EFFECTS OF AMINO ACID IMBALANCE ON POLYSOME PROFILES AND ^{14}C -LABELED AMINO ACID INCORPORATION INTO TISSUE PROTEINS IN RATS

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Summary Feeding of rats on a low casein diet supplemented with a small amount of methionine caused an enlargement of liver size and an accumulation of lipids in the liver. Experiments were conducted to examine the effects induced by the amino acid-imbalanced diet on the metabolism of dietary amino acids with special reference to protein synthesis. Protein and DNA contents of the liver per 100 g body weight with rats fed on the imbalanced diet were apparently higher than those of rats fed on the basal diet. Ingestion of the imbalanced diet clearly stimulated hepatic ribosome aggregation but not skeletal muscle ribosome aggregation. Incorporation of [^{14}C]leucine into liver protein was markedly increased in the group consisting of rats fed on the imbalanced diet (imbalanced group) and that into skeletal muscle was similar to the result with the group receiving the basal diet (basal group). Relative content of [^{14}C]leucine in plasma albumin was higher in rats of the imbalanced group, whereas that in plasma of very low-density lipoprotein was reduced. These results indicate that the unbalanced inter-organ or intra-organ utilizations of dietary amino acids for protein synthesis were produced by the condition of the amino acid imbalance. Such metabolic effects of the amino acid imbalance may result in the enlargement of liver size and the accumulation of lipids in the liver.

Keywords amino acid imbalance, polysome profile, methionine, threonine, protein synthesis

Supplementation of a small amount of methionine in a low casein diet stimulates the growth of rats but produces an accumulation of lipids in the liver and an enlargement of liver size (1). Additionally, a decrease in the concentration of the limiting amino acid threonine in the blood plasma is the one of the earliest biochemical changes observed in rats fed on an amino acid-imbalanced diet (1-3).

Yoshida *et al.* (4) indicated that in amino acid imbalance, created by adding to a low casein diet a large amount of a mixture of all but one of the indispensable amino acids, accelerated incorporation of the most limiting amino acid into liver protein led to the decrease in the limiting amino acid concentration in plasma. In a recent study, Yoshida (24) examined the utilization of the limiting amino acid for protein synthesis in the amino acid imbalance created by feeding a low casein diet supplemented with a small amount of methionine and found the unbalanced distribution of [^{14}C]threonine, the limiting amino acid, in various organs or in cell components. We further examined the effects of feeding a similar imbalanced diet on the metabolism of dietary amino acids with special reference to protein synthesis.

METHODS

Experiment 1. Young male Wistar strain rats of about 70 g body weight were used, six animals per group. They were individually housed in suspended wire-bottomed cages in an air-conditioned room which was maintained at a temperature of approximately 23°C, and were allowed free access to water. Experimental diets were an 8% casein diet (8C), an 8% casein diet supplemented with 0.3% L-methionine (8CM), an 8% casein diet supplemented with 0.3% L-methionine and 0.3% L-threonine (8CMT), and a 10% casein diet (10C). In all diets, sucrose was used as the sole source of carbohydrate because of its high lipogenic capability, and a 5.0% salt mixture (5), 0.85% vitamin mixture (5), 5.0% corn oil and 0.2% choline-Cl were included. Retinyl palmitate, ergocalciferol and α -tocopheryl acetate were also present (0.4 mg, 1.5 μg and 10 mg per 100 g diet, respectively). Dietary casein or supplemented amino acids were replaced by sucrose. Animals were fed on one of these diets *ad libitum* for 15 days. During the course of feeding, body weight change and food intake were determined. On day 15, rats were killed and liver, gastrocnemius muscle and small intestine (from pylorus to cecum) were immediately excised and weighed. Liver protein and lipid contents were determined by Lowry's method (6) and Folch's method (7), respectively.

Experiments 2, 3, and 4. Male Wistar rats (Table 2) were fed on a 25% casein diet for a 4-hr period (13:00–17:00) once a day for 10 days to adapt them to a meal-feeding regimen. They were then divided into two groups and were meal-fed on the 8C or 8CM diet (experiments 2 and 3). In experiment 4, adapted animals were fed on the 10C or 8CM diet for about 10 days. On the final day, rats were killed four hours after the start of the meal-feeding and the tissues were immediately excised and weighed.

Preparation of PMS from rat liver and skeletal muscle. A weighed portion of the liver was homogenized in 2 volumes of TKM-buffer (containing 0.025 M KCl, 0.05 M Tris-HCl pH 7.6 at 4°C, 0.05 M MgCl_2 and 0.25 M RNase-free sucrose) (8) with a loosely-fitting Potter-Elvehjem type teflon homogenizer. On the other hand,

skeletal muscle was cut with scissors and placed in 3 volumes of HIB-buffer (containing 0.25 M KCl, 0.01 M MgCl₂ and 0.01 M Tris-HCl pH 7.6 at 4°C) (9) and homogenized for 15 sec with the aid of a Polytron homogenizer (Kinematica H.m.b.H., Type PT 10/35, dial 6). The homogenates of liver and skeletal muscle were then centrifuged for 10 min at 9,000 *g* (average) in an RPR 18B rotor of a Hitachi 18 PR-3 Automatic High-Speed Centrifuge at 4°C to remove nuclei, unbroken cells and cell debris. The supernatant (PMS) was promptly frozen in liquid nitrogen and stored at -80°C until required for analysis. The effect of this operation on polysome aggregation was negligible.

Analysis of polysome sedimentation profile. Polysome profiles were obtained from the prepared PMS by the method previously reported (10). 0.2 ml of the liver PMS and 0.5 ml of the skeletal muscle PMS were used for each analysis.

Ribosomal distribution between free and membrane-bound states in rat liver. Free and membrane-bound ribosomes were isolated from rat liver PMS according to the method described by Blobel and Potter (11). Quantitative analysis of RNA in the isolated pellets containing free ribosomes was done by the method of Fleck and Munro (12). The percentage distribution of free ribosomal RNA to total ribosomal RNA was calculated on the assumption that 80% of the tissue RNA was ribosomal RNA.

Estimation of nucleic acids in tissues. RNA was quantitatively estimated by the orcinol method as modified by Kerr and Seraidarian (13), yeast RNA being used as a standard. DNA was determined by the modified diphenylamine method, with highly polymerized calf thymus DNA used as a standard.

Experiment 5. Male rats of the Sprague-Dawley strain (110 g) adapted to the same meal-feeding regimen as described above were separated into four groups (in groups of six animals), and were meal-fed 8C, 8CM, 8CMT, or 10C for 9 days. The compositions of these diets were essentially the same as those used in experiment 1 except for two changes: a) Corn oil was not included in these diets in order to diminish the plasma level of chylomicrons. Adjustments were made by changing the amount of sucrose present (by weight). b) For the preparation of agar-gel diet, each diet was dispersed in an equal quantity of hot water containing 3% of agar and was cooled at room temperature.

On day 9, animals were fed for two hours only (13.00–15.00) on one of these diets into which [*U*-¹⁴C]leucine (17.5 μCi/rat) was incorporated. Two hours after the meal-feeding, rats were anesthetized with ethyl ether and blood was taken by heart puncture. Then liver and leg muscle were rapidly removed, frozen and stored at -20°C until analyses were performed.

Spilled food and gastrointestinal contents were suspended in 1N HCl separately, heated for 30 min, filtered and aliquots were used for the determination of radioactivity with Bray's solution (14).

Tissues were homogenized in water, TCA-soluble and insoluble fractions were obtained and the radioactivity of each fraction was measured according to the method previously reported (15).

Very low-density plasma lipoproteins (VLDL) were purified by the method of Lindgren *et al.* (16) and incorporated radioactivity was determined. The protein content of VLDL was determined by Lowry's method. Plasma albumin was also purified according to the method of Swick and Ip (17) and incorporated radioactivity was measured. The albumin concentration of blood plasma was determined by the method of Rutstein *et al.* (18).

RESULTS

Daily food intake was unchanged between the groups fed on either 8C or 8CM but body weight of rats fed on 8CM markedly increased compared with that of rats fed on 8C (Table 1). Liver weight per 100 g body weight and protein content in the liver per 100 g body weight of rats fed on 8CM were significantly higher than those of the 8C group (Table 1).

Gain in body weight of rats fed on 10C was similar to that of the 8CM group but there was no difference in both liver weight per 100 g body weight and protein content of the liver per 100 g body weight between the 10C and 8C groups (Table 1).

These results indicate that the increase in the hepatic protein content of the 8CM group is greater than that in body weight.

The lipid accumulation in the liver of rats fed on 8CM was suppressed by the further supplementation of threonine to this diet without suppression of an increase in liver protein.

When rats were provided with these diets for nearly 10 days by the meal-feeding regimen, liver weight per 100 g body weight and protein content of the liver per 100 g body weight of rats fed on 8CM were also higher than those of rats fed on 8C (Table 2).

The ratios of RNA to DNA and of protein to DNA in the liver of the 8C group did not differ from those of the 8CM group (Table 3). However, it was found that total DNA content in the liver of rats fed on 8CM was significantly higher than that of the 8C group (Table 3). These results seem to indicate that the enlargement of liver size observed in the 8CM group mainly resulted from the increase of the number of hepatic cells.

Liver and skeletal muscle polysome aggregation levels of rats fed on these diets using the meal-feeding regimen were examined. Representative polysome profiles obtained from liver (Fig. 1) and skeletal muscle (Fig. 2) of rats fed on either 8C or 8CM (experiment 3) are shown. The data from these experiments are summarized in Table 4 as a percentage of each profile accounted for by monosomes and disomes. The percentage of monosomes and disomes per total ribosomes in the liver of rats fed on 8CM was significantly lower than that of rats fed on 8C or 10C in every case, indicating a clear effect of the imbalanced diet on hepatic polysome aggregation.

Nevertheless, there was no change in the percentages of monosomes and

Table 1. Effect of *ad libitum* feeding of an 8% casein diet supplemented with 0.3% Met or Met, Thr on tissue weight and liver composition (experiment 1).

Diet	Daily wt gain	Daily food intake	Tissue wt			
			Liver	Gastrocnemius muscle	Intestine ²	Liver lipids ¹
	(g)	(g)	(g)	(g/100 g body wt)	(g/100 g body wt)	(mg/100 g body wt)
8C	1.1 ± 0.1 ³	10.5 ± 0.6	4.37 ± 0.14	0.52 ± 0.02	4.58 ± 0.08	4.93 ± 0.07
8CM	2.1 ± 0.2 ⁴	10.8 ± 0.5	5.05 ± 0.23 ⁵	0.54 ± 0.02	4.09 ± 0.29	7.26 ± 0.51 ^{4,6}
8CMT	2.8 ± 0.1 ^{4,6}	10.9 ± 0.4	5.30 ± 0.24 ⁵	0.54 ± 0.03	4.32 ± 0.13	5.18 ± 0.26
10C	2.1 ± 0.2 ⁴	11.4 ± 0.5	4.70 ± 0.16	0.50 ± 0.01	4.42 ± 0.24	1,003 ± 10 ⁴
						822 ± 40

¹ Wet weight basis. ² From pylorus to cecum. ³ Means ± SEM. ⁴ Significantly different from 8C ($p < 0.01$). ⁵ Significantly different from 8C ($p < 0.05$). ⁶ Significantly different from 10C ($p < 0.01$).

Table 2. Effect of meal-feeding (13:00–17:00) of an 8% casein diet supplemented with 0.3% Met on weight gain and tissue weight (experiments 2, 3 and 4).

Exp. No.	Diet	Initial body wt	Daily wt gain	Daily food intake	Body wt at sacrifice	Tissue weight		
						Liver	Gastrocnemius muscle	Intestine ¹
Exp. 2	8C	(g) 57.3±2.9 ²	(g) 0.1±0.1	(g) 4.9±0.4	(g) 66.1±2.9	3.47±0.10	(g/100 g body wt) 1.22±0.04	0.45±0.01
	8CM	57.6±2.0	0.5±0.1 ³	5.0±0.2	72.6±2.1	3.76±0.03 ⁴	1.18±0.03	0.46±0.01
Exp. 3	8C	93.8±1.9	0.1±0.2	5.7±0.2	106.4±1.6	3.53±0.04		
	8CM	93.4±1.8	0.6±0.2	6.0±0.2	111.4±3.3	3.93±0.06 ³		
Exp. 4	10C	103.8±2.3	0.5±0.04	7.0±0.2	119.3±3.8	3.67±0.10		
	8CM	104.2±2.2	0.8±0.1 ⁵	7.2±0.4	122.9±3.7	3.85±0.05		

¹ A quarter of the length from pylorus to cecum. ² Means ± SEM. ³ Significantly different from 8C ($p < 0.01$). ⁴ Significantly different from 8C ($p < 0.05$). ⁵ Significantly different from 10C ($p < 0.05$).

Table 3. Effect of Met supplementation of an 8% casein diet on tissue composition (experiment 2).

Tissue	Diet	RNA	Protein (mg/g tissue)	DNA	RNA/DNA	Protein/DNA	Total DNA (mg)	Total protein (mg/100 g body wt)
Liver	8C	9.77 ± 0.25 ¹	176 ± 4	3.79 ± 0.19	2.60 ± 0.11	46.7 ± 1.8	8.64 ± 0.40	597 ± 21
	8CM	9.09 ± 0.17	168 ± 2	3.57 ± 0.14	2.56 ± 0.11	47.5 ± 8.8	9.69 ± 0.37 ⁴	632 ± 6
G. muscle ²	8C	1.85 ± 0.03	198 ± 2	1.12 ± 0.06	1.67 ± 0.10	178 ± 9	0.33 ± 0.01	88.5 ± 2.2
	8CM	2.15 ± 0.05 ³	189 ± 1 ³	0.98 ± 0.02	2.11 ± 0.11 ³	186 ± 9	0.33 ± 0.01	87.1 ± 1.3
Intestine ⁵	8C	5.05 ± 0.05	160 ± 2	3.11 ± 0.05	1.63 ± 0.03	51.3 ± 1.5	2.57 ± 0.08	194 ± 7
	8CM	4.67 ± 0.07 ³	161 ± 3	3.00 ± 0.06	1.56 ± 0.03	50.4 ± 1.3	2.57 ± 0.06	178 ± 2

¹ Means ± SEM. ² Gastrocnemius muscle. ³ Significantly different from 8C ($p < 0.01$). ⁴ Significantly different from 8C ($p < 0.05$). ⁵ A quarter of the length from pylorus to cecum.

Fig. 1. Representative hepatic polysome profiles of rats that were meal-fed (13:00–17:00) on 8C or 8CM diet for 12 days.

Fig. 2. Representative muscle polysome profiles of rats that were meal-fed (13:00–17:00) on 8C or 8CM diet for 12 days.

disomes for skeletal muscle polysome profiles between groups fed on 8C and 8CM and also between groups fed on 10C and 8CM.

Additionally, ribosomal distribution between free and membrane-bound states in the liver of rats fed on these diets was examined (experiment 3). However, as shown in Table 4, there was no difference in percentages of free ribosomal RNA between groups fed on either 8C or 8CM.

An isotope experiment *in vivo* was carried out to examine effects induced by

Table 4. Effect of Met supplementation of an 8% casein diet on ribosome-distributions between monosomes-disomes and polysomes in rat liver and skeletal muscle and between free and membrane-bound states in rat liver.

Exp. No.	Diet	Liver		Skeletal muscle
		Monosomes-disomes area	Free r-RNA/ Total r-RNA ¹	Monosomes-disomes area
		(%)	(%)	(%)
Exp. 2	8C	47.4 ± 2.0 ²		
	8CM	33.8 ± 1.0 ³		
Exp. 3	8C	37.2 ± 1.3	13.4 ± 0.5	39.1 ± 3.3
	8CM	30.4 ± 1.1 ³	14.1 ± 0.3	39.9 ± 1.1
Exp. 4	10C	38.4 ± 0.8		42.5 ± 0.8
	8CM	32.0 ± 0.7 ⁴		41.3 ± 0.6

¹ Values calculated on the assumption that 80% of tissue RNA were ribosomal RNA.

² Means ± SEM. ³ Significantly different from 8C ($p < 0.01$). ⁴ Significantly different from 10C ($p < 0.01$).

feeding the imbalanced diet on the metabolism of dietary amino acids, especially in relation to protein synthesis.

Uniformly labeled L-leucine, the plasma level of which is not affected by feeding the imbalanced diet, in contrast to the drastic reduction in the plasma level of the limiting amino acid (3), was administered orally with each diet for two hours (13:00–15:00). Rats were killed four hours after the beginning of the meal.

Percent absorption of [¹⁴C]leucine was calculated from the difference between the amount of radioactivity ingested and that recovered from the gastrointestinal tract. The value for the 8CM group was slightly lower than that for other three groups, but the difference was not significant.

Distribution of absorbed radioactivity in liver and skeletal muscle is shown in Table 5. Values for the percentage of absorbed radioactivity incorporated into the TCA-insoluble fraction of liver were significantly higher in the 8CM, 8CMT and 10C groups than in the 8C group, the 8CM group displaying the highest value. The value for the percentage of absorbed radioactivity in the TCA-soluble fraction of liver was significantly higher for the 8CM group than for the 8C group, and values for the 8CMT and 10C groups were intermediate.

On the other hand, values for the percentage absorbed radioactivity incorporated into the TCA-insoluble fraction of skeletal muscle were similar for all groups, and in the TCA-soluble fraction were slightly lower for the 8CM group than for the other three groups.

The incorporation of [¹⁴C]leucine into plasma albumin and plasma VLDL, which are two typical secretory proteins synthesized in liver, was also examined.

Table 5. Distributions of absorbed radioactivity in liver and skeletal muscle.¹

Liver			
	a) TCA insoluble fraction	b) TCA soluble fraction	a/b
(% of absorbed radioactivity)			
8C	6.75 ± 0.62 ²	0.86 ± 0.07	7.89 ± 0.67
8CM	16.6 ± 2.35 ³	1.18 ± 0.11 ⁴	14.3 ± 1.94 ⁴
8CMT	12.5 ± 0.87 ³	0.95 ± 0.09	13.6 ± 1.11 ⁴
10C	10.7 ± 1.04 ⁴	0.94 ± 0.04	11.4 ± 0.83 ⁴
Skeletal muscle			
8C	24.5 ± 2.4	0.86 ± 0.08	28.7 ± 1.3
8CM	24.1 ± 2.1	0.75 ± 0.06	32.0 ± 0.7
8CMT	22.2 ± 1.9	0.81 ± 0.04	27.9 ± 2.9
10C	22.7 ± 2.9	0.90 ± 0.08	25.3 ± 2.4

¹ Leg muscle. ² Means ± SEM. ³ Significantly different from 8C ($p < 0.01$) ⁴ Significantly different from 8C ($p < 0.05$).

Table 6. Distributions of absorbed radioactivity in plasma VLDL apoprotein and albumin.

Diet	Plasma VLDL		Plasma albumin		a/b
	Concentration	a) % of absorbed ¹⁴ C	Concentration	b) % of absorbed ¹⁴ C	
	(μg protein/ml ¹)	(%)	(mg albumin/ml)	(%)	(× 10 ⁻³)
8C	68.7 ± 1.9 ²	1.05 ± 0.13	48.1 ± 1.4	4.45 ± 0.20	2.39 ± 0.38
8CM	56.3 ± 0.2 ^{3,4}	0.93 ± 0.06	51.4 ± 2.4	6.87 ± 0.11 ^{3,4}	1.35 ± 0.07 ^{3,5}
8CMT	74.7 ± 8.7	1.23 ± 0.20	46.6 ± 3.1	5.86 ± 0.51	2.10 ± 0.02
10C	69.2 ± 3.9	1.02 ± 0.06	47.5 ± 5.3	5.43 ± 0.63	1.93 ± 0.32

¹ Plasma ml. ² Means ± SEM. ³ Significantly different from 8C ($p < 0.05$). ⁴ Significantly different from 10C ($p < 0.05$). ⁵ Significantly different from 8CM ($p < 0.01$).

The plasma albumin level of rats fed on 8CM was slightly higher than that of the other three groups and a marked increase in the percentage of absorbed radioactivity incorporated into the plasma albumin of rats fed on 8CM was observed as compared with that of rats fed on 8C or 10C (Table 6).

In contrast, the protein content of VLDL prepared by the floatation technique

from plasma of rats fed on 8CM was significantly lower than that of the 8C or 10C group and a slight decrease in the percentage of absorbed radioactivity incorporated into the plasma VLDL apoprotein of the 8CM group was observed as compared with that of the other three groups (Table 6).

Therefore, the ratio of the percentage of absorbed radioactivity in the plasma VLDL apoprotein to that in the plasma albumin of rats fed on 8CM was markedly lower than the ratios of the other three groups (Table 6).

DISCUSSION

Amino acid imbalances are commonly created by adding to a low protein diet a large amount of a mixture of all but one of the indispensable amino acids.

Altered plasma and tissue amino acid patterns, especially a significant reduction in the concentration of the most limiting amino acid, are considered to be related to the decreased food intake and growth retardation (1).

Yoshida *et al.* (4) showed that a single ingestion of the imbalanced diet increased incorporation of the limiting amino acid into liver proteins and, in contrast, reduced incorporation of that amino acid into muscle proteins.

On the other hand, Ip and Harper (19) found that ingestion of the threonine-imbalanced diet stimulated hepatic polysome aggregation and [^{14}C]leucine incorporation into liver protein more than did ingestion of the basal diet, when the tissue threonine pool had not been depleted prior to feeding of the adequate diets.

From these results, it has been suggested that the amino acid imbalance leads to acceleration of protein synthesis in certain organs, the limiting amino acid being consumed for this purpose, with the resultant decrease in the blood plasma concentration within a few hours after ingestion of the imbalanced diet. This would cause a deficiency of the limiting amino acid for protein synthesis in other organs.

We further examined effects induced by feeding the imbalanced diet on the metabolism of dietary amino acids with special reference to protein synthesis.

Ingestion of the imbalanced diet clearly stimulated hepatic polysome aggregation but the polysome aggregation in skeletal muscle was not affected. The isotope-incorporation experiments are consistent with the polysome-aggregation studies in that the imbalanced diet stimulates hepatic protein synthesis but the muscle protein synthesis is relatively unaffected (Table 5).

Thus the amino acid imbalance appears to affect the utilization of dietary amino acids for protein synthesis in liver and muscle in a different fashion. Furthermore, the incorporation of [^{14}C]leucine into plasma albumin was higher in rats of the imbalanced group whereas the incorporation into plasma VLDL was reduced (Table 6).

Thus, it appears that the utilization of dietary amino acids for synthesis of various proteins in liver is also differently affected by the present condition of amino acid imbalance.

It may be especially significant in relation to the fat accumulation in the liver

of rats fed on the imbalanced diet that incorporation of [^{14}C]leucine into plasma VLDL is relatively reduced in the imbalanced group. It is well known that the production of fatty liver after the administration of ethionine(20) or orotic acid(21) is ascribed to a decrease in lipoprotein synthesis or secretion of lipoprotein into blood plasma. It is generally considered that the plasma VLDL functions primarily in the transport of glycerides of endogenous origin out of the liver. There may be a possibility of relatively insufficient transport of lipids from the liver to extrahepatic tissue in the present experimental conditions.

In our previous experiments (22, 23), plasma levels of esterified fatty acids or VLDL were not reduced by feeding animals on the amino acid-imbalanced diet, *ad libitum*. In the present study, animals were meal-fed and a fairly large amount of carbohydrate was ingested in a short period leading to the stimulated lipogenesis in the liver. This may be a reason why the difference in the plasma level of VLDL appeared between the control and the imbalanced groups.

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