.4	4.2 ± 0.7[c]	4.4 ± 0.6	<0.05
Flow-mediated dilatation (%)	2.0 (−1.5–11.1)[e]	4.8 (−0.4–9.7)[c]	2.7 (−1.5–11.6)[f]	0.79
Post-nitroglycerine dilatation (%)	12.7 ± 7.1	11.5 ± 4.4[c]	12.1 ± 7.6	0.87
Post-ischaemic max flow rate/baseline flowrate ratio	5.1 ± 1.5	4.6 ± 1.0[c]	4.2 ± 0.9	0.08

Data are mean ± SD or median (range). [a]$p < 0.003$ versus patients with increased UOER; [b]$p < 0.003$ versus patients with normal UOER; [c]$n=16$; [d]$n=23$; [e]$n=19$; [f]$n=22$.

Table V. Markers of cardiac function and echocardiography.

Variable	Control persons (n=21)	Patients with normal UOER (n=17)	Patients with increased UOER (n=23)	ANOVA (p-value)
Plasma pro-brain natriuretic peptide (pmol/L)	3.1 (<0.2–17)	3.6 (<0.2–25)	3.0 (<0.2–15)[a]	0.61
Plasma homocystein (μmol/L)	10.1±3.1	11.3±4.0	10.3±3.4[a]	0.52
Left atrium diameter index (mm/m^2)	18.1±1.9	18.5±2.3	18.5±2.3	0.79
Left ventricular mass index (g/m^2)	70±18	75±15	79±18	0.26
Ejection fraction (%)	61 (55–61)	61 (55–61)	61 (45–61)	0.08
E/A ratio	1.2±0.3	1.1±0.3	1.0±0.3	0.08
E wave deceleration time (ms)	200 (155–305)	210 (125–325)	220 (160–410)	0.50
Tei index	0.33±0.08[b]	0.39±0.13	0.40±0.13[c]	0.11
Diastolic function, abnormal (%)	14	35	30	0.30

Data are mean±SD or median (range). [a]n=24; [e]n=20; [b]n=19; [c]n=22.

compared to healthy individuals and that UOER was positively correlated with serum orosomucoid, CRP and IL-6. However, the inflammatory markers orosomucoid, CRP and IL-6 in plasma were within or just above the upper reference limits in individual patients. This subclinical increase is indicative of a chronic inflammatory condition and not an acute phase response [32]. In connection with our previous observation that UOER independently of classical risk markers predicts cardiovascular mortality [11], our new results are supported by the hypothesis that low-grade inflammation is involved in the development of atherosclerosis [14,33]. This notion also finds support in observations of other studies showing that subclinical elevations of serum orosomucoid are present in patients with type 2 diabetes [13] and are associated with the development of cardiovascular disease and cardiovascular mortality [34]. Furthermore, subclinical increases of CRP and IL-6 were shown to predict the development of cardiovascular diseases [35], cardiovascular mortality [36] and the development of type 2 diabetes [12,37].

Concerning endothelial function, we found increased levels of sICAM and tPA in patients with increased UOER compared to healthy individuals. Increased levels of both sICAM and tPA were found in patients with type 2 diabetes [38,39] and both markers were predictive of the development of cardiovascular disease in diabetic patients [40] and in patients with angina pectoris [39]. These studies and our findings of increased levels of sICAM and tPA in patients with increased UOER support the hypothesis that increased UOER could be linked to early endothelial dysfunction.

We found that UOER correlated to HbA_1c and that patients with increased UOER had higher levels of HbA_1c compared to patients with normal UOER; the differences between the two subgroups of patients could therefore be partly explained by differences in glycemic control. There were no differences in the markers of inflammation and endothelial function between the two subgroups of patients with diabetes. However, we found that UOER predicted markers of inflammation and endothelial dysfunction independently of HbA1c and other risk factors in patients with type 2 diabetes. This finding supports the hypothesis that chronic low-grade inflammation and endothelial dysfunction explain at least part of the pathophysiologic link between increased UOER and cardiovascular mortality.

In the multivariate analysis, UOER was positively associated with tPA and UAER negatively with tPA. We found a significant correlation between tPA and UOER, but not between tPA and UAER. These differences might reflect that UOER and UAER are related differently to the fibrinolytic system. Increased tPA reflects fibrinolytic activity [39] and decreased tPA reflects decreased fibrinolytic or thrombogenic activity. In studies of patients with diabetes, the stimulated increase in tPA was lower in the presence of diabetic complications compared to patients without complications [41,42]. Diabetic complications, including increased UAER, therefore seem to promote a thrombogenic response. To the best of our knowledge, there are no published data on a possible connection between tPA and UOER. However, subclinical increased levels of orosomucoid in serum were found together with increased levels of tPA in men with angina pectoris [43]. Altogether, these findings might reflect differences in the pathophysiology behind the occurrence of UOER and UAER.

We found no differences in the relative increases of the arterial dilatory capacity between the three subgroups in the post-ischaemic phase (endothelium-dependent) or after nitroglycerine administration (endothelium-independent). Other studies of flow-mediated arterial dilatory capacity have shown impaired function in patients with type 2 diabetes [44,45]. The differences could be explained by the fact

that patients in these studies were younger and had lower BMI compared to the patients in our study.

Left ventricular mass has been shown to be a powerful predictor of mortality in patients who are clinically free of cardiovascular disease [17]. We did not find any differences in the dimensions of the left ventricular mass and the left atrium when corrected for body surface area, nor did we find any differences in diastolic function between the three subgroups. However, we found a high prevalence of diastolic dysfunction in all three groups of participants, the prevalence being comparable to that of other studies [46–48]. The prognostic value of diastolic dysfunction in asymptomatic persons remains to be evaluated.

In summary, we did not find any signs of cardiovascular deterioration as measured by echocardiography in diabetic patients selected to be without history of cardiovascular diseases.

The renal pathophysiologic mechanism behind increased UOER is unknown. There may be different pathogenic factors behind increased UOER and UAER. We found an amplified signal of UOER compared to UAER in this and in other studies [11,49]. The difference might be explained by a separate pathophysiologic pathway for increased orosomucoid excretion in urine. Local renal production of orosomucoid is a possibility, since it has been shown that human endothelial cells produce orosomucoid [50] and that orosomucoid performs a very important role in maintaining the normal glomerular capillary barrier [51]. Despite a positive correlation between serum orosomucoid and UOER the subclinical increase in serum orosomucoid could only explain part of the increased UOER, since there were no differences in the serum orosomucoid between patients with normal and those with increased UOER. In this study, UOER was independently associated with IL-6, and a significant correlation was found.

Hence, a local renal production of orosomucoid could be regulated by IL-6 or other proinflammatory cytokines, since the normal hepatic production of orosomucoid is regulated by IL-6 and IL-1, among others [32].

The study is limited by not withholding the medication of patients treated with ACE inhibitors or statins before the investigations. It is well known that ACE inhibitors can improve endothelial function [52] and that statins have anti-inflammatory effects [53]. However, we found no differences in endothelial function or inflammatory markers between patients treated with ACE inhibitors and persons without treatment, and we found no differences in LDL cholesterol between patients with normal and those with increased UOER.

Conclusions

Previously, we found UOER to be a powerful, independent predictor of cardiovascular mortality in patients with type 2 diabetes. This study shows increased levels of markers of subclinical inflammation and endothelial dysfunction in asymptomatic patients with type 2 diabetes and increased UOER. Furthermore, UOER was independently associated with increased levels of inflammatory and endothelial markers. The study suggests that the relation between increased UOER and cardiovascular mortality may be caused by chronic low-grade inflammation and early endothelial dysfunction. Further prospective studies are needed to evaluate this hypothesis.

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