Quick Responses of Plasma Amino Acid Concentration to Changes in Dietary Amino Acid Levels in Rats

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Abstract: In order to evaluate the availability of plasma amino acid (AA) concentration as a parameter to determine AA requirements of rats within a short period, the response of the plasma AA concentration to changes in dietary AA levels was studied. In Experiment 1, to clarify whether the plasma AA concentration responded to changes in dietary AA levels and how long the response of the plasma AA concentration was maintained, 10 male rats were fed a casein diet for a 7 day period, and then the food was changed to AA diets. The serine (Ser), glycine (Gly) and threonine (Thr) levels were decreased from 120, 90 to 60% of the casein diet at 7-day intervals successively. Blood samples were taken at 2, 4 and 7 days after changing the diet, from the vena caudalis by vein puncture. In all cases, the plasma AA concentration responded to changes in dietary AA within 2 days, and the response was maintained until the 7th day. Experiment 2 was conducted to confirm that the plasma concentration of each AA responded to the changes in dietary AA levels at the first day and the responses of the plasma concentration of AA were maintained until the 4th day after changing the dietary AA levels, when the combination of metabolically unrelated AA was added. Five rats in A groups were fed diets with added arginine + Thr, histidine + methionine, lysine or isoleucine + Ser, and five rats in B groups were fed diets with added Gly + valine, leucine + phenylalanine or Thr + tyrosine+valine for a 4-day period successively. Blood samples were taken at 1 and 4 days after changing diets. Although the plasma concentrations of histidine, leucine, methionine, phenylalanine and Ser did not respond to changes in dietary AA levels, those of other AA responded at 1 day and the responses were maintained until the 4th day.

Key words: dietary amino acid levels, plasma amino acid concentration, young adult rats

Introduction

Since the 1970s, there have been no reports available on systematic studies of the amino acid (AA) require-

ments of rats, because a feeding trial to determine AA requirements is time-consuming and laborious, and requires a large number of animals. Furthermore, only one AA requirement is determined from one feeding

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trial. Although in feeding standards of livestock, only one pattern of AA requirement is presented [1, 10], the AA requirement is affected by many factors such as strain, age and physiological conditions of the animals, dietary protein, AA and metabolizable energy, and environmental conditions [6]. Because it is impossible to determine the AA requirements proportionate to the such factors by means of feeding trials, it has been desirable to develop a new, easy and exact method to determine the AA requirement. The plasma AA concentration is one of them. It has been recognized that the AA requirement determined from the plasma AA concentration agrees well with that determined in feeding trials in growing rats [8, 14], chicks [17], laying hens [5], young pigs [9] and young men [16]. In all of the above cited studies, since plasma samples are taken on the last day of feeding trials, there is no merit in shortening the trial period. Recently, it was reported that the plasma AA concentration responded to changes in dietary AA levels within 2 days, and the response of the plasma AA concentration was maintained for 3 weeks [7], and that it was possible to distinguish the response of the plasma concentration of each AA in laying hens when the multiple metabolically unrelated AA were added simultaneously [15]. These results suggested that it was possible to determine the multiple AA requirements simultaneously within a short period by using a small number of animals repeatedly.

In this study, therefore, to determine the AA requirement of rats and to confirm the usefulness of the plasma AA concentration as a parameter in determining the AA requirement in a short period, two experiments were conducted on young adult rats.

Materials and Methods

Experiment 1: Male Wistar rats (Japan SLC, Inc., Shizuoka, Japan) were given a commercial diet (Nihon Nosan Kogyo K.K., Kanagawa, Japan) and water *ad libitum* in individual wire cages in an environmentally controlled ($22 \pm 2^\circ\text{C}$ and lights on from 4:00 to 18:00) room. At 11 weeks of age, 10 rats weighing 300 ± 9 g were chosen, and fed an AA diet containing the same levels of essential AAs as those in the casein diet for 7 days. The dietary serine (Ser), glycine (Gly) and threonine (Thr) levels were changed from 120, 90 to 60% of the casein diet successively at 7-day intervals. Three

AA were chosen to elucidate the effects of dietary AA on the plasma concentration of other metabolically related AA.

Experiment 2: The objective of this experiment was to confirm the possibility of distinguishing the response of the plasma concentration of multiple AA to changes in dietary levels of multiple AA, when the metabolically unrelated AA were combined. The same rats at 27 weeks of age used in Experiment 1 were divided into 2 groups of 5 rats, A and B, the rats weighing 453 ± 16 and 456 ± 13 g, respectively, and fed the casein diet as above *ad libitum* for 4 days. In group A, the casein diet was switched to the casein diet supplemented with arginine (Arg) + Thr, histidine (His) + methionine (Met), lysine (Lys) and isoleucine (Ile) + Ser successively at 4-day intervals, and in group B the casein diet was switched to the casein diet supplemented with Gly + valine (Val), leucine (Leu) + phenylalanine (Phe), and Thr + tyrosine (Tyr) + Val, respectively, at 4-day intervals. Because the changes in dietary AA affected the plasma concentration of metabolically related AA in Experiment 1, the metabolically unrelated AA were combined.

Plasma concentration of AA and body weight: At 13:00 to 14:00, about 0.4 ml blood samples were taken from the *vena caudalis* by vein puncture with heparinized syringes at 7 days on the AA diet and at 2 and 4 days after switching diets in Experiment 1. The plasma AA concentration responded to changes in dietary AA levels at 2 days and the response of the plasma AA concentration was maintained for 7 days in Experiment 1. Blood samples were therefore taken at 1 and 4 days after switching diets to investigate whether the responses of the plasma AA concentration to changes in dietary AA were quicker in Experiment 2. The plasma AA concentration was determined with a HPLC as described by Fujimura *et al.* [4]. Body weight and diet intake were recorded before every blood sampling, and the switching of each experimental diet was conducted after blood sampling.

Diet: The compositions of the casein and AA diets, and the mixture of AA (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) are shown in Table 1. The casein diet was formulated to contain 20% casein and 0.3% cystine (Cys) as an AA source, to which other nutrients were added as described by Reeves *et al.* [12]. In the AA diet, casein and Cys were replaced by 17.64% of

AA mixture containing the same amount of essential AA except Ser, Gly and Thr as in the casein diet and 5.22% glutamic acid (Glu). The levels of Ser, Gly and Thr were adjusted to 60, 90 and 120% of the casein

diet by replacing the same amount of Glu. In Experiment 2, 50% of each AA contained in the casein diet was added to the casein diet without replacing Glu. In group A, Arg + Thr, His + Met, Lys and Ile + Ser was added, and in group B, Gly + Val, Leu + Phe and Thr + Tyr + Val was added.

Table 1. Compositions of casein and amino acid diets, and amino acid mixture (%)

Ingredients	Casein diet	AA diet	³ AA mixture	
Corn starch	52.95	55.80	Arg	0.721
Casein (87.2% CP) ¹⁾	20.00	—	Cys	0.341
Sucrose	10.00	10.00	Gly	0.358
Cellulose powder	5.00	5.00	His	0.556
Soybean oil	7.00	7.00	Ile	0.964
Mineral mixture ²⁾	3.50	3.50	Leu	1.800
Vitamin mixture ²⁾	1.00	1.00	Lys	1.600
Choline bitartate	0.25	0.25	Met	0.536
2-t-Butylhydroquinone	0.0014	0.0014	Phe	0.992
L-Cystine	0.30	—	Ser	1.162
Amino acid mixture ³⁾	—	17.64	Thr	0.858
			Trp	0.210
Analytical (calculated)			Tyr	1.074
CP (%)	17.66	14.66	Val	1.292
ME (kcal/g)	3.20	3.19	Glu	5.220

¹⁾From Nihon Nosan Kogyo K. K., Kanagawa, Japan.

²⁾See Reeves *et al.* [12] From Nihon Nosan Kogyo K. K., Kanagawa, Japan.

Statistical analysis: For statistical significance, the two-way ANOVA was employed with the General Linear Models procedure in SAS® [13]. Significant differences between means were assayed by Duncan's new multiple range test [3].

Results

Experiment 1: As shown in Table 2, when dietary Ser decreased from 120, 90 to 60% in the AA diet, the diet intake decreased, but it was not reflected in body weight. The plasma Ser concentration remained constant, but those of Gly and Thr increased with decreasing dietary Ser levels. When dietary Gly decreased, body weight changed, diet intake and plasma concentration of Ser and Thr were not affected, but the plasma Gly concentration decreased with decreasing dietary Gly levels. When dietary Thr decreased, the body weight change, diet intake and plasma concentration of Ser

Table 2. Plasma amino acid concentration of male rats two and four days after switching diets successively at four-day intervals in Experiment 1

Treated Amino acid	Ratio to Casein (%)	Body weight change (g/d)	Diet intake (g/d)	Plasma amino acid concentration (Days after changing diet)								
				Ser (nmol/ml)			Gly (nmol/ml)			Thr (nmol/ml)		
				0	2	4	0	2	4	0	2	4
Control (None)	100	1.40	17.9	210.3			165.2			233.1		
Ser	60	0.92	14.5 ^y		211.1	203.4		176.3 ^a	160.9 ^{ab}	242.0 ^a	249.7 ^a	
	90	0.62	16.0 ^{xy}		206.1	219.8		163.5 ^{ab}	180.4 ^a	214.9 ^{ab}	230.8 ^{ab}	
	120	0.63	17.8 ^x		205.5	198.7		132.3 ^b	144.4 ^b	187.2 ^b	184.1 ^b	
	Pooled SE	0.04	0.5			14.7			13.2		10.3	
Gly	60	1.08	16.4		217.6	206.5		141.2 ^b	138.0 ^b	238.8	235.9	
	90	0.87	16.2		208.6	217.0		156.6 ^{ab}	152.3 ^{ab}	231.7	242.2	
	120	1.10	15.9		209.8	201.5		179.0 ^a	164.6 ^a	233.6	216.8	
	Pooled SE	0.06	0.4			12.4			8.3		13.0	
Thr	60	0.03	16.2		205.0	200.4		161.4	171.3	128.7 ^c	122.1 ^c	
	90	1.02	16.8		210.9	210.1		154.6	162.3	212.8 ^b	208.2 ^b	
	120	1.10	15.8		212.2	210.9		157.7	163.2	303.7 ^a	280.2 ^a	
	Pooled SE	0.05	0.5			14.7			10.0		17.9	

Each value is the mean for 10 rats. ^{xy}Means of body weight change and diet intake in the same column in the same group and ^{a-c}means in the same column of amino acid concentration in rats in the same dietary groups with different superscripts differ significantly ($p < 0.05$).

Table 3. Plasma amino acid concentration of male rats one and four days after changing dietary amino acid level successively at four-day intervals in Experiment 2

Group	Added amino acid	Body weight change (g/d)	Diet intake (g/d)	Plasma amino acid	Before addition		After addition		After removal		Pooled SE
					4 days	1 day	1 day	4 days	1 day	4 days	
					(nmol/ml)		(nmol/ml)		(nmol/ml)		
A	Arg + Thr	−1.03	24.0 ^x	Arg Thr	66.7 ^c	75.7 ^{bc}	97.2 ^a	87.1 ^{bc}	82.3 ^{ab}	69.6 ^c	6.8
					197.7 ^b	229.3 ^b	388.2 ^a	374.1 ^a	234.0 ^b	206.6 ^b	16.4
	His + Met	−0.82	20.5 ^x	His Met	61.7	58.4	63.4	62.9	63.4	61.8	4.1
					21.2	20.0	23.4	25.5	23.7	24.0	17.8
	Lys	−1.89	16.7 ^y	Lys Arg	264.3 ^b	234.2 ^b	308.9 ^a	299.8 ^a	228.9 ^b	233.8 ^b	17.2
					87.1	82.9	86.8	80.2	72.5	73.5	6.0
	Ile + Ser	−1.40	18.9 ^{xy}	Ile Ser	46.4 ^{ab}	42.4 ^b	55.5 ^a	51.1 ^{ab}	48.0 ^{ab}	42.6 ^b	3.4
					234.0	216.8	247.0	233.0	229.0	219.9	10.3
	Pooled SE		0.05	1.1							
	B	Gly + Val	−1.03	18.7 ^x	Gly Val	173.0 ^{ab}	154.3 ^b	182.9 ^a	173.6 ^{ab}	144.7 ^b	173.3 ^{ab}
125.0 ^{ab}						121.3 ^b	130.4 ^{ab}	146.1 ^a	120.5 ^b	119.6 ^b	9.8
Leu + Phe		−0.94	19.6 ^x	Leu	76.1	81.7	72.4	96.2	85.1	83.7	5.7
				Phe	34.3	31.7	30.1	36.1	32.3	34.8	3.0
				Tyr	53.8 ^b	57.9 ^b	68.5 ^a	74.7 ^a	68.0 ^a	73.8 ^a	3.9
Thr + Tyr + Val		−1.36	16.1 ^y	Thr	229.3 ^b	234.8 ^b	401.1 ^a	413.9 ^a	245.9 ^b	211.2 ^b	21.1
				Tyr	68.5 ^a	74.7 ^a	68.0 ^a	73.8 ^a	48.9 ^b	54.5 ^b	4.2
				Phe	30.1	36.1	32.3	34.8	36.4	37.0	2.3
				Val	115.7 ^b	114.9 ^b	136.3 ^a	127.5 ^{ab}	118.8 ^{ab}	120.4 ^{ab}	9.1
Pooled SE		0.07	0.7								

Each value is the mean for five rats. ^{xy}Means of body weight change and diet intake in the same column in the same group and ^{a-c}means in the same row of amino acid concentrations in rats in the same dietary groups with different superscripts differ significantly ($p < 0.05$).

and Gly were not affected, but that of Thr decreased with decreasing dietary Thr levels. In all cases, there were no differences between the plasma AA concentrations at 2 and 4 days after switching diets.

Experiment 2: The results obtained are summarized in Table 3. Only when Arg + Thr and Thr + Tyr + Val diets were fed, body weights and diet intakes decreased.

To avoid complexity, the plasma concentrations of added and metabolically related AA are presented. When the Arg + Thr, Lys, Gly + Val and Thr + Tyr + Val diets were fed, the plasma concentration of all added AA *per se* increased at day 1, and remained at high until 4 days after adding AA. When the added AAs were removed, the plasma concentration of removed AAs decreased at day 1 and remained low 4 day after removing added AA. There were no differences in the plasma AA concentrations at 1 and 4 days after changing dietary AA levels. When the Ile + Ser diet was fed,

only the Ile responded in a similar manner to the abovementioned AA. When His + Met or Leu + Phe was added or removed from the casein diet, the plasma concentration of all AAs remained unaffected. When the Lys or Thr + Tyr + Val diet was fed, the plasma concentration of metabolically related Arg or Phe remained constant, but when the Leu + Phe diet was fed, the plasma Tyr concentration responded to the change in the dietary Leu + Phe level.

Discussion

Although the dietary Ser, Gly and Thr were replaced with the same amount of Glu in Experiment 1, the added AA was not replaced with Glu in Experiment 2, so that the dietary crude protein increased ranging from 0.62% by adding His + Met to 1.06% by adding the mixture of Thr, Tyr and Val. These small differences (1.06–0.62%)

in crude protein might not affect the performance and plasma AA concentrations. Because blood samples were repeatedly taken 19 times in 36 days in Experiment 1, and 12 times in 22 days in Experiment 2, the young adult rats which were old enough to overcome blood sampling stress, were used. During the experimental period, body weight change ranged from 0.03 to 1.10 and -0.82 to -1.89 gram per day, in Experiments 1 and 2, respectively, which were less than that in control rats fed a commercial diet, 1.96 and 0.04 gram per day at 11 and 27 weeks of age. The poor body weight gain might be due to stress caused by frequent blood samplings and changes in AA levels.

When the dietary Gly and Thr decreased, the plasma Thr concentration decreased lineally, and those of other AA remained constant but when the dietary Ser decreased, the plasma Ser concentration remained constant, those of Gly and Thr had decreased at 2 days and the response of their plasma concentration remained constant until 7 days after changing dietary Ser levels. These results indicated that the quick response of the plasma concentration of these AA made it possible to determine AA requirements at 2 days after changing dietary AA levels using the same rats repeatedly, if the stress of frequent blood sampling and changing dietary AA levels were lightened.

In a metabolic map [2], Thr dehydratase converts Thr to α -ketobutyric acid, Thr aldolase converts Thr to Gly and acetaldehyde, and Thr dehydrogenase converts Thr to 2-amino-3-oxobutyric acid. Among them, the degradation of Thr by Thr aldolase to Gly is reported to be most important in mammals. Gly and Ser are interconvertible. Therefore, it was very curious that the plasma concentration of Gly and Thr decreased when the dietary Ser increased.

As observed in laying hens [15], the plasma concentration of seven AAs (Arg, Gly, Ile, Lys, Thr, Tyr and Val) responded to changes in their dietary levels the first day after these AA were added or removed, and the response of the plasma concentration of these AA was maintained for 7 days, so that it might be possible to estimate AA requirements within 2 days, repeatedly using a small number of rats by changing dietary AA levels at 2-day intervals. If a large number of rats were put into 5 or 6 dietary groups, the AA requirement might be estimated from the plasma AA concentration at 2 day after changing diets. Furthermore, if the meta-

bolically unrelated AAs were combined, it might be possible to determine the AA requirements from one trial.

Because the plasma concentration of five AAs (His, Leu, Met, Phe and Ser) did not respond to changes in their dietary levels, it was impossible to determine requirements of these AA from their plasma concentration. The observation that the plasma Met concentration did not respond to changes in the dietary Met level in rats, might indicate that Met was limiting in the Cys-added casein diet. In broilers [11] and laying hens [15] the plasma Met concentration did not respond to changes in dietary Met levels, when the dietary Met was less than the requirement level, and increased lineally with increasing dietary Met levels, when the dietary Met was higher than the requirement level.

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