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A study on the estimation of sulfur-containing amino acid metabolism by the determination of urinary sulfate and taurine

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Summary. Sulfate and taurine are major end products of sulfur-containing amino acid metabolism in mammals including humans, and they are excreted in urine. Average excretions (μmol/mg of creatinine) in the morning urine of 58 female college students were: total (free plus ester) sulfate (a), 12.53 ± 3.85; free sulfate, 11.57 ± 3.69; taurine, 0.78 ± 0.53. Ratio of total sulfate and taurine was 10 : 0.6. Regression lines obtained by plotting total sulfate, free sulfate, or total sulfate plus taurine against urea have shown that the former excretions are significantly correlated with urea excretion. Excretion of total sulfate at zero point of urea excretion (b) was 5.30, which corresponded to 42.3% of average excretion (12.53) and was assumed to be derived from dietary sulfate. The difference 7.23 (a − b) seemed to be derived from sulfur-containing amino acids. It was pointed out that the difference of average sulfate excretion and sulfate excretion at zero urea excretion, namely a − b, was appropriate for the metabolic index of sulfur-containing amino acids of the group examined. As free sulfate constituted 92.3% of total sulfate, excretion of ester sulfate was at a constant level, and that of taurine was not significantly correlated with urea excretion, the value of free sulfate corresponding to the value a − b of total sulfate mentioned above seemed to be a reliable and convenient index in the assessment of sulfur-containing amino acid metabolism.

Keywords: Amino acids – Sulfur-containing amino acids – Sulfate – Taurine – Metabolic index

Introduction

Inorganic sulfate and taurine are major end products of sulfur-containing amino acid metabolism in mammals including humans. Inorganic sulfate is important as a raw material for the synthesis of tissue proteoglycan and for the detoxication reaction by sulfate conjugation. Taurine is contained in muscle and brain tissues at high concentrations and it is also utilized for the synthesis of taurine conjugates of bile acids. Sulfur-containing amino acids, methionine and cysteine, are taken up by mammals as constituents of proteins in foods. Through the transsulfuration pathway the sulfur atom of methionine is transferred to cysteine, which is further metabolized (Griffith, 1987) through the oxidation (Yamaguchi et al., 1973) and 3-mercaptopyruvate (or transamination) (Ubuka et al., 1990, 1992) pathways, or by cystathionase reaction (Stipanuk et al., 1982; Drake et al., 1987). A schematic summary of these pathways is shown in Fig. 1. The main final metabolites of cysteine-sulfur in these pathways, inorganic sulfate and taurine, are excreted in urine. The sulfate in urine is present mainly as free (inorganic) and partly as bound (ester) sulfate. The sum of free and ester sulfate (total sulfate) constitutes approximately 90% of sulfur compounds in the human urine, and taurine content is less than 10% (Mårtensson, 1982).

Another source of inorganic sulfate and taurine in humans is diet. Inorganic sulfate is contained in foods and drinking water and can be absorbed from the intestine (Morris and Murer, 2001). Foods high in sulfate contents include commercial breads, dried fruits and vegetables, nuts, sausage, shellfish, beer and wine (Florin et al., 1993). Florin et al. (1991) estimated that dietary sulfate accounted for up to 42% of available sulfate in humans. It was reported that tracer dose of sulfate is well absorbed and 80% of an oral dose was recovered in the urine over 24 h (Bauer, 1976) and a dose of 0.75 mmol/kg sodium sulfate administered in 4 hourly divided doses had a average bioavailability of 43.5% (Cocchetto and Levy, 1981). Magnesium sulfate is also absorbed, but less completely than sodium sulfate (Morris and Levy, 1983).

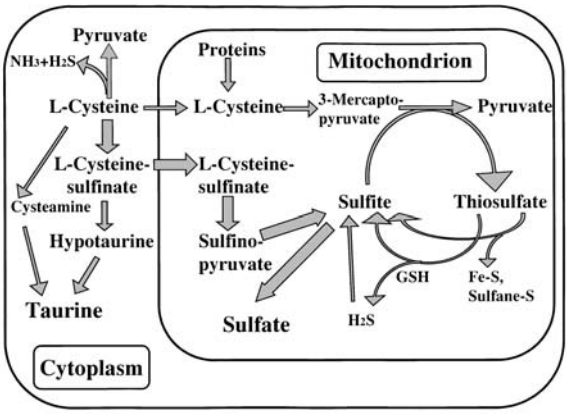

Fig. 1. Schematic summary of cysteine metabolism leading to sulfate and taurine formation through oxidation and 3-mercaptopyruvate pathways and cystathionase reaction in mammalian cells

Table 1. Urinary excretions of sulfate and taurine in human urine (mean ± SD)

	a	%	b	a − b
Sulfate (μmol/mg Cr)				
Total	12.53 ± 3.85	(100.0)	5.30	7.23
Free	11.57 ± 3.69	92.3	4.56	7.01
Ester	0.96 ± 0.94	7.7	0.74	0.22
Taurine (μmol/mg Cr)	0.78 ± 0.53	6.2	–	–
Creatinine (Cr, mg/ml)	2.01 ± 0.78	–	–	–
Urea (μmol/mg Cr)	187.71 ± 66.13	–	–	–

a: Mean excretions in morning urine of 58 female college students. b: Mean values at zero point of urea excretion, which are assumed to be derived from sulfate in the diet (see text). a − b: Mean values of sulfate excretion, which are assumed to be derived from metabolism of sulfur-containing amino acids (see text)

Taurine is contained in foods such as shellfish, meat of turkey and chicken, and fish (Laidlaw et al., 1990). It has been reported that taurine corresponding to 40 to 70% of orally administered dose was excreted in the urine (Evered et al., 1969; Sturman et al., 1975; Wang and Zhao, 1998).

In our previous studies (Yukihiro et al., 1998; Tomozawa et al., 1998), we investigated excretions of sulfate and taurine in rats fed synthetic diets containing various levels of casein and devoid of sulfate and taurine. In these studies it was shown that rats fed 25 and 40%-casein diets exhibited similar growth curves, and these rats excreted sulfate and taurine at ratios of 10 to 2–3.

In the present study, we dtermined sulfate and taurine in the urine of female college students and discussed relation to the metabolism of sulfur-containing amino acids in this group and significance of determining urinary sulfate and taurine in human nutrition.

Materials and methods

Morning urines of 58 female college students aged 20.2 ± 1.2 years were used in this study. Urine samples were collected at 9:00 a.m. after 12 h of starvation following taking usual meals and frozen at −20°C until analyses. After thawing, urine samples were centrifuged at 1,200 × g for 15 min at 4°C and resulting clear supernatants were used for analyses of sulfate, taurine, urea and creatinine.

Free sulfate was determined with ion chromatography according to previous report (Ubuka et al., 2001) with some modification using a Hitachi LaChrom system (Hitachi Company, Tokyo) consisted of an isocratic pump L-7110, a conductivity detector L-7470, column oven CA-202 (Tokyo Rika Company, Tokyo) and data processor D-2500. The column used was a Shodex IC I-524A (4.6 mm diameter × 100 mm length) with a guard column Shodex IC IA-G (4.6 mm diameter × 10 mm length) of Showa Denko Company (Tokyo). One hundred μl of 1 : 100 diluted urine was applied to the system and chromatography was performed with 2.5 mM sodium phthalate adjusted to pH 4.20 with Tris. Total sulfate was determined as above after hydrolysis with 0.2 M hydrochloric acid at 80°C for 2 h. Sulfate contents in urine samples were obtained by comparison with the peak area of a standard potassium sulfate solution.

Taurine was determined according to our previous report (Futani et al., 1994) with some modification by reversed-phase high-performance liquid chromatography using Hitachi LaChrom HPLC (Hitachi Company, Tokyo) after dabsylation of urine samples. The system was consisted of a gradient pump L-7100, a UV-visible detector L-7420, column oven 505 (FROM Company, Tokyo) and data processor D-7500. The column used was TSKgel ODS-80Ts (4.6 mm diameter × 150 mm length) with a guard column TSKguardgel ODS-80Ts (3.2 mm diameter × 15 mm length) of Toso Company (Tokyo). A typical chromatogram of authentic dabsyl-amino acids is shown in Fig. 2A and that of urine in Fig. 2B. L-Homoserine was used as an internal standard in the present study. Taurine was calculated from the regression line obtained by plotting the molar ratio (y) against the peak area ratio (x) of taurine and L-homoserine. The regression line was $y = 0.551x + 0.002$ and correlation coefficient was 1.000 ($p < 0.001$).

Urea was determined by indophenol formation after urease treatment, and creatinine was determined with picric acid.

Results and discussion

Table 1 shows average excretions of sulfate, taurine, urea and creatinine. The average values (mean ± SD, μmol/mg of creatinine) were: total sulfate, 12.53 ± 3.85; free sulfate, 11.57 ± 3.69; ester sulfate 0.96 ± 0.94, and taurine 0.78 ± 0.53. Free sulfate constituted 92.3% of total sulfate. Taurine excretion corresponded to 6.2% of total sulfate excretion. Thus, the ratio of total sulfate and taurine was 10 : 0.6. The ratio of taurine to sulfate in the human urine was much smaller than that in the rat urine (Yukihiro et al., 1998; Tomozawa et al., 1998), indicating the production of

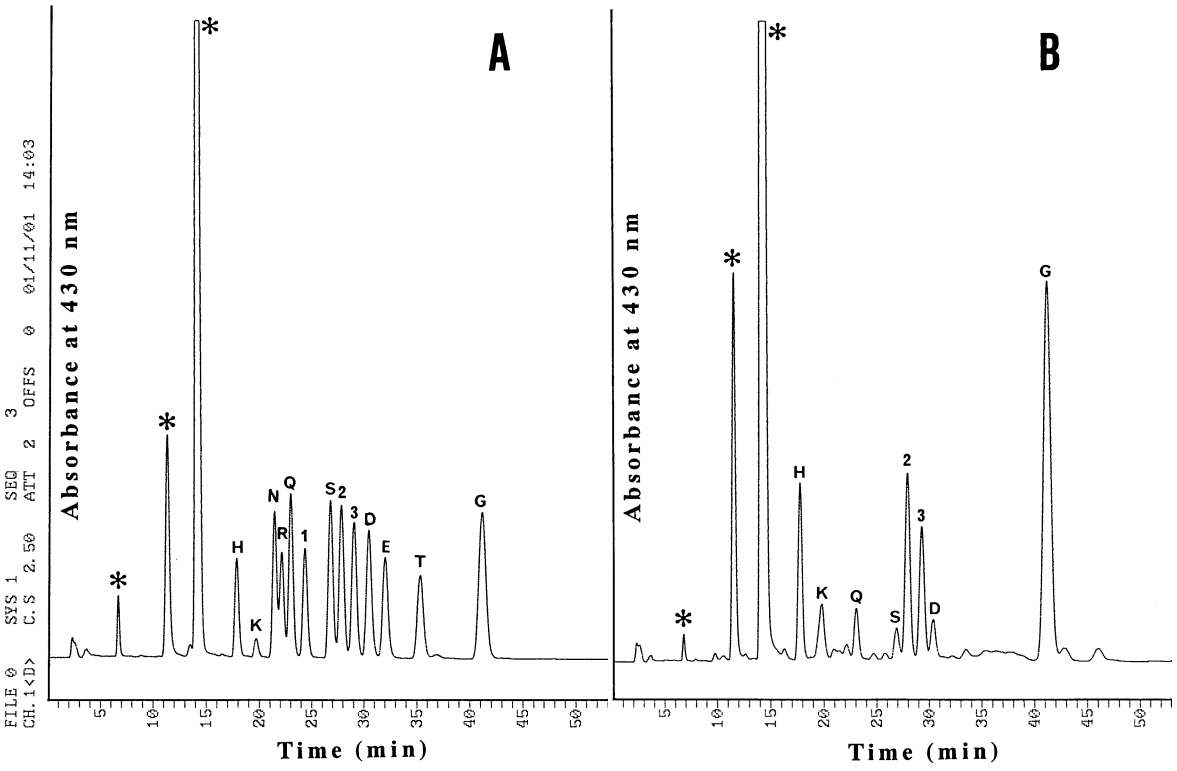

Fig. 2. Reversed-phase high performance liquid chromatography of dabsylated authentic (**A**) and urinary (**B**) amino acids. *1*, hypotaurine; *2*, L-homoserine added as an internal standard; *3*, taurine; *, reagent peak

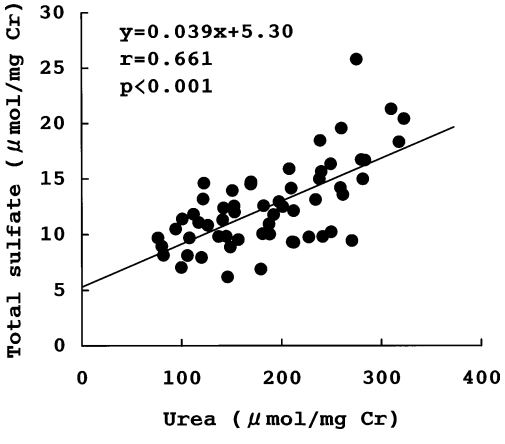

Fig. 3. Relation between urinary total (free + ester) sulfate and urea. *Cr*, creatinine

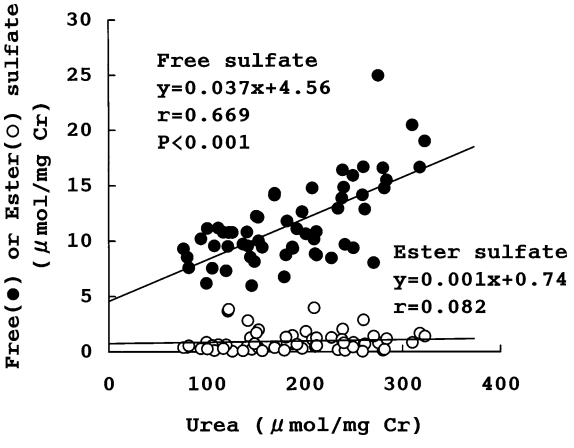

Fig. 4. Relation between urinary free or ester sulfate and urea. *Cr*, creatinine

sulfate is dominant than that of taurine in humans compared to rats.

Figure 3 shows the relation of total sulfate and urea excretions (μmol/mg of creatinine), in which sulfate excretion was plotted against urea excretion in each subject. The regression line was y = 0.039x + 5.30 and correlation coefficient was 0.661. The relation between excretion of free sulfate, ester sulfate, taurine, or total sulfate plus taurine, respectively, and that of urea is shown in Figs. 4, 5 and 6. The regression line of the relation between excretions of free sulfate and urea was y = 0.037x + 4.56 and that between excretions of total sulfate plus taurine and urea was y = 0.040x + 5.75. The correlation coefficients were 0.669 and 0.675

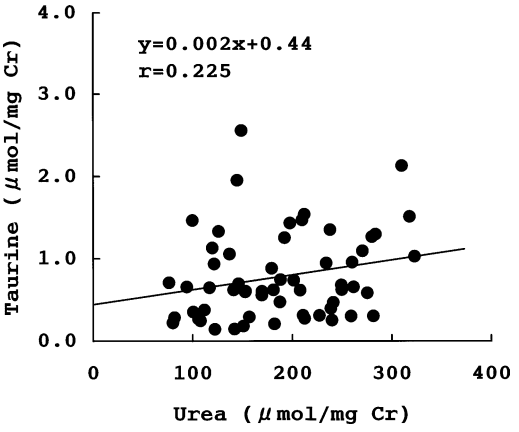

Fig. 5. Relation between urinary taurine and urea. *Cr*, creatinine

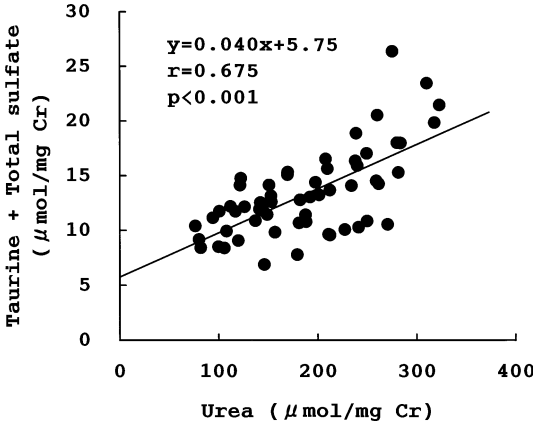

Fig. 6. Relation between urinary taurine plus total sulfate and urea. *Cr*, creatinine

($p < 0.001$), respectively. Thus, excretions of free sulfate, total sulfate and total sulfate plus taurine were significantly correlated with urea excretion. It has been shown that total sulfate or the sum of total sulfate plus taurine in the urine constitutes over 90% of sulfur excretion in rats (Yukihiro et al., 1998; Tomozawa et al., 1998) and humans (Mårtensson, 1982; Yuasa et al., 1990). Therefore, total sulfate or the sum of total sulfate plus taurine can be an index of sulfur-containing amino acid metabolism. However, as shown in Fig. 5, taurine excretion did not correlate significantly with urea excretion and exhibited wide distribution with a 68% standard deviation. This seems to indicate that taurine excretion is influenced by taurine content in the diet as reported (Wang and Zhao, 1998). Average excretion of free sulfate constituted 92.3% of total sulfate excretion, and as shown in Fig. 4, excretion of ester sulfate is independent of urea excretion, showing sulfate conjugation occurs at a constant level in normal subjects. These results seem to show that free sulfate in urine is a reliable measure of sulfur-containing amino acid metabolism.

In Figs. 3 and 4, the intercepts of the ordinate of total and free sulfate excretions were 5.30 and 4.56, respectively. These values, also shown in column b in Table 1, are the average excretions of total or free sulfate in the present group studied at the point where urea excretion was zero. We considered the significance of these values. As urea excretion reflects amino acid metabolism, it seems reasonable to assume that the sulfate excretion at null urea excretion did not derive from the metabolism of sulfur-containing amino acids, but derived from sulfate in the diet, or sulfur compounds which was metabolized to sulfate. As mentioned above, inorganic sulfate is contained in foods such as commercial bread, dried fruits and vegetables, nuts, beer and wine (Florin et al., 1993). Connective tissue contained in meats contains proteoglycans, which contain sulfated mucopolysaccharides. Inorganic sulfate taken can be easily absorbed from intestine (Morris and Murer, 2001). It was reported that net absorption of dietary sulfate exceeded 16 mmol/day in normal subjects (Florin et al., 1991), and 97% of dietary sulfate is excreted in urine. The values, 5.30 and 4.56 μmol per mg of creatinine in the present study correspond 42.3 and 39.4%, respectively of total and free sulfate excretions. These results are in accordance with the study of Florin et al. (1991), which reported that dietary sulfate accounted for up to 42% of urinary sulfate in humans and rest is formed from sulfur-containing amino acids. These results also suggest that contribution to urinary sulfate of sulfur compounds other than dietary sulfate and sulfur-containing amino acids is small.

The values, 7.23 and 7.01, in column a − b of Table 1 show the difference between the average value of excretion and that at the point of urea excretion was zero. These values seem to express the extent of average metabolism of sulfur-containing amino acids in this group, in accordance with the assumption of Florin et al. (1991) as mentioned above. Thus, these values seem to be more appropriate than values of average total urinary excretions (in the present study, 12.53 and 11.57) for an index of sulfur-containing amino acid metabolism. As mentioned above, free sulfate level may be a reliable measure, and the determination of free sulfate can be done by a simple turbidimetric method. Therefore, the use of value a − b of

free sulfate (7.01 in this study) may be a reliable and convenient index in the assessment of sulfur-containing amino acid metabolism of the group examined. The present study was undertaken in human subjects who had taken uncontrolled diets. Further study using controlled diets is needed.

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