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Original Article

Urinary Excretion of Sulfate after Protein Intake

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Urinary free sulfate was measured after protein intake in order to examine the possibility of biological monitoring using ion chromatography. Urinary free sulfate excretions reached their maximum values in 6 hours after administration in three levels of beef doses. Free sulfate excretions in the urine samples for 16-hour periods and for the first 6 hours after administration were related to the doses beef administered. Free sulfate excretion, adjusted using the sulfate-creatinine ratio, in the urine 6 hours after the administration was related to the dose of beef administered. These experimental results showed that the amount of protein intake could be estimated by urinary free sulfate.

Key Words: Protein intake, Urinary sulfate, Biological monitoring, Ion chromatography

Introduction

Sulfate is a major metabolite of L-cysteine in mammals [1, 2] and plays various important roles such as a constituent of proteoglycans [3] and as a detoxicating agent [4]. Taurine is another major metabolite of L-cysteine in mammals [5] and is involved in the production of taurobile acids [6] and membrane protection [7].

Mammals take up sulfur mainly as methionion and cysteine in proteins [1]. After digestion and absorption, these sulfur-containing amino acids enter the amino acid pool and are metabolized.

Methionine is converted to cysteine by the transsulfuration reaction, and the sulfur of cysteine is ultimately oxidized to sulfate and taurine [1, 2], which are utilized in the body and finally excreted in the urine [6, 8]. The sum of these metabolites constitutes more than 90% of total urinary sulfur when cysteine is administered to mammals [9, 10].

Medes reported that almost 100% of sulfur from ingested L-cysteine and L-methionine was excreted as sulfate in the urine of human subjects [9]. In rats, 95% of sulfur from intraperitoneally injected L-cysteine was excreted as free sulfate and taurine [10].

The present study was undertaken to examine the possibility that the amount of protein intake can be estimated by determining the urinary free sulfate using ion chromatography.

Materials and Methods

Materials

Australian beef purchased in the supermarket was used. All reagents used in this study were of analytical grade and obtained from Wako Pure Chemical Ind., Osaka, Japan.

Administration of beef

Beef was administered orally to a healthy male adult (48 year-old) in doses of 100 g, 200 g and 300 g separately. The urine sample from 2 hours before the administration of beef was discarded. The urine after administration of beef was collected at 2-hour intervals. Food intake was restricted during this experiment and water ad libitum.

Determination of urinary sulfate

Sulfate is excreted in the urine as free sulfate (inorganic sulfate) and bound sulfate (ester sulfate). In the

present study, total sulfate (free + ester) was determined by ion chromatography after hydrolysis of ester sulfate as follows: Urine (one ml) was heated at 80 °C with one ml of 0.4 M hydrochloric acid for 2 hours. The hydrolyzed urine was diluted 100 times with water and 20 μl of the diluted urine was subjected to ion chromatography. The ion chromatography system used in this study consisted of a Dionex 2000 i/SP ion chromatograph, a Dionex HPIC-AS4A column (4 × 250 mm) with a IonPac AG4A guard column (4 × 50 mm) and a Chromatocorder 12 integrator. The mobile phase was composed of 1.8 mM sodium carbonate, 1.7 mM sodium hydrogencarbonate at a flow rate of 1.0 ml per min at 30 °C. Sulfate content was calculated from the standard curve prepared with standard potassium sulfate solutions.

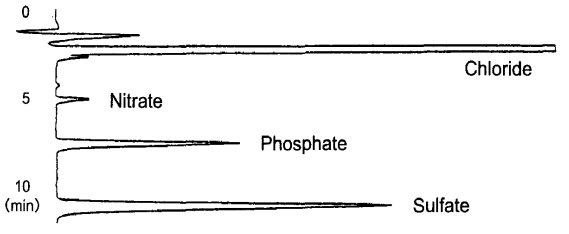

Fig. 1 A typical ion chromatogram of urine diluted 100 times with water after administration of beef.

Determination of urinary creatinine [11]

Urine was diluted 50 times with water and 20 μl of the diluted urine was subjected to high-performance liquid chromatography. Separation of urinary creatinine was achieved with a Shodex DS-4 liquid chromatography apparatus equipped with a Rheodyne Model 7125 injector. Column effluents were monitored at 265 nm with a Shodex UV-41 variable-wavelength detector. A Shodex RSpak DE-413 column (6 × 150 mm) was used. The mobile phase was composed of 8 mM phosphate buffer, pH 6.8, containing 3 mM tetra-n-butylammonium bromide. The flow rate was 0.8 ml/min. Creatinine contents were calculated with SIC480 datastation

Results and Discussion

Fig. 1 shows a typical ion chromatogram of urine after administration of beef. Sulfate was eluted at 11.3 min and was completely separated from other peaks. This ion chromatography was a very useful method for the analysis of urinary sulfate. The detection limit of sulfate was 0.24 μg/ml.

Fig. 2 shows the time-dependent curves of urinary free sulfate and total sulfate (free + ester) excretions after administration of 200 g of beef. The urinary excretion of free sulfate in total sulfate ranged from 87.6% to 96.6% and the average was 91.6 ± 3.1 (average ± SD)%. Free sulfate was the major compound excreted in urine in

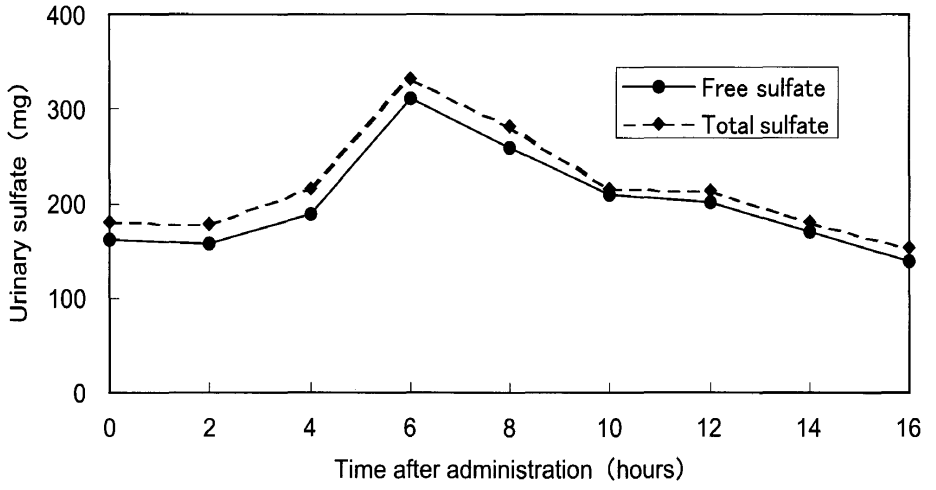

Fig. 2 The time-dependent curves of urinary total sulfate (free + ester) and free sulfate excretion after administration of beef (200 g). Total sulfate was determined after hydrolysis of ester sulfate.

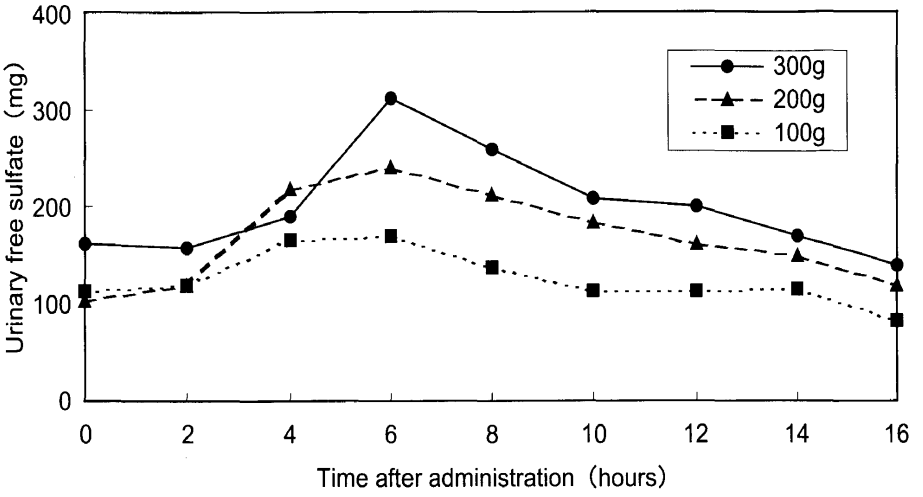

Fig. 3 The time-dependent curves of urinary free sulfate excretion after administration of beef (100 g, 200 g and 300 g).

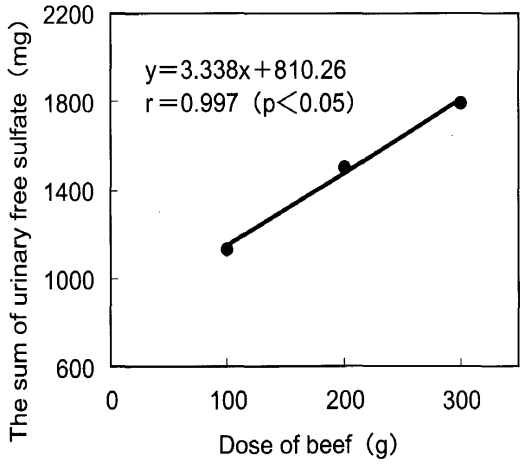

Fig. 4 The correlation between the doses of beef administered and the sum of urinary free sulfate for 16-hour periods.

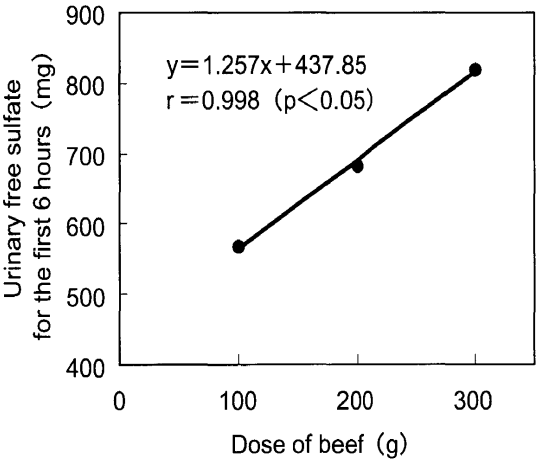

Fig. 5 The correlation between the doses of beef administered and the sum of urinary free sulfate excretion for the first 6 hours.

comparison with ester sulfate. Sulfate and taurine are two major end products of sulfur metabolism in mammals [1, 2]. Medes reported that almost 100% of sulfur from ingested L-cysteine and L-methionine was excreted as sulfate in the urine of human subjects [9]. Therefore, the measurement of free sulfate was effective to monitor the amount of protein intake.

Fig. 3 shows the time-dependent curves of urinary free sulfate excretion after administration of 100 g, 200 g and 300 g of beef. Urinary free sulfate excretions reached their maximum values in 6 hours after administrations in three levels of beef doses. They decreased gradually after that and they returned to the first level on average after 16 hours.

Fig. 4 shows the correlation between the doses of beef administered and the sum of urinary free sulfate excretion for 16-hour periods. The relationship between both was described by a regression line $y = 3.338x + 810.26$, $r = 0.997$ ($p < 0.05$), where x represents dose of beef administered and y represents the sum of urinary free

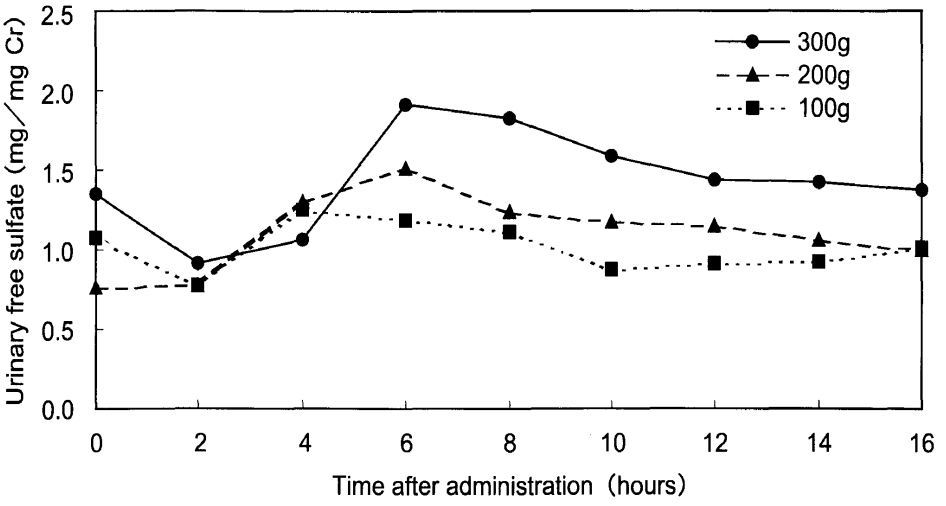

Fig. 6 The time-dependent curves of urinary free sulfate excretion corrected for creatinine (Cr) after administration of beef (100 g, 200 g and 300 g).

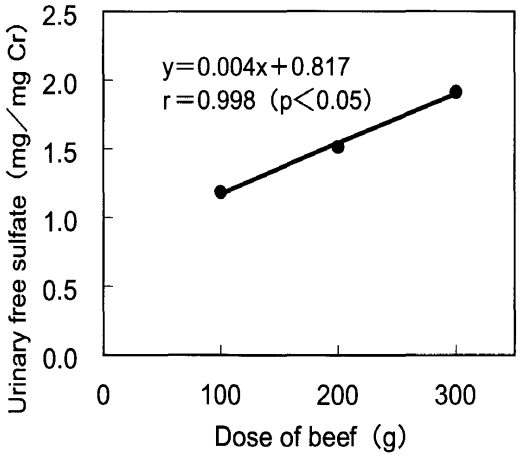

Fig. 7 The correlation between the doses of beef administered and urinary free sulfate excretion corrected for creatinine (Cr) in the 6 th hour sample.

sulfate excretion.

These results indicate that the analysis of urinary free sulfate for 16-hour periods after a meal can estimate the amount of protein intake.

Fig. 5 shows the correlation between the doses of beef administered of beef and the sum of urinary free sulfate excretion for the first 6 hours. The relationship between both was described by a regression line $y = 1.257x + 437.85$, $r = 0.998$ ($p < 0.05$), where x represents dose of beef administered and y represents the sum of urinary free sulfate excretion.

These results indicate that the analysis of urinary free sulfate for the first 6 hours after a meal can estimate the amount of protein intake.

Fig. 6 shows the time-dependent curves of urinary free sulfate excretion corrected for creatinine after administration of 100 g, 200 g and 300 g of beef. Creatinine adjustment has been thought to be an effective measure in case of the spot urine specimen, which is very concentrated or diluted. Urinary free sulfate excretions corrected for creatinine reached their maximum values in 6 hours in doses of 200 g and 300 g of beef and reached its maximum value in 4 hours in dose of 100 g of beef. They decreased gradually after that and they returned to the first level on average 16 hours after.

Fig. 7 shows the correlation between dose of beef administered of beef and urinary free sulfate excretion corrected for creatinine in the 6 th hour sample. The relationship between both was described by a regression line $y = 0.004x + 0.817$, $r = 0.998$ ($p < 0.05$), where x represents the doses of beef administered of beef and y represents urinary free sulfate excreted 6 hours after the administration and adjusted using the sulfate-creatinine ratio in the urine.

These results indicate that urinary free sulfate excretion corrected for creatinine also can estimate the amount of protein intake.

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