Urinary Creatine and Kidney Transaminase Activity of Rats Fed Diets Supplemented with Arginine, Glycine and Creatine

Hisanao TAKEUCHI, Ryuzo NAGANO,
Takao SAKABE and
Keiichiro MURAMATSU

Laboratory of Food and Nutrition,
Faculty of Agriculture, Shizuoka
University, Oya, Shizuoka 422

Received August 28, 1975

Some workers have reported that the activity of kidney transaminase was decreased in fasted rats,^{1,2)} or rats fed a protein-free diet,³⁾ protein-depleted diets,^{4,5)} restricted normal diets,⁵⁾ or casein diet supplemented with L-arginine and/or glycine.⁶⁾ The enzyme activity was depressed by dietary creatine,^{1,4,7,8)} but the depressive effect of dietary creatine was partially prevented by the supplementation of L-arginine and/or glycine.^{6,9,10)} The decrease in enzyme activity was accompanied by the increased ratio of urinary creatine to creatinine.¹¹⁾ Some reports have indicated that the amount of urinary excreted creatine was increased when the enzyme activity was decreased in fasted rats²⁾ or rats fed the casein diets containing L-arginine and/or glycine which are involved in creatine synthetic system.^{12,13)} In the present study, when the activity of kidney transaminase was decreased by the feeding of the protein-free diet and the creatine-added diet, the effects of the supplementation of L-arginine and glycine to these diets on the creatine metabolism were investigated.

Animals and diets

After male weanling rats of the Donryu strain were fed a stock diet (25% casein) for 5 to 10 days, they were divided and used in three series of experiments.

Exp. I. Rats weighing about 65 g were separated into 10 groups, 5 of which were fed the 10% casein diet containing 3% L-arginine·HCl with 0, 2, 4, 6 and 8% of glycine and 5 of which were fed the 10% casein diet containing 3% glycine with 0, 2, 4, 6 and 8% of L-arginine·HCl for 5 days.

Exp. II. Animals weighing about 100 g were divided into 3 groups, which were fed the 10% casein diet, the protein-free diet and the protein-free diet supplemented with 1% L-arginine and 3% glycine for 5 days.

Exp. III. Rats having about 70 g of body weight

were divided into 4 groups, which were given *ad libitum* the 10% casein diet and the same casein diets supplemented with either 1% L-arginine·HCl plus 3% glycine, 1% creatine and both for 5 days.

The composition of the basal diet (10% casein) was as described previously.¹²⁾ To neutralize hydrochloride in L-arginine·HCl, equimolar NaHCO₃ was added. Ammonium citrate was supplemented to some diets so that the nitrogen content was the same in all diets. Amino acids, creatine, NaHCO₃ and ammonium citrate were added at the expense of α -corn starch. Each food intake was controlled on the basis of the group having the least amount of meal in each experiment. Each group was consisted of 3 to 4 rats.

Analysis

The urine was collected into a vessel with 10 ml of 0.1 N H₂SO₄ for the last 2 days of each experimental period and diluted with water to a final volume of 250 ml. After the feeding of test diets for the experimental period, animals were treated as described

FIG. 1. Changes in Urinary Excreted Creatine (●) and Kidney Transaminase Activity (▲) of Rats Fed the 10% Casein Diets Containing 3% L-Arginine or Glycine to Which Glycine or L-Arginine was Added (Exp. I).

Open symbols (○, △) express the enzyme activity and the amount of urinary excreted creatine of rats fed the 10% casein diet, respectively. Vertical lines indicate the standard errors of means.

previously.^{10,13)} Urinary creatine and creatinine,^{2,12)} and kidney transaminase activities¹²⁾ were determined by the methods previously described.

Figure 1 shows the result of Exp. I. Body weight gain, food intake and kidney weight were not so much different among each group (5~10 g/5 days, 35~42 g/5 days and 0.70~0.85 g, respectively). By the addition of 1% L-arginine to the 10% casein diet containing 3% glycine or of 1% glycine to the 10% casein diet containing 3% L-arginine, the amount of urinary excreted creatine was increased, while the activity of kidney transaminase was decreased. This result was similar to that reported previously.^{2,11-13)} However, the increase of supplemented L-arginine or glycine did not result in an extreme change of the enzyme activity and the amount of urinary creatine. The amount of urinary creatinine was almost constant in every group.

higher than that of the other groups. The transaminase activity in the kidney of the group fed the protein-free diet was lower than that of the group fed the 10% casein diet, which agreed with the observation of Van Pilsum.⁴⁾ However, the supplementation of both amino acids to the protein-free diet did not enhance the decreased enzyme activity of the group fed the protein-free diet. This result was not different from that reported by Van Pilsum and Canfield⁵⁾ who observed that the kidney from rats fed the protein-free diet supplemented with either L-arginine or glycine had higher transaminase activity than that of rats fed the protein-free diet. The present observations may indicate that the creatine synthesis is accelerated by the supplementation of L-arginine and glycine to the diet and that synthesized creatine is intactly excreted in the urine, though the enzyme activity is kept to be

TABLE I. BODY WEIGHT CHANGE, FOOD INTAKE, KIDNEY WEIGHT, THE AMOUNTS OF URINARY EXCRETED CREATINE AND CREATININE, AND KIDNEY TRANSAMINASE ACTIVITY OF RATS FED THE 10% CASEIN AND PROTEIN-FREE DIETS WITH OR WITHOUT L-ARGININE, GLYCINE AND CREATINE (Exp. II and III)

Dietary group	Body weight change (g/5 days)	Food intake (g/5 days)	Kidney weight (g)	Urinary creatine (mg/100 g body wt./day)	Urinary creatinine (mg/100 g body wt./day)	Kidney transaminase activity (total units ^{a)} /100g body wt.)
Exp. II ^{b)}						
10% Casein (10C)	-1.3±2.6 ^{c)}	42.7±1.5	0.82±0.01	0.96±0.15 ^a	1.47±0.17 ^a	1.24±0.12 ^a
Protein-free (PF)	-14.6±1.1	34.2±2.0	0.68±0.03	3.96±0.68 ^{bc}	1.78±0.09 ^a	0.72±0.15 ^b
PF+1% L-Arg·HCl+3% Gly	6.0±0.5	44.7±1.3	0.86±0.04	8.54±0.95 ^d	2.35±0.04 ^b	0.66±0.18 ^b
Exp. III						
10% Casein (10C)	10.8±4.3	41.6±3.6	0.72±0.05	0.80±0.17 ^a	1.27±0.12 ^a	7.26±0.35 ^a
10C+1% L-Arg·HCl+3% Gly	7.7±1.5	39.9±1.7	0.77±0.02	3.47±0.22 ^{bc}	0.98±0.25 ^a	3.69±0.18 ^{bc}
10C+1% Creatine	4.6±1.2	32.3±3.5	0.67±0.03	35.17±5.50 ^{de}	1.60±0.25 ^a	1.31±0.12 ^{de}
10C+1% L-Arg·HCl+3% Gly						
1% Creatine	4.3±1.3	36.1±2.7	0.72±0.05	49.40±4.00 ^f	3.55±0.32 ^b	2.10±0.20 ^f

^{a)} Total units per 100 g of body weight means total units per whole kidney per 100 g of body weight. One unit is 1 μ mole of hydroxyguanidine formed per hour under the assay condition.

^{b)} Each food intake was controlled on the basis of the group having the least amount of meal.

^{c)} Mean±SE. Significant difference between the superscripts of a and b, and c and d, and e and f at $P<0.05$.

The results of Exp. II are shown in Table I. The amount of urinary creatine in the group fed the protein-free diet supplemented with L-arginine and glycine was larger than that in the group fed the protein-free diet. This increased creatine might be synthesized from added L-arginine and glycine and might not be due to the enhanced excretion of muscle creatine to the urine which should result in the decrease of body weight,²⁾ because the body weight of the group fed the protein-free diet containing both amino acids increased a little while that of the group fed the protein-free diet decreased. The amount of creatinine in the urine of the group fed the protein-free diet containing both amino acids was

low.

Table I also shows the results of Exp. III. The amount of urinary creatine in the group fed the creatine-containing diet supplemented with both amino acids was greater than that of the group fed the creatine-containing diet, though the enzyme activity was also kept to be low. This increased amount of creatine was generally similar to that enhanced by the addition of both amino acids to the 10% casein diet. The transaminase activity in the kidney of the 10C group in Exp. II was extremely smaller than that of the same group in Exp. III. This result may be due to the decrease of body weight in the 10C group of Exp. II,

because the enzyme activity was decreased in the kidney of rats fed protein-free diets^{4,5)} and restricted normal diets,⁵⁾ of which body weights were diminished, as compared with that of rats fed *ad libitum* a normal diet.

Though many works^{1,4,7,8,11)} have suggested that the activity of kidney transaminase may be regulated by creatine, it should be further investigated whether creatine truly regulates the creatine metabolism or not, since this metabolite is formed from added L-arginine and glycine in the presence of the extremely low enzyme activity as shown in the present experiment. It was also observed that the enzyme activity was not so much decreased by dietary creatine supplementation using the other method for estimating the activity¹⁴⁾ (unpublished data).

REFERENCES

- 1) J. B. Walker *J. Biol. Chem.*, **236**, 493 (1961).
 - 2) H. Takeuchi, K. Hukaya, K. Narita and K. Muramatsu, *Agr. Biol. Chem.*, **34**, 1610 (1970).
 - 3) J. F. Van Pilsum, B. Olsen, D. Taylor, T. Rozycki and J. C. Pierce, *Arch. Biochem. Biophys.*, **100**, 520 (1963).
 - 4) J. F. Van Pilsum, *J. Biol. Chem.*, **228**, 145 (1957).
 - 5) J. F. Van Pilsum and T. M. Canfield, *ibid.*, **237**, 2574 (1962).
 - 6) C. D. Fitch, C. Hsu and J. S. Dinning, *Biochem. Biophys. Acta*, **52**, 194 (1961).
 - 7) J. B. Walker, *ibid.*, **36**, 574 (1959).
 - 8) C. D. Fitch, C. Hsu and J. S. Dinning, *J. Biol. Chem.*, **235**, 2362 (1960).
 - 9) D. L. Coleman and M. E. Ashworth, *Am. J. Physiol.*, **199**, 927 (1960).
 - 10) J. F. Van Pilsum, *J. Nutr.*, **101**, 1085 (1971).
 - 11) C. D. Fitch, C. Hsu and J. S. Dinning, *J. Biol. Chem.*, **236**, 490 (1961).
 - 12) H. Takeuchi, T. Tanaka and K. Muramatsu, *Agr. Biol. Chem.*, **33**, 1161 (1969).
 - 13) H. Takeuchi and K. Muramatsu, *ibid.*, **35**, 1298 (1971).
 - 14) J. F. Van Pilsum, D. Taylor, B. Zakis and P. McCormick, *Anal. Biochem.*, **35**, 277 (1970).
-