Effect of Protein Intake Level on Urinary Energy/Nitrogen Ratio in Japanese

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Summary Urinary energy/nitrogen ratios were determined in 179 female and 14 male subjects given protein from various sources and at various intake levels. The ratio decreased with increasing protein intake from zero to 1 g/kg/day but was constant when protein intake was between 1 to 1.8 g/kg/day. The ratio was not affected by the variety of protein source. There was no difference between the data for semisynthetic diet and conventional diet. Mean values and standard deviations of the ratio in men and women given the diet containing 1.0 to 1.8 g protein/kg/day were 9.06 ± 0.56 (n=14) and 8.19 ± 0.81 (n=37) kcal/kg N, respectively. The difference between two figures in men and women was significant (p<0.05). The mean values of urinary E/N ratio actually measured did not approach those of urea (5.34 kcal/g N), the principal nitrogenous compound in urine, the proportion of which increased at higher protein intake level. Characteristically high ratios were obtained in the ma-konbu (Laminaria japonica) and enokitake (Flammulina velutipes) diet groups. The results suggest that urinary energy originates not only from nitrogen-containing compounds but also from other organic compounds containing no nitrogen. Therefore, further investigation is necessary to evaluate the urinary E/N ratio applicable to the urinary loss of incompletely oxidized nitrogenous compounds.

Key Words urinary energy loss, urinary E/N ratio, protein intake

Physiological fuel values of foods are calculated as available energy from digested nutrients, carbohydrate, fat and protein (1). Available energy of carbohydrate and fat is determined by the coefficient of digestibility and the physical fuel values. For protein (nitrogenous compound), in addition to these factors, it is necessary to correct for the urinary loss of incompletely oxidized nitrogenous compounds such as urea, creatinine, creatine and uric acid. To do this, the ratio of the heat of combustion of urine to nitrogen (urinary E/N ratio) should be determined.

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In 1900, Atwater and Bryant (2) determined this ratio in 46 male subjects. The ratios varied from 5.22 to 10.54 kcal/g nitrogen and averaged 7.9 kcal/g N equivalent to 1.26 kcal/g of absorbed protein (7.9 ÷ 6.25 = 1.26). The rounded figure of 1.25 kcal/g protein had been used to calculate available energy of foods in the standard food table of Japan (3) until 1982.

In 1982, the National Institute of Resources (4) published the results of a series of experiments using 164 male and 60 female Japanese subjects and proposed the Japanese figure of 8.16 kcal/g N or 1.30 kcal/g of absorbed protein, which was higher than Atwater's. The report showed that the urinary E/N ratio decreased with increasing protein intake from 0.4 to 1.5 g/kg/day and that there was a significant difference between the figures for men and women, 8.62 and 7.69 kcal/g N, respectively. In the report, two problems were pointed out for further study. The first was that more data is needed, especially with those taking more dietary protein, and the second to investigate the reason for the sex difference found.

This study provides further information on the urinary E/N ratio determined for male and female subjects given protein from various and of intake levels, from zero to 1.8 g/kg/day.

MATERIALS AND METHODS

One hundred and seventy-nine female and fourteen male college students were divided into twelve groups and the subjects were given test diets listed in Table 1 for more than seven days. Urine was collected for the last three days of each experimental period. Urinary nitrogen was determined by the Kjeldahl method and gross energy of lyophilized urine was measured with a bomb calorimeter.

| Experiment | Protein source                  | Protein intake (g/kg BW) | Subject |
|------------|---------------------------------|--------------------------|---------|
|            |                                 |                          | Number  | Sex |
| 1          | Protein-free                    | 0                        | 7       | F   |
| 2          | Egg                             | 0.3–0.7                  | 23      | F   |
| 3          | Rice, soy protein isolate       | 0.45                     | 5       | F   |
| 4          | Rice, egg                       | 0.5–1.0                  | 34      | F   |
| 5          | Rice, egg, soy products         | 0.7–1.2                  | 18      | F   |
| 6          | Rice, egg, milk                 | 0.7–1.1                  | 8       | F   |
| 7          | Rice, egg, red bean or peanut   | 0.7–0.9                  | 8       | F   |
| 8          | Rice, egg, soy curd             | 0.7–0.9                  | 14      | F   |
| 9          | Rice, chicken                   | 0.5–0.8                  | 12      | F   |
| 10         | Rice, egg, ma-konbu or purple laver or enokitake | 0.7–1.2 | 19 | F |
| 11         | Conventional diet               | 0.9–1.8                  | 31      | F   |
| 12         | Conventional diet               | 1.0–1.7                  | 14      | M   |

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The protein sources and intake levels for each experiment are shown in Table 1. In experiment 1, subjects were given a protein-free diet composed of corn starch, sucrose, shortening, mineral mixture, vitamin mixture and agar. The composition of test diets of experiments 2 to 9 was the same as for the protein-free diet except that one or more foods listed in the table were incorporated at several protein levels. In experiment 10, ma-konbu (*Laminaria japonica*) at 20 or 40 g dry weight/day, purple laver (*Porphyra tenera*) at 20 or 40 g dry weight/day or enokitake (*Flammulina velutipes*) at 140 or 280 g wet weight/day was incorporated into the same diet as in experiment 9. In experiments 11 and 12, subjects were given a conventional Japanese diet containing rice, milk, egg, meat, fish, soy bean and its products, and vegetables.

Energy intake was about 40 kcal/kg/day for women and about 45 kcal/kg/day for men. Protein content of foods was calculated by multiplying the total nitrogen content determined by the Kjeldahl method by 6.25.

### RESULTS AND DISCUSSION

To determine available energy of protein, the urinary E/N ratio should be evaluated in the subjects who maintained nitrogen equilibrium. In the report of the National Institute of Resources (1982) (4), the urinary E/N ratio decreased with increasing protein intake from 0.4 to 1.5 g/kg/day. However, there were not enough data on sufficient intake levels of protein, at more than 1.1 g/kg/day, so that it was not clear whether the ratio decreased further at higher intake levels. In the present study, we firstly investigated the change in the urinary E/N ratio with protein intake at submaintenance to maintenance levels. We then determined the urinary E/N ratio from the data of male and female subjects receiving sufficient protein to maintain nitrogen equilibrium. Apparent nitrogen balances (difference between nitrogen intake and urinary and fecal excretions) were negative in the subjects receiving less than 0.8 g/kg/day of protein. The subjects receiving more than 0.8 g/kg/day of protein maintained apparent nitrogen equilibrium.

Urinary E/N ratios of various protein sources and at protein intake levels are summarized in Table 2 and a correlation between urinary E/N ratio and protein intake level is shown in Fig. 1. The results for women showed that the urinary E/N ratio was not affected by the variety of protein sources but was affected by protein intake level with the exception of the ma-konbu or enokitake diets. There was no difference between the data for the semisynthetic diet and the conventional diet (○ and ● in Fig. 1, respectively). It was evident from Fig. 1 that the urinary E/N ratio decreased with an increasing protein intake of zero to about 1 g/kg/day, and this decrease occurred constant at a level in a range of protein intake of 1.0 to 1.8 g/kg/day. Urinary urea excretion decreased in the protein-free or low-protein diet, whereas creatinine and uric acid, having a much higher E/N ratio than urea, maintained constant excretion levels. This would be one of the reasons for the higher E/N ratio found for the protein-free or low-protein diet. A wide difference in
| Exp. | Protein intake (g/kg) |
|------|-----------------------|
|      | No 0 0.3 0.45 0.6 0.8 1.0 1.2 1.4 1.6 |
| 1    | 13.8 ± 1.8(7)                       |
| 2    | 12.5 ± 2.9(8) 11.2 ± 1.5(6) 9.7 ± 0.9(9) |
| 3    | 11.5 ± 0.9(5)                       |
| 4    | 8.8 ± 1.1(24) 8.7 ± 0.6(10)         |
| 5    | 8.7 ± 0.6(9) 8.9 ± 0.7(8) 7.7 ± 0.6(9) |
| 6    | 8.9 ± 0.7(8) 9.0 ± 0.3(8)            |
| 7    | 8.6 ± 0.5(14)                       |
| 8    | 9.1 ± 0.7(12)                       |
| 9    | 10.6 ± 0.7(6)                       |
| 10-1 | 8.8 ± 0.5(8)                        |
| -2   | 14.4 ± 1.6(5)                       |
| -3   | 9.2 ± 1.4(4) 8.5 ± 1.1(9) 8.1 ± 0.6(9) 8.1 ± 0.5(9) |
| 11   | 8.8 (1) 9.4 ± 0.8(6) 9.1 (2) 8.8 ± 0.7(5) |
| 12   |                                     |

*a kcal/g nitrogen. *b Mean ± SD; figures in parentheses are the number of subjects. *c Figures are the data for the diets containing enokitake, purple laver and ma-korbu, respectively.
Urinary energy/nitrogen ratio for protein from various sources and at various intake levels. ○ shows the data for semisynthetic diet used in experiments 1 to 10 except for those subjects given either ma-konbu (□) or enokitake (△). ● and × respectively show the data for female and male subjects given a conventional diet. A and B are the regression lines suggested in the 1982 report of the National Institute of Resources, for male and female, respectively (see RESULTS AND DISCUSSION).

Fig. 1 Urinary energy/nitrogen ratio for protein from various sources and at various intake levels. ○ shows the data for semisynthetic diet used in experiments 1 to 10 except for those subjects given either ma-konbu (□) or enokitake (△). ● and × respectively show the data for female and male subjects given a conventional diet. A and B are the regression lines suggested in the 1982 report of the National Institute of Resources, for male and female, respectively (see RESULTS AND DISCUSSION).

Individual urinary E/N ratios was also observed for protein-free and low-protein diet.

Urinary E/N ratios for male and female subjects given diets containing 1.0 to 1.8 g protein/kg/day (experiments 5, 11 and 12) were 9.06 ± 0.56 (n = 14) and 8.19 ± 0.81 (n = 37) kcal/g N, respectively. The figures were slightly higher than those proposed by the National Institute of Resources (1982). In the report, the fuel values per g of urinary nitrogen for men and women were 8.62 and 7.69, respectively, which were calculated from the regression equations (A and B shown in Fig. 1) obtained from 164 male and 60 female subjects on the assumption that they consumed 1.18 g protein/kg/day, which was regarded as the recommended daily protein allowance for Japanese adults. However, these figures might be underestimated, since they were calculated on the assumption that the urinary E/N ratio decreased with an increasing protein intake at above 1 g/kg/day.
The difference between the urinary E/N ratios for male and female subjects was significant ($p < 0.05$) in the present study. However, this difference (0.8 kcal/g N) was almost the same as that reported in the 1982 report of the National Institute of Resources. Further investigation into the reason for the sex difference is necessary. Assuming that appreciable portions of urinary energy derive from unknown compounds containing no nitrogen, as discussed later, excretion of these compounds may differ between men and women in terms of both quantity and variety. However, at present it is essential to use the average value of the urinary E/N ratio for men and women for the purpose of practical calculation of the available energy of protein.

The higher the level of protein intake, the more urea is excreted in the urine, whereas other nitrogenous compounds are excreted at an almost constant level. So, theoretically, the value of the urinary E/N ratio would approximate that for urea (5.34 kcal/g N) at higher protein intake. However, our data showed it had reached a constant level at 8 to 9 kcal/g N. This suggests that the urinary energy originates not only from nitrogen-containing compounds but also from other organic compounds containing no nitrogen. Nagao and Yoshida (6) measured E/N ratio by analysis of the composition of nitrogenous compounds in urine of female students fed on a conventional diet. The urinary E/N ratio calculated as the sum of urea, ammonia, uric acid, creatinine and hippuric acid was 65% of the value actually measured. From both our results and those of Nagao and Yoshida, it was assumed that appreciable amounts of urinary energy derived from organic compounds containing no nitrogen. What are these compounds? Do they come from dietary protein? These questions should be elucidated. Then the question should be discussed of whether urinary E/N ratio is applicable to the urinary loss of incompletely oxidized nitrogenous compounds.

Characteristically high values of urinary E/N ratio were obtained in subjects fed ma-konbu or enokitake. Their urinary urea excretion was almost the same as for other diet groups fed at about the same protein level. Therefore, urinary excretion of non-nitrogenous energy-containing compounds was assumed to increase on consumption of these diets. Takezoe et al. (7) also observed a higher E/N ratio and identified a phenolsulphate-positive compound in the urine of subjects fed on the ma-konbu diet. However, it is not yet certain whether only or mainly this compound gives rise to the high ratio. Furthermore, it is not certain how either ma-konbu or enokitate affect the excretion of this compound. These questions should be elucidated in the future.

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