Effect of the Quality of Dietary Amino Acids Composition on the Urea Synthesis in Rats

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Summary We have shown that urinary urea excretion increased in rats given a lower quality protein. The purpose of present study was to determine whether the composition of dietary amino acids affects urea synthesis. Experiments were done on three groups of rats given diets containing a 10% gluten amino acid mix diet or 10% casein amino acid mix diet or 10% whole egg protein amino acids mix diet for 10 d. The urinary excretion of urea, the liver concentration of N-acetylglutamate, and the liver concentration of free serine, glutamic acids and alanine were greater in the group given the amino acid mix diet of lower quality. The fractional and absolute rates of protein synthesis in tissues declined with a decrease in quality of dietary amino acids. The hepatic concentration of ornithine and the activities of hepatic urea-cycle enzymes were not related to the urea excretion. These results suggest that the increased concentrations of amino acids and N-acetylglutamate seen in the liver of rats given the amino acid mix diets of lower quality are likely among the factors stimulating urea synthesis. The protein synthesis in tissues is at least partly related to hepatic concentrations of amino acids. The composition of dietary amino acids is likely to be one of the factors regulating urea synthesis when the quality of dietary protein is manipulated.

Key Words dietary amino acids, urea synthesis, protein synthesis, N-acetylglutamate, rats

Schimke (1, 2) has suggested that the concentrations of urea-cycle intermediates are 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 are 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 the diet containing high quality protein was replaced by the isonitrogenous diet with low quality protein (3–7).

We demonstrated that urinary excretion of urea increased with the 10% casein diet and still more with the 10% gluten diet as compared with the 10% whole egg protein diet (5). Gluten is known to be a lower quality protein than whole egg protein because of a deficiency of lysine (8). The concentrations of threonine and sulfur amino acids in gluten are also lower than in whole egg protein. On the other hand, the concentration of glutamic acid in gluten is markedly higher than in whole egg protein. Thus, the changes in amino acid composition of dietary protein may be included in regulating the urea synthesis in rats given proteins of different quality (gluten, casein or whole egg protein). However, little documentation for the effects of the amino acid composition in dietary proteins of different quality on urea synthesis is available.

Substrate availability normally may limit the rate of urea synthesis (9). When substrates for urea production are present in excess, urea formation has been shown to be stimulated by adding ornithine, a urea cycle intermediate, in perfused liver (10, 11) and in isolated hepatocytes (12). Thus, at least two factors other than enzyme activity—substrate and urea cycle intermediates—may regulate the rate of urea synthesis. We (5, 13) demonstrated that the hepatic concentration of ornithine was not affected by the dietary protein quality, and that liver concentrations of some free nonessential amino acids rose in rats fed the lower quality protein. Protein synthesis is an important flux of protein turnover and affects the nitrogen balance together with proteolysis. In a previous study, Hayase and Yoshida (14) reported the increase of degradation rate of hepatic protein in rats fed a low quality protein like a gluten. On the other hand, the quality of dietary protein is known to affect the protein synthesis in tissues (13, 15–17). The possible effects of the dietary amino acid composition on tissue protein synthesis are of nutritional importance in understanding the role of amino acid availability in urea synthesis.

Urea formation has been shown to be stimulated by adding N-acetylglutamate in perfused liver (10, 11, 18) and in isolated hepatocytes (12) when substrates for

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urea production were present in excess. N-Acetylglutamate, an essential activator of carbamylphosphate synthetase (EC 6.3.4.16), plays a critical role in the regulation of urea synthesis in mammalian liver (19, 20). Shigesada and Tatibana (21) have demonstrated that N-acetylglutamate synthetase (EC 2.3.1.1) catalyzed the acetylation of glutamate in liver. Thus, the supply of N-acetylglutamate may limit the rate of urea synthesis. In the our previous report (6, 22), a positive correlation existed between the liver concentration of N-acetylglutamate and urea excretion when the dietary protein quality was manipulated. However, little documentation for the effects of the dietary amino acid composition on the concentration of N-acetylglutamate in the liver is available.

The purpose of this present study was to determine the mechanism by which the composition of dietary amino acids affects urea synthesis in rats fed a 10% gluten amino acid mix diet, 10% casein amino acid mix diet or 10% whole egg protein amino acid mix diet. Four questions were considered in the present study: 1) whether the dietary amino acid composition might control the activities of hepatic urea cycle enzymes and regulate urea synthesis, 2) whether the concentration of hepatic ornithine might regulate the urea synthesis when the dietary amino acid composition was manipulated, 3) whether decreased protein synthesis in rats fed the low quality amino acid mix diet resulted in greater amino acid availability and urea synthesis than in rats fed the high quality amino acid mix diet and 4) whether the dietary amino acid composition might affect the N-acetylglutamate concentration in the liver.

Therefore, we examined the urinary excretion of urea, the hepatic concentrations of the free amino acids, ornithine, and N-acetylglutamate, and protein synthesis in liver, small intestine and skeletal muscle. In the present study, the activities of argininosuccinate synthetase (EC 6.3.4.5), a rate-limiting enzyme in the urea cycle, and carbamylphosphate synthetase (EC 6.3.4.16) were also determined. Thus, in this experiment, we used a 10% gluten amino acid mix diet, 10% casein amino acid mix diet or 10% whole egg protein amino acid mix diet. The composition of amino acids in gluten, casein or whole egg protein were chosen to represent different amino acid mixes in the present study.

### MATERIALS AND METHODS

**Chemicals.** L-Tyrosine deccarboxylase, leucylalanine, β-phenylalanine and N-acetylglutamic acid were purchased from Sigma Chemical (St. Louis, MO, USA). L-[2,6-3H]Phenylalanine (1.5 TBq/mmol) and NaH14CO3 (1.85 GBq/mmol) were obtained from GE Healthcare Bio-Sciences Japan (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

**Animals and diets.** Young male Wistar rats (100–110 g, Japan SLC, Inc., 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 contained 10% gluten amino acid mix diet, 10% casein amino acid mix diet or 10% whole egg protein amino acid mix diet (Table 1) after being fed a commercial nonpurified diet (MF, Oriental Yeast Co., Ltd., 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 experimental, animals were fed the experimental diet for 10 d. On days 6–9, urine and feces were collected for 3 d, filtered and used for the analysis of urea. After the experimental period, the rats were decapitated and the plasma was collected in glass tubes and stored at −20°C. Liver, 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 pylorus was cut.

In Experiment 1, the effects of quality of dietary amino acid composition on the activity of hepatic argininosuccinate synthetase and free amino acids, urinary excretion of urea and plasma concentration of urea in rats were investigated. In Experiment 2, the effects of the quality of dietary amino acid composition on fractional and absolute protein synthesis rates in liver, small intestine and gastrocnemius muscle, and plasma concentration of urea were determined. In Experiment 3, the effect of the quality of dietary amino acid composition on the hepatic concentrations of free amino acids in rats was examined. In Experiment 2, the effects of the quality of dietary amino acid composition on fractional and absolute protein synthesis rates in liver, small intestine and gastrocnemius muscle, and plasma concentration of urea were examined. In Experiment 3, the effect of the quality of dietary amino acid composition on the activity of hepatic carbamylphosphate synthetase, the liver concentration of N-acetylglutamate and plasma concentration of urea were investigated.

Analytical procedures. The plasma concentration and urinary excretion of urea were measured by the method of Archibald (23). The activities of argininosuccinate synthetase and carbamylphosphate synthetase in the liver were determined by the method of Schimke (1). For measuring the concentrations of free amino acids, liver was treated with ice-cold 120 mmol/L sulfosalicylic acid to precipitate the protein (24). The
Table 4. Effect of the quality of dietary amino acid composition on fractional and absolute protein synthesis rates in liver, gastrocnemius muscle, and small intestine and plasma concentration of urea in rats.1

<table>
<thead>
<tr>
<th></th>
<th>Gluten amino acid mix</th>
<th>Casein amino acid mix</th>
<th>Whole egg protein amino acid mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>125.8±1.6</td>
<td>125.8±2.3</td>
<td>125.8±1.5</td>
</tr>
<tr>
<td>Body weight gain (g/10 d)</td>
<td>23.8±0.6a</td>
<td>49.0±1.4a</td>
<td>44.6±3.0a</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>18.6±0.3</td>
<td>18.5±0.5</td>
<td>18.0±0.4</td>
</tr>
<tr>
<td>Plasma urea (mmol/L plasma)</td>
<td>5.58±0.20a</td>
<td>4.71±0.27b</td>
<td>3.79±0.24c</td>
</tr>
<tr>
<td>Liver weight (g/100 g of body weight)</td>
<td>4.08±0.07b</td>
<td>4.44±0.05a</td>
<td>4.22±0.09ab</td>
</tr>
<tr>
<td>Small intestine (g/20 cm)</td>
<td>0.86±0.08b</td>
<td>1.03±0.15a</td>
<td>0.84±0.10b</td>
</tr>
<tr>
<td>Gastrocnemius muscle</td>
<td>1.05±0.02ab</td>
<td>1.02±0.09b</td>
<td>1.08±0.01a</td>
</tr>
</tbody>
</table>

1 Values are means±SE, n=6. Means with different superscript letters are significantly different (p<0.05).
2 Fractional rate of protein synthesis.

amino acid concentrations were measured by an amino acid analyzer (L-8500, Hitachi, Tokyo, Japan). The concentration of protein in the liver, kidney, small intestine and gastrocnemius muscle was measured according to the method of Lowry et al. (25) with bovine serum albumin as a standard. The N-acetylglutamate concentration was measured by the method of McGivan et al. (26) as described in our previous report (27).

Fractional rate of protein synthesis in tissues. The fractional rates of protein synthesis in tissues were determined using the method of Garlick et al. (28). 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 L-[3H]phenylalanine in tissue samples were determined according to the method described in our previous report (29). Tissue samples were homogenized with 10 volumes of cold 0.2 mol/L perchloric acid and then centrifuged at 2,800 g × 9 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 reprecipitating 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 evaporated to dryness, and the amino acids were dissolved in citrate buffer (pH 6.3). The determination of the specific radioactivity of L-[3H]phenylalanine involved its enzymatic conversion into phenylalanine, followed by a radioactivity count (LS 5000TD, Beckman Japan, Tokyo, Japan) and fluorometric determination (F-3000, Hitachi).

Statistical analysis. The means and SE values are reported. Duncan’s multiple-range test was used to compare means after one-way ANOVA (30, 31). Differences were considered significant at p<0.05.

RESULTS

Liver concentrations of amino acids and ornithine, urinary excretion of urea, plasma concentrations of urea and the activity of hepatic argininosuccinate synthetase (Experiment 1)

The rats fed the 10% gluten amino acid mix diet gained less body weight than the other two groups, which did not differ. The relative food intake and liver weight were not different among the three groups. Urinary excretion and the plasma concentration of urea increased significantly with the 10% casein amino acid mix diet and still more with the 10% gluten amino acid mix diet as compared with the 10% whole egg protein amino acid mix diet. The activity of hepatic argininosuccinate synthetase, a urea cycle enzyme, decreased significantly with the 10% gluten amino acid mix diet as compared with the 10% whole egg protein amino acid mix diet (Table 2). The liver concentrations of free serine, glutamic acids and alanine were significantly higher in the 10% gluten amino acid mix diet. The quality of dietary amino acids did not affect the hepatic concentration of ornithine (Table 3).

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

As in Experiment 1, the rats fed the 10% gluten amino acid mix diet grew less than the 10% casein mix amino acid diet or 10% whole egg protein amino acid mix diet rats. Compared to the case of rats fed the 10%
whole egg protein amino acid mix diet, the plasma concentration of urea was significantly higher in rats fed the 10% casein amino acid mix diet and higher still in rats fed the 10% gluten amino acid mix diet (Table 4). Fractional (Ks) and absolute rates of protein synthesis in liver, small intestine and gastrocnemius muscle decreased significantly with the 10% casein amino acid mix diet and still more with the 10% gluten amino acid mix diet as compared with the 10% whole egg protein amino acid mix diet (Table 4).

Effect of the quality of dietary amino acid composition on the activity of hepatic carbamylphosphate synthetase, the liver concentration of N-acetylglutamate and plasma concentration of urea in rats (Experiment 3)

The hepatic activities of carbamylphosphate synthetase, a urea cycle enzyme, were proportional to the quality of dietary amino acids. The plasma concentration of urea and the hepatic concentration of N-acetylglutamate increased significantly with the 10% casein amino acid mix diet and still more with the 10% gluten amino acid mix diet as compared with the 10% whole egg protein amino acid mix diet (Table 4).

**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 this present study was to determine whether the composition of dietary amino acids affects urea synthesis in rats fed a 10% gluten amino acid mix diet, 10% casein amino acid mix diet or 10% whole egg protein amino acid mix diet. In the present study, the activities of argininosuccinate synthetase, a rate-limiting enzyme of the urea cycle, and carbamylphosphate synthetase were proportional to the quality of dietary amino acid composition. Moreover, urea excretion was greater in the group given the diet with low quality amino acids. Therefore, the results suggest that regulation of urea synthesis by dietary amino acid composition may not be attributable to changes in activities of urea cycle enzymes, thus corroborating the findings of Das and Waterlow (4).

Ornithine is an intermediate of the urea cycle. Katunuma et al. (32) reported that an elevated hepatic ornithine concentration could be involved in activating the urea cycle under certain physiological conditions. Urea formation has been shown to be stimulated by adding ornithine in vivo (33), in perfused liver (10, 11), and in isolated hepatocytes (12) when substrates for urea production were present in excess. Hayase et al. (5) has shown that the levels of free serine and ornithine in the liver of rats fed a methionine-free diet were found to increase markedly as compared with those of rats fed the basal diet. Therefore, we assumed that the concentration of ornithine in the liver may limit the rate of urea synthesis. But in this study, the hepatic concentration of ornithine did not differ among the three groups (Table 3). In a previous report, we (5) demonstrated that the concentration of ornithine in the liver was not affected by the dietary protein quality in rats fed gluten, casein or whole egg protein as dietary protein. Therefore, the changes in liver concentration of ornithine may not be a factor affecting urea synthesis when the dietary composition of amino acids is manipulated.

The metabolic response to dietary protein includes marked changes in protein synthesis, especially in liver, muscle and brain (15, 17, 34, 35). Glutamic acid in gluten is markedly decreased in gluten compared with those of gluten, casein or whole egg protein as dietary protein. Therefore, the changes in liver concentration of ornithine may not be a factor affecting urea synthesis when the dietary composition of amino acids is manipulated.
acid mix diet as compared with the 10% gluten amino acid mix diet in the present experiment (Table 4). The concentrations of free serine, glutamic acid and alanine in the liver were significantly higher in the 10% gluten amino acid mix diet compared with those in 10% whole egg protein amino acid mix diet (Table 3). These results reflect changes in tissue protein synthesis. Ishikawa et al. (36), examined the arteriovenous difference of the plasma concentrations of various amino acids, and demonstrated the importance of alanine, serine and glutamine as major end products of the degradation of amino acids in rat tissues. Serine supplies the nitrogen by dehydratase directly (37, 38). The lower protein synthesis in tissues of rats given the amino acid mixture of lower quality may have stimulated the release of these amino acids from tissues and regulated the liver concentrations of amino acids.

We (13) also reported the decreased protein synthesis in tissues and increased concentration of glutamic acid, serine and alanine in liver of rats given glutamine as a low quality protein. In the present study, when amino acid mixtures were used as the source of dietary protein, we indicated the same results for the protein synthesis and amino acid concentrations as in our previous findings (13) in rats fed protein of different quality. Therefore, these results may suggest that the regulation of the supply of amino acids by protein synthesis is mediated through changes in the composition of dietary amino acids when the quality of dietary protein is manipulated.

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 (39). 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, proline, glutamine and histidine in the liver (39, 40). In the present study, the 10% whole egg protein amino acid mix diet increased significantly the hepatic concentrations of free valine and methionine. These results suggest that proteolysis in tissues may decline with a high quality amino acid mix diet, although the role of protein degradation in urea synthesis remains unknown under our physiological conditions. This is a possibility to consider in detail in further examination of the mechanism by which the quality of dietary protein and the composition of dietary amino acids alter urea synthesis.

N-Acetylglutamate is a physiological activator of carbamylphosphate synthetase (12, 41). Variation in the N-acetylglutamate concentration has correlated well with urea synthesis in intact animals (19, 21, 22, 42, 43) and in isolated hepatocytes (18, 44). Previously, we (22) demonstrated that the positive correlation between the urea excretion and the liver concentration of N-acetylglutamate was induced when the quality of dietary protein was changed. Therefore, we also assumed that the concentration of N-acetylglutamate in the liver might control urea synthesis in rats given amino acid mixtures of different quality. In this present study, the liver concentration of N-acetylglutamate and plasma concentration of urea increased significantly with the 10% casein amino acid mix diet and still more with the 10% gluten amino acid mix diet as compared with the 10% whole egg protein amino acid mix diet (Table 5). The higher concentration of liver N-acetylglutamate in rats fed the glutaminated casein mixture or casein amino acid mix diet enhanced urea synthesis in these groups. Therefore, the changes in N-acetylglutamate concentration may be one of the factors affecting urea synthesis in this study.

Mitochondrial carbamylphosphate synthetase in the liver has an absolute requirement for N-acetylglutamate as an activator. Shigesada et al. (45) reported that the increase of N-acetylglutamate concentration under physiological conditions stimulated urea synthesis in isolated hepatocytes. The Ka value (0.11 mM) of the carbamylphosphate synthetase for N-acetylglutamate was reported to be close to its physiological concentration in mitochondria (19). From the observed hepatic concentration of N-acetylglutamate (Table 5), its average concentration in hepatic mitochondria of rats fed the glutaminated amino acid mix diet is calculated to be about 0.1 mM based on the estimate of the mitochondrial space in the cellular volume (19). The magnitude concentration appear to be adequate for the function of N-acetylglutamate. These results strongly indicate the control mechanism for the urea synthesis was mediated by the hepatic concentration of N-acetylglutamate when the quality of dietary amino acid composition was changed.

As described above, in the previous studies, we (6, 13, 22) demonstrated the increased concentrations of hepatic amino acids and N-acetylglutamate in rats given a lower-quality protein as such as glutamine stimulated urea synthesis. In the present study, when dietary protein was replaced by amino acid mixtures as protein sources, we also show that an increased supply of amino acids and increased concentration of N-acetylglutamate in rats given a lower quality of amino acid mixture stimulate urea synthesis, thus corroborating our finding (6, 13, 22). Therefore, the composition of dietary amino acids may be a factor regulating urea synthesis when the quality of dietary protein is changed.

These results suggest that the increased concentrations of amino acids and N-acetylglutamate seen in the liver of rats given the amino acid mix diets of lower quality are likely among the factors stimulating urea synthesis. The protein synthesis in tissues is at least partly related to hepatic concentrations of amino acids. The composition of amino acids is likely to be one of the factors regulating urea synthesis when the quality of dietary protein is manipulated.

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Dietary Amino Acids Composition and Urea Synthesis


