UK Prospective Diabetes Study (UKPDS)

IX: Relationships of urinary albumin and N-acetylglucosaminidase to glycaemia and hypertension at diagnosis of Type 2 (non-insulin-dependent) diabetes mellitus and after 3 months diet therapy

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Summary. In 672 newly-diagnosed, Type 2 (non-insulin-dependent) diabetic patients without urinary infection, aged 51 (9) years, mean (1 SD), 28 % of patients had raised albuminuria, defined as albumin excretion greater than 25 mg/l and 66 % raised urinary N-acetylglucosaminidase excretion defined as greater than 300 $\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$ (both urinary analytes corrected by linear regression on urinary creatinine to 10 mmol/l). In a univariate analysis, urinary N-acetylglucosaminidase and albumin excretion correlated with each other ($r_s = 0.42$, $p < 0.001$), and with fasting plasma glucose ($r_s = 0.46$ and $r_s = 0.27$, $p < 0.001$, respectively). The association of urinary albumin and N-acetylglucosaminidase remained significant after taking the fasting plasma glucose levels into account, partial $r_s = 0.34$, $p < 0.001$. After 3 months of dietary therapy BMI decreased from 29.7 (5.9) kg/m^2 to 28.8 (5.8) kg/m^2 , fasting plasma glucose levels from 12.2 (3.8) mmol/l to 9.8 (3.8) mmol/l, and systolic blood pressure from 143 (21.8) mmHg to 131 (20.3) mmHg, $p < 0.001$ for each variable. There were concomitant decreases in urinary N-acetylglucosaminidase, geometric mean (1 SD interval), 397

$\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$ (216 to 728) to 291 $\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$ (160 to 528), $p < 0.001$ and in albumin excretion 16 mg/l (5 to 51) to 13 mg/l (4 to 40), $p < 0.001$. The decrease in urinary N-acetylglucosaminidase, but not the decrease in urinary albumin excretion, was associated with the initial degree of glycaemia and the decrease in glycaemia in response to diet. Urinary albumin excretion correlated with systolic blood pressure at diagnosis ($r_s = 0.23$, $p < 0.001$) and was higher in both treated and untreated patients with hypertension, $p < 0.001$, whereas urinary N-acetylglucosaminidase excretion was only slightly raised in patients with hypertension on therapy, $p < 0.05$. In conclusion, urinary albumin excretion was associated with hyperglycaemia and hypertension, whereas urinary N-acetylglucosaminidase was primarily associated with hyperglycaemia.

Key words: Urine albumin excretion, plasma glucose, urine N-acetylglucosaminidase excretion, blood pressure, type 2 diabetes, UK Prospective Diabetes Study

Early detection and monitoring of diabetic complications is desirable in Type 2 (non-insulin-dependent) diabetic patients. Microalbuminuria is associated with an increased risk of progressive renal dysfunction [1, 2] and excessive cardiac mortality [3–6]. Raised urinary albumin excretion indicates glomerular damage in the kidney and is associated with hyperglycaemia and hypertension in both Type 1 (insulin-dependent) [7, 8] and Type 2 diabetic patients [2, 9, 10].

An alternative measure of renal dysfunction is provided by the assay of urinary N-acetylglucosaminidase, a lysosomal enzyme involved in the breakdown of glycoproteins. Increased urinary N-acetylglucosaminidase excretion is thought to reflect cellular damage in the proximal tubule of the kidney, and has been used to assess renal disease and detect nephrotoxic damage [11, 12]. Increased urinary N-acetylglucosaminidase excretion has been re-

ported in both Type 1 [12–14] and Type 2 [15, 16] diabetic patients. It has also been reported to be raised in normoglycaemic patients with essential hypertension but it correlated only with systolic and not diastolic blood pressure [17]. Urinary N-acetylglucosaminidase has also been reported not to differ between hypertensive and normotensive Type 2 diabetic patients in a study of 125 randomly selected patients [18].

We have studied the relationship of urinary N-acetylglucosaminidase and albumin excretion in 716 Type 2 patients at diagnosis of diabetes and 195 age-matched control subjects. We have investigated Type 2 diabetic patients with urinary infection and excluded them from further analyses. The relationships of urinary N-acetylglucosaminidase and albumin excretion with hyperglycaemia, plasma N-acetylglucosaminidase and blood pressure have been studied both at diagnosis of diabetes and after

3 months of diet therapy. The relationship of urinary albumin and N-acetylglucosaminidase excretion with the hypertensive status of the patients at diagnosis, (either normotensive or hypertensive, treated or untreated) has also been studied.

Subjects and methods

Patients

Seven hundred and sixteen newly-diagnosed Type 2 diabetic patients who had been recruited into the UK Prospective Diabetes Study (UKPDS) [19] between 1988 and 1990 were studied; the patients were aged 51 (9) years, mean (1 SD). Patients were referred by general practitioners or other physicians to 23 centres in the United Kingdom and were seen in a UKPDS clinic within 3 weeks. Diabetes was confirmed by two fasting plasma glucose values 6.0 mmol/l or greater. Fasting plasma glucose greater than 7.8 mmol/l, was found in 88% of patients, the mean fasting plasma glucose was 12.2 (3.8) mmol/l. Patients with renal failure (plasma creatinine > 175 µmol/l), accelerated hypertension, a recent myocardial infarct, heart failure, or angina were excluded. Tablet therapy for hypertension was used in 18% of patients.

The patients came to the clinic after an overnight fast from 22.00 hours the previous night, with only water to drink. A urine sample was taken at the clinic and blood samples for fasting plasma glucose, HbA_{1c} and plasma N-acetylglucosaminidase. After diabetes was confirmed, patients were given dietary advice for a high carbohydrate, low saturated fat and moderately high fibre diet with a reduced energy content for obese patients. The patients were seen monthly for 3 months by a doctor and a dietitian. Blood pressure was taken at the initial visit (diagnosis) and at the 2-month visit (after diet). Blood and urine samples were taken at the first and 3-month visit. Urinary infection was assessed as more than 10⁵ bacteria/ml at the initial visit.

All patients gave their informed consent and the study received approval from the ethics committee of each centre.

Control subjects

An age-matched reference range for urinary albumin and N-acetylglucosaminidase excretion was provided by 195 non-diabetic volunteers (spouses or friends of the UKPDS patients), mean age 52 (9) years. The subjects attended the clinic after an overnight fast and had blood and urine samples taken as for the patients in the study. Exclusion criteria were fasting plasma glucose greater than 6 mmol/l or a first degree relative with diabetes.

Measurements

Fasting plasma glucose was measured from a venous sample at the centres. The glucose measurements were monitored by an external UKPDS glucose quality assurance scheme with between centre coefficients of variation (CVs) of 4.5% at 4.4 mmol/l, 4.9% at 8.2 mmol/l and 3.9% at 10.2 mmol/l glucose.

Blood pressure was measured in the sitting position, after a 5-min rest with the supported forearm semi-flexed and palm upwards. Either a Copal UA-251 or Takeda UA-751 electronic, automatic, auscultatory machine (Andrew Stephens Co, Brighouse, West Yorkshire, UK) was used, but if the arm circumference was greater than 33 cm a Hawksley random zero sphygmomanometer (Hawksley and Sons Ltd, Lancing, Sussex, UK) was employed. In each patient four consecutive readings were taken with intervals of at least 2 min and the mean of the last three readings recorded. A blood pressure quality control scheme was used to measure inter-machine and between centre variation. Hypertension at diagnosis was defined as blood pressure ≥ 160 systolic or ≥ 90 diastolic mm Hg or treatment with anti-hypertensive therapy.

Blood and urine samples taken at diagnosis and at the 3-month visit were transported overnight at 4°C from the centres to a central laboratory in Oxford. Samples were stored for up to 7 days at 4°C before assay. HbA_{1c} was measured by HPLC using a Biorad Diamat HbA_{1c} analyser (Biorad Laboratories Ltd, Hemel Hempstead, Herts, UK) with inter-assay CVs 1.9% at 6.2% HbA_{1c} and 1.8% at 10.3% HbA_{1c}. The reference range was 4.4 to 6.4% (2 SD range) in 250 non-diabetic subjects, aged 56 (11) years, mean (1 SD). The labile aldime fraction was removed by 30-min incubation in borate haemolysing buffer at 37°C before analysis. A urine sample was taken in the clinic into a tube (6 ml) containing 50 µl of 0.1% thiomersal as a preservative. Albumin was determined by an immunoturbidimetric method [20] using goat anti-human albumin antiserum (INCSTAR Ltd, Womersley, Berks, UK) and human serum protein calibrator (Dakopatts, High Wycombe, Bucks, UK). Samples were first centrifuged at 1500 g for 10 min to clear them of particulate matter and screened with Albustix (Ames Division, Miles Laboratories Ltd, Stoke Poges, Slough, Berks, UK) to indicate samples with excess albumin which required dilution. Quality control was monitored using tri-level quality controls by an "in house" computer package, QSTAT, applying Westgard rules [21] for assay rejection. The lower limit of the assay was 2 mg/l albumin and inter-assay CVs 5.6% at 4.4 mg/l, 3.1% at 31.3 mg/l and 6.5% at 136.7 mg/l albumin. External quality control was monitored by UKEQAS, UK External Quality Assurance Scheme (Queen Elizabeth Hospital, Birmingham, UK). There was a good correlation between our laboratory results and designated values for UKEQAS samples, $r_s = 0.997$.

Urinary N-acetylglucosaminidase was measured by methoxynitrovinylphenol release [22] (Cortecs Diagnostics Ltd, Deeside,

Table 1. Characteristics of normal subjects and Type 2 diabetic patients (with and without urinary infection) at diagnosis of diabetes and the effect of 3 months diet in patients without urinary infection

	Normal subjects (n = 195)	Patients without infection (n = 672)		Patients with infection (n = 44)
		At diagnosis	After diet	At diagnosis
Age (years)	52 (9)	50.6 (9.3)	—	52.8 (10.4)
BMI (kg/m ²)	26.2 (4.0)	29.7 (5.9)	28.8 (5.8) ^c	30.2 (6.8)
Systolic BP (mm Hg)	—	143 (21.8)	131 (20.3) ^c	146 (27.5)
Diastolic BP (mm Hg)	—	86.3 (12.1)	79.9 (11.9) ^c	88.2 (13.0)
FPG (mmol/l)	5.0 (0.6)	12.2 (3.8)	9.8 (3.8) ^c	12.6 (3.0)
HbA _{1c} (%)	5.4 (0.4)	9.1 (2.5)	7.2 (1.7) ^c	9.6 (2.2)
Urinary NAGase (µmol · h ⁻¹ · l ⁻¹) ^a	195 (129 to 295)	397 (216 to 728)	291 (160 to 528) ^c	447 (246 to 814)
Urinary albumin (mg/l) ^a	8.0 (3.5 to 18.4)	15.9 (5.0 to 51.4)	12.8 (4.1 to 40.4) ^c	25.3 (7.1 to 90.1) ^b
Plasma NAGase (µmol · h ⁻¹ · l ⁻¹) ^a	599 (476 to 754)	662 (508 to 863)	574 (458 to 770) ^c	659 (502 to 866)

^a indicates geometric mean and 1 SD interval, otherwise mean and SD; ^b $p < 0.05$, ^c $p < 0.001$ for the significance: between patients with and without infection at diagnosis, and patients without infection

at diagnosis and after 3 months diet. BP, Blood pressure; NAGase, N-acetylglucosaminidase

Clywd, UK), and creatinine by the endpoint Jaffé [23, 24] method. All three assays were performed on the same urine sample using a centrifugal analyser (Cobas FARA, Roche Diagnostica, Welwyn Garden City, Herts, UK). Inter-assay CVs for urinary N-acetylglucosaminidase were 4.7% at $235 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$, 4.5% at $726 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$ and 5.5% at $1914 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$ and for creatinine 6.1% at 3.3 mmol/l , 3.3% at 8.9 mmol/l and 3.9% at 17.0 mmol/l .

Plasma N-acetylglucosaminidase was measured on Cobas MIRA by methoxynitrovinylphenol release with a smaller sample volume (10 μl) and longer incubation time (900 s) than for urine and sample blanking i.e. assay buffer without substrate on a separate run [25]. Inter-assay CVs for plasma N-acetylglucosaminidase were 15.2% at $100 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$, 2.8% at $1064 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$ and 2.6% at $1900 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$.

Statistical analysis

The statistical computer programs MINITAB Statistical Software, SAS and CSTAT (Oxtech 1991), were used for calculations, and SLIDEWRITE PLUS (Advance Graphics Software Inc.) for graphs. Urinary protein excretion was corrected for urine dilution by linear regression on creatinine concentration. Values were adjusted to a standard creatinine of 10 mmol/l , the mean creatinine concentration of all study patients ($n = 5102$) at diagnosis [26]. Comparison with the more conventional urine analyte/creatinine ratios showed no significant differences. Urinary and plasma N-acetylglucosaminidase and albumin were log transformed to provide a normal distribution. For log transformed data geometric mean (1 SD interval) have been quoted, otherwise mean (1 SD) are shown.

Results

Control subjects

Urinary N-acetylglucosaminidase and albumin concentrations were $195 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$ (129 to 295) and 8 mg/l (3.5 to 18.4), corrected by linear regression to 10 mmol/l creatinine, in an age-matched normoglycaemic reference population, $n = 195$. Raised urinary N-acetylglucosaminidase expressed as greater than $300 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$ was the 87th percentile of this population and raised urinary albumin greater than 25 mg/l , the 90th percentile. Urinary N-acetylglucosaminidase and albumin did not correlate with fasting plasma glucose in normal subjects. However, they correlated with HbA_{1c} , $r_s = 0.19$ and $r_s = 0.17$, $p < 0.05$, respectively.

Urinary infection

Urinary infection greater than 10^5 bacteria/ml was found in 6% of Type 2 diabetic patients recruited into the study at diagnosis of diabetes. These patients had increased albumin excretion (Table 1) and were excluded from subsequent analyses. There were no statistical differences in age, BMI, blood pressure or glycaemic control between patients with urinary infection and those without. Urinary N-acetylglucosaminidase was slightly higher in patients with infection but the difference was not statistically significant. Plasma N-acetylglucosaminidase was similar in both groups.

Table 2. Proportion of 672 patients with urinary albumin greater than 25 mg/l (high albumin) and N-acetylglucosaminidase, greater than $300 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$ (high NAGase) at diagnosis and after 3 months diet

Diagnosis of Type 2 diabetes			
Urinary excretion	Normal albumin	High albumin	Total
Normal NAG	30%	4%	34%
High NAG	42%	24%	66%
Total	72%	28%	
After 3 months diet			
Urinary excretion	Normal albumin	High albumin	Total
Normal NAG	56%	6%	62%
High NAG	27%	11%	38%
Total	83%	17%	

The chi-square for the distributions were 39.9 ($p < 0.001$) at diagnosis and 45.0 ($p < 0.001$) after 3 months diet

Table 3. Relationship of urinary albumin and N-acetylglucosaminidase excretion by Spearman rank correlation with patient characteristics and biochemical variables at diagnosis and after 3 months diet

Characteristic	Urinary albumin		Urinary N-acetylglucosaminidase	
	Diagnosis	After diet	Diagnosis	After diet
Age	0.09 ^a	NS	0.09 ^a	NS
BMI	NS	0.14 ^c	NS	NS
Systolic BP	0.23 ^c	0.17 ^b	NS	NS
Diastolic BP	0.20 ^c	0.15 ^a	0.11 ^a	NS
Fasting plasma glucose	0.27 ^c	0.25 ^c	0.46 ^c	0.43 ^c
HbA _{1c}	0.25 ^c	0.23 ^c	0.48 ^c	0.45 ^c
Urinary albumin	—	—	0.42 ^c	0.40 ^c
Plasma NAGase	0.16 ^c	NS	0.28 ^c	0.19 ^c

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$; BP, blood pressure; NAGase N-acetylglucosaminidase

Fig. 1. Comparison of urinary albumin and N-acetylglucosaminidase (NAGase) excretion in newly-presenting Type 2 diabetic patients. Solid line indicates linear regression and dotted lines represent upper limit of normal for albumin, 25 mg/l , and for proteinuria, 250 mg/l , and N-acetylglucosaminidase, $300 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$

Fig. 2. Relationship of urinary albumin and N-acetylglucosaminidase (NAGase) with fasting plasma glucose at diagnosis. Solid line indicates linear regression and dotted lines represents upper limit of normal for albumin, 25 mg/l, or N-acetylglucosaminidase, 300 $\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$

Fig. 3. Relationship of urinary albumin and N-acetylglucosaminidase (NAGase) excretion with HbA_{1c} at diagnosis. Solid line indicates regression and dotted lines represents upper limit of normal for albumin, 25 mg/l, or N-acetylglucosaminidase, 300 $\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$

At diagnosis of diabetes

Patients studied after exclusion of those with urinary infection ($n = 672$) were aged 51 (9) years with BMI 29.7 (5.9) kg/m^2 and had plasma urate and creatinine levels, geometric mean (1 SD interval), of 0.40 mmol/l (0.17 to 0.92) and 79 $\mu\text{mol}/\text{l}$ (64 to 97), respectively. Fasting plasma glucose and HbA_{1c} levels were 12.2 (3.8) mmol/l and 9.1 (2.5) %, respectively (Table 1) and urinary N-acetylglucosaminidase and albumin higher than the age-matched normoglycaemic population.

Of the Type 2 diabetic patients, 66 % had raised urinary N-acetylglucosaminidase, ($> 300 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$), and 28 % raised albumin excretion ($> 25 \text{ mg}/\text{l}$) (Table 2). Of those with raised albumin 86 % also had raised N-acetylglucosaminidase excretion whereas only 36 % of those with raised N-acetylglucosaminidase had raised albumin

excretion. Only 4 % of patients had raised albumin with normal N-acetylglucosaminidase excretion. Urinary albumin excretion above 250 mg/l was found in 2.5 % of diabetic patients.

In a univariate analysis, urinary N-acetylglucosaminidase and albumin excretion were associated, $r_s = 0.42$, $p < 0.001$ (Table 3 and Fig. 1). Both urinary N-acetylglucosaminidase and albumin correlated with the degree of glycaemia, as assessed by fasting plasma glucose (Fig. 2) and HbA_{1c} (Fig. 3), and with plasma N-acetylglucosaminidase. Albumin excretion also correlated with systolic blood pressure (Fig. 4), whereas N-acetylglucosaminidase did not. In a multivariate analysis (Table 4) urinary albumin excretion was associated with fasting plasma glucose (6 % explained variance), systolic blood pressure (6 %) and waist/hip ratio (1.3 %) and urinary N-acetylglucosaminidase excretion with fasting plasma

Fig. 4. Relationship of urinary albumin with systolic blood pressure at diagnosis. Solid line indicates linear regression and dotted lines represents upper limit of normal for albumin, 25 mg/l

glucose (20 %) and systolic blood pressure (1 %). The association of urinary N-acetylglucosaminidase with plasma N-acetylglucosaminidase in the univariate analysis (Table 3) was no longer found when fasting plasma glucose, systolic blood pressure, waist/hip ratio and height were taken into account. However, the association between urinary N-acetylglucosaminidase and albumin excretion remained significant after the same variables were taken into account, partial $r_s = 0.32$, $p < 0.001$.

Response to diet

After 3 months of diet therapy, the patients showed a reduction in BMI, systolic and diastolic blood pressures and hyperglycaemia (Table 1); urinary protein excretion (albumin and N-acetylglucosaminidase) and plasma N-acetylglucosaminidase were also lowered, all these changes on diet were significant at $p < 0.001$.

The decreases in N-acetylglucosaminidase and micro-albuminuria after 3 months diet were assessed by multi-

Table 4. Multivariate linear regression analysis: stepwise model relating urinary albumin and N-acetylglucosaminidase at diagnosis of diabetes to patient characteristics and biochemical variables

	Urinary albumin at diagnosis ($n = 610$)				Urinary NAGase at diagnosis ($n = 620$)			
	N	β	p	Proportion explained variance R^2 (%)	N	β	p	Proportion explained variance R^2 (%)
Age at entry			NS				NS	
Gender			NS				NS	
Height	4	-0.004	< 0.05	0.6			NS	
BMI at entry			NS				NS	
Waist/hip ratio	3	0.937	< 0.01	1.3			NS	
Systolic BP at entry	2	0.005	< 0.0001	6.0	2	0.001	< 0.01	1.0
Fasting plasma glucose at entry	1	0.034	< 0.0001	6.3	1	0.031	< 0.0001	20.6
Plasma NAGase at entry			NS				NS	
Total R^2				14.2				21.6

N, The order of significant associations in the model; β , slope; p , significance of addition; BP, blood pressure; NAGase, N-acetylglucosaminidase

Table 5. Multivariate linear regression analyses: stepwise model relating the differences in urinary excretion between diagnosis and after diet with patient characteristics and biochemical variables at diagnosis and the difference between diagnosis and after diet

	Difference between urinary albumin at diagnosis and after 3 months diet ($n = 484$)				Difference between urinary NAGase at diagnosis and after 3 months diet ($n = 492$)			
	N	β	p	Proportion explained variance R^2 (%)	N	β	p	Proportion explained variance R^2 (%)
BMI at entry			NS				NS	
BMI difference			NS		5	-19.83	< 0.05	0.5
Systolic BP at entry			NS				NS	
Systolic BP difference			NS				NS	
Fasting plasma glucose at entry			NS		3	19.18	< 0.0001	3.4
Fasting plasma glucose difference			NS		2	32.26	< 0.0001	7.5
Plasma NAGase at entry			NS		6	249.7	< 0.05	0.5
Plasma NAGase difference			NS		4	0.407	< 0.0001	2.0
Urinary analyte at entry	1	-50.1	< 0.0001	7.9	1	-586.3	< 0.0001	35.4
Total R^2				7.9				49.3

N, The order of significant associations in the model; β , slope; p , significance of addition; BP, blood pressure; NAGase, N-acetylglucosaminidase

Table 6. Association at diagnosis of diabetes of the patient blood pressure status with urinary albumin and N-acetylglucosaminidase (NAGase) excretion, assessed by analysis of variance with age, BMI and gender as co-variables

Patient group	<i>n</i>	Albumin (mg/l)	<i>n</i>	NAGase ($\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$)
Non-hypertensive	308	13.2 (4.4 to 39.2)	311	376 (205 to 691)
Hypertensive (not on therapy)	212	19.5 (3.3 to 65.2) ^b	216	403 (227 to 715)
Hypertensive (on therapy)	114	18.4 (5.4 to 63.4) ^b	117	441 (229 to 850) ^a

The urine concentrations are expressed as geometric mean and 1 SD interval. ^a $p < 0.05$, ^b $p < 0.001$ compare the untreated and treated hypertensive groups with the normotensive group

variate analysis (Table 5). Urine albumin at diagnosis was the only determinant of the decrease in urine albumin (8% explained variance) whereas the decrease in urine N-acetylglucosaminidase was determined by the initial value (35%), the fall in fasting plasma glucose (8%), and fasting plasma glucose at entry (3%).

Association of hypertension with N-acetylglucosaminidase and albumin excretion

Of the patients studied 49% were normotensive, 18% hypertensive on therapy and 33% hypertensive not being treated for hypertension. The relationships of urinary N-acetylglucosaminidase and albumin excretion with hypertension were established after correction for age, BMI and gender with the fasting plasma glucose not being significantly different between the three groups. Albumin excretion was significantly higher in hypertensive patients in both treated and untreated groups ($p < 0.001$) with urinary N-acetylglucosaminidase only being elevated in hypertensive patients on therapy ($p < 0.05$) and not in those who were untreated (Table 6).

Discussion

Urinary excretion of N-acetylglucosaminidase and albumin was higher in patients at diagnosis of Type 2 diabetes than in an age-matched normoglycaemic reference population. Type 2 diabetic patients with urinary infection had higher albumin excretion but neither raised urine nor plasma N-acetylglucosaminidase than those without infection. The Type 2 diabetic patients with infection were excluded from subsequent analyses.

If raised urinary N-acetylglucosaminidase and microalbuminuria were defined, as in other studies, greater than $300 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$ and 25 mg/l, respectively, these values corresponded to the 87th and 90th percentiles of the age-matched normoglycaemic population. Using these criteria, 66% of the Type 2 diabetic population had raised N-acetylglucosaminidase and 28% raised albumin excretion at diagnosis. The majority (86%) of those patients with raised urinary albumin excretion had raised urine N-acetylglucosaminidase excretion, whereas a minority (36%) of those with raised urinary N-acetylglucosaminidase excretion has raised urinary albumin excretion.

At diagnosis of Type 2 diabetes urinary albumin correlated with urinary N-acetylglucosaminidase excretion and

both were associated with the degree of glycaemia. The association between urinary albumin and N-acetylglucosaminidase was reduced by taking into account fasting plasma glucose and other variables but was still significant at $r_s = 0.34$, ($p < 0.001$) suggesting that 12% of the variability is explained by association between the two analytes. This association may relate to a common factor or factors such as nephropathy. The excretion of urinary albumin and N-acetylglucosaminidase decreased in response to 3 months diet treatment which lowered the fasting plasma glucose. The relationship with the degree of glycaemia was stronger for urinary N-acetylglucosaminidase than for urinary albumin, as only urine N-acetylglucosaminidase reduction correlated with the degree of fall of the glucose levels. It seems likely that the degree of glycaemia has a greater effect on renal excretion of N-acetylglucosaminidase than on albumin in these newly-diagnosed Type 2 diabetic patients. Increased urinary N-acetylglucosaminidase activity has been shown to correlate with poor long-term blood glucose control in Type 1 diabetes [27]. The relationship between elevated urinary N-acetylglucosaminidase and hyperglycaemia may indicate that the lysosomes of renal tubular cells are sensitive to plasma glucose or increased tubular glucose reabsorption, or both. Although urinary N-acetylglucosaminidase excretion correlated with the plasma N-acetylglucosaminidase concentration in a univariate analysis, this was not significant after taking the fasting plasma glucose into account. This suggests that increased glycaemia affects release of the enzyme from the tissues into the plasma and from the kidney tubular cells into the urine. The enzyme is unlikely to pass from the plasma through the glomerular basement membrane to the urine as it is too large a molecule (~190,000 Da).

Raised urinary albumin excretion was also associated with an increase in systolic blood pressure, whereas urinary N-acetylglucosaminidase was not. Raised blood pressure can be associated with microvascular renal disease in Type 1 diabetes [28, 29] but it is not certain whether the hypertension or microvascular disease is primary. The association of urinary albumin with hypertension suggests that increased microalbuminuria but not increased urinary N-acetylglucosaminidase provides an index of microvascular disease at this stage of diabetes. The increased urinary albumin excretion found in patients with both treated and untreated hypertension ($p < 0.001$) probably indicates disturbed glomerular function, whereas increased urinary N-acetylglucosaminidase excretion only reflects tubular dysfunction. Urinary N-acetylglucosaminidase excretion was only weakly associ-

ated ($p < 0.05$) with treated and not with untreated hypertension.

Both glomerular and tubular microproteinuria precede the development of overt nephropathy in Type 1 diabetes [1, 2, 12–14], whereas in Type 2 diabetic patients this has not been clearly established. In 20 newly-diagnosed Type 2 diabetic patients, Martin et al. [16] found improved fasting plasma glucose, HbA_{1c} and systolic blood pressure at 2 months after diagnosis, with a significant decrease in urinary albumin excretion but not in urine N-acetylglucosaminidase. Schnoell et al. [18] found urinary N-acetylglucosaminidase excretion did not differ significantly between 125 hypertensive and normotensive Type 2 diabetic patients, whereas urinary albumin and urinary α -microglobulin excretion were raised in the hypertensive subjects. Urinary N-acetylglucosaminidase excretion was higher in patients with severe vascular disease compared to those without atherosclerosis [18].

Clinical relevance of the improvement in urine albumin and N-acetylglucosaminidase accompanying diet treatment of hyperglycaemia is uncertain. Since the UK Prospective Diabetes Study is a long-term, prospective study, assessing factors relevant to the development of complications, including the effect of improved control of blood glucose and blood pressure, the study will be able to determine whether the response of urinary albumin or N-acetylglucosaminidase excretion has any predictive value for the development of renal disease or other microvascular or macrovascular complications of Type 2 diabetes.

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