Influence of time lag in amino acid absorption on nitrogen retention in rats

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Depending on the form of nitrogen sources, there is a time lag in amino acid absorption. However, the influence of such a time lag on nitrogen retention is not known. In the present experiment we created a time lag in amino acid absorption and observed nitrogen retention in rats. Two unbalanced amino acid mixtures were prepared. The equal amount of combination of these mixtures makes the amino acid pattern of egg protein. Rats were given these two unbalanced amino acid mixture diets alternatively at 08:00–09:30 hr, 11:00–12:30 hr, 14:00–15:30 hr, and 17:00–18:30 hr for 14 days. Control rats were given egg-protein-pattern amino acid mixture diet at the same time and periods as the experimental group. Dietary amino acid levels were 5% and 10%. Although the control and experimental rats ate similar amounts of each amino acid, there was a time lag in the amino acid absorption. Plasma aminograms, hematologic values, body protein concentration, and growth were very similar. The results indicate that nitrogen utilization is not influenced by a relatively small time lag in amino acid absorption.

Keywords: amino acids; absorption; nitrogen retention; elemental diet; rats

Introduction

In the late 1940s some researchers studied the effect of ingestion time of protein and amino acids on nitrogen retention by the body[1–5] and concluded that all the essential amino acids must be available simultaneously for effective protein synthesis. Recent studies on the absorption of peptides and amino acids have shown that the amount of amino acids absorbed from a peptide diet is greater and the pattern of absorption is better balanced than from an amino acid-mixture diet.[6–10] The results of the above studies led to the impression that nitrogen retention is more efficient with a peptide diet than with a free amino acid-mixture diet and peptide from a nitrogen source is recommended rather than free amino acids in elemental diets.[10] However, our previous study showed that the efficiency of nitrogen retention in protein, peptide, and amino acid-mixture diets were similar among normal, protein-deficient, gastrectomized, or hepatectomized rats.[11] It was therefore not clear whether the time lag in amino acid absorption really affects nitrogen retention by the body and we designed to elucidate this.

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Materials and methods

Diets

We made two unbalanced pattern amino acid mixtures as follows. Amino acids of egg protein[12] were categorized into two groups (A and B) according to their absorption rates as reported by Silk et al.[10] Group A included the rapidly absorbable amino acids (ile, leu, met, cys, tyr, arg, ala, glu, and pro) and group B included those that are absorbed slowly (lys, phe, thr, val, his, trp, asp, gly, and ser). The first unbalanced mixture contained two-thirds of group A and one-third of group B (group A rich mixture) and the second unbalanced mixture contained one-third of group A and two-thirds of group B (group B rich mixture). The amino acid pattern of the control diet simulated egg protein. With these amino acid mixtures, 5% and 10% amino acid diets were prepared as shown in *Table 1*. All the diets contained 5% corn oil, 2% cellulose, 5% mineral mixture (Oriental Yeast Co., Tokyo, Japan), and 1% vitamin mixture (Oriental Yeast Co.). The remaining part was carbohydrates (α-starch: sugar = 2:1). Lysine was added as hydrochloride.

Animals

Male Wistar rats (purchased from Tokushima Experimental Animal Research Center, Tokushima, Japan) weighing about 80 g, were kept in separate cages in an air-conditioned room at 25 ± 2° C with a 12-hr light period from 08:00–20:00 hr. The rats were divided into four groups of six animals. They were 5% and 10% unbalanced amino acid-mixture diet groups and their controls. The feeding times of the diets were from 08:00–09:30 hr, 11:00–12:30 hr, 14:00–15:30 hr, and 17:00–18:30 hr, giving a 90 min. interval between each feeding time. Prior to the experimental diets, the animals were fed a commercial stock diet (Clea Japan Inc., Tokyo, Japan) on this feeding schedule for 9 days. Group A and group B rich mixture diets were fed alternatively at the four feeding times for 14 days. The control diets were also fed at the same feeding time. Body weight was measured daily at 10:00 hr. Food intake of each meal was weighed. Tap water was supplied automatically. At 11:00 hr on day 15, 90 min. after the first diet, half of the animals in each group were anesthetized with ether and blood was withdrawn from the vena cava into a heparinized syringe. Part of the whole blood was used to measure hematocrit and hemoglobin, the rest was centrifuged, and the plasma was stored at −20° C for protein analysis. The contents of the stomach and small intestine were washed out with cold saline and stored at −20° C for analyses. The carcass was also stored at −20° C for body protein determination. At 14:00 hr on the same day, 90 min. after the second diet, the remaining three animals of each group were killed and samples were treated in the manner as above.

Analyses

Sulfosalicylic acid was added to the contents of the stomach and small intestine to make the final concentration of the acid 5%, and the mixture was centrifuged at 2500g for 15 min. The supernatant was used for amino acid analysis with a high speed automatic amino acid analyzer (IRICA Model A-330, IRICA Kogyo Co., Kyoto, Japan) and also for nitrogen determination by the Kjeldahl method.[13] Hematocrit was measured by centrifugation[14] and hemoglobin by the cyanmethemoglobin method.[15] The method of analysis of the plasma amino acids was similar to that of the gastrointestinal contents. The carcass was dried at 110° C for 24 hrs and then

Table 1 Composition of experimental diets

Ingredient	5% amino acid diet: Control	5% amino acid diet: Group A rich mixture	5% amino acid diet: Group B rich mixture	10% amino acid diet: Control	10% amino acid diet: Group A rich mixture	10% amino acid diet: Group B rich mixture
			g/kg			
Free amino acids[a]						
Group A						
L-Ile	3.1	3.9	2.0	6.1	7.8	3.9
L-Leu	4.3	5.5	2.7	8.6	10.9	5.4
L-Met	1.6	2.1	1.0	3.3	4.2	2.1
L-Cys	1.2	1.5	0.8	2.4	3.0	1.5
L-Tyr	2.0	2.6	1.3	4.0	5.1	2.6
L-Arg	3.0	3.8	1.9	5.9	7.5	3.8
L-Ala	2.9	3.7	1.8	5.7	7.3	3.7
L-Glu	6.2	7.9	3.9	12.4	15.7	7.9
L-Pro	2.0	2.6	1.3	4.0	5.1	2.6
Group B						
L-Lys·HCL	4.2	3.0	5.9	8.6	5.9	11.8
L-Phe	2.8	1.0	3.9	5.6	3.9	7.8
L-Thr	2.5	1.7	3.5	5.0	3.5	6.9
L-Trp	0.7	0.5	1.0	1.4	1.0	2.0
L-Val	3.3	2.3	4.6	6.6	4.6	9.3
L-His	1.2	0.8	1.7	2.4	1.7	3.3
L-Asp	4.6	3.3	6.5	9.3	6.5	13.0
Gly	1.6	1.1	2.2	3.2	2.2	4.5
L-Ser	3.7	2.6	5.2	7.4	5.2	10.4
Carbohydrate[b]	769	769	769	818	819	819
Corn oil	50	50	50	50	50	50
Mineral[c]	50	50	50	50	50	50
Cellulose + vitamins[d]	10	10	10	10	10	10
Cellulose	20	20	20	20	20	20

[a]Donated by Otsuka Pharmaceutical Co., Tokushima, Japan.
[b]α-starch:sucrose, 2:1 ratio.
[c]Obtained from Oriental Yeast Co., Tokyo, Japan. The mixture consisted of (mg/kg of diet): $CaHPO_4 \cdot 2H_2O$, 7,280; KH_2PO_4, 12,860; NaH_2PO_4, 4,680; NaCl, 2330; Ca-lactate, 17,550; Fe-citrate, 1,590; $MgSO_4$, 3,590; $ZnCO_3$, 55; $MnSO_4 \cdot 4\text{-}6H_2O$, 60; $CuSO_4 \cdot 5H_2O$, 15; KI, 5.
[d]Obtained from Oriental Yeast Co. Composition expressed units or mg of vitamins per kg of diet: thiamin·HCl,12; riboflavin, 40; pyridoxine·HCl, 8; vitamin B-12, 0.005; ascorbic acid, 300; D-biotin, 0.2; folic acid, 2; calcium pantothenate, 50; ρ-aminobenzoic acid, 50; niacin, 60; inositol, 60; choline chloride, 2,000; retinyl acetate, 5,000 IU; ergocalciferol, 1,000; tocopheryl acetate, 50; menadione, 52. The cellulose was used to increase the bulk of the vitamin mixture.

homogenized with a kitchen mixer and its nitrogen content was determined by the Kjeldahl method.[13]

Statistics

Student *t* test was used to determine the statistical significance among the means. Values were considered different at a significance level of $P < 0.05$.

Results

Figure 1 shows the food intakes of the rats fed the 5% and 10% amino acid diets for 14 days. The food intakes of the experimental and control groups were similar. The intakes of the group A and B amino acid rich mixtures were also similar.

Figure 2 shows the changes in the body weight of the rats when the commercial stock diet and the amino acid diets were fed. The changes were very similar among the experimental and control groups when the 5% and 10% amino acid diets were fed.

Table 2 shows the amount of amino acids absorbed between 08:00 hr–11:00 hr and 11:00 hr–14:00 hr in the rats fed 5% or 10% amino acid diets. Between 08:00 hr–11:00 hr, absorbed amino acids of group A tended to be higher while those of group B tended to be lower in the experimental groups than in the control groups for both 5% and 10% amino acid diets. The reverse was observed in the results between 11:00 hr–14:00 hr. The pattern of amino acids absorbed therefore reflected that of the amino acids ingested. That is, the absorption patterns in the control groups were similar to that of egg protein, and in the experimental groups the two unbalanced amino acid patterns alternated every 3 hr.

Table 3 shows the plasma aminograms 90 min. after the first and second diets on day 15. Although the absorbed amino acid patterns were different between the experimental and control groups, the plasma aminograms were similar for each killing time and each amino acid diet with some exceptions that did not reflect the absorbed amino acid pattern.

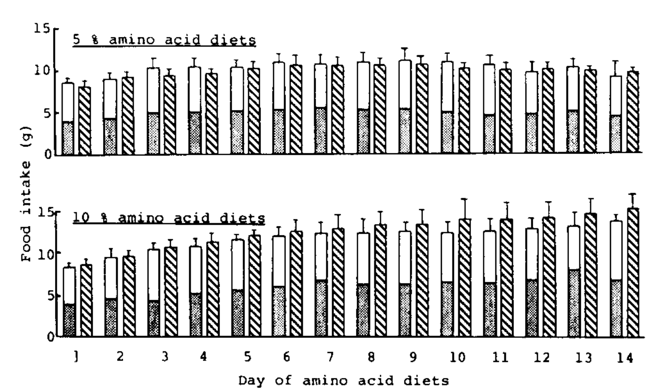

Figure 1 Daily intake by rats of group A amino acid rich mixture diet (dotted area of the left column), group B amino acid rich mixture diet (open area of the left column), or the control diet (hatched column) when they were given 5% (upper figure) or 10% (lower figure) amino acid diets. Values are means ± SD for six rats per group.

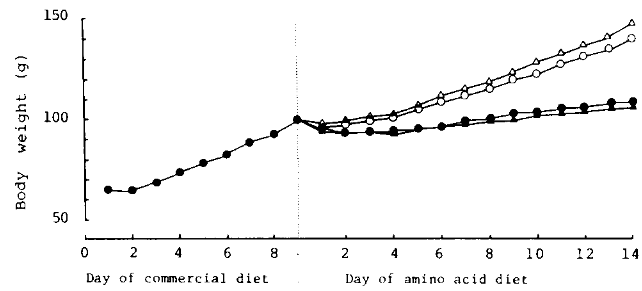

Figure 2 Changes of body weight in rats when they were fed the commercial stock diet (●), the experimental diet of 5% (▲) or 10% (△) amino acid mixture, and the control diet of 5% (●) or 10% (○) amino acid mixture. Values are means ± SD of six rats per group.

Table 4 shows the hematocrit values and the concentrations of hemoglobin, plasma protein, and carcass protein. The values of all the items were similar among the experimental and control groups.

Discussion

Although in the present study the patterns of amino acids absorbed in the experimental groups changed every 3 hr from 08:00–18:30 hr and were different from those of the control groups, nitrogen retention in the experimental groups was similar to that of the control groups, indicating that a 3-hr delay in absorbing some of the egg-pattern amino acids does not affect nitrogen retention at all. However, before we make this conclusion, it should be assured that the dietary amino acid levels were far above the requirement level. If so, even the amino acid absorbed least is quantitatively enough to support maximum growth, and the effect of the absorbed pattern of amino acids on nitrogen retention by the body cannot be observed. Barness et al.[16] reported that the 10% egg-pattern amino acid diet is just enough to support optimal growth in growing rats. Therefore, 5% and 10% dietary amino acid levels used in this study were proper.

Some researchers studied the effect of the ingestion time of an amino acid diet on nitrogen retention by the body.[2-5] Schaeffer and Geiger,[2,3] fed rats tryptophan-, lysine-, or methionine-deficient diets for 12 hr and then tryptophan, lysine, or methionine diets for the subsequent 12 hr. Geiger et al.[4] observed in rats the effect of 12-hr delayed supplementation with protein to be superior in quality. On the other hand, Leverton and Gram[5] compared the effect of the supplementation of animal proteins to vegetable proteins contained in lunch and dinner, and to those contained in all three meals. All of these researchers observed the poor utilization of nitrogen in the animals or subjects given the amino acid-deficient diet and the supplementary diet separately, compared with those given the complete amino acid diets, and concluded that all the amino acids must be available in tissues simultaneously. However, in these studies the supplementation of the deficient amino acids was delayed greatly.

Table 2 Amount of amino acid (mg) absorbed between 08:00 hr and 11:00 hr and 11:00 hr and 14:00 hr in rats fed 5% or 10% amino acid diets

	5% amino acid diet groups				10% amino acid diet groups			
	08:00 hr–11:00 hr		11:00 hr–14:00 hr		08:00 hr–11:00 hr		11:00 hr–14:00 hr	
Amino acids	Cont[a]	Exp[a]	Cont	Exp	Cont	Exp	Cont	Exp
Group A amino acids								
Ile	5 ± 1[b]	6 ± 1	13 ± 2	6 ± 2[c]	14 ± 4	24 ± 4*	39 ± 5	27 ± 3*
Leu	8 ± 1	9 ± 1	19 ± 2	10 ± 2*	25 ± 2	34 ± 6	55 ± 9	41 ± 14
Met	5 ± 1	5 ± 1	8 ± 2	5 ± 1	12 ± 1	14 ± 1	24 ± 4	17 ± 3
Cys	4 ± 1	3 ± 1	6 ± 1	4 ± 1	11 ± 1	14 ± 3	18 ± 4	14 ± 4
Tyr	4 ± 1	4 ± 1	9 ± 1	5 ± 1*	14 ± 1	20 ± 5	26 ± 5	20 ± 7
Arg	5 ± 1	7 ± 1	13 ± 2	7 ± 1*	16 ± 1	21 ± 2*	37 ± 6	29 ± 6
Ala	2 ± 1	5 ± 1*	11 ± 2	4 ± 1*	12 ± 1	16 ± 1*	33 ± 6	24 ± 10
Glu	9 ± 1	13 ± 2*	24 ± 3	12 ± 1*	34 ± 2	44 ± 3*	77 ± 9	58 ± 9
Pro	2 ± 1	4 ± 2	7 ± 1	4 ± 1*	8 ± 4	10 ± 2	21 ± 3	18 ± 8
Group B amino acids								
Lys	5 ± 1	4 ± 1	14 ± 2	23 ± 2*	22 ± 3	14 ± 1	42 ± 7	81 ± 12*
Phe	3 ± 1	3 ± 1	12 ± 1	19 ± 1*	14 ± 1	11 ± 2	33 ± 5	66 ± 12*
Thr	5 ± 2	3 ± 1	10 ± 1	15 ± 2*	10 ± 1	7 ± 1	28 ± 4	57 ± 10*
Val	3 ± 1	2 ± 1	13 ± 2	19 ± 3*	14 ± 1	11 ± 1*	37 ± 4	73 ± 15*
His	8 ± 1	6 ± 1	5 ± 1	7 ± 1	6 ± 1	5 ± 1	14 ± 2	27 ± 5*
Asp	2 ± 1	5 ± 1*	19 ± 1	32 ± 1*	26 ± 1	20 ± 2*	58 ± 8	112 ± 17*
Gly	6 ± 1	4 ± 2	5 ± 1	9 ± 2*	6 ± 1	4 ± 1	17 ± 3	35 ± 12*
Ser	24 ± 1	15 ± 1*	15 ± 1	24 ± 1*	21 ± 2	15 ± 1*	46 ± 8	88 ± 17*

[a]Cont and Exp stand for the control and experimental groups, respectively.
[b]Values are means ± SD for three rats.
[c]Statistical analysis was done by Student *t* test. Comparison was made between the values of control and experimental groups in each killing time and each dietary group.
*Significant difference ($P < 0.05$).

Table 3 Plasma aminograms ($\times 10^{-1}$ μmol/100 mL) of rats fed 5% or 10% amino acid diets for 14 days and killed at 11:00 hr or 14:00 hr

	5% amino acid diet groups				10% amino acid diet groups			
	Killed at 11:00 hr		Killed at 14:00 hr		Killed at 11:00 hr		Killed at 14:00 hr	
Amino acids	Cont[a]	Exp[a]	Cont	Exp	Cont	Exp	Cont	Exp
Group A amino acids								
Ile	24 ± 10[b]	24 ± 1	52 ± 4	37 ± 9	61 ± 14	75 ± 18	84 ± 13	46 ± 20
Leu	27 ± 3	36 ± 5	67 ± 5	40 ± 10*[c]	51 ± 23	100 ± 21	88 ± 7	35 ± 3*
Met	22 ± 2	29 ± 2*	45 ± 11	24 ± 1	55 ± 11	66 ± 4	55 ± 13	30 ± 15
Cys	37 ± 10	51 ± 9	61 ± 13	51 ± 5	62 ± 27	69 ± 15	85 ± 6	49 ± 21*
Tyr	28 ± 5	30 ± 4	36 ± 9	34 ± 3	66 ± 8	68 ± 26	103 ± 18	65 ± 10*
Arg	21 ± 2	19 ± 8	28 ± 7	25 ± 5	34 ± 12	62 ± 13	53 ± 14	41 ± 6
Ala	325 ± 80	383 ± 70	500 ± 24	530 ± 96	705 ± 87	727 ± 47	864 ± 74	855 ± 47
Glu	73 ± 15	68 ± 11	72 ± 15	85 ± 10	122 ± 40	176 ± 57	137 ± 47	148 ± 42
Pro	150 ± 27	149 ± 21	140 ± 66	149 ± 10	251 ± 39	255 ± 66	217 ± 11	151 ± 14*
Group B amino acids								
Lys	84 ± 10	69 ± 5	118 ± 9	99 ± 18	135 ± 41	91 ± 39	56 ± 7	98 ± 25*
Phe	30 ± 6	18 ± 7	43 ± 11	47 ± 6	50 ± 8	36 ± 5	52 ± 6	51 ± 8
Thr	124 ± 31	166 ± 15	171 ± 27	171 ± 20	468 ± 38	376 ± 83	774 ± 93	522 ± 88*
Val	56 ± 5	89 ± 18*	137 ± 44	154 ± 38	156 ± 15	168 ± 19	227 ± 71	382 ± 96*
His	54 ± 8	37 ± 11	65 ± 24	53 ± 11	39 ± 12	36 ± 9	56 ± 7	49 ± 7
Asp	13 ± 4	18 ± 3	20 ± 5	27 ± 12	26 ± 2	28 ± 9	27 ± 6	25 ± 3
Gly	228 ± 7	215 ± 17	211 ± 16	229 ± 25	289 ± 50	199 ± 55*	329 ± 28	268 ± 24*
Ser	285 ± 44	283 ± 40	424 ± 83	418 ± 82	468 ± 91	350 ± 71	627 ± 76	575 ± 34

[a]Cont and Exp stand for the control and experimental groups, respectively.
[b]Values are means ± SD for three rats.
[c]Statistical analysis was done by Student *t* test. Comparison was made between the values of control and experimental groups in each killing time and each dietary group.
*Significant difference ($P < 0.05$).

Table 4 Hematologic values and carcass protein[a]

Group	Hematocrit %	Hemoglobin g/L	Plasma protein g/L	Carcass protein %
5% amino acid diet				
experimental group	35 ± 3 (6)	111 ± 54	44 ± 2	17.7 ± 0.8
control group	37 ± 2 (6)	112 ± 8	42 ± 3	17.6 ± 0.4
10% amino acid diet				
experimental group	36 ± 8 (6)	103 ± 25	48 ± 4	18.0 ± 0.5
control group	32 ± 3 (6)	96 ± 11	45 ± 2	17.9 ± 0.6

[a]Values are mean ± SD for the number of rats indicated in parenthesis.

Our previous study shows that the absorption of amino acid and protein diets that contained 2.4% nitrogen was completed in about 5 hr even when the rats ate a large amount of diet after 24-hr starvation.[17] From the available data, the absorption rate of an amino acid mixture is about 70% of the rate of absorption of peptides.[10] Therefore the difference in time required for absorption between peptide and amino acid may be less than 2 hr. This indicates that the conclusion drawn from the studies dealing with delayed supplementation of deficient amino acids[2–5] cannot be a proper reference for comparison of nitrogen retention between peptide and amino-acids diets.

The effect of a relatively short time delay in amino acid supply on the nitrogen retention was observed in rats by Cannon et al.[1] They divided the essential amino acids into two groups, offered them at alternate hours, and observed a decrease in body weight, although the control animals that were fed the complete diet gained weight. However they did not describe the food intake. In our previous study,[18] we divided the egg-pattern amino acids into two groups and fed them to rats alternately at 3-hr intervals for 7 days. The food intake was only from one-third to one-half of that observed in the control group, which was fed the egg-pattern amino acid mixture diet. This indicates that when the amino acid pattern is drastically unbalanced, animals cannot eat enough food, which would not occur if the pattern is slightly unbalanced. Therefore the study of Cannon et al.[1] cannot be a good reference to properly evaluate the effect of amino acid absorption rates of peptide and amino acid mixture diets on nitrogen retention.

It is quite reasonable that the time lag in amino acid absorption did not affect the nitrogen retention because the plasma aminograms were similar at all times between the experimental and control groups. This indicates a modification of the unbalanced pattern of absorbed amino acids by some tissues. The gut is known to have little modifying role on the amino acid composition of diet.[19] The liver is the most likely organ to play such a role judging from the study of Elwyn.[19] He showed that the amino acids absorbed were kept in the liver for awhile and then released gradually into the circulating system, as the absorbed amino acid would not impact the peripheral tissues. However we do not know the mechanism of the modification in the liver and have to study it in the future.

The results of this study indicate that nitrogen retention is not influenced by a relatively small time lag of amino acid absorption that is observed among the different nitrogen sources.

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