Elevation of Urinary Trehalase and Maltase Activities with Maturity-Onset Diabetes Mellitus

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(Received February 18, 1987)

Summary In the case of maturity-onset diabetes mellitus, urinary trehalase and urinary maltase activities were significantly elevated as compared with those of healthy individuals. The urinary trehalase activity in non glucosuric and non proteinuric patients was higher than that in healthy individuals, and the trehalase activity in those diabetics with proteinuria was significantly higher than in those without it ($p < 0.05$). Both urinary trehalase and maltase activities were elevated with increased concentration of urinary β_2 -microglobulin in this diabetics. Furthermore, urinary trehalase was elevated in disease of short duration (1–4 years), whereas in that of relatively long duration (more than 13 years) the activity was rather low. These results suggest that renal brush borders are damaged in the early stages of the disease and that urinary trehalase is a good indicator of renal tubular damage.

Key Words: urinary trehalase, urinary maltase diabetes mellitus, renal tubules, renal brush borders

With any disease it is important to detect the disorder as early as possible. In diabetes mellitus, proteinuria, an important parameter used to measure renal function, is also a clinical sign of diabetic nephropathy. However, the diabetic kidney is known to undergo many functional and morphological changes in the early stages of disease prior to the appearance of clinical nephropathy [1, 2]. Parving *et al.* [3], focusing on tubular proteinuria [4] in diabetes mellitus, measured the renal excretion of β_2 -microglobulin. In healthy persons, the small molecular weight protein β_2 -microglobulin is freely filtered through the glomerulus and

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completely reabsorbed at a tubular site [5].

It is well known that in HgCl_2 -induced nephrotoxicity damage to tubular brush borders occurs [6]. In this case the brush border enzymes maltase, LAP and γ -GTP are excreted in the urine [7–9]. Recently we reported that urinary trehalase activity is elevated in HgCl_2 -induced nephrotoxicity [10], and in chronic toxicity due to prolonged exposure to low levels of cadmium [11]. The enzyme trehalase [E.C. 3.2.1.28] splits trehalose (α -D-glucopyranosyl- α -D-glucopyranoside) to two glucose moieties. It has been proved by biochemical [12–14] and immunohistochemical procedures [15] that renal trehalase is localized in the brush borders of proximal tubules. Of the brush border enzymes, maltase, leucine aminopeptidase (LAP), and γ -glutamyl transpeptidase (γ -GTP) are solubilized by papain, but not trehalase [14]. Thus, it is suggested that trehalase has a different localization in the membranes from other brush border enzymes such as maltase, LAP, and γ -GTP [16]. Urinary trehalase shows the same antigenicity as renal trehalase. It has been proved biochemically and immunohistochemically that the trehalase content in kidney tubules is significantly decreased in acute tubular necrosis [10] and in the case of chronic cadmium poisoning [11]. More recently, we reported that inhabitants of cadmium-polluted areas [17] and patients with Itai-itai disease [18] show renal tubular damage. In fact, the renal tubular damage seen in Itai-itai disease is extremely severe [19]. Higuchi *et al.* [20] reported that normal pregnant women showed an elevation of urinary trehalase activity and that after delivery the activity was quickly restored to the level of healthy individuals, suggesting repair of the renal tubular damage.

This paper deals with the elevation of urinary trehalase and urinary maltase activities in patients with maturity-onset diabetes, and with the relationship between urinary enzymes and such factors as urinary sugars, urinary protein, β_2 -microglobulin, and duration of disease.

EXPERIMENTAL PROCEDURES

Urine collection. (a) Pathological subjects: Twenty-four-hours or two-hours morning urine (7:00–9:00) specimens were collected from patients with diabetes mellitus (maturity onset type), who were admitted to Nagoya University Hospital. The 24-h urine specimens were collected into glass bottles without preservative, and the urine was kept at 4°C during the collection period. (b) Normal subjects: We studied 200 clinically healthy individuals for normal specimens of the 2-h morning urine. Only experimental data from subjects who had normal results of urinalysis were selected for the estimation of normal limits. Thus, 32 subjects were excluded for the reason just stated above. The remaining sample consisted of 87 men (mean age, 48 years; range, 29–68 years) and 81 women (mean age, 45 years; range, 25–68 years).

Pretreatment of urine. Five milliliter of the urine was dialyzed thoroughly against distilled water as described previously [10]. The dialyzed urine was then

concentrated 10-fold (to 0.5 ml) with a Minicon B-15 (Amicon Co.), and this concentrate was used as the enzyme solution.

Assay for enzyme activity. Trehalase activity was determined by the method described previously [21, 22]. The reaction mixture contained enzyme solution, 40 μmol of trehalose, and 20 μmol of phosphate buffer (pH 6.2), in a total volume of 0.25 ml. After incubation at 37°C for 60 min, the reaction was stopped by addition of the Tris-glucose oxidase-peroxidase reagent of Dahlqvist [23] which was used for the determination of the liberated glucose. Maltase activity was assayed similarly with maltose as the substrate. The activity of trehalase and maltase was expressed as μmol glucose formed per h per g creatinine.

Analytical procedures. Sugar and protein contents in the urine were determined by automated methods. Urinary creatinine content was determined by the Jaffe method. Urinary β_2 -microglobulin was assayed with an enzyme immunoassay KIT (FUJIREBIO Co., Tokyo) based on an enzyme-immunoassay method [24]. There were no urine specimens below pH 5.5 in which β_2 -microglobulin was measured.

Statistics. Differences between means were tested by Student's *t*-test. Relationships between variables were tested with linear regression lines and correlation coefficients.

RESULTS

Trehalase is quite stable in urine, and no significant loss of activity was observed during storage at 4°C or at -80°C for at least 2 weeks. Satisfactory reproducibility was obtained with sample from both healthy and pathological individuals.

As can be seen in Fig. 1, trehalase activity in the 24-h urine showed a good correlation with that in the 2-h morning urine. Similar results were obtained in the activity of urinary maltase (data not shown). Thus, we employed the 2-h morning urine specimens in the following experiments.

As shown in Table 1, both urinary trehalase and maltase activities were significantly higher in diabetes mellitus patients than in healthy controls ($p < 0.01$). No significant difference, however, was observed in urinary trehalase activity between male and female patients with diabetes mellitus: male ($n=36$), 14.3 ± 1.35 ; female ($n=43$), 15.2 ± 1.42 . Nor were there any age-related differences (data not shown). Urinary maltase activity in the diabetics also was not sex- or age-related (data not shown).

As shown in Fig. 2, trehalase activity in the diabetic group without glucosuria ($n=20$) and in that with glucosuria ($n=38$) was 11.3 ± 2.41 and 19.4 ± 2.94 , respectively, but the difference was not significant. However, both groups were observed to have significantly higher trehalase activity than that of healthy individuals ($p < 0.01$). On the other hand, trehalase activity in the diabetics without proteinuria ($n=27$) and in those with proteinuria ($n=31$) was 11.9 ± 2.17 and $21.3 \pm$

Fig. 1. Correlation between trehalase activity in 24-h urine and that in 2-h morning urine. The 2-h morning urine was collected from 7 a.m. to 9 a.m. Urine specimens were collected from 40 patients (mean age, 52 years; range, 30–78 years).

Table 1. Urinary trehalase and maltase activities in diabetes mellitus.

	Trehalase	Maltase
Control ($n=168$)	2.89 ± 0.25	163 ± 11.0
Diabetes mellitus ($n=82$)	$11.14 \pm 1.19^*$	$269 \pm 20.7^*$

*Significant difference, $p < 0.01$.

3.29, respectively, and this difference was significant ($p < 0.05$). The trehalase activity in urine of diabetics with neither glucosuria nor proteinuria was significantly higher than that in urine specimens from healthy individuals ($p < 0.01$).

As shown in Fig. 3, urinary trehalase activity in the diabetics showing more than 200 $\mu\text{g/liter}$ of β_2 -microglobulin was significantly higher than that in the healthy group. Significant difference was observed in the group with a lower level of β_2 -microglobulin (0–200 $\mu\text{g/liter}$). In the case of urinary maltase (Fig. 4), the activity in all three groups of patients (0–200, 200–1,000, and more than 1,000 $\mu\text{g/liter}$ of β_2 -microglobulin) was also significantly higher than that in the healthy group.

As shown in Fig. 5, the plotting of urinary trehalase activity against the duration of disease yielded a parabolic curve. The activity in the 5–8 year group was the highest seen in any other group. No significant difference was observed between normals and patients having the disease for less than one year. Those patients with the disease of long duration (more than 13 years) showed relatively low urinary

Fig. 2. Urinary trehalase activity of diabetics with glucosuria (A) or with proteinuria (B). Urine specimens were collected from 58 patients (mean age, 49 years; range 35–71 years). Significant difference: * $p < 0.05$.

Fig. 3. Correlation between urinary trehalase activity and urinary β_2 -microglobulin. Urine specimens were collected from 56 patients (mean age, 58 years; range 33–78 years). Significant difference: * $p < 0.05$; ** $p < 0.01$.

Fig. 4. Relationship between urinary maltase activity and urinary β_2 -microglobulin. Urine specimens were collected from 66 patients (mean age, 59 years; range 30–77 years). Significant difference: * $p < 0.05$; ** $p < 0.01$.

Fig. 5. Correlation between urinary trehalase activity and duration of diabetes mellitus. Urine specimens were collected from 81 patients (mean age, 57 years; range 33–76 years). Significant difference: * $p < 0.05$; ** $p < 0.01$.

Fig. 6. Relationship between urinary maltase activity and duration of diabetes mellitus. Number of cases; 87 (mean age, 56 years; range 30–78 years). Significant difference: * $p < 0.05$; ** $p < 0.01$.

trehalase activity, but it was still significantly higher than that in the healthy individuals. In the case of urinary maltase activity (Fig. 6), the activity in diabetics with a duration of less than one year was not observed to be significantly different from the level in healthy individuals. Patients with a longer history of the disease showed significantly high urinary maltase activity as compared with the healthy normals.

DISCUSSION

Proteinuria is one clinical sign of nephropathy. Thus, it is noteworthy that urinary trehalase activity was elevated in the case of diabetics displaying neither proteinuria nor glucosuria (Fig. 2), even though the activity in those without proteinuria was lower ($p < 0.05$) than that in those with it. Parving *et al.* [3] reported that urinary β_2 -microglobulin was elevated in patients with diabetes mellitus. As can be seen in Fig. 3, urinary trehalase activity was high at stages of the disease during which β_2 -microglobulin was still at a low level (0–200 $\mu\text{g/liter}$). Generally, it is thought that functional changes can be demonstrated in the disease after the development of morphological changes. Excretion of β_2 -microglobulin is caused by failure of the tubular reabsorption mechanism [5], a functional failure. Renal trehalase is localized in the tubular brush borders [12–15], and urinary trehalase has the same antigenicity as the renal one [10, 11]. Thus, the excretion of urinary trehalase is probably caused by damage to tubular brush borders, which appears as morphological changes [10, 11, 17, 18, 25]. Elevation of urinary trehalase was observed in patients suffering from Itai-itai disease and in inhabitants of a cadmium-polluted area (Jinzu River basin in Japan) [17, 18]. Furthermore, in the

case of normal pregnancy, urinary trehalase activity was elevated significantly at the first trimester, and elevated further at the third trimester [20]. However, the elevated activity seen during pregnancy was completely restored to the normal level by one month after delivery [20]. From these results, it is inferred that the elevation of urinary trehalase seen in diabetes mellitus is caused by damage to renal tubules.

The urinary trehalase activity in patients with short duration of disease was higher than that in diabetics with long-term disease (Fig. 5). Patients with Itai-itai disease, who show severe tubular dysfunction, have low urinary trehalase activity [18]. Therefore, low urinary trehalase activity in disease of long duration (more than 13 years) might be due to the severe damage to tubular brush borders. That is, a gradual loss of trehalase from the tubules could be occurring during the long duration of disease.

Every brush border enzyme examined showed similar behavior in urine (Fukui *et al.*, personal communication). After centrifugation of the urine, much of the enzyme activities (about 70%) were recovered in the particulate fraction, indicating urinary excretion of brush borders. Brush border enzymes except trehalase have a wide distribution in the organ and broad substrate specificity [26–29]. Generally, it is thought that an indicator (a marker enzyme) should have a specific localization and a narrow substrate specificity. Trehalase has a specific localization and a extremely narrow substrate specificity [12–15]. Therefore we propose urinary trehalase to be a good indicator of renal tubular damage.

This work was partially supported by the Ishida Foundation. The authors express their thanks to the members of the expert clinical team in the 3rd Department of Internal Medicine, Nagoya University School of Medicine, for their helpful discussion. The excellent technical assistance of Mr. Takashi Sugita is gratefully acknowledged.

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