Amino Acids (2005) 28: 297–303
DOI 10.1007/s00726-005-0187-8

Amino Acids
Printed in Austria

Effect of adding dietary L-lysine, L-threonine and L-methionine to a low gluten diet on urea synthesis in rats*

K. Tujioka[1], **S. Lyou**[1], **Y. Fukaya**[2], **A. Sano**[2], **K. Hayase**[2], and **H. Yokogoshi**[1]

[1] Laboratory of Nutritional Biochemistry, School of Food and Nutritional Sciences, COE Program in the 21st Century, The University of Shizuoka, Yada, Shizuoka, Japan
[2] Department of Home Economics, Aichi University of Education, Kariya, Aichi, Japan

Received December 14, 2004
Accepted February 3, 2005
Published online March 25, 2005;

Summary. We have shown that urinary urea excretion increased in rats fed a low quality protein. The purpose of present study was to determine whether an addition of dietary limiting amino acids affected urea synthesis in rats fed a low gluten diet. Experiments were done on three groups of rats given diets containing 10% gluten, 10% gluten + 0.5% L-lysine or 10% gluten + 0.5% L-lysine, 0.2% L-threonine and 0.2% L-methionine for 10 d. The urinary excretion of urea, and the liver concentrations of serine and ornithine decreased with the addition of dietary L-lysine, L-threonine and L-methionine. The fractional and absolute rates of protein synthesis in tissues increased with the treatment of limiting amino acids. The activities of hepatic urea-cycle enzymes was not related to the urea excretion. These results suggest that the addition of limiting amino acids for the low gluten diet controls the protein synthesis in tissues and hepatic ornithine and decline urea synthesis.

Keywords: Dietary limiting amino acids – Urea synthesis – Protein synthesis – Amino acids – Ornithine – Rats

Introduction

Shimke (1962, 1963) 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 the diet containing high quality protein was replaced by the isonitrogenous diet with low quality protein (Kiriyama et al., 1967; Das and Waterlow, 1974; Hayase et al., 1980; Tujioka et al., 2002).

In the previous report, 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 (Hayase et al., 1980). Gluten is known to be a lower quality protein than whole egg protein because of deficiency in lysine (Rama Rao et al., 1961). The concentrations of threonine and sulfur amino acids in gluten are also lower than in the whole egg protein. However, little documentation for the effects of supplementation of dietary limiting amino acids to the low quality protein on urea synthesis is available. Therefore, the purpose of this present study was to determine whether the addition of dietary L-lysine, L-threonine and L-methionine affects urea synthesis in rats fed a low gluten diet.

Substrate availability normally may limit the rate of urea synthesis (Meijer et al., 1990). When substrates for urea production are present in excess, urea formation has been shown to be stimulated by adding ornithine, urea cycle intermediates, in perfused liver (Kramer, 1972; Saheki and Katunuma, 1975) and in isolated hepatocytes (Briggs and Freedland, 1976). Thus, at least two factors, substrate and urea cycle intermediates, may regulate the rate of urea synthesis other than enzyme activity. We (Hayase et al., 1980) 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 elevated in rats fed the lower quality protein. Protein synthesis is an important flux of protein turnover and affects the nitrogen balance together

* Supported in part by a Nutrition and Food Science Fund of the Japanese Society of Nutrition and Food Science and by a 21st Century COE program and city area promoting program from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

with proteolysis. In the previous study, we already reported the increase of degradation rate of hepatic protein in rats fed the low quality protein like a gluten (Hayase and Yoshida, 1980). On the other hand, the quality of dietary protein are known to affect the protein synthesis in tissues (Yokogoshi et al., 1980, 1992; Tanaka et al., 2002). The possible effects of the addition of dietary limiting amino acids to the low quality protein on tissue protein synthesis are of nutritional importance in understanding the role of the amino acid availability in urea synthesis.

Four questions were considered in the present study: 1) whether the addition of dietary limiting amino acids to the low gluten diet might improve the nitrogen balance and decrease the urea excretion, 2) whether increased protein synthesis in rats fed the higher quality protein by the dietary addition of limiting amino acids resulted in decreased amino acid availability and urea synthesis than in rats fed the lower quality protein, 3) whether the concentration of ornithine might regulate the urea synthesis when dietary limiting amino acid were supplemented to the low gluten diet and 4) whether the addition of limiting amino acids to the low gluten diet might control the activities of hepatic urea cycle enzymes and regulate urea synthesis. Therefore, we examined the nitrogen balance, urinary excretion of urea, plasma concentration of ammonia, the hepatic concentrations of free amino acids and ornithine, and protein synthesis in liver, kidney, 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 diet and 10% gluten + 0.5% L-lysine diet, 10% gluten + 0.5% L-lysine, 0.2% L-threonine and 0.2% L-methionine diet as the experimental diet. The composition of amino acids in dietary proteins was considered to decide the ratio of addition of dietary L-lysine, L-threonine and L-methionine to the 10% gluten diet.

Materials and methods

Chemicals

L-Tyrosine decarboxylase, L-leucyl-L-alanine and β-phenethylamine were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). L-[2,6-^{3}H]Phenylalanine (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 contained 10% gluten, 10% gluten + 0.5% L-lysine or 10% gluten + 0.5% L-lysine, 0.2% L-threonine and 0.2% L-methionine (Table 1) after being fed a commercial nonpurified 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 6–9, urine and feces were collected for 3 d, filtered and used for the analysis of urea and nitrogen. 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 pylorus was cut. In Experiment 1, the effects of the addition of dietary amino acids to a low gluten diet on the urinary excretion of urea, the plasma concentration of ammonia, the nitrogen balance and the concentrations of free amino acids in liver were examined. In Experiment 2, the effects of the addition of dietary amino acids to a low gluten diet on the hepatic activities of argininosuccinate synthetase and carbamylphosphate synthetase were investigated. In

Table 1. Composition (g/100 g of diet) of experimental diets

Ingredient	10% Gluten	10% Gluten + 0.5% L-Lysine	10% Gluten + 0.5% L-Lysine + 0.2% L-Threonine + 0.2% L-Methionine
Gluten	10.0	10.0	10.0
L-Lysine	0.0	0.5	0.5
L-Threonine	0.0	0.0	0.2
L-Methionine	0.0	0.0	0.2
Cornstarch[1]	50.2	49.9	49.6
Sucrose[1]	25.1	24.9	24.8
Corn oil	5.0	5.0	5.0
AIN-93G mineral mix[2]	3.5	3.5	3.5
AIN-93VX vitamin mix[2]	1.0	1.0	1.0
Cellulose[1]	5.0	5.0	5.0
Choline chloride	0.2	0.2	0.2

[1] Supplied by Oriental Yeast, Tokyo, Japan
[2] Supplied by Nihon Nosan K. K., Yokohama, Japan (American Institute of Nutrition, 1993)

Experiment 3, the effects of the addition of dietary amino acids to a low gluten diet on the fractional and absolute rates of protein synthesis in liver, kidney, small intestine and gastrocnemius muscle were determined.

Analytical procedures

The plasma concentration and urinary excretion of urea were measured by the method of Archibald (1945). The levels of ammonia in plasma was determined according to the modified method of Seligson (1951), and Okuda and Fujii (1966) respectively. The nitrogen in urine, feces and diets were determined by semimicro-Kjeldahl method (Nagahara et al., 1967). For measuring the concentrations of free amino acids, liver was treated with ice-cold sulfosalicyclic acid to precipitate the protein (Millward et al., 1974). The amino acid concentrations were measured by an amino acid analyzer (L-8500, Hitachi, Tokyo, Japan). The activities of argininosuccinate synthetase and carbamylphosphate synthetase in the liver were determined by the method of Schimke (1962). The concentration of protein in the liver, kidney, small intestine and gastrocnemius muscle were measured according to the method of Lowry et al. (1951) with bovine serum albumin as a 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. (1980). Radioactive L-[2,6-^{3}H]phenylalanine was combined with unlabelled L-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 [^{3}H]phenylalanine in tissue samples were determined according to the method described in our previous report (Hayase et al., 1998). Tissue samples were homogenized with 10 volumes of cold 0.2 mol/L perchloric acid and then centrifuged at 2800 × 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 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 [^{3}H]phenylalanine involved its enzymatic conversion into phenethylamine, followed by a radioactivity counting (LS 5000TD, Beckman Japan, Tokyo, Japan) and fluorometric determination (F-3000, Hitachi Co., Tokyo, Japan). The absolute rates of protein synthesis in tissues were calculated from the results for fractional rates of protein synthesis and protein contents in tissues.

Statistical analysis

The means and pooled SEM are reported. Duncan's multiple-range test was used to compare means after one-way ANOVA (Duncan, 1955; Snedecor and Cochran, 1967). Differences were considered significant at $p < 0.05$.

Results

Nitrogen balance, ammonia concentration in plasma, and free amino acid concentrations in liver (Experiment 1)

The rats fed the 10% gluten diet gained less body weight than the other two groups, which did not differ. Compared with the rats fed the 10% gluten diet alone, rats fed 10% gluten + L-lysine, L-threonine and L-methionine diet or 10% gluten + L-lysine diet had food intake which were significantly higher. The relative liver weight was not different among three groups. Urinary excretion of urea and nitrogen decreased significantly with the 10% gluten + L-lysine diet and still more with the 10% gluten + L-lysine, L-threonine and L-methionine diet as compared with the 10% gluten diet alone (Table 2). Similarly the addition of L-lysine alone, or L-lysine, L-threonine and L-methionine to the 10% gluten diet increased significantly the nitrogen balance (Table 2). The plasma concentrations of ammonia did not differ among groups. Compared with the rats fed the 10% gluten diet, the liver concentrations of free serine

Table 2. Effect of the addition of dietary amino acids to a low gluten diet on the nitrogen balance, urinary excretion of urea and plasma concentration of ammonia in rats[1]

	10% Gluten	10% Gluten + 0.5% L-Lysine	10% Gluten + 0.5% L-Lysine + 0.2% L-Threonine + 0.2% L-Methionine	Pooled SEM
Initial body weight (g)	123.5	123.0	121.9	1.9
Body weight gain (g/10 days)	19.1^b	49.8^a	47.6^a	2.9
Food intake (g/3 days)	48.1^c	59.5^a	53.4^b	1.6
Liver weight (g/100 g of body weight)	3.91	3.82	4.15	0.12
Urinary urea (mmol/day)	5.47^a	3.99^b	1.92^c	0.18
Plasma NH_3 (mmol/L)	0.171	0.164	0.175	0.006
Intake N (mg/3 days)	674^b	857^a	817^a	24
Urinary N (mg/3 days)	501^a	414^b	267^c	16
Feces N (mg/3 days)	64^b	74^a	66^b	2
Nitrogen balance (mg/3 days)	108^c	369^b	484^a	12

[1] Values are means and pooled SEMs, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$)

Table 3. Effect of the addition of dietary amino acids to a low gluten diet on the hepatic concentration of free amino acid in rats[1]

	(μmol/g of liver)			Pooled SEM
	10% Gluten	10% Gluten + 0.5% L-Lysine	10% Gluten + 0.5% L-Lysine + 0.2% L-Threonine + 0.2% L-Methionine	
Aspartic acid	1.95	1.97	2.09	0.21
Threonine	0.51[b]	0.11[c]	0.64[a]	0.04
Serine	2.51[a]	2.91[a]	1.43[b]	0.13
Glutamic acid + Glutamine	7.91[c]	9.11[b]	10.69[a]	0.31
Alanine	4.18	3.47	4.25	0.45
Valine	0.16	0.18	0.17	0.01
Methionine	0.18[c]	0.33[b]	0.41[a]	0.02
Isoleucine	0.082	0.090	0.100	0.006
Leucine	0.18	0.21	0.22	0.01
Tyrosine	0.061[b]	0.073[b]	0.096[a]	0.006
Phenylalanine	0.072	0.076	0.088	0.005
Lysine	0.13[b]	0.65[a]	0.55[a]	0.05
Histidine	0.46	0.51	0.51	0.03
Ornithine	0.33[a]	0.22[b]	0.18[b]	0.03

[1] Values are means and pooled SEMs, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$)

and ornithine were significantly lower in the L-lysine, L-threonine and L-methionine-treated rats (Table 3).

Urea cycle enzyme activities in liver (Experiment 2)

The body weight gain and liver weight increased significantly with the 10% gluten + L-lysine diet and still more with the 10% gluten + L-lysine, L-threonine and L-methionine diet as compared with the 10% gluten diet alone (Table 4). Compared with the case of rats fed the 10% gluten diet or 10% gluten + L-lysine diet, the plasma concentration of urea was significantly lower in rats fed the 10% gluten + L-lysine, L-threonine and L-methionine diet. The hepatic activities of argininosuccinate synthetase and carbamylphosphate synthetase, urea cycle enzymes, were not affected by the addition of amino acids to the 10% gluten diet (Table 4).

Fractional and absolute rates of protein synthesis in tissues (Experiment 3)

As in Experiments 1 and 2, the rats fed the 10% gluten diet alone grew less than the L-lysine-treated, or L-lysine, L-threonine and L-methionine-treated rats. The concen-

Table 4. Effect of the addition of dietary amino acids to a low gluten diet on the activities of hepatic urea cycle enzymes and plasma concentration of urea in rats[1]

	10% Gluten	10% Gluten + 0.5% L-Lysine	10% Gluten + 0.5% L-Lysine + 0.2% L-Threonine + 0.2% L-Methionine	Pooled SEM
Initial body weight (g)	135.4	133.0	133.8	1.3
Body weight gain (g/10 days)	17.2[c]	47.6[b]	57.6[a]	3.0
Food intake (g/day)	15.2[b]	20.3[a]	18.7[a]	1.0
Liver weight (g/100 g of body weight)	3.60[c]	4.11[b]	4.40[a]	0.07
Plasma urea (mmol/L)	3.64[a]	3.42[a]	3.05[b]	0.09
Liver argininosuccinate synthetase[2](U/g of liver)	0.174	0.172	0.199	0.018
Liver caramylphosphate synthetase[3](U/g of liver)	0.285	0.266	0.288	0.020

[1] Values are means and pooled SEMs, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$)
[2] Unit of enzyme activity: mmol of urea produced per hour
[3] Unit of enzyme activity: mmol of citrulline produced per hour

Table 5. Effect of the addition of dietary amino acids to a low gluten diet on fractional and absolute synthesis rates in liver, small intestine, kidney and gastrocnemius muscle, and plasma concentration of urea in rats[1]

	10% Gluten	10% Gluten + 0.5% L-Lysine	10% Gluten + 0.5% L-Lysine + 0.2% L-Threonine + 0.2% L-Methionine	Pooled SEM
Initial body weight (g)	146.4	146.4	146.0	2.1
Body weight gain (g/10 days)	18.6[b]	49.6[a]	54.8[a]	3.1
Food intake (g/day)	18.4	19.4	19.6	0.5
Liver weight (g/100 g of body weight)	3.69[b]	3.93[a]	4.01[a]	0.08
Small instine weight (g/20 cm)	0.81	0.87	0.90	0.04
Kidney weight (g/100 g of body weight)	0.81	0.79	0.80	0.06
Gastrocnemius muscle weight (g/100 g of body weight)	1.04	1.01	1.00	0.02
Plasma urea (mmol/L)	5.28[a]	4.95[ab]	4.55[b]	0.17
Protein synthesis (Ks)[2] (%/day)				
Liver	71.3[c]	84.0[b]	100.9[a]	1.9
Small intestine	74.6[c]	89.4[b]	108.5[a]	2.3
Kidney	55.2[c]	64.9[b]	76.5[a]	1.2
Gastrocnemius muscle	7.6[b]	8.8[b]	11.2[a]	0.5
Absolute protein synthesis (mg protein synthesized/(tissue · day))				
Liver	858[c]	1315[b]	1630[a]	67
Small intestine	83[c]	114[b]	132[a]	3
Kidney	119[c]	160[b]	196[a]	4
Gastronemius muscle	28[c]	34[b]	46[a]	2

[1] Values are means and pooled SEMs, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$)
[2] Fractional rate of protein synthesis

tration of plasma urea decreased with the addition of L-lysine, L-threonine and L-methionine to the 10% gluten diet (Table 5). Fractional (*Ks*) and absolute rates of protein synthesis in liver, kidney small intestine and gastrocnemius muscle increased significantly with the 10% gluten + L-lysine diet and still more with the 10% gluten + L-lysine, L-threonine and L-methionine diet as compared with the 10% gluten diet (Table 5). In case of gastrocnemius muscles, the addition of dietary lysine, L-threonine and L-methionine resulted in the fractional rate of protein synthesis that was significantly higher than in the other two groups, which did not differ (Table 5).

Discussion

The purpose of the present experiments was to elucidate the mechanism by which the addition of dietary limiting amino acids to the low quality protein alters urea synthesis.

In the present study, the activities of argininosuccinate synthetase, a rate-limiting enzyme of urea cycle, and carbamylphosphate synthetase were not affected by the dietary addition of limiting amino acids. While L-lysine, L-threonine and L-methionine treatment to the gluten diet decreased urea excretion and improved the nitrogen balance. Therefore, the results suggest that regulation of urea synthesis by the addition of dietary limiting amino acids to the low quality protein may not be attributable to changes in activities of urea cycle enzymes, thus corroborating the findings of Das and Waterlow (1974).

Ammonia is a substrate of carbamylphosphate synthetase in urea synthesis. Therefore, the determination of ammonia concentration in plasma also served as a measurement of the substrate for urea synthesis. In this study, the concentration of ammonia in plasma did not differ among all three groups. Katunuma et al. (1966) 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.

L-Ornithine is an intermediate of urea cycle. Katunuma et al. (1966) reported that an elevated hepatic ornithine concentration could be involved in activating the urea cycle under the physiological conditions. Urea formation has been shown to be stimulated by adding ornithine *in vivo* (Greenstein et al., 1956), in perfused liver (Kramer

et al., 1972; Saheki et al., 1975), and in isolated hepatocytes (Briggs et al., 1976) when substrates for urea production were present in excess. Hayase et al. (1980) has shown that the levels of free serine and ornithine in the liver of rats fed the 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. In the present experiment, compared with the rats fed the 10% gluten diet, the liver concentrations of free ornithine was significantly lower in rats fed the 10% gluten + L-lysine, L-threonine and L-methionine. The decrease in liver ornithine may decrease urea synthesis in groups treated with L-lysine, L-threonine and L-methionine. The changes in liver concentration of ornithine may be one of the factors affecting urea synthesis without altering urea-cycle enzyme activities when the dietary limiting amino acids were added to the low quality protein.

The metabolic response to dietary protein includes marked changes in protein synthesis, especially in liver, muscle and brain (Yokogoshi et al., 1980; Tanaka et al., 2002; Millward et al., 1976; Symmons et al., 1972). The L-lysine is the first limiting amino acid of gluten for requirement in mammals (Rama Rao et al., 1961). The concentrations of threonine and sulfur amino acids in gluten are also lower. Koie et al. (2000) reported the addition of lysine to the low gluten diet elevated the protein synthesis rates in the brain. 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. We hypothesized that the higher tissue protein synthesis in rats fed the gluten diet supplemented with limiting amino acids might result in the lower concentration of amino acids and decreased urea synthesis compared with those fed the gluten diet alone in the present experiment. In the liver, small intestine, kidney and gastrocnemius muscle, the fractional and absolute rates of protein synthesis increased with the addition of dietary L-lysine, L-threonine and L-methionine. The concentrations of free serine in the liver was significantly lower in the L-lysine, L-threonine and L-methionine treatment compared with those in the group given the 10% gluten diet alone. These results reflected the changes in tissue protein synthesis. Ishikawa et al. (1972), 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. Serine supplies the nitrogen by dehydratase directly (Goldstein et al., 1962; Imai et al., 2003). The higher protein synthesis in tissues of rats given the low quality protein diet supplemented with dietary limiting amino acids may have decreased the release of these amino acids from tissues and regulate the liver concentrations of amino acids.

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 (Kadowaki and Kanazawa 2003). The major proteolytic pathways in tissues include the autophagic/lysosome pathway. In autophagic proteolysis, several regulatory amino acids inhibit deprivation-induced proteolysis; leucine, tyrosine, methionine, tryptophan, proline, glutamine and histidine in the liver (Kadowaki and Kanazawa 2003; Mortimore et al., 1987). In the present study, the addition of L-lysine, L-threonine and L-methionine for low gluten diet increased significantly the hepatic concentrations of free glutamic acid + glutamate and methionine. These results suggest that proteolysis in tissues may decline with the dietary addition of limiting amino acids, although the role of protein degradation in urea synthesis remains unknown under our physiological condition. This is the 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 alters urea synthesis.

These results suggest that the increased protein synthesis in tissues of rats given the addition of dietary limiting amino acids is likely to be one of the factors to decrease the concentrations of amino acids and decline urea synthesis, and that the hepatic concentration of ornithine is at least partly related to the urea synthesis.

Acknowledgments

The authors are grateful to T. Tuchiya and C. Yamada for their valuable assistance.

References

American Institute of Nutrition (1993) AIN purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J Nutr 123: 1939–1951

Archibald RM (1945) Colorimetric determination of urea. J Biol Chem 157: 507–518

Briggs S, Freedland RA (1976) Effect of ornithine and lactate on urea synthesis in isolated hepatocytes. Biochem J 160: 205–209

Das TK, Waterflow JC (1974) The rate of adaptation of urea cycle enzymes in rat liver. Br J Nutr 32: 353–373

Duncan DB (1967) Multiple range and multiple F tests. Biometrics 11: 1–42

Garlick PJ, McNurlan MA, Preedy VR (1980) A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of [^{3}H]phenylalanine. Biochem J 192: 719–723

Goldstein L, Knox WE, Behrman EJ (1962) Studies on the nature, inducibility, and assay of the threonine and serine dehydratase activities of rat liver. J Biol Chem 237: 2855–2860

Greenstein JP, Winitz M, Gullino P, Bilnbaum SM, Otey MC (1956) Studies on the metabolism of amino acids and related compounds in vivo. III. Prevention of ammonia toxicity by arginine and related compounds. Arch Biochem Biophys 64: 342–354

Hayase K, Koie M, Yokogoshi H (1998) The quantity of dietary protein affects brain protein synthesis rate in aged rats. J Nutr 128: 1533–1536

Hayase K, Yokogoshi H, Yoshida A (1980) Effect of dietary proteins and amino acid deficiencies on urinary excretion of nitrogen and the urea synthesizing systems in rats. J Nutr 110: 1327–1337

Imai S, Kanamoto R, Yagi I, Kotaru M, Saheki T, Iwami K (2003) Response of the induction of rat liver serine dehydratase to changes in the dietary protein requirement. Biosci Biotechnol Biochem 67: 383–387

Ishikawa E, Aikawa T, Matsutaka H (1972) The role of alanine as a major precursor among amino acids for hepatic gluconeogenesis and as a major end product of the degradation of amino acids in rat tissues. J Biochem 71: 1097–1099

Kadowaki M, Kanazawa T (2003) Amino acids as regulators of proteolysis. J Nutr 133: 2052S–2056S

Katunuma N, Okada M, Nishi Y (1966) Reguration of the urea cycle and TCA cycle by ammonia. In: Weber G (ed) Advances in enzyme reguration, vol 4. Pergamon Press, New York, pp 317–335

Kiriyama S, Iwao H, Ashida K (1967) Dietary protein quality and liver arginase activity of rats. Agric Biol Chem 31: 671–674

Koie M, Tanaka M, Hayase K, Yokogoshi H (2000) Effects of adding dietary lysine to a low gluten diet on the brain protein synthesis rate in aged rats. Biosci Botechnol Biochem 64: 1466–1471

Kramer JW, Freedland RA (1972) Possible rate-limiting factors in urea synthesis by the perfused rat liver. Proc Soc Exp Biol Med 141: 833–835

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275

Meijer AJ, Lamers WH, Chamuleau RAFM (1990) Nitrogen metabolism and ornithine cycle function. Physiol Rev 70: 701–748

Millward DJ, Nnanyelugo DO, James WPT, Garlick PJ (1974) Protein metabolism in skeletal muscle: the effect of feeding and fasting on muscle RNA, free amino acids and plasma insulin concentrations. Br J Nutr 32: 127–142

Millward DJ, Garlick PJ, Nnanyelugo DO, Waterlow JC (1976) The relative importance of muscle protein synthesis and breakdown in the regulation of muscle mass. Biochem J 156: 185–188

Mortimore GE, Pöso R, Kadowaki M, Wert JC Jr (1987) Multiphasic control of hepatic protein degradation by regulatory amino acids. J Biol Chem 262: 16322–16327

Nagahara T, Iwao H, Kubo S (1967) Analytical methods for foods. Shibata Publishing Inc., Tokyo, pp 100–107

Okuda H, Fujii S (1966) Direct colorimetric determination of blood ammonia. Saishin Igaku 21: 622–627

Rama Rao PB, Norton HW, Johnson C (1961) The amino acid composition and nutritive value of proteins. IV. Phenylalanine, tyrosine, methionine and cystine requirements of the growing rat. J Nutr 73: 38–42

Saheki T, Tsuda M, Katunuma N (1975) Analysis of regulatory factors for urea synthesis by isolated perfused liver. II. Comparison of urea synthesis in livers of rats subjected to different dietary conditions. J Biochem 77: 671–678

Schimke RT (1962) Adaptive characteristics of urea cycle enzymes in the rat. J Biol Chem 237: 459–468

Schimke RT (1963) Studies on factors affecting the levels of urea cycle enzymes in rat livers. J Biol Chem 238: 1012–1018

Snedecor GW, Cochran WG (1967) Statistical methods, 6th edn. Iowa State University Press, Ames, pp 135–171

Symmons RA, Maquire EJ, Rogers QR (1972) Effect of dietary protein and feeding schedule on hepatic polysome pattern in the rat. J Nutr 102: 639–646

Tanaka M, Lyou S, Hayase K, Okuyama S, Yokogoshi H (2002) Effect of dietary protein quantity and quality on the brain protein synthesis rate in overiectomized female rats. J Agric Food Chem 50: 1731–1735

Tujioka K, Lyou S, Hirano E, Sano A, Hayase K, Yoshida A, Yokogoshi H (2002) Role of *N*-acetylglutamate concentration and ornithine transport into mitochondria in urea synthesis of rats given proteins of different quality. J Agric Food Chem 50: 7467–7471

Yokogoshi H, Sakuma Y, Yoshida A (1980) Relationships between nutritional quality of dietary proteins and hepatic polyribosome profiles in rats. J Nutr 110: 383–387

Yokogoshi H, Hayase K, Yoshida A (1992) The quality and quantity of dietary protein affect brain protein synthesis in rats. J Nutr 122: 2210–2217

Authors' address: Kazutoshi Hayase, Department of Home Economics, Aichi University of Education, Kariya, Aichi 448-8542, Japan,
Fax: 81-566-26-2476, E-mail: khayase@auecc.aichi-edu.ac.jp