Changes in Tissue Protein Synthesis Are Involved in Regulating Urea Synthesis in Rats Given Proteins of Different Quality

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Summary The purpose of present study was to determine whether the regulation of urea synthesis is mediated through changes in supply of amino acids by protein synthesis and whether the concentration of ammonia, or activities of amino acid catabolizing enzymes, regulate urea synthesis when the dietary protein quality is manipulated. Experiments were done on three groups of rats given diets containing 10g gluten, 10g casein or 10g whole egg protein/100g for 10d. The urinary excretion of urea, and the liver concentrations of glutamate, serine and alanine increased with a decrease in quality of dietary protein. The fractional and absolute rates of protein synthesis in tissues declined with the decrease in quality of dietary protein quality. The ammonia concentration in plasma and liver, and activities of hepatic amino acid catabolizing enzymes was not related to urea excretion under these conditions. These results suggest that the lower protein synthesis seen in tissues of rats given the lower quality of protein is likely to be one of the factors to increasing the supply of amino acids and stimulating urea synthesis.

Key Words dietary protein quality, urea synthesis, protein synthesis, amino acids, rats

Shimke (1, 2) has suggested that the concentrations of urea-cycle intermediates were unchanged under conditions affecting the rate of urea excretion (e.g., ingestion of a high-protein diet) and concluded that the activities of various urea-cycle enzymes were regulatory factors of urea synthesis. However, many investigators have previously reported that there was an increase in urinary urea excretion without a comparable increase in the enzyme activities when a diet containing high quality protein was replaced by an isonitrogenous diet with low-quality protein (3-6).

Substrate availability normally may limit the rate of urea synthesis (7). When substrates for urea production are present in excess, an additional supply of urea cycle intermediates has been shown to stimulate urea production in liver perfusion (8, 9) and in isolated hepatocytes (10). We (5) demonstrated that the hepatic concentration of ornithine was not affected by the dietary protein quality. Thus, at least two factors, the tissue concentrations of substrates and amino acid catabolism may limit the rate of urea synthesis. Protein synthesis is an important flux of protein turnover and affects the nitrogen balance together with proteolysis. In the previous study, we already reported the increase of degradation rate of hepatic protein in rats fed the low-quality protein (11). On the other hand, the quality of dietary protein is known to affect protein synthesis in tissues (12-14). The possible effects of the dietary protein quality on tissue protein synthesis are of nutritional importance in understanding the role of the amino acid availability in urea synthesis.

The purpose of this present study was to elucidate the mechanism by which dietary protein affects urea synthesis. Three questions were considered in the present study: 1) whether decreased protein synthesis in rats fed the low-quality protein might result in elevated amino acid availability and urea synthesis, 2) whether the concentration of substrates might regulate the urea synthesis when the dietary protein quality was manipulated, and 3) whether the dietary protein quality might control the activities of amino acid-catabolizing enzymes in the liver and regulate urea synthesis. Therefore, we examined the protein synthesis in the liver, kidney, small intestine and skeletal muscle, the hepatic concentration of free amino acids, the concentrations of ammonia in plasma and urine, and the activities of argininosuccinate synthetase (EC 6.3.4.5), a rare-limiting enzyme in the urea cycle, was also determined in the present study.

MATERIALS AND METHODS

Chemicals. L-Tyrosine decarboxylase, leucylalanine and β-phenethylamine were purchased from Sigma Chemical (St. Louis, MO, USA). L-[2,6-3H]Phenylala-
Dietary Protein Quality and Urea Synthesis

Table 1. Composition of experimental diets.

| Ingredient                  | 10% Gluten | 10% Casein | 10% Whole egg protein |
|-----------------------------|------------|------------|-----------------------|
|                             | (g/kg diet) |            |                       |
| Whole egg protein           | 100        |            |                       |
| Casein                      | 100        |            |                       |
| Gluten                      | 100        |            |                       |
| Cornstarch                  | 502        | 502        | 502                   |
| Sucrose                     | 251        | 251        | 251                   |
| Corn oil                    | 50         | 50         | 50                    |
| AIN-93G mineral mix         | 35         | 35         | 35                    |
| AIN-93VX vitamin mix        | 10         | 10         | 10                    |
| Cellulose                   | 50         | 50         | 50                    |
| Choline chloride            | 2          | 2          | 2                     |

1. Supplied by Taiyo Kagaku, Yokkaichi, Japan. 2. Supplied by Oriental Yeast, Tokyo, Japan. 3. Supplied by Nihon Nosan K. K., Yokohama, Japan (34).

nine (1.5 TBq/mmol) was obtained from Amersham (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemical (Osaka, Japan).

**Animals and diets.** Young male Wistar rats (110–120 g, Japan SLC, Hamamatsu, Japan) were individually housed at 24°C in a room with a 12-h light/dark cycle. The rats were transferred to the experimental diets containing 10% gluten, 10% casein or 10% whole egg protein (Table 1) after being fed a commercial non-purified diet (MF, Oriental Yeast, Tokyo, Japan) for 2 d. All rats were provided free access to food and water. The approval of Aichi University of Education Animal Care and Use Committee was given for our animal experiments.

**Experimental design.** Three experiments were done, with 18 rats being divided randomly into three groups. In each experiment, animals were fed the experiment diet for 10 d. On d 7–10, urine was collected for 3 d, filtered and used for urea analysis. After the experimental period, the rats were decapitated and the plasma was collected in glass tubes and stored at −20°C. Liver, kidney, small intestine and gastrocnemius muscle were quickly removed and frozen in liquid nitrogen. The small intestine was slit longitudinally after rinsing with cold saline. A jejunal segment representing the second 20 cm segment distal from the pylorus was cut.

In Experiment 1, the effects of dietary protein quality on the urinary excretion of urea, the hepatic activity of argininosuccinate synthetase, the concentrations of free amino acids in the liver, and the concentration of ammonia in plasma and the liver were investigated.

In Experiment 2, the effects of dietary protein quality on the fractional and absolute rates of protein synthesis in liver, kidney, small intestine and gastrocnemius muscle were determined.

In Experiment 3, the effects of dietary protein quality on the hepatic activities of serine dehydratase, aspartate transaminase and alanine transaminase were investigated.

**Analytical procedures.** The plasma concentration and urinary excretion of urea were measured by the method of Archibald (15). The levels of ammonia in liver and plasma were determined according to the modified method of Seligson (16), and Okuda and Fujii (17), respectively. The activity of argininosuccinate synthetase in the liver was determined by the method of Schimke (1). The activity of serine dehydratase in the liver was assayed according to the method of Goldstein et al. (18). The activities of GOT and GPT in the liver were measured by the method of Das and Waterlow (4). For measuring the concentrations of free amino acids, liver was treated with ice-cold sulfosalicylic acid to precipitate the protein (19). The amino acid concentrations were measured using an amino acid analyzer (L8500, Hitachi, Tokyo, Japan). The concentration of protein in the liver, kidney, small intestine and gastrocnemius muscle were measured according to the method of Lowry et al. (20) with bovine serum albumin as the standard.

**Fractional rate of protein synthesis in tissues.** The fractional rates of protein synthesis in tissues were determined using the method of Garlick et al. (21). Radioactive L-[2,6-3H]phenylalanine was combined with unlabelled phenylalanine to yield a dose of 1.85 MBq and a concentration of 150 μmol/mL saline. Rats were injected with the radioisotope through the tail vein at a dose of 1 mL/100 g body weight. At 10 min after injection, rats were quickly decapitated. Specific radioactivities of [3H]phenylalanine in tissue samples were determined according to the method described in our previous report (22). Tissue samples were homogenized with 10 volumes of cold 0.2 mol/L perchloric acid and then centrifuged at 2,800 × g for 15 min at 4°C. The supernatant was used for the measurements of specific radioactivity after adjusting the pH to 6.0–7.0 with saturated potassium citrate. The precipitate containing protein was washed three times with 5 mL of 0.2 mol/L perchloric acid, suspended in 10 mL of 0.3 mol/L NaOH and incubated at 37°C for 1 h.

Protein-bound phenylalanine was obtained by precipitating the protein with 2 mL of 2 mol/L perchloric acid, washing the pellet with 5 mL of 0.2 mol/L perchloric acid twice and hydrolyzing the protein in 10 mL of 6 mol/L HCl for 24 h at 110°C. The HCl was evapo-
Table 2. Effect of quality of dietary protein on the activity of hepatic urea cycle enzymes and the composition of urine and plasma in rats.

|                      | 10% Gluten | 10% Casein | 10% Whole egg protein | Pooled SEM |
|----------------------|------------|------------|-----------------------|------------|
| Initial body weight (g) | 128.4      | 128.4      | 128.4                 | 1.7        |
| Body weight gain (g/10 d) | 24.0\textsuperscript{a} | 45.5\textsuperscript{b} | 58.5\textsuperscript{c} | 2.0        |
| Food intake (g/d)     | 18.7       | 19.1       | 19.8                  | 0.9        |
| Liver weight (g/100 g body weight) | 3.87\textsuperscript{b} | 4.00\textsuperscript{c} | 4.16\textsuperscript{c} | 0.10       |
| Urinary urea (mmol/d) | 4.37\textsuperscript{c} | 2.19\textsuperscript{c} | 0.97\textsuperscript{c} | 0.14       |
| Liver NH\(_3\) (\textmu mol/g) | 1.30       | 1.35       | 1.51                  | 0.11       |
| Plasma NH\(_3\) (nmol/L) | 0.043      | 0.042      | 0.044                 | 0.002      |
| Liver arginosuccinate synthetase\textsuperscript{2} (U/g liver) | 0.130\textsuperscript{a} | 0.161\textsuperscript{b} | 0.190\textsuperscript{a} | 0.006 |

\textsuperscript{1} Value are means and pooled SEM, n=6. Means with different superscript letters are significantly different (p<0.05).

Table 3. Effect of quality of dietary protein on the hepatic concentrations of free amino acid in rats.

|                      | 10% Gluten | 10% Casein | 10% Whole egg protein | Pooled SEM |
|----------------------|------------|------------|-----------------------|------------|
| Asp                   | 2.11       | 2.04       | 2.13                  | 0.09       |
| Thr                   | 0.57       | 0.83       | 0.75                  | 0.08       |
| Ser                   | 2.13\textsuperscript{a} | 2.10\textsuperscript{ab} | 1.64\textsuperscript{b} | 0.15       |
| Glu+Gln               | 4.00\textsuperscript{a} | 3.33\textsuperscript{b} | 3.25\textsuperscript{b} | 0.16       |
| Gly                   | 2.28       | 2.85       | 2.88                  | 0.26       |
| Ala                   | 5.73\textsuperscript{a} | 4.06\textsuperscript{b} | 3.92\textsuperscript{b} | 0.24       |
| Val                   | 0.15\textsuperscript{b} | 0.19\textsuperscript{ab} | 0.23\textsuperscript{b} | 0.02       |
| Met                   | 0.13\textsuperscript{b} | 0.12\textsuperscript{b} | 0.15\textsuperscript{b} | 0.004      |
| Ile                   | 0.13\textsuperscript{b} | 0.18\textsuperscript{a} | 0.19\textsuperscript{a} | 0.01       |
| Leu                   | 0.20\textsuperscript{b} | 0.28\textsuperscript{a} | 0.29\textsuperscript{a} | 0.01       |
| Tyr                   | 0.12       | 0.15       | 0.12                  | 0.02       |
| Phe                   | 0.10       | 0.12       | 0.10                  | 0.009      |
| Lys                   | 0.12\textsuperscript{b} | 0.74\textsuperscript{a} | 0.70\textsuperscript{a} | 0.04       |
| His                   | 0.56\textsuperscript{b} | 0.61\textsuperscript{*} | 0.52\textsuperscript{a} | 0.03       |

\textsuperscript{1} Value are means and pooled SEM, n=6. Means with different superscript letters are significantly different (p<0.05).

Statistical analysis. The means and pooled SEM are reported. Duncan’s multiple-range test was used to compare means after one-way ANOVA (23, 24). Differences were considered significant at p<0.05.

RESULTS

Urea cycle enzyme activities, ammonia concentrations in liver and plasma, and free amino acid concentration in liver (Experiment 1)

The rats fed the 10% gluten diet gained less body weight and had less food intake than the other two groups, which did not differ. Compared with the rats fed the 10% whole egg protein or 10% casein diets, rats fed the 10% gluten diet had relative liver weights which were significantly lower. Urinary excretion of urea increased significantly with the 10% casein diet and still more with the 10% gluten diet as compared with the 10% whole egg protein diet (Table 2). The activities of arginosuccinate synthetase, urea cycle enzymes, were proportional to the dietary protein quality. The hepatic and plasma concentrations of ammonia did not differ among groups (Table 2). Compared with the rats fed the 10% whole egg protein, the liver concentrations of free glutamic acid + glutamine, serine and alanine were significantly higher in rats fed the 10% gluten diet (Table 3).

Fractional and absolute rates of protein synthesis in tissues (Experiment 2)

As in Experiment 1, the group fed the 10% gluten diet grew less than the group fed the 10% casein diet or the 10% whole egg protein diet. Compared to the case of rats fed the 10% whole egg protein diet, the plasma concentration of urea was significantly higher in rats fed the 10% casein diet, and higher still in rats fed the 10% gluten diet (Table 4). Fractional (Ks) and absolute rates of protein synthesis in liver, kidney, small intestine and gastrocnemius muscle decreased significantly with
Table 4. Effect of quality of dietary protein on fractional and absolute protein synthesis rates in liver, small intestine, kidney and gastrocnemius muscle, and plasma concentration of urea in rats1.

| Protein Synthesis (Ks) (%/d) | 10% Gluten | 10% Casein | 10% Whole egg protein | Pooled SEM |
|-----------------------------|------------|------------|-----------------------|------------|
| Liver                       | 71.3       | 87.6       | 102.4                 | 1.7        |
| Small intestine             | 74.6       | 88.8       | 113.4                 | 3.5        |
| Kidney                      | 55.2       | 64.1       | 76.7                  | 1.2        |
| Gastrocnemius muscle        | 7.6        | 9.6        | 12.0                  | 0.4        |
| Absolute protein synthesis (mg protein synthesized/(tissue-d)) | 858      | 1,326      | 1,633                 | 56         |

1 Value are means and pooled SEM, n=6. Means with different superscript letters are significantly different (p<0.05).
2 Fractional rate of protein synthesis.

Table 5. Effect of quality of dietary protein on the hepatic activities of amino acid-catabolizing enzymes and plasma concentration of urea in rats1.

| Enzyme                     | 10% Gluten | 10% Casein | 10% Whole egg protein | Pooled SEM |
|---------------------------|------------|------------|-----------------------|------------|
| Initial body weight (g)   | 131.4      | 131.2      | 131.6                 | 1.2        |
| Body weight gain (g/10 d) | 22.4       | 53.8       | 68.0                  | 2.7        |
| Food intake (g/d)         | 18.0       | 19.0       | 20.7                  | 0.8        |
| Liver weight (g/100 g body weight) | 3.85       | 4.27       | 4.54                  | 0.09       |
| Plasma urea (mmol/L)      | 8.05       | 6.53       | 4.58                  | 0.22       |
| Liver enzymes (U/g liver) | 4.35       | 4.96       | 4.64                  | 0.25       |
| Alanine transaminase2     | 93.1       | 92.0       | 94.0                  | 5.6        |
| Serine dehydratase3       | 8.86       | 8.38       | 7.58                  | 1.32       |

1 Value are means and pooled SEM, n=6. Means with different superscript letters are significantly different (p<0.05). 2 Unit of enzyme activity: mmol of NAD produced per minute. 3 Unit of enzyme activity: mmol of pyruvate produced per minute.

The 10% casein diet and even more with the 10% gluten diet as compared with the 10% whole egg protein diet (Table 4).

Amino acid-catabolizing enzyme activities in liver (Experiment 3)

The concentration of plasma urea elevated gradually with the decreasing quality of the dietary protein. The dietary protein quality did not affect the hepatic activities of serine dehydratase, GOT and GPT (Table 5).

DISCUSSION

We (5, 6), along with Das and Waterlow (4), have reported that an increase in excretion of urea occurred without a concomitant change in the activities of urea cycle enzymes when a diet containing high quality protein was replaced by an isonitrogenous diet with low quality protein. The purpose of the present experiments was to elucidate the mechanism by which the dietary protein quality alters urea synthesis. In the present study, the activities of argininosuccinate synthetase, a rate-limiting enzyme of the urea cycle, were proportional to the dietary protein quality. Also, urea excretion was greater in the group given the diet with low quality protein. Therefore, the results suggest that regulation of urea synthesis by dietary protein quality may not be attributable to changes in activities of urea cycle enzymes, thus corroborating the findings of Das and Waterlow (4). Ammonia is a substrate of carbamylphosphate synthetase (EC 2.7.2.5) in urea synthesis. Therefore, the determination of ammonia concentration in plasma and liver also served as a measurement of the substrate for urea synthesis. In this study, the concentration of ammonia in plasma and liver did not differ among all
three groups. Katunuma et al. (25) reported that the differences in ammonia level in the liver of mice fed the low or the high protein diet were quite small, and that there were great changes in urea excretion. Under our experimental conditions, there were no correlations between the ammonia concentration and urea excretion of groups. The concentration of ammonia may not have regulated urea synthesis in the present investigation.

Amino acids supply nitrogen for urea synthesis by various amino acid-catabolizing enzymes. Many investigators reported that the activities of GOT, GPT and serine dehydratase in liver was induced by the ingestion of high-protein diet, and that a significant correlation was found between the activities of these amino acid-catabolizing enzymes and urea excretion when the quantity of dietary protein was varied (1, 18). Therefore, we assumed that the activities of amino acid-catabolizing enzymes in liver might be important in regulating urea synthesis when the quality of dietary protein was changed. However, the activities of liver GOT, GPT and serine dehydratase were not correlated to the urea concentration in the present study. The activities of glutamate dehydrogenase (EC 1.4.1.2) in liver have been known to depend on the quality of dietary protein (4). The changes in hepatic activities of amino acid-catabolizing enzymes are not considered to be the factor leading to the greater urea synthesis in rats fed the low quality protein.

The metabolic response to dietary protein includes marked changes in protein synthesis, especially in liver, muscle and brain (13, 14, 26, 27). The possible effects of the dietary protein quality on tissue protein synthesis are of nutritional importance in understanding the role of the amino acid availability in urea synthesis. We hypothesized that the lower tissue protein synthesis in rats fed the low-quality protein might result in the higher concentration of amino acids and increased urea synthesis compared with those fed the high-quality protein in the present experiment. In the liver, small intestine, kidney and gastrocnemius muscle, the fractional and absolute rates of protein synthesis declined with a decrease of dietary protein quality. The concentrations of some amino acids in the liver significantly increased in rats fed the diet with low-quality protein. The liver concentrations of free serine, alanine, and glutamic acid + glutamine in the group given the 10% gluten diet, particularly increased as compared with those in the group given the 10% whole egg protein. These results reflected the changes in tissue protein synthesis. Ishikawa et al. (28), examined the arteriovenous difference of the plasma concentrations of various amino acids, and demonstrated the importance of alanine, serine and glutamine as a major end product of the degradation of amino acids in rat tissues. Garber et al. (29) proposed that the release of alanine reflected the de novo synthesis of amino acids in skeletal muscle and did not come from the selective proteolysis of an alanine-rich protein. The lower protein synthesis in tissues of rats given the low quality of protein may be one of the factors to stimulate the release of these amino acids from tissues and regulate the liver concentrations of amino acids.

The first limiting amino acid of wheat gluten for the requirement of amino acids is lysine. Recently we (30) reported that the addition of lysine to the gluten diet elevated the rate of protein synthesis in the brain of rats. However, little documentation for the effects of supplementation of dietary limiting amino acids to the low quality protein on protein synthesis of visceral organs and skeletal muscle is available. Further studies for the effect of the addition of dietary limiting amino acids to low-quality protein on tissue protein synthesis should be included in the examination of the mechanism by which the quality of dietary protein affects urea synthesis.

Proteolysis is a major flux of protein turnover and affects the nitrogen balance together with protein synthesis. It is quite sensitive to physiological regulation by amino acids as well as hormones (31). The major proteolytic pathways in tissues include the autophagic/lysosome pathway. In autophagic proteolysis, several amino acids have a direct regulatory potential; leucine, tyrosine, methionine, tryptophan, proline, glutamine and histidine in the liver (31, 32), and leucine in the skeletal muscle (33). In the present study, the ingestion of low-quality protein, such as gluten diet, decreased significantly the hepatic concentrations of free leucine and methionine. In the previous study, we demonstrated the increase of degradation rates of liver protein in rats fed the low quality protein (11). These results suggest that proteolysis in tissues may be increased with the reduction of dietary protein quality, although the role of protein degradation in urea synthesis remains unknown under our physiological conditions. This is the possibility to consider in detail in further examination of the mechanism by which the dietary protein quality alters urea synthesis.

These results suggest that the decreased protein synthesis in tissues of rats given the lower quality of protein is likely to be one of the factors to increase the concentrations of amino acids and stimulate urea synthesis.

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