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Short communication

Transcapillary escape rate and albuminuria in Type II diabetes. Effects of short-term treatment with low-molecular weight heparin

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Summary The relation between urinary albumin excretion rate (UAE), transcapillary escape rate of albumin (TER_{alb}), haemostatic factors, ambulatory blood pressure, and metabolic variables was investigated in 45 Type II (non-insulin-dependent) diabetic patients without overt nephropathy or uncontrolled blood pressure. We enrolled 44 patients in a placebo controlled study to test the effects of 3 week long treatment with low-molecular weight heparin (tinzaparin) on the same variables. BMI, 24 h systolic and diastolic blood pressure, plasma concentrations of triglycerides, fasting glucose, factor VIII, von Willebrand factor (vWf), fibrinogen, α-2 macroglobulin, and fibronectin were notably higher in patients with increased albuminuria compared with normoalbuminuric patients, whereas the TER_{alb} was similar in the two groups. TER_{alb} correlated with fasting plasma glucose. UAE correlated more closely than TER_{alb} with 24 h ambulatory blood pressure, vWf, and factor VIII. Urinary albumin excretion rate was unchanged during tinzaparin [28.9 ± 5.6 vs 28.1 ± 6.0 μg/min (geometric mean (antilog SD)] vs placebo (18.0 ± 5.4 vs 17.6 ± 5.3 μg/min), and no change was found in TER_{alb} [6.3 ± 1.6 vs 6.0 ± 1.5 %/h (means ± SD), and 6.3 ± 1.5 vs 5.6 ± 1.8 %/h; tinzaparin versus placebo, respectively]. Only minor changes were observed in blood pressure, lipids, glycaemic control and haemostatic factors. This study shows no correlation between albuminuria and transcapillary escape rate in Type II diabetic patients without overt nephropathy or uncontrolled blood pressure. UAE is related to markers of atherosclerosis, endothelial injury and dysfunction, and haemostatic factors. Moreover, UAE correlates much more than TER_{alb} with 24 h ambulatory blood pressure, von Willebrand factor, and factor VIII. Finally, short-term treatment with tinzaparin does not change the transvascular or glomerular leakage of albumin. These results indicate that TER_{alb} is not a sensitive marker of microvascular dysfunction in such patients and that factors other than abnormal glycosaminoglycan metabolism may contribute to the vascular damage of these patients. [Diabetologia (1999) 42: 60–67]

Keywords Transcapillary escape rate of albumin, microalbuminuria, albuminuria, low-molecular weight heparin, heparin.

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Abbreviations: UAE, Urinary albumin excretion rate; TER_{alb}, transcapillary escape rate of albumin; IVM_{alb}, intravascular mass of albumin; J_{alb}, outflux of albumin; HS, heparan sulfate; GAG, glycosaminoglycan; LMWH, low-molecular weight heparin; AMBP, ambulatory blood pressure; PV, plasma volume; J_{alb}, flow of albumin; vWf, von Willebrand factor; $U_{alb/cre}$, albumin to creatinine ratio.

Type II (non-insulin-dependent) diabetic patients with abnormal urinary albumin excretion rate (UAE), even in the microalbuminuric range (UAE 20–200 μg/min), have an increased prevalence of vascular disease and risk of cardiovascular events compared with patients with normoalbuminuria (UAE < 20 μg/min) [1–5]. The association has raised the hypothesis that patients with increased albuminuria do not only have glomerular disease but also extensive vascular damage [6, 7]. Increased transcapillary escape rate of albumin (TER_{alb}) [i. e. the fraction

of intravascular mass of albumin (IVM_{alb}) passing to the extravascular space per unit of time] has been found in Type II diabetic subjects; however, the precise relation with the degree of albuminuria remains unclear [7–9].

One underlying mechanism of increased TER_{alb} is suggested to be an alteration in either the production or sulphation degree of heparan sulfate (HS), a glycosaminoglycan (GAG) sidechain of proteoglycans normally found in basement membranes and extracellular matrix. The presence of normally sulphated HS GAG is associated with a diminished permeability of negatively charged plasma proteins across the vascular wall bed due to charge repulsion and size exclusion [6, 10]. Moreover, HS GAG stimulates normal extracellular matrix protein synthesis, inhibits mesangial cell proliferation and possesses anticoagulative properties [6, 10–12]. Treatment with HS like molecules such as unfractionated or low-molecular weight heparin (LMWH) [13], heparinoid [14], and an orally available GAG [15] has been shown to reduce albuminuria in diabetic patients with abnormal UAE.

This study was undertaken to evaluate the relation between UAE, TER_{alb}, haemostatic factors and metabolic variables in Type II diabetic patients without clinically overt nephropathy and uncontrolled blood pressure. Moreover, we examined the effect of short-term treatment with a LMWH on these variables.

Subjects and methods

Subjects. We recruited 20 Type II diabetic patients with a urinary albumin to creatinine ratio ($U_{alb/cr}$) less then 2.0 mg/mmol and 25 Type II diabetic patients with a ratio of 2.0–25 mg/mmol from the outpatient clinic. All patients were to have a serum creatinine less than 150 μmol/l and no proliferative retinopathy. This approach was used to obtain a wide range of normo- and microalbuminuria as well as low-range macroalbuminuria without other evidence of overt clinical nephropathy. Other criteria of inclusion were: Caucasian, aged 40 to 70 years, age at diagnosis 30 years or older and diabetes duration longer than 1 year, blood pressure less than 160/90 mmHg irrespective of ongoing treatment, HbA_{1c} less than 12%, body mass index (BMI) less than 35 kg/m^2. Excluded from the study were patients with severe heart failure, unstable angina pectoris, untreated hypertension, known endocrine or renal disease unrelated to diabetes, known hepatic, haematological or malignant disease, as well as pregnant or lactating women and women of childbearing potential not using medical contraceptives. We also included patients with a spontaneous prothrombin time less than 0.80 (ref.: 0.80–1.20) or on ongoing anticoagulative therapy, or taking medication (other than antidiabetic or antihypertensive drugs) known to affect glucose homeostasis or blood pressure, as well as patients who had had a stroke or acute myocardial infarction within the past 6 months. Sodium intake was unrestricted and none of the patients was on a low protein diet. Antihypertensive medication was used by 22 patients, of these 14 used an ACE-inhibitor either alone ($n = 11$) or in combination with other agents ($n = 3$). The remaining 8 patients used diuretics, beta-blocking agents, and calcium antagonists either alone or as combination therapy. The study was approved by the local ethics committee and informed consent was obtained from all participating patients.

Methods. UAE was measured by radioimmunoassay [16] and calculated as the geometric mean of 3 consecutive 24 hour urine collections. Urinary sterility was checked using a dipstick method (Multistix 8 SG Ames, Bayer Denmark A/S, Frederiksberg, Denmark) followed by a urinary culture if infection could not be excluded.

TER_{alb} was assessed as described by Parving [17]. The patients were studied in the morning between 8 and 10 o'clock after an overnight fast. A catheter was inserted in an antecubital vein in each arm for tracer injection and blood sampling and the patients then rested for 1 h in the supine position. Hereafter a 10 ml baseline blood sample was obtained without stasis and followed by a bolus injection (30 s) containing 0.2 MBq ^{125}I-albumin. The exact amount of tracer was assessed by weighing the syringes before and after injection. Additional 10 ml blood samples were collected without stasis after 10, 15, 20, 30, 40, 50, 55, and 60 min to count plasma radioactivity and to measure the total plasma protein concentration in duplicate by refractometry (Bellingham and Stanley, Turnbridge Wells, UK). Radiotracer activity was corrected for the total plasma protein concentration and the slope of the linear regression of radioactivity on time was used to calculate TER_{alb} (i.e. the plasma tracer disappearance rate). Plasma volume (PV) was calculated using the intercept of the regression line extrapolated to time zero and the injected amount of radiotracer and corrected to 1.73 m^2 body surface area. The IVM_{alb} was derived by multiplying PV by the serum albumin concentration and the outflow of albumin (J_{alb}) from multiplying IVM_{alb} by TER_{alb}.

Twenty-four hour ambulatory blood pressure (AMBP) was measured using a portable blood pressure monitor (SpaceLabs model 90202, Redmond, Wash., USA) using oscillometry [18]. The device was programmed to measure blood pressure every 20 min between 06.00 and 24.00 hours and every hour during the night. Patients recorded actual time for going to bed and rising in the morning for correct assessment of day and night blood pressures.

HbA_{1c} was measured by HPLC and plasma glucose by a glucose oxidase technique. Plasma cholesterol was assessed by continuous-flow analysis, plasma triglycerides by an enzymatic technique, and plasma HDL-cholesterol by a dextran sulfate-Mg^{2+} precipitation procedure. Serum creatinine was measured by a modified Jaffe's reaction. Von Willebrand factor (vWf) was measured by ELISA, as reported previously [19]. Factor VIII was quantitated by ELISA [20], and plasma α-2 macroglobulin, fibrinogen, and total fibronectin by nefelometry (BNA, Behring AG, Germany) using monospecific rabbit antibodies (Dakopatts, Roskilde, Denmark). Procoagulant factor VII function (F VII:C) was recorded by a one-stage coagulometric method employing Simplastin Excel (Organon Tekika, Turnhout, Belgium) as the tissue factor containing reagent. The prothrombin fragment F1 + 2 was quantitated by an ELISA method, Enzygnost F1 + 2 micro (Behring, Marburg, Germany).

Protocol. The study was designed as a cross-sectional study followed by a double-blind, randomized, placebo controlled intervention trial.

Cross-sectional study. All patients completed three 24 h urine samples and a 24 h AMBP measurement, this was followed by assessment of TER_{alb} and baseline blood samples for haemostatic and metabolic variables.

Double-blind study. Following each TER_{alb} measurement all but one patients were randomized in a double-blind, placebo controlled design for treatment with subcutaneous (thigh) injections of tinzaparin 50 IU/kg once daily (maximum 5000 IU) or with an equal amount of 0.9 % saline. Block randomization was used. Thus, patients with a $U_{alb/cr}$ below and above 2.0 mg/mmol were separated into two groups and randomized in blocks of 4 (2 tinzaparin, 2 placebo) in order to obtain equivalent numbers of patients with elevated albuminuria in the two treatment arms. The patients were asked to return the study medication vials and injection equipment (single-use syringes and needles) for evaluation of compliance. At the end of the treatment 3 24 h urine collections were obtained and the patients returned for blood sampling, measurement of TER_{alb} and 24 h AMBP. We withdrew two placebo treated patients from the study during the treatment period: one patient was excluded due to unsatisfactory injection of the study drug. A second patient was excluded because of a non-fatal myocardial infarction after 19 days of placebo treatment. This event was unprovoked and was not found to be related to the subject's participation in the study. A total of 22 tinzaparin treated and 20 placebo treated patients completed the study. Among these patients the 3 weeks measurement of TER_{alb} was technically insufficient in one patient (placebo) and the urine collection was incomplete in another patient (placebo). The proportion of patients using antihypertensive medication, including ACE-inhibitors, was not different between the groups.

Statistics. Values are given as means ± SD unless otherwise indicated except UAE which was log(10) transformed due to the positively skewed distribution and therefore given as the geometric mean (× /÷ (i. e. multiplied/divided by) antilog SD). Statistical analyses at baseline were done using Student's *t*-test, the Mann-Whitney two sample test, Fisher's exact test or the χ^2 test. For longitudinal data Student's *t*-test for paired data and Wilcoxon test for matched pairs were used for within group analysis. Two-way analysis of variance (ANOVA) for repeated measurements was used for between group comparisons. Multiple regression analysis was done by stepwise linear regression analysis. Correlations were evaluated by Pearson's *r*. To compare the strength of the relationships between risk factors and TER_{alb} or UAE, Choi's approach [21] was used, which involves regression analysis of the ranked risk factor level(s) vs the ranked TER_{alb} minus the ranked UAE for each patient. A *p* value less than 0.05 was considered statistically significant.

Power calculation. The probability of detecting a decline in UAE of (means ± SD) 17 ± 25 % (values obtained from Myrup et al. [13]) using a paired *t*-test ($n = 20$) is 82 %. The probability of detecting a change in TER_{alb} of 20 % (SD 20 %) using a paired *t*-test is greater than 0.98 ($n = 19$) and greater than 0.80 ($n = 10$).

Results

Cross sectional study. A total of 45 patients [21 with normal UAE (range 1.9–19.9 μg/min) and 24 with increased UAE (range 31.3–1019 μg/min)] were enrolled. They were 27 men and 18 women, aged 58 ± 8 years, with BMI 27.9 ± 4.1 kg/m^2, HbA_{1c} 8.4 ± 1.6 %, and serum creatinine 81 (60–146) μmol/l [mean (range)]. Compared with normoalbuminuric patients these variables were higher in patients with abnormal albuminuria: BMI (26.6 ± 4.0 vs 29.5 ± 3.8 kg/m^2, $p < 0.02$), 24 h systolic (125 ± 13 vs 142 ± 15 mmHg, $p < 0.001$) and diastolic (74 ± 7 vs 81 ± 8 mmHg, $p < 0.005$) AMBP, triglycerides ([median (25^{th}–75^{th} percentile] 1.41 (0.89–2.06) vs 2.25 (1.49–2.94) mmol/l, $p < 0.03$), fasting plasma glucose [8.3 (6.9–9.2) vs 9.9 (7.8–12.5) mmol/l, $p < 0.04$], factor VIII (1.27 ± 0.37 vs 1.76 ± 0.62 IU/l, $p = 0.002$), vWf [1.18 (0.99–1.61) vs 1.48 (1.22–2.14) IU/l, $p < 0.02$], fibrinogen 4.9 (4.4–5.7) vs 5.6 (5.0–6.7) g/l, $p < 0.03$), α-2 macroglobulin (1.78 ± 0.51 vs 2.35 ± 0.76 g/l, $p = 0.005$), and fibronectin (0.44 ± 0.09 vs 0.51 ± 0.11 g/l, $p = 0.02$); normal vs abnormal albuminuria, respectively. TER_{alb} was similar in the two groups (6.2 ± 1.8 vs 6.6 ± 1.3 %/h). Likewise, no difference was found with respect to PV, IVM_{alb}, J_{alb}, total and HDL-cholesterol, or serum concentrations of creatinine and albumin, HbA_{1c}, and age.

UAE correlated closely with 24 h systolic and diastolic AMBP, BMI, total cholesterol, HDL cholesterol, and triglyceride concentrations (Table 1). Moreover, correlations were found between UAE and vWf, factor VIII, α-2 macroglobulin, and fibronectin.

Table 1. Univariate correlations of UAE, TER_{alb} in Type II diabetic patients without overt nephropathy or uncontrolled blood pressure

	UAE		TER_{alb}	
	r	*p*-value	*r*	*p*-value
Systolic AMBP	0.49	< 0.001	– 0.04	0.79
Diastolic AMBP	0.41	< 0.01	0.10	0.51
Total cholesterol	0.31	< 0.05	0.17	0.27
HDL cholesterol	– 0.30	< 0.05	– 0.07	0.63
Triglycerides	0.40	< 0.01	0.25	0.09
Fasting plasma glucose	0.29	0.06	0.35	< 0.05
HbA_{1c}	0.23	0.13	0.15	0.33
von Willebrand factor	0.38	< 0.05	– 0.10	0.54
α-2 macroglobulin	0.41	< 0.01	0.17	0.27
F VII : C	0.26	0.08	– 0.08	0.61
Factor VIII	0.40	< 0.01	– 0.08	0.62
Fibronectin	0.33	< 0.05	0.04	0.79
Fibrinogen	0.24	0.12	– 0.07	0.66
BMI	0.38	< 0.01	– 0.03	0.87
TER_{alb}	0.14	0.36		

Table 2. Characteristics of patients randomized to treatment with tinzaparin or placebo

	Tinzaparin ($n = 22$)	Placebo ($n = 22$)
Age (years)	58 ± 7	58 ± 8
Sex (men/women)	16/6	9/11
Diabetes duration (years)	10 ± 9	10 ± 9
BMI (kg/m^2)	28.4 ± 4.5	27.2 ± 3.8
HbA_{1c} (%)	8.6 ± 1.8	8.1 ± 1.4
24 h AMBP (mm Hg)	133/78 ± 16/8	133/76 ± 17/7
Antihypertensive treatment (yes/no)	15/7	9/13
Antidiabetic treatment (d/o/i)	4/11/7	2/10/10

Values are means ± SD
d = diet, o = oral hypoglycaemic agents, i = insulin

UAE and TER_{alb} did not correlate very much and no important relation was observed between TER_{alb} and AMBP, lipids or haemostatic measurements. TER_{alb} correlated closely with fasting plasma glucose but not with HbA_{1c}. There was no relation between night-time (resting) blood pressure and TER_{alb}. The pronounced correlations were consistent after controlling for HbA_{1c} and lipid concentrations in a multiple linear regression analysis (data not shown). When the data were subjected to Choi's analysis, we found that systolic AMBP ($p < 0.01$), vWf ($p < 0.02$), factor VIII ($p < 0.04$) were much better correlated with UAE than with TER_{alb}.

Double blind study. Table 2 shows the baseline characteristics of patients in the tinzaparin and placebo groups. Except for a slight, not significant majority of men in the tinzaparin group, the groups were well matched for age, UAE, glycaemic control, and blood pressure. UAE was comparable at baseline in the two groups and did not change much during treatment with tinzaparin [from $28.9\times/\div5.6$ to $28.1\times/\div6.0$ μg/min (geometric mean ×/÷antilog SD)] compared with placebo (from $18.0\times/\div5.4$ to $17.6\times/\div5.3$ μg/min) (Fig. 1). Moreover, baseline concentrations of TER_{alb}, IVM_{alb}, J_{alb}, and PV were similar (Table 3). Although PV and IVM_{alb} decreased in both groups, no change in TER_{alb} was noted and the change in J_{alb} found in the placebo group was not statistically different from the change observed in the tinzaparin group. Figure 2 shows the changes in TER_{alb} during treatment with tinzaparin and placebo. Dividing patients into tertiles of baseline UAE did not show any differences in treatment effects of tinzaparin versus placebo on UAE or TER_{alb} (data not shown). Thus, the baseline concentration of UAE was not associated with different treatment responses. Furthermore, no change was observed in any of the haemostatic variables (Table 4). In both groups 24 h AMBP was unchanged and changed barely during treatment (Table 5). Finally, fasting plasma glucose and HbA_{1c} improved slightly during tinzaparin treatment but did not reach the level of statistical significance when compared with placebo. Total cholesterol increased significantly during tinzaparin compared with placebo and minor fluctuations were observed in plasma triglycerides during tinzaparin treatment (Table 6). Uncomplicated moderate haematoma was reported by three patients at the injection site but otherwise no adverse events were reported. Patients' compliance of the self-administrated injection regime was excellent based on evaluation of the returned vials and injection equipment.

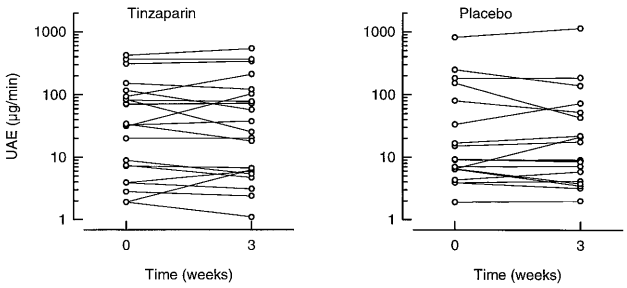

Fig. 1. Urinary albumin excretion rate in Type II diabetic patients with normal or controlled blood pressure before and after 3 weeks of treatment with tinzaparin (50 IU/kg once daily) and placebo (0.9 % saline)

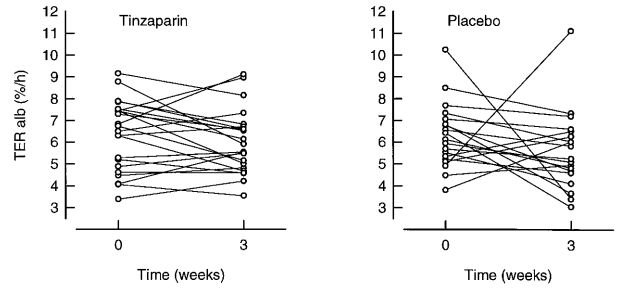

Fig. 2. Transcapillary escape rate of albumin in Type II diabetic patients with normal or controlled blood pressure before and after 3 weeks of treatment with tinzaparin (50 IU/kg once daily) and placebo (0.9 % saline)

Discussion

We examined the relation between UAE, TER_{alb} and cardiovascular risk factors as well as the effect of short term treatment with the LMWH, tinzaparin on

Table 3. Intravascular mass and dynamics of intravascular albumin during tinzaparin and placebo treatment

	Tinzaparin		Placebo	
	Baseline	3 weeks	Baseline	3 weeks
TER_{alb} (%/h)	6.3 ± 1.6	6.0 ± 1.5	6.3 ± 1.5	5.6 ± 1.8
Plasma volume (ml)	3082 ± 602	2867 ± 576[a]	2994 ± 466	2693 ± 379[c]
Plasma volume (ml/1.73 m^2)	2713 ± 342	2515 ± 295[b]	2815 ± 323	2533 ± 260[c]
Serum albumin (g/l)	41 ± 2	41 ± 3	41 ± 3	40 ± 4
IVM_{alb} (g/1.73 m^2)	112 ± 14	103 ± 15[b]	115 ± 14	99 ± 21
J_{alb} (g/h · 1.73 m^2)	709 ± 200	614 ± 168[b]	718 ± 167	572 ± 188[a]

Values are means ± SD
[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ compared with baseline

Table 4. Haemostatic factors during tinzaparin and placebo treatment

	Tinzaparin		Placebo	
	Baseline	3 weeks	Baseline	3 weeks
α-macroglobulin (g/l)	2.00 ± 0.74	2.11 ± 0.74	2.11 ± 0.67	2.12 ± 0.61
F VII : C (IU/l)	1.39 ± 0.26	1.46 ± 0.29	1.41 ± 0.33	1.47 ± 0.29
Factor VIII (IU/l)	1.62 (1.11–1.97)	1.71 (1.45–2.46)[ab]	1.31 (1.14–1.50)	1.33 (1.21–1.71)
Prothrombin fragment 1 + 2 (nmol/l)	1.44 (0.95–1.80)	1.58 (1.02–2.05)	1.63 (1.32–1.85)	1.61 (1.32–1.80)
Fibrinogen (g/l)	5.6 (5.1–6.0)	5.2 (4.8–6.2)	4.9 (4.3–6.1)	5.2 (4.6–5.9)
Fibronectin (g/l)	0.50 (0.38–0.55)	0.53 (0.47–0.75)[c]	0.44 (0.40–0.48)	0.47 (0.42–0.52)
von Willebrand factor (IU/l)	1.43 (1.05–2.07)	1.54 (1.25–2.18)	1.23 (1.03–1.51)	1.25 (1.05–1.66)

Values are means ± SD; median (25^{th}–75^{th} percentile)
[a] $p < 0.01$ compared with baseline; [b] $p < 0.05$, [c] $0.05 < p < 0.10$ change from baseline compared with placebo

Table 5. Twenty-four hour ambulatory blood pressure during tinzaparin and placebo treatment

	Tinzaparin		Placebo	
	Baseline	3 weeks	Baseline	3 weeks
Systolic daytime (mm Hg)	136 ± 16	135 ± 17	136 ± 18	138 ± 16
Systolic nighttime (mm Hg)	124 ± 18	123 ± 20	124 ± 19	126 ± 20
Systolic diurnal (mm Hg)	133 ± 16	132 ± 17	133 ± 17	134 ± 17
Diastolic daytime (mm Hg)	81 ± 9	79 ± 10	80 ± 7	80 ± 6
Diastolic nighttime (mm Hg)	69 ± 10	67 ± 11	68 ± 10	69 ± 10
Diastolic diurnal (mm Hg)	78 ± 8	75 ± 9[a]	76 ± 7	77 ± 7

Values are means ± SD
[a] $p < 0.05$ compared with baseline

Table 6. Metabolic parameters and BMI during tinzaparin and placebo treatment

	Tinzaparin		Placebo	
	Baseline	3 weeks	Baseline	3 weeks
Total cholesterol (mmol/l)	5.4 ± 1.0	6.1 ± 1.4[bc]	5.8 ± 1.2	5.8 ± 1.0
HDL cholesterol (mmol/l)	1.1 (0.9–1.2)	1.0 (0.9–1.4)	1.3 (1.0–1.5)	1.1 (0.9–1.5)
Triglycerides (mmol/l)	1.96 (1.02–2.69)	1.82 (1.23–3.91)[a]	1.56 (1.05–2.10)	1.61 (1.16–2.93)
Fasting plasma glucose (mmol/l)	10.2 ± 3.0	9.3 ± 2.9[a]	8.6 ± 1.9	9.2 ± 2.0
HbA_{1c} (%)	8.6 ± 1.8	8.3 ± 1.6[a]	8.1 ± 1.5	8.2 ± 1.4
Serum creatinine (μmol/l)	78 (67–95)	75 (64–100)	77 (71–82)	74 (70–83)
BMI (kg/m^2)	28.4 ± 4.5	28.6 ± 4.6[a]	27.2 ± 3.7	27.3 ± 3.6

Values are means ± SD; median (25^{th}–75^{th} percentile)
[a] $p < 0.05$, [b] $p < 0.01$ compared with baseline, [c] $p < 0.05$ change from baseline compared with placebo

these variables in Type II diabetic patients with normal or low grade abnormal albuminuria and normal or controlled blood pressure. The results showed that 24 h AMBP, lipids, BMI, homeostatic factors and markers of endothelial injury are higher in patients with abnormal UAE compared with normal UAE. Moreover, 24 h systolic AMBP, vWf, and factor VIII were more closely correlated with UAE than with TER_{alb}. The TER_{alb} was similar in patients with normal and raised UAE. Moreover, we found that treatment with tinzaparin for 3 weeks had no effect on UAE or TER_{alb} and only minor effects on cardiovascular risk factors.

Our finding of a relation between UAE and many cardiovascular risk factors confirms that raised UAE is a cardiovascular risk marker in Type II diabetes. Previous studies have reported a similar increase of TER_{alb} in Type II diabetic patients with normoalbuminuria and overt proteinuria [7, 8]. In another study TER_{alb} was found to be increased in microalbuminuric but not normoalbuminuric patients [9]. Higher fasting plasma glucose concentration in the microalbuminuric patients, however, may have confounded the relation between TER_{alb} and UAE since both variables increase during hyperglycaemia [22–26]. In our study of patients with normal or low grade abnormal albuminuria we found no association between UAE and TER_{alb}. We did not include patients with uncontrolled blood pressure because hypertension enhances UAE and TER_{alb} [17, 22, 27–29]. TER_{alb} is normal in moderate hypertension [22, 30] and coexistence of microalbuminuria does not increase TER_{alb} compared with microalbuminuria alone [30]. On the other hand microalbuminuria is associated with in-

creased TER_{alb} in non-diabetic subjects and normotensive, long-term Type I (insulin-dependent) diabetic patients [30–32]. Moreover, we excluded patients with proliferative retinopathy to minimize the risk of retinal bleeding during LMWH treatment. Absence of retinopathy indicates a higher proportion of non-diabetic renal disease in Type II diabetic patients with abnormal albuminuria; however, the exact proportion is being debated [33–35]. None of our patients had been diagnosed with non-diabetic renal disease and none had had a kidney biopsy. In theory, the absence of a correlation between UAE and TER_{alb} could be due to a high proportion of patients using ACE-inhibitors since these drugs may have renal effects independent of their effects on systemic blood pressure [36]. Exclusion of this potential confounder did not improve the relation between UAE and TER_{alb} nor did it improve the correlations between UAE and other risk factors.

We found higher concentrations of Factor VIII, vWF, fibrinogen, α-2 macroglobulin, and fibronectin in patients with raised UAE. Plasma concentrations of fibronectin and vWf are considered markers of endothelial injury and associated with glomerular disease in diabetes [37–40]. α-2 macroglobulin is increased in patients with diabetic complications [41, 42]. Moreover, fibrinogen, vWf, F VII:C, and factor VIII (a liver synthesized protein bound to vWf in the circulation) are associated with a higher incidence of ischaemic heart disease. The finding of higher vWf and fibrinogen in patients with abnormal UAE confirms earlier results [40, 43]. Increased concentrations of haemostatic factors along with endothelial injury in Type II diabetic patients with abnormal UAE possibly contributes to the increased cardiovascular risk in these patients. None of the haemostatic factors were related to TER_{alb}. In conclusion, TER_{alb}, as opposed to UAE, is not a sensitive marker of the degree of microvascular/endothelial dysfunction in microalbuminuric or low grade proteinuric Type II diabetic subjects with normal or controlled blood pressure.

It has been hypothesized that early phases of increased albuminuria in diabetes could represent a renal manifestation of generalized vascular damage [6, 7]. The pathogenic mechanism was first formulated in Type I diabetes [6] and involves a genetically based abnormal metabolism of HS proteoglycan of extracellular matrix and basement membranes with a decrease of normally sulphated, negatively charged HS GAG sidechains. These changes provide the biochemical basis for increased transvascular permeability of negatively charged plasma proteins which promotes extravascular coagulation and structural damage of the vasculature and glomeruli [6, 10–12, 44]. Animal [45–47] and human studies [13–15] suggest that treatment with heparin and orally available GAGs improve GAG metabolism, prevent renal morphological changes, and prevent progression of albuminuria. Thus, treatment with LMWH, danaparoid sodium, and sulodexide reduced microalbuminuria in Type I [13, 14] and Type II [15] diabetic patients with abnormal albuminuria. In the latter study [15], a decrease in blood pressure and glycaemic control was also observed during sulodexide treatment which may have confounded the ability to find a positive effect. We found no effect on UAE or TER_{alb} during 3 weeks treatment with tinzaparin in Type II diabetic patients with varying degrees of albuminuria. This does, however, not exclude a long-term treatment effect. The lack of effect on TER_{alb} or UAE could be due to a type 2 error. The probability of detecting a change of $20 \pm 20\,\%$ (paired *t*-test) is greater than 0.80 ($n = 10$). Moreover, tinzaparin was given in conventional anticoagulative doses which may be different from doses required to affect extracellular matrix metabolism. Different formulas of low-molecular weight and unfractionated heparins may differ in chemical composition, especially in their sulphation ratio, which could have an important effect on GAG metabolism. Plasma volume decreased similarly in both groups. Plasma albumin concentrations were unchanged and, consequently, lower IVM_{alb} was found after treatment. Thus, the total outflow of albumin (J_{alb}) decreased in both groups. The reason for these findings is not clear. There was no difference between the groups and the fraction of the IVM_{alb} disappearing from the circulation (i.e. TER_{alb}) was similar and unchanged in the two groups. The lack of effect of tinzaparin treatment as well as the lack of an association between UAE and TER_{alb} indicate that abnormal GAG metabolism either might not be uniformly present or might not be a cause of the widespread vascular disease of Type II diabetic patients with abnormal albuminuria. Whether quantitative differences caused by other cardiovascular risk factors such as hyperglycaemia or hypertension or both on UAE and TER_{alb} exist in Type II diabetes remains to be defined.

Heparin treatment could lower blood pressure by decreasing blood viscosity [48] and stimulates endothelial lipoprotein lipase activity which possibly decreases plasma triglycerides. The lack of effect of tinzaparin on TER_{alb} and UAE cannot be explained by changes in blood pressure or glycaemic control. Tinzaparin treatment was associated with a slight decrease in 24 h diastolic AMBP. Glycaemic control improved slightly during tinzaparin. Therefore, if anything, the reduction in blood pressure and plasma glucose would have tended to decrease TER_{alb} and UAE. Only minor changes were observed in haemostatic factors and plasma lipids during treatment with tinzaparin, although a slight increase in total cholesterol was noted.

In conclusion our study shows no association between TER_{alb} and UAE in microalbuminuric or low

grade proteinuric Type II diabetic subjects with normal or controlled blood pressure. This was also true after exclusion of patients using ACE-inhibitors. The degree of albuminuria, but not TER_{alb}, is a sensitive marker of microvascular dysfunction and correlates more closely than TER_{alb} with systolic blood pressure, vWf, and factor VIII. Moreover, our results do not show that short-term treatment with tinzaparin exerts a potentially beneficial effect on glomerular and vascular leakage of albumin in Type II diabetic subjects. Our findings indicate that factors other than abnormal GAG metabolism contribute to the vascular damage in these patients.

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