

Tubular dysfunction in type I diabetes mellitus

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SUMMARY Tubular function was investigated in patients with diabetic ketoacidosis and those with poorly controlled type I diabetes. Urinary excretion of beta₂ microglobulin and that of certain enzymes: γ glutamyltransferase, leucine aminopeptidase, and N-acetyl- β -D-glucosaminidase activities were significantly raised during ketoacidosis in 11 patients compared with healthy controls. In 13 poorly controlled diabetics, tubular electrolyte transport was studied and a significant reduction in tubular phosphate and sodium reabsorption was found. Tubular dysfunction occurring during diabetic ketoacidosis and in poorly controlled diabetics may contribute to the development of diabetic nephropathy.

In an earlier study we found reversible tubular proteinuria in diabetic children during hyperglycaemic ketoacidosis.¹ In the present study further aspects of tubular dysfunction were investigated.

Patients

Urinary excretion of beta₂ microglobulin and of certain enzymes was investigated in three groups of patients:

(i) Eleven diabetics with ketoacidosis, aged mean (SD) 9.6 (3.7) years, who had had diabetes for 1.4 (1.7) years. Six of the patients were newly diagnosed and five had been diabetic for less than five years. At the start of our investigation the blood glucose concentration for the entire group was mean (SD) 22.2 (8.8) mmol/l. Glycosuria of 520 (330) mmol/day was found. Mean blood pH was 7.12 (0.10) with a base excess of -21.8 (5.2) mmol/l.

(ii) Thirteen well controlled diabetic children who had a HbA_{1c} of less than 9.5%. Their age was 10.3 (3.0) years, and the mean duration of diabetes in this group was 2.4 (2.7) years.

(iii) Eighteen healthy children aged 12.0 (3.4) years, who served as controls.

In 13 poorly controlled diabetics renal tubular electrolyte transport was tested during water loading. Their age was 12.7 (2.25) years, and the mean duration of diabetes in this group was 4.3 (3.4) years. These children had a HbA_{1c} of 12.5 (2.7)%. Seven of 13 were just recovering from ketoacidosis. Water loading was also performed in 11 healthy children.

Methods

Urinary beta₂ microglobulin concentrations. These values were determined by ELISA.²

Urinary enzyme excretion. The following three enzymes were measured.

(i) Gamma glutamyltransferase (γ -GT EC. 2.3.2.2.) activity in the urine was determined by the MERCK 14302 test following the method of Szász.³

(ii) The assay of leucine aminopeptidase (LAP EC. 3.4.1.1.) was performed according to the method of Nagel by a Boehringer 204-323 test.⁴

(iii) The activity of N-acetyl- β -D-glucosaminidase (NAG EC. 3.2.1.30.) in the urine was determined by the method of Maruhn.⁵

A 24 hour urine specimen was collected in most cases, but during diabetic ketoacidosis the collection period was four hours. Mean and 2 SDs were calculated on logged data.

Investigations of renal tubular electrolyte transport.

Water loading was performed according to standard methods.⁶ Calculations for proximal and distal electrolyte clearances were carried out according to Haycock *et al.*⁷

Creatinine. Creatinine concentration was determined by enzymatic creatinine test (Boehringer).⁸

Statistical analysis. For statistical evaluation, the paired Student's *t* test was used.

Results

Beta₂ microglobulin excretion. Excretion in 13 well controlled diabetics was similar to that of controls, but it was raised significantly during ketoacidosis compared with values pertaining in well controlled diabetics. Results are shown in Table 1. Excretion 8 to 10 days after ketoacidosis did not differ significantly from either the values of well controlled diabetics or from those of the healthy controls.

Urinary enzyme excretion. Gamma glutamyltransferase activity did not differ significantly in well controlled diabetics and healthy controls, but excretion rose significantly during ketoacidosis. The urinary leucine aminopeptidase and N-acetyl-β-D-glucosaminidase activities in well controlled diabetics were significantly higher than those in healthy controls. In ketoacidosis further increase in the urinary activity of these enzymes occurred. Data are summarised in Table 1. Eight to 10 days later the urinary excretion of all three enzymes decreased, but the difference was not statistically significant.

Renal function and tubular electrolyte handling. There was no statistical difference in endogenous

creatinine clearance during water diuresis in diabetic and control children. Free water clearance and fractional water and sodium excretion were significantly increased in diabetics. Sodium reabsorption was decreased in the proximal tubule, but it was enhanced at the distal part of the nephron: the overall effect was of increased sodium loss. The tubular phosphate reabsorption was significantly lower in diabetic than in the control children. Data are given in Table 2 and calculations in Table 3.

Table 3 Calculations

C_{cr}	$= U_{cr} \times V / P_{cr}$	DD_{Na}	$= P_{Na} \times (C_{Na} + C_{H_2O})$
C_{H_2O}	$= V - C_{osm}$	PR_{Na}	$= F_{Na} - DD_{Na}$
$Fe_{H_2O}^2 \%$	$= V / C_{cr} \times 100$	DR_{Na}	$= DD_{Na} - E_{Na}$
$Fe_{Na} \%$	$= E_{Na} / F_{Na} \times 100$	$TPR \%$	$= 1 - \frac{U_p \times P_{cr}}{P_p \times U_{cr}}$
$FPR_{Na} \%$	$= PR_{Na} / F_{Na} \times 100$		
$FDR_{Na} \%$	$= DR_{Na} / F_{Na} \times 100$		

U=urinary concentration, P=plasma concentration, C=clearance concentration, cr=creatinine, Na=sodium, osm=osmolality, V=urine flow rate, F_{Na}=sodium filtration rate, E_{Na}=sodium excretion rate, DD_{Na}=rate of delivery of sodium to the distal nephron, DR_{Na}=distal tubular sodium reabsorption rate, PR_{Na}=proximal tubular sodium reabsorption rate. The F—prefix indicates 'fractional'—that is the proportion of the filtered load reabsorbed or excreted, TPR=tubular phosphate reabsorption.

Table 1 Urinary β₂ microglobulin (β₂M), gamma glutamyltransferase (γ-GT), leucine aminopeptidase (LAP), N-acetyl-β-D-glucosaminidase (NAG) excretion in the different patient groups (values, mean (range) calculated on logged data)

Groups	β ₂ M (μg/min per m ²)	γ-GT	LAP (μg/mmol creatinine)	NAG
Controls (n=18)	0.08 (0.05-0.12)	2.72 (1.46-5.01)	0.14 (0.11-0.19)	0.26 (0.18-0.39)
Well controlled diabetics (n=13)	0.09 (0.05-0.15)	3.31 (1.62-6.76)	0.19‡ (0.14-0.27)	1.11‡ (0.73-1.50)
Diabetics in coma (n=11)	3.89* (1.70-8.90)	9.68* (4.85-19.3)	0.32‡ (0.21-0.48)	2.57* (1.48-4.47)
Diabetics after coma (n=11)	0.10* (0.05-0.21)	5.75 (2.29-14.40)	0.28 (0.18-0.44)	2.19 (1.23-3.89)

*P<0.001; †P<0.01; and ‡P<0.02.

Table 2 Tubular electrolyte handling in poorly controlled diabetics (values mean (SD))

Group	Creatine clearance (ml/min per 1.73 m ²)	Water clearance	Fractional water excretion (%)	Fractional sodium excretion (%)	Fractional PR _{Na} (%)	Fractional DR _{Na} (%)	Tubular phosphate reabsorption (%)
Diabetics n=13	137.4 (26.6)	14.1 (5.3)	15.7 (4.7)	2.9 (1.2)	86.5 (4.8)	10.5 (4.1)	78.6 (9.0)
Controls n=11	132.7 (35.1)	8.9 (2.6)	9.3 (2.9)	0.8 (0.3)	92.1 (2.6)	7.1 (2.4)	93.7 (3.3)
P	ns	<0.01	<0.001	<0.001	<0.01	<0.05	<0.001

PR_{Na}=proximal tubular sodium reabsorption rate; DR_{Na}=distal tubular sodium reabsorption rate.

Discussion

Tubular proteinuria is a consequence of tubular impairment. In our earlier study we found reversible tubular proteinuria in diabetic ketoacidosis even when this was of recent onset.¹ We therefore investigated other parameters of tubular function in diabetic ketoacidosis.

In coma, raised beta₂ microglobulin excretion was found, but this resolved 8 to 12 days after ketoacidosis. Parving *et al*⁹ also found higher excretion of this in poorly controlled diabetics.

In diabetic ketoacidosis we observed raised γ glutamyltransferase leucine aminopeptidase, and N-acetyl- β -D-glucosaminidase excretion. These high molecular weight enzymes originate from the tubules and are not products of glomerular filtration. Gamma glutamyltransferase is a glycoprotein-type enzyme, localised in the brush border of the proximal tubules and in the loop of Henle. Leucine aminopeptidase is a proteolytic enzyme, raised urinary activity was observed in tubular impairment.¹⁰ N-acetyl- β -D-glucosaminidase is detectable in the lysosomal fraction of the proximal tubular cells, and increased excretion in diabetics has been reported by several authors.¹¹ Increased excretion of these urinary enzymes during diabetic ketoacidosis indicates tubular dysfunction.

Changes in the electrolyte transport of the renal tubular system may occur from the onset of diabetes. In poorly controlled diabetics we observed increased urinary phosphate excretion, confirming earlier findings.¹² In juvenile diabetics, significantly increased urinary sodium excretion was found in relation to body surface area. When related to filtered load of sodium, however, excretion has been found higher only in newly diagnosed patients. Ditzel *et al*¹² showed increased sodium reabsorption closely related to the increased glomerular filtration rate in well controlled diabetics. In our study, a reduced proximal tubular sodium reabsorption rate was observed in children with poor glycaemic control not compensated for by the enhanced distal sodium reabsorption.

In conclusion, a reversible tubular dysfunction may be found in diabetic ketoacidosis as well as in poorly controlled diabetes. As early as 1939, McCance and Widdowson¹³ had suggested that tubular injury occurred in coma.

In diabetes, there are some pathological changes in the tubules. Five to six days after the onset of hyperglycaemia, Armani-Ebstein cells occur in

the distal part of the proximal convolute.¹⁴ In streptozotocin-induced diabetes hypertrophy of the kidneys may be observed. Both the glomerular and tubular masses are proportionately enlarged.¹⁵

The relation between changes in the tubular system developing in the early phase of diabetes and the pathological changes occurring later in the glomeruli, has not, as yet, been elucidated.¹⁶

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