Metabolism of L-Leucine-U-¹⁴C in Young Rats Fed Excess Glycine Diets

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Received April 7, 1975

As reported previously,¹⁾ while the growth-depressive effect of excess glycine was prevented by the supplementation of L-arginine and L-methionine, the degradation of glycine-U-¹⁴C into the expired CO₂ was not accelerated by the supplementation of both amino acids. However, the incorporation of the isotope into lipids of the liver and carcass was found to increase in the rats fed the excess glycine diet containing both amino acids.²⁾ The lipid synthesis utilizing excess glycine may be accelerated by adding L-arginine and L-methionine to the 10% casein diet containing excess glycine. In the experiments reported in the present paper, the metabolic fate of L-leucine-U-¹⁴C was studied in rats fed the excess glycine diets with or without L-arginine and L-methionine.

Male weanling rats of the Donryu strain weighing 70 to 75 g fed a stock diet (25% casein) for 3 to 4 days were divided into three groups of 4 to 5 rats. Each was fed one of the following diets: 10% casein (10C), 10% casein diet containing 7% glycine (10C7G), and 10C7G supplemented with 1.4% L-arginine·HCl and 0.9% L-methionine (10C7GArgMet). The composition of the 10C diet in detail and the amounts of supplemented amino acids were as described in the previous reports.^{1,2)} Animals were fed *ad libitum* for 14 days and then fasted for 12 hr before the last feeding. Fasted rats were force-fed 10 ml of the diet suspension containing 4 μ Ci of L-leucine-U-¹⁴C per 100 g of body weight. Other treatments of animals were as described previously,²⁾ except that the expired CO₂ was collected at 2- to 4-hr of intervals for 12 hr after the last feeding. The radioactivities²⁾ in the expired CO₂, trichloroacetic acid (TCA) soluble fraction, protein, glycogen, lipids and urine, and the concentrations^{3,4)} of free amino acids in the blood plasma, liver and urine were measured by the previously described methods.

The body weight gain and food intake of the 10C7G group (19.7 ± 2.4 g) were much smaller than those of the 10C (57.5 ± 2.3 g) and the 10C7GArgMet (66.0 ± 9.9 g) groups.¹⁻³⁾ The recovery of ¹⁴C-radioactivity

Fig. 1. Changes in Radioactivity Recovered in Expired CO₂ after an Oral Loading Dose of L-Leucine-U-¹⁴C.

After rats were fed *ad libitum* the 10% casein diets containing 7% glycine with or without L-arginine and L-methionine for 14 days, they were fasted for 12 hr and then force-fed 10 ml of the diet suspensions containing 4 μ Ci of L-leucine-U-¹⁴C per 100 g body weight. ○, 10% casein; ●, +7% Gly; △, +7% Gly +1.4% L-Arg·HCl +0.9% L-Met. L-Arg·HCl (1.4%) and L-Met (0.9%) equal the contents of L-arginine and L-methionine in a 30% casein diet. Vertical lines indicate the standard errors of means.

in the expired CO₂ of rats fed the 10C7GArgMet diet was much lower than that of the other groups (Fig. 1). The similar result was obtained in the previous experiment²⁾ using glycine-U-¹⁴C in place of L-leucine-U-¹⁴C, though the degree of the decrease of the expiratory ¹⁴CO₂ in the 10C7GArgMet group force-fed glycine-¹⁴C was smaller than that in the same group force-fed L-leucine-¹⁴C. The reduced recovery of radioactivity in the expired CO₂ in the present experiment using L-leucine-¹⁴C compared to that in the previous experiment using glycine-¹⁴C might be due to the dilution of glycine-¹⁴C by body free glycine, the concentration of which is higher than that of leucine. The ¹⁴C-radioactivity in the expired CO₂ of the 10C7GArgMet group force-fed L-leucine-¹⁴C was in an inverse proportion to that in the protein of plasma, liver and muscle (Table I), though that of the same group force-fed glycine-¹⁴C was inversely proportional to the incorporation of the isotope into liver and carcass lipids as reported previously.²⁾ The supplementation of L-arginine and L-methionine may accelerate the utilization of amino acids. The recovery of ¹⁴C-radioactivity in the TCA soluble fraction of plasma, liver and muscle, in the glycogen of liver and muscle, in lipids of the liver and in the urine were less than 2% in all groups, and were not so much different among each group (Table I). Six to nine percent of recoveries of radioactivity were observed in lipids of the carcass of each group (Table I). The recovery in the TCA soluble fraction of plasma and

TABLE I. RECOVERIES OF RADIOACTIVITY IN TCA SOLUBLE FRACTION AND PROTEIN OF PLASMA, LIVER AND MUSCLE, LIVER- AND MUSCLE-GLYCOCEN, LIVER- AND CARCASS-LIPIDS, AND URINE AT 12 HR AFTER FORCE-FEEDING OF L-LEUCINE-U-¹⁴C

Dietary group (symbol)	Recovery of radioactivity									
	Plasma		Liver		Muscle		Carcass		Urine ²⁾	
	TCA solu. ¹⁾	Protein %	TCA solu.	Protein %	TCA solu.	Protein %	Lipids %	Glycogen %	Lipids %	Urine ²⁾ %
10% Casein (10C)	0.56 ±0.13 ⁴⁾	2.12 ±0.40 ^a	1.88 ±0.38	12.28 ±0.78 ^a	0.36 ±0.05	14.56 ±2.28 ^a	1.44 ±0.26	0.52 ±0.07	9.07 ±0.22 ^a	1.00 ±0.08
+7% Gly (10C7G)	0.44 ±0.06	2.08 ±0.06 ^a	1.38 ±0.18	13.78 ±0.52 ^a	0.24 ±0.16	16.04 ±3.64 ^a	1.40 ±0.10	0.45 ±0.05	5.65 ±0.45 ^a	1.14 ±0.16
+7% Gly+1.4% L-Arg·HCl ³⁾	0.46 ±0.05	3.92 ±0.66 ^b	1.42 ±0.08	17.28 ±1.86 ^b	0.25 ±0.02	27.74 ±1.44 ^b	1.40 ±0.08	0.35 ±0.05	6.62 ±0.29 ^b	0.88 ±0.38
+0.9% L-Met ³⁾ (10C7GArgMet)										

¹⁾ TCA = trichloroacetic acid.²⁾ The urine in the urinary bladder was collected and combined with the excreted urine.³⁾ L-Arg·HCl (1.4%) and L-Met (0.9%) were similar to the contents of L-arginine and L-methionine in a 30% casein diet.⁴⁾ Mean ± SE. Significant difference between the superscripts of ^a and ^b at *P* < 0.05.

TABLE II. FREE AMINO ACID CONCENTRATIONS IN THE BLOOD PLASMA AND LIVER 12 HOURS AFTER THE FORCE-FEEDING OF THE 10% CASEIN DIETS CONTAINING 7% GLYCINE WITH OR WITHOUT L-ARGININE AND L-METHIONINE

Dietary group (symbol)	Free amino acid concentration ¹⁾													
	Thr	Lys	Arg	His	Leu	Val	Ile	Phe	Met	Tyr	Ser	Glu	Ala	Pro
	Plasma μmoles/100 ml plasma													
10% Casein (10C)	73.7	40.2	15.4	14.0	8.4	7.6	3.7	3.4	2.5	t ³⁾	129.6	68.0	62.5	29.4
+7% Gly (10C7G)	17.9	36.7	11.3	7.4	6.4	4.6	2.1	2.7	3.1	t	140.0	36.2	38.5	17.8
+7% Gly+1.4% L-Arg·HCl ²⁾	17.8	36.7	21.1	13.0	9.1	7.9	3.8	4.8	5.8	t	137.0	43.2	41.1	22.9
+0.9% L-Met ²⁾ (10C7GArgMet)														
	Liver μmoles/g liver													
10C	3.4	1.8	nd ⁴⁾	0.8	0.9	0.7	0.4	0.4	0.4	0.4	7.7	4.0	6.1	2.4
10C7G	2.9	2.1	nd	0.7	1.0	0.7	0.4	0.4	0.4	0.4	8.4	4.0	6.6	2.1
10C7GArgMet	2.7	1.8	nd	0.7	0.7	0.5	0.3	0.3	0.3	0.3	6.8	3.2	3.8	1.3

¹⁾ Values were obtained from groups of each 3 to 4 animals pooled. Asp and Cys were not detected.²⁾ L-Arg·HCl (1.4%) and L-Met (0.9%) equal the contents of L-arginine and L-methionine in a 30% casein diet.³⁾ Trace.⁴⁾ None detectable.

tissues was extremely lower than that found in the previous experiment²⁾ using glycine-¹⁴C. That the dilution of L-leucine-¹⁴C by body free leucine might be smaller than that of glycine-¹⁴C could explain this difference, because the concentration of glycine was higher than that of leucine in the plasma and liver (Table II). The concentrations of free amino acids in the plasma and the liver in Table II were generally similar to those of the previous reports.^{3,4)} The decrease of plasma threonine concentration in both of the excess glycine groups may suggest that the degradation of threonine is accelerated by the enhancement of serine dehydratase activity, because the enzyme activity was increased by

the supplementation of L-arginine and L-methionine to the 10% casein diet with or without glycine.^{3,4)}

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