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Tubular dysfunction and microalbuminuria in insulin dependent diabetes

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SUMMARY The nature of microproteinuria in the early years of insulin-dependent diabetes was investigated in a cross sectional study of 80 children with insulin-dependent diabetes and 40 normal children. Urinary excretion of three low molecular weight proteins: α-1-microglobulin, β-2-microglobulin and κ light chains was used as an index of proximal renal tubular function. The first urine samples of the morning were collected and excretion of proteins measured was expressed as ratio of protein to creatinine. There was a strong correlation between excretion of α-1-microglobulin and κ light chains and their excretion was significantly higher in diabetic children indicating decreased proximal tubular reabsorbtion. The excretion of β-2-microglobulin was found to be an unsatisfactory index of proximal tubular function. Urinary albumin excretion was not significantly raised in diabetic children and did not correlate with urinary α-1-microglobulin or κ light chain excretion. Glycaemic control might influence proximal tubular function as both urinary glucose concentration and glycosylated haemoglobin showed correlations with urinary α-1-microglobulin excretion and with urinary κ light chain excretion.

Proetinuria results from increased glomerular filtration or impaired tubular reabsorption. Interest in microproteinuria in diabetes mellitus has focused mainly on albumin excretion. The interest was aroused by the finding that in adults with insulin-dependent diabetes (IDDM) microalbuminuria (overnight albumin excretion rate >30 μg/minute) subsequently progressed to persistent proteinuria detectable with Albustix.[1]

Excretion of low molecular weight proteins serves as an index of proximal renal tubular function as proteins with molecular weigh less than 40 000 are freely filtered by the glomerulus and more than 95% is then reabsorbed and catabolised by the proximal tubule. The view that the microproteinuria of diabetes is solely of glomerular origin is based on observations that β-2-microglobulin, a protein of molecular weight 11 800, is excreted normally.[2] This is not upheld, however, by the results of analyses using sodium dodecyl sulphate polyacrylamide gel electrophoresis of urinary proteins, which have disclosed patterns consistent with tubular proteinuria.[3 4] Furthermore, Teppo and Groop have reported increased excretion of the κ light chains of immunoglobulin (molecular weight 23 000, dimer 46 000) in adults with IDDM.[5]

Accordingly we decided to investigate proximal renal tubular function in patients with diabetes by measuring excretion of three proteins with low molecular weight: α-1-microglobulin (molecular weight 31 000), β-2-microglobulin, and κ light chains. The study was carried out in children with IDDM so that the confounding variables that are found in many adult diabetics, who are often receiving concomitant drug treatment and where there is the possibility of other diseases, could be excluded. This approach also examined the prospect that the assays might prove sensitive markers of abnormal renal function in diabetes, preceding the appearance of microalbuminuria.

Patients and methods

Eighty children with IDDM (40 boys and 40 girls) seen consecutively at diabetic clinics in Leeds and Huddersfield and a control group of 40 normal children (19 boys and 21 girls) who were not attending hospital and who had no family history of diabetes in a first degree relative, took part in the study. All children were less than 16 years old, with a mean (SD) age of 10·5 (3·5) years for children with IDDM and 9·2 (3·3) years for children in the

control group. The mean (SD) duration of diabetes was 3·8 (2·9) years. None of the children had symptoms of urinary tract infection.

The first urine samples of the morning were collected prospectively from all subjects and stored at −20° with 0·1% sodium azide as preservative. Urinary protein concentrations were measured and expressed as ratio to urinary creatinine concentration. The first urine samples of the morning were preferred to timed overnight collections because in children urinary albumin:creatinine ratios in these morning samples do not change with age.[6] In contrast, albumin excretion rates increase with age and correlate with height, weight, and surface area.[7] In this paper we use the term urinary excretion of a protein to indicate the ratio of concentration of that protein to creatinine in the first urine sample of the morning.

The concentrations of α-1-microglobulin were measured by radial immunodiffusion[8] using an antiserum and standards supplied by Behringwerke Ag (Marburg, Lahn, West Germany). The concentrations of κ light chains were measured by radial immunodiffusion using an antiserum against pooled free κ light chains and standards supplied by the Department of Immunology, University of Birmingham. The standards used are prepared from pooled κ Bence Jones protein and the results are expressed as milliunits (mU) where one mU is equivalent to one milligram of standard. Milliunits are used because free light chains vary in their degree of polymerisation and antigenicity. Radioimmunoassays were used to measure concentrations of albumin (Pharmacia, Albumin RIA 100) and β-2-microglobulin (Pharmacia, β_2m RIA 100); β-2-microglobulin is degraded at pH <6·0 and was therefore only measured in urine samples of pH ≥6·0. The limit of sensitivity of the assays were respectively: α-1-microglobulin, 1 mg/l; κ light chains, 10 mU/l; albumin, 0·8 mg/l; and β-2-microglobulin, 0·2 mg/l. Interassay coefficients of variation were respectively: α-1-microglobulin, 6·8%; κ light chains, 13%; albumin, 8·9%; and β-2-microglobulin, 11%. Creatinine was measured by the Jaffe reaction and glucose by an autoanalyser using a glucose oxidase method. Venous blood samples collected within 24 hours of collection of urine samples were assayed for glycosylated haemoglobin by isoelectric focusing.[9] Statistical analysis was performed using the Mann-Whitney U test and Spearman Rank Correlation test and a two tailed value was taken for estimates of p. Values are expressed as median (range).

Results

Urinary excretion of α-1-microglobulin and κ light chains was significantly higher in children with IDDM compared with normal children (figs 1 and 2). Median urinary α-1-microglobulin:creatinine ratio was 0·57 (0·11–1·92) mg/mmol in children with IDDM and 0·23 (0·11–0·90) mg/mmol in normal children (p<0·0002). Median urinary κ light chain: creatinine ratio was 2·4 (0·4–7·4) mU/mmol in children with IDDM and 1·3 (0·6–4·8) mU/mmol in normal children (p<0·0002). In contrast there was no significant difference in urinary albumin excretion (fig 3); urinary albumin:creatinine ratios were 0·89 (0·06–6·85) mg/mmol for children with IDDM and 0·82 (0·11–3·67) mg/mmol for normal children. Twenty six urine samples from diabetic children and

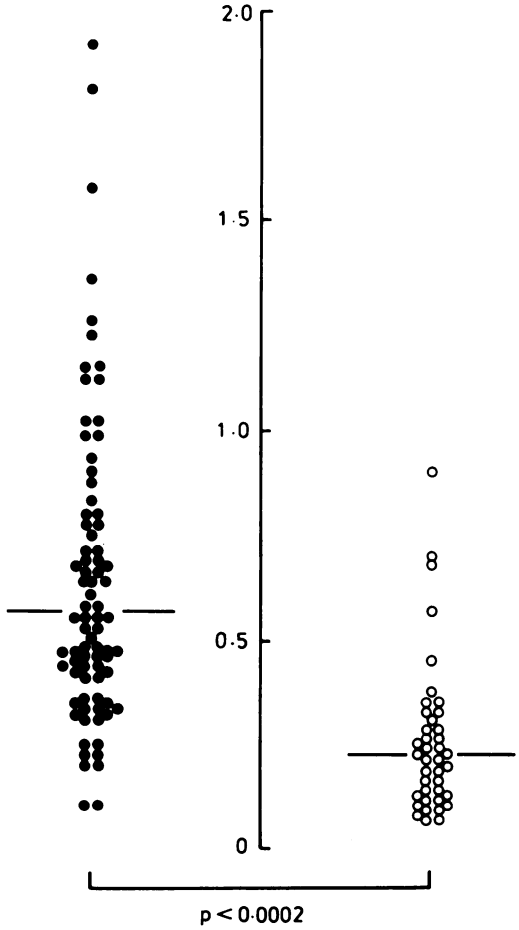

Fig 1 *Urinary α-1-microglobulin excretion (mg/mmol creatinine) in 80 children with insulin-dependent diabetes (closed circles) and 40 normal children (open circles). Bars indicate median values.*

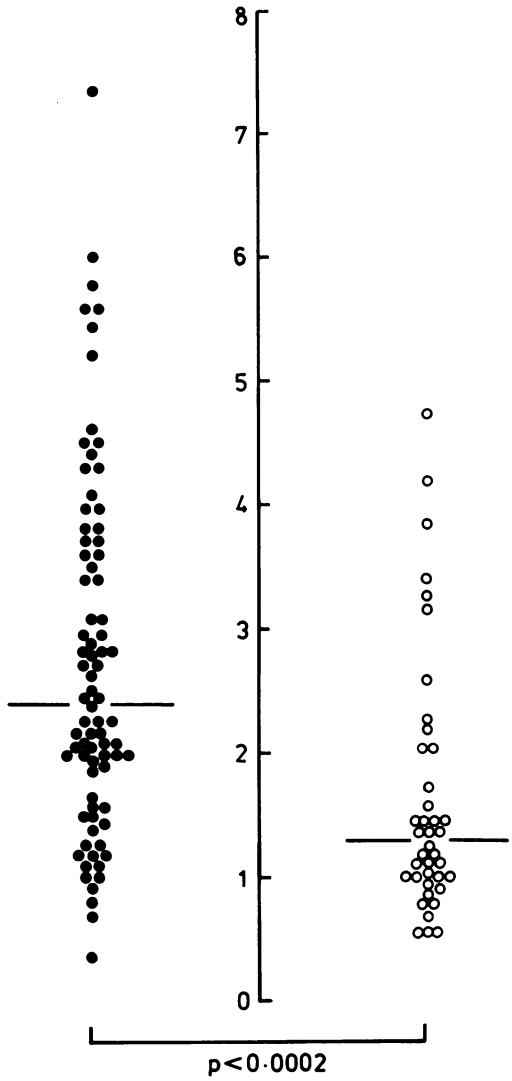

Fig 2 *Urinary κ light chain excretion (mU/mmol creatinine) in 80 children with insulin-dependent diabetes (closed circles) and 40 normal children (open circles). Bars indicate median values.*

11 urine samples from normal children were of pH ≥6·0. Detectable concentrations of β-2-microglobulin were present only in five of 26 urines from the diabetic and in one of 11 urines from normal children.

There was a highly significant correlation between urinary excretion of α-1-microglobulin and urinary excretion of κ light chains in both children with IDDM (r_s=0·68, p<0·0002) and normal children (r_s=0·78, p<0·0002). Urinary albumin excretion did not correlate significantly with urinary excretion of α-1-microglobulin or κ light chains either in normal or diabetic children.

In children with IDDM the glycosylated haemoglobin correlated with urinary excretion of α-1-microglobulin (r_s=0·34, p<0·0005, fig 4), κ light chains (r_s=0·27, p<0·05, fig 5), and albumin

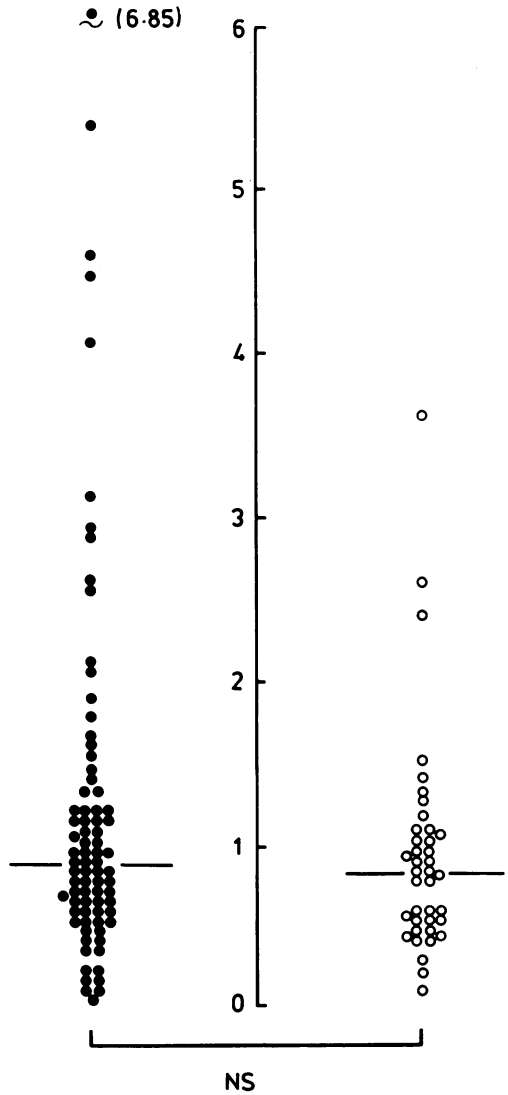

Fig 3 *Urinary albumin excretion (mg/mmol creatinine) in 80 children with insulin-dependent diabetes (closed circles) and 40 normal children (open circles). Bars indicate median values.*

(r_s=0·27, p<0·05). Urinary glucose concentration in children with IDDM correlated with urinary excretion of α-1-microglobulin (r_s=0·52, p<0·0002, fig 6), and κ light chains (r_s=0·51, p<0·0002, fig 7), but not with urinary albumin excretion.

Neither age nor duration of diabetes correlated with urinary excretion of α-1-microglobulin, κ light chains, or albumin in children with IDDM. In normal children there was a trend towards a negative correlation of age with urinary excretion of κ light chains (r_s=−0·34, p=0·06) and age with urinary excretion of α-1-microglobulin (r_s=−0·31, p=0·10). There were no significant differences between boys and girls in urinary excretion of albumin, α-1-microglobulin, or κ light chains either in children with IDDM or in normal children (table).

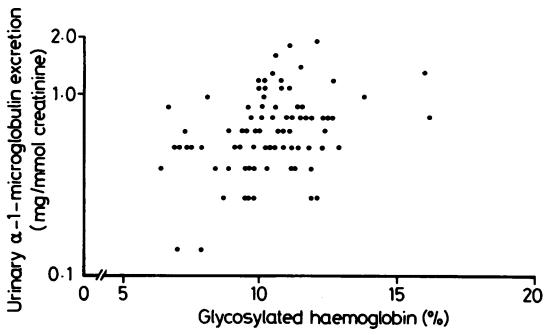

Fig 4 *Scatter diagram of urinary α-1-microglobulin excretion (logarithmically transformed to compensate for positively skewed distribution) and glycosylated haemoglobin in diabetic children.*

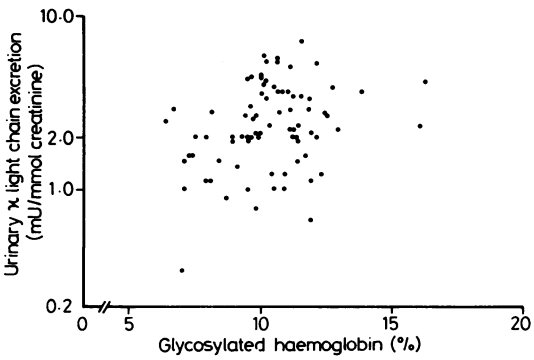

Fig 5 *Scatter diagram of urinary κ light chain excretion (logarithmically transformed to compensate for positively skewed distribution) and glycosylated haemoglobin in diabetic children.*

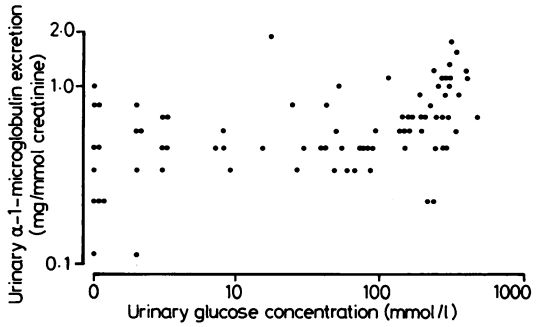

Fig 6 *Scatter diagram of logarithmically transformed data for urinary α-1-microglobulin excretion and urinary glucose concentration in diabetic children.*

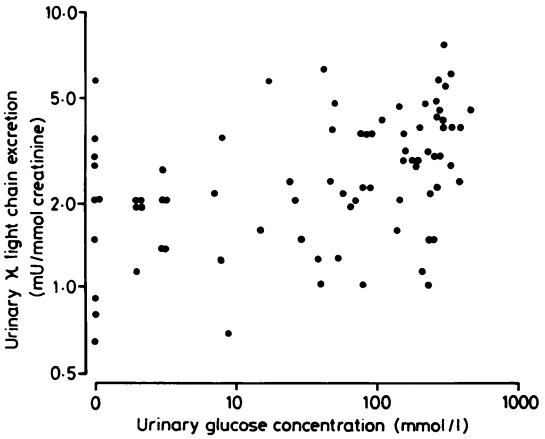

Fig 7 *Scatter diagram of logarithmically transformed data for urinary κ light chain excretion and urinary glucose concentration in diabetic children.*

Discussion

Children with IDDM have a significantly raised urinary excretion of α-1-microglobulin and κ light chains without a significant rise in albumin excretion, which indicates impaired tubular reabsorbtion of low molecular weight proteins. There was a strong correlation between urinary excretion of α-1-microglobulin and κ light chains in both the normal and diabetic children but neither correlated with urinary albumin excretion. This is consistent with the expected renal handling of α-1-microglobulin and κ light chains as proteins that are freely filtered by the glomerulus and greater than 95% reabsorbed and catabolised by the proximal tubule.

Previous studies of proximal renal tubular function in diabetes have used urinary excretion of β-2-

Table *Urinary excretion of albumin, α-1-microglobulin, and κ light chains in children with insulin-dependent diabetes and in normal children*

	Diabetic children			*Normal children*		
	Boys (n=40)	*Girls (n=40)*	*Boys v girls*	*Boys (n=19)*	*Girls (n=21)*	*Boys v girls*
Albumin (mg/mmol creatinine)						
Median	0·81	0·97	NS	0·57	1·00	NS
Range	0·12–6·85	0·06–5·39		0·11–1·40	0·21–3·67	
α-1-microglobulin (mg/mmol creatinine)						
Median	0·60*	0·55*	NS	0·26	0·23	NS
Range	0·23–1·70	0·11–1·92		0·14–0·90	0·11–0·68	
κ light chains (mU/mmol creatinine)						
Median	2·26	2·43*	NS	1·47	1·24	NS
Range	0·68–7·35	0·36–5·54		0·57–4·75	0·57–3·39	

*p=<0.0002 compared with normal children of same sex.
NS=Not significant, two tailed p>0.05.

microglobulin as an index of proximal tubular function. Normal urinary excretion of β-2-microglobulin in adult diabetics has been reported in several studies.[10–12] Parving *et al*,[13] however, found raised urinary excretion of β-2-microglobulin when metabolic control of diabetes was poor and Christiansen *et al* showed a fall in its excretion during control of glycaemia by an intravenous insulin infusion.[14] Ellis *et al* reported a raised excretion of β-2-microglobulin in 67 children with IDDM.[15] Miltenyi *et al* found a raised urinary excretion of β-2-microglobulin in 11 children with diabetic ketoacidosis, but after 10 days its excretion was no longer significantly raised compared with either 13 children with well controlled diabetes or 18 non-diabetic children.[16] Part of the discrepancy between the results of these studies might be due to the properties of β-2-microglobulin. Degradation occurs in vitro at pH <6·0. We confined our radioimmunoassay estimation of β-2-microglobulin, however, to urines of pH ≥6·0 but found measurable quantities of β-2-microglobulin in only five of 26 diabetic samples. This is in keeping with Teppo and Groop who found that adult insulin-dependent diabetics with normal urinary albumin excretion have increased excretion of κ light chains despite normal excretion of β-2-microglobulin.[5] Therefore β-2-microglobulin might be an insensitive indicator of proximal tubular reabsorptive function in diabetes.

There are few low molecular weight proteins other than β-2-microglobulin whose excretion has been studied as an index of tubular function in diabetes. The excretion of enzymes, however, which are present in the renal tubular cells but are not in the glomerular filtrate has been investigated. Excretion of N-acetyl-β-D-glucosaminidase (NAG) a lysosomal enzyme, is increased in IDDM.[17] In children with IDDM urinary leucine aminopeptidase (a brush border enzyme) and urinary NAG activities were higher in diabetic children, even when well controlled, than in non-diabetic children.[16]

The possibility of a relation between poor glycaemic control and impaired tubular reabsorption is raised by the weak but significant correlation of glycosylated haemoglobin and the stronger correlation of urinary glucose, with both urinary excretion of α-1-microglobulin and κ light chains. Such a relation would be consistent with experimental evidence. In the rat kidney increases in urinary flow rate reduce tubular protein reabsorption[18]: in man a similar effect might result from the osmotic diuresis associated with hyperglycaemia. In children with IDDM glycosuria is associated with alterations in the tubular transport of sodium, calcium, and phosphate[19] and might directly alter the transport of other substances. It is clearly possible, however, that the relation between urinary glucose and tubular protein reabsorption is one of effect rather than cause, a defect in tubular reabsorption accounting for part of the glycosuria as well as the loss of low molecular weight proteins in urine.

Pathological changes in the proximal convoluted tubule consisting of glycogen-containing vacuoles (Armanni-Ebstein change) have long been known to occur in diabetes.[20] Impaired tubular reabsorption of low molecular weight proteins in children with IDDM, but without evidence of microalbuminuria, indicates that proximal renal tubular function may

be one of the earliest detectable abnormalities of renal function in IDDM. Its relation to other early functional changes in the diabetic kidney, such as increased glomerular filtration, is unknown, and it will require prospective longitudinal studies for proper evaluation.

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