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Changes in Blood and Tissue Free Amino Acid Concentrations in Cats Adapted to Low-and High-protein Diets

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ABSTRACT

Changes in free amino acid concentrations in blood and various tissues were evaluated in cats adapted to the low-protein diet(20% protein, LPD) or the high-protein diet(60% protein, HPD) for 5 weeks. Cumulative body weigth gain for the 5 week period was 463 ± 43g, and -128 ± 40g for cats fed HPD and LPD, respectively. Feeding HPD significantly increased the sizes of liver and kidney. Cats adapted to HPD for 5 weeks have significantly elevated plasma concentrations of essential amino acids (branched-chain amino acids, threonine, tryptophan, phenylalanine and methionine), whereas plasma levels of non-essential amino acids(alanine, asparagine, glycine, glutamine and serine) were significantly reduced in animals adapted to HPD($p < 0.01$, or $p < 0.001$) compared to the values for the cats fed LPD. Changes in free amino acid concentrations in whole blood induced by the variations in dietary level of protein closely reflect the pattern seen in plasma. Amino acids such as branched-chain amino acids, proline and threonine were most difficult to maintain homeostasis and consistantly elevated in liver, kidney, skeletal muscle and brain, as well as in blood of cats adapted to HPD($p < 0.01$ or $p < 0.001$). All of the free amino acids in jejunum, excluding taurine and ornithine, were significantly elevated in animals adapted to HPD, most probably due to the rapid absorption of large amount of amino acids across the epithelium of small intestine.

KEY WORDS : free amino acids · dietary protein · tissue · adaptation · cat.

Introduction

Long-term and short-term effects of consuming inadequate amounts of protein on free amino acid concentrations and their metabolism in plasma and tissues have been extensively studied[1-3]. General consensus is that most mammals show adaptive response of the metabolism to large fluctuations in dietary level of protein in order to keep their amino acid concentrations within a relatively narrow range. With low-protein diets, free amino acids are mainly utilized for protein synthesis and rarely go through the catabolic pathways. Whereas high-protein intakes above the levels required to sustain maximum growth induce in-

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tracellular metabolizing enzymes[4)5)] and amino acid transport systems[6)] in the liver, which result in a concomittant increase in free amino acid catabolism favoring amino acid homeostasis. In the course of this adaptation, amino acid imbalances produced during the first days by the consumption of large amounts of protein[2)] become less profound as the amino acid catabolism has been adjusted to the new nutritional conditions.

The purpose of this study was to evaluate the adaptive response of amino acid metabolism to the dietary level of protein in cats, a species which had been known to be lacking hepatic enzymatic adaptation[7)]. Changes in free amino acid concentrations in blood and various tissues were evaluated in cats adapted to low- and high-protein diets for 5 weeks. Major differences in the regulation of amino acid homeostasis in cats compared to other species have been discussed.

Materials and Methods

1. Experimental Animals and Diets

Twelve female cats(1950±75g, 17~19 week old) from the specific pathogen-free colony at University of California, Davis, U.S.A. were randomly selected from a larger group maintained on a purified diet containing 400g protein and 1.5g taurine/kg diet. Half of them were fed 200g protein/kg diet(the low protein diet, LPD), and the other half were fed 600g protein/kg diet(the high protein diet, HPD) for 5 weeks. The composition of experimental diets are shown in Table 1.

All animals were housed in indivisual metal cages in a room having a light cycle from 06:00 to 22:00 daily and maintained at 24℃ to 26℃. Food and water were provided ad libitum and changed each morning (09:00~10:00). Animals were weighed twice a week, and the daily food intake was measured.

2. Blood and Tissue Collection

Blood sampls were collected from the jugular vein into heparinized 3ml syringes right before the animals were killed. Animals were killed between 9:00~11:00 without preceding fasting. A portion of the heparinized blood was centrifuged at 3,000×g for 10 min for the seperation of plasma. Both whole blood and plasma samples were kept at −20℃ until amino acid determination.

Table 1. Composition of experimental diets

	LPD	HPD
	g/kg diet	
Soy protein	100	300
Casein	100	300
Animal tallow	100	100
Sucrose	150	150
Starch	484	84
Mineral mix[1)]	50	50
Vitamin mix[2)]	10	10
70% choline	4.3	4.3
Taurine	1.5	1.5

1) The mineral mixture contained(g/100g) $CaHPO_4$ 39.0 ; K_2HPO_4 9.0 ; $CaCO_3$ 11.0 ; $MgSO_4$ 4.5 ; KC1 10.0 ; $KHCO_3$ 10.0 ; $NaHCO_3$ 14.0 ; $MnSO_4 \cdot H_2O$ 0.384 ; $ZnSO_4 \cdot 7H_2O$ 0.445 ; $CuSO_4 \cdot 5H_2O$ 0.080 ; $FeC_6H_5O_7 \cdot 3H_2O$ 1.000 ; KI 0.003 ; $SnCl_2 \cdot 2H_2O$ 0.010 ; Na_2SeO_3 0.003 ; $(NH_4)_6Mo_7O_4 \cdot 4H_2O$ 0.004 ; $CrCl_3 \cdot 6H_2O$ 0.026 ; $NiCl_2 \cdot 6H_2O$ 0.030 ; NaF 0.014 ; $NH_4VO_3 \cdot 4H_2O$ 0.002 ; NaCl 0.499

2) The vitamin mixture contained(g/kg) cobalamine 0.005 ; riboflavin 1.002 ; nicotinic acid 10.022 ; calcium d-pantothenic acid 2.004 ; menadione sodium bisulfite complex 1.353 ; folic acid 1.002 ; pyridoxine · HC1 1.000 ; thiamin mononitrate 2.436 ; myo-inositol 20.042 ; d-biotin 0.100 ; ascorbic acid 40.084 ; retinyl acetate 2,004,182 I.U./kg ; cholecalciferol 200,418 I.U./kg ; DL-alpha-tocopheryl acetate 16,034 I.U./kg.

The whole blood samples were repeated freeze/thaw cycle more than twice for the disruption of blood cells prior to amino acid analysis.

Liver, kidney, skeletal muscle, heart, spleen, brain and small intestine(jejunum) were removed form the cats under pentobarbital anesthesia and immediately placed on ice. The contents of intestine were washed out with ice-cold saline before placing the intestine on ice. A portion of fresh tissue was homogenized in 0.05M potassium phosphate buffer, PH 6.8 using a polytron homogenizer(model PT 10/35, Brinkman Instrument Co., Westbury, NY, U.S.A.) set at No. 6 speed to form a 20%(w/v) homogenate. Homogenates were centrifuged at 20,000×g for 30 minutes at 4℃, and the supernatants were kept at -20℃ until amino acid determination.

3. Determination of Amino Acid Concentration

Samples of whole blood, plasma and tissue supernatants were analyzed for free amino acid concentrations by ion exchange chromatography(Beckman model 121-MB) of sulfosalicylic acid extracts.

4. Statistical Analysis

The values in the tables and figures represent the mean±SEM of 6 cats. The significances of the effect of dietary protein on organ weights, blood and tissue concentrations of free amino acids were tested by Student's t-test.

Results

1. Body and Organ Weights

Cumulative body weight gains of cats fed experimental diets are shown in Fig. 1. The low-protein diet containing 10% casein plus 10% soyprotein did not support the growth of young cats, producing a negative balance in weight gain from the third week of experiment. At the end of 5 weeks of feeding period, cats fed the high-protein diet gained 463±43g, while those fed the low-protein diet lost 128±40g of body weights compared to their initial weights. Protein levels in the diet did not influence the average daily food intake(data not shown).

Organ weights and the ratios of organ weight to body weight from cats fed the high- and low-protein diets are shown in Table 2. Feeding HPD significantly enhanced the liver weight(67% increase, $p < 0.001$), as well as the ratio of liver weight to body weight(30% increase, $p < 0.01$) compared to the values for the low-protein diet group. Kidney size was even more greatly affected by the dietary level of protein, showing 96% increase in the organ weigth, and 37% increase in its relative ratio to body weight in animals fed the high-protein diet than the values for the low-protein diet group ($p < 0.001$).

In contrast to liver and kidney, brain and heart weights were resistant to the changes in dietary level of protein, and their relative ratios to body weight decreased 24~26% in cats fed the high-protein diet because of the concomittant increases in their body weights.

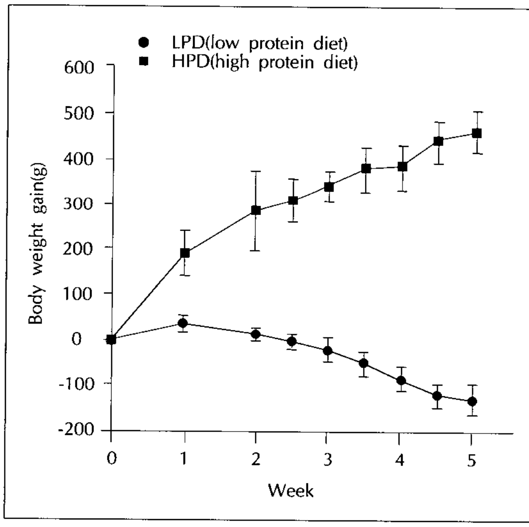

Fig. 1. Cumulative body weight gain over 5 week period.

Table 2. Organ weights of cats fed experimentral diets

	Liver		Kidney		Brain		Heart	
	organ wt(g)	organ wt(g)/ body wt(kg)	organ wt(g)	organ wt(g)/ body wt(kg)	organ wt(g)	organ wt(g)/ body wt(kg)	organ wt(g)	organ wt(g)/ body wt(kg)
LPD	59 ± 6	30 ± 2	9.6 ± 1.2	5.1 ± 0.3	24 ± 2	13 ± 2.2	6.3 ± 1.8	3.3 ± 0.2
HPD	98** ± 4	39* ± 3	19** ± 1.1	7.0** ± 0.3	24 ± 1	9.1 ± 0.2	6.1 ± 0.8	2.5 ± 0.3

Values are mean ± SEM of 6 animals

* $p < 0.01$ compared with LPD(low protein diet) group

** $p < 0.001$ compared with LPD(low protein diet) group

2. Free Amino Acid Concentrations in Plasma and Whole Blood

Changes in free amino acid concentrations in plasma and whole blood are summarized in Table 3. Feeding the high-protein diet for 5 weeks significantly elevated the majority of essential amino acid concentrations, and reduced several non-essential amino acid concentrations in the plasma of cats compared to the values for the low-protein diet group. Among the essential amino acids, concentrations of branched-chain amino acids increased most substantially(180~250% increases) in plasma by the large consumption of dietary protein, followed by threonine(160% increase), tryptophan(140% increase), phenylalanine(50% increase) and methionine(48% increase). Plasma levels of non-essential amino acids such as alanine, asparagine, glutamine, glycine and serine were reduced by the high-protein diet to a relatively consistant extent(30~53% decrease) compared to the values for the low-protein diet group.

Table 3. Free amino acid concentrations in plasma, whole blood and liver of cats fed the low-protein diet(LPD) and the high-protein diet(HPD)

	Plasma		Whole blood		Liver	
	LPD	HPD	LPD	HPD	LPD	HPD
	nmole/ml			μmole/g wet tissue		
EAA[1]						
Arg	105 ± 20	132 ± 11	225 ± 16	265 ± 36	0.26 ± 0.02	0.12 ± 0.05*
His	149 ± 13	142 ± 10	158 ± 9	163 ± 6	2.1 ± 0.2	2.4 ± 0.1
Ile	56 ± 6	159 ± 25**	82 ± 5	207 ± 12**	2.1 ± 03	2.5 ± 0.2
Leu	99 ± 10	287 ± 42**	168 ± 12	396 ± 22**	5.3 ± 0.8	6.2 ± 0.4
Lys	159 ± 18	176 ± 10	234 ± 19	268 ± 23	3.8 ± 0.5	4.8 ± 0.4
Met	46 ± 5	68 ± 7*	-	-	1.4 ± 0.2	1.5 ± 0.1
Phe	76 ± 4	114 ± 14*	96 ± 4	141 ± 8**	2.2 ± 0.3	2.5 ± 0.2
Thr	143 ± 13	374 ± 54**	273 ± 60	567 ± 93**	3.3 ± 0.4	5.0 ± 0.4*
Trp	34 ± 7	82 ± 10**	14 ± 4	24 ± 7	-	-
Val	130 ± 29	451 ± 61**	201 ±14	556 ± 28**	3.4 ± 0.5	4.5 ± 0.3
Tau[2]	156 ± 6	78 ± 6**	448 ± 28	602 ± 34**	11 ± 1.6	16 ± 1.1*
NEAA[3]						
Ala	592 ± 53	409 ± 36*	703 ± 62	583 ± 74	13 ± 1.8	13 ± 0.9
Asn	106 ± 15	65 ± 7*	70 ± 9	57 ± 4	1.8 ± 0.2	2.1 ± 0.1
Asp	34 ± 3	33 ± 4	79 ± 7	101 ± 11	3.0 ± 0.7	3.7 ± 0.5
Cit	14 ± 1	20 ± 2*	15 ± 1	22 ± 2*	0.08 ± 0.03	0.05 ± 0.01
Gln	701 ± 38	480 ± 59*	698 ± 64	487 ± 37*	5.3 ± 0.6	3.2 ± 0.1**
Glu	286 ± 40	213 ± 11	349 ± 63	329 ± 42	4.4 ± 0.6	4.4 ± 0.6
Gly	390 ± 27	275 ± 29*	532 ± 10	460 ± 116	4.8 ± 0.5	6.1 ± 0.6
Orn	62 ± 15	70 ± 17	73 ± 26	77 ± 9	4.6 ± 0.6	5.6 ± 0.5
Pro	229 ± 42	266 ± 49	238 ± 33	357 ± 22*	2.6 ± 0.2	3.9 ± 0.3*
Ser	259 ± 28	121 ± 9*	268 ± 51	219 ± 41	4.3 ± 0.5	5.1 ± 0.4
Tyr	52 ± 5	97 ± 13*	168 ± 9	241 ± 7**	2.0 ± 0.3	2.1 ± 0.1

Values are mean ± SEM of 6 animals
* $p < 0.01$ compared with LPD group
** $P < 0.001$ compared with LPD group
1) Essential amino acid
2) Taurine is an essential amino acid in feline species, and its conditional essentiality has been proposed in human infants.
3) Non-essential amino acid

Changes in free amino acid concentrations in whole blood induced by the variations in dietary level of protein closely reflected the pattern seen in plasma(Table 3). Feeding the high-protein diet reduced plasma taurine concentration by 47%, but elevated whole blood taurine concentration by 42% compared to the values for the low-protein diet group($p < 0.001$).

3. Free Amino Acid Concentrations in Tissues

The high-protein diet significantly increased the concentrations of taurine(45%), threonine(52%) and proline(50%), but decreased arginine(54%) and glutamine (40%) concentrations in the liver of cats(Table 3). Changes in free amino acid concentrations in kidney induced by feeding the high-protein diet generally resemble the phenomenon seen in plasma and whole blood, but to a much less extent. Significant increases in the concentrations of branched-chain amino acids (57~80% increases) and threonine(58% increase), along with the concentrations of lysine, methionine, proline and serine(23~60% increases) were obseved

Table 4. Free amino acid concentrations in kidney, skeletal muscle and heart of cats fed the low-protein diet(LPD) and the high-protein diet(HPD)

	Kidney		Skeletal muscle		Heart	
	LPD	HPD	LPD	HPD	LPD	HPD
	nmole/ml		μmole/g wet tissue			
EAA[1]						
Arg	0.6 ± 0.1	0.8 ± 0.2	0.7 ± 0.3	0.6 ± 0.1	1.0 ± 0.2	0.9 ± 0.2
His	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.3	0.8 ± 0.1	1.1 ± 0.06	1.1 ± 0.1
Ile	0.5 ± 0.04	0.9 ± 0.1**	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Leu	1.4 ± 0.1	2.2 ± 0.2**	0.8 ± 0.1	0.8 ± 0.04	1.2 ± 0.2	1.1 ± 0.2
Lys	1.3 ± 0.09	1.9 ± 0.2*	1.0 ± 0.2	0.9 ± 0.1	1.3 ± 0.2	1.2 ± 0.2
Met	0.5 ± 0.04	0.8 ± 0.1*	0.3 ± 0.06	0.3 ± 0.03	0.4 ± 0.1	0.4 ± 0.1
Phe	0.7 ± 0.1	1.0 ± 0.1	0.4 ± 0.07	0.4 ± 0.02	0.6 ± 0.1	0.6 ± 0.1
Thr	1.2 ± 0.1	1.9 ± 0.2*	0.4 ± 0.05	1.4 ± 0.2**	1.1 ± 0.1	1.5 ± 0.2
Val	1.0 ± 0.1	1.7 ± 0.2*	0.6 ±0.1	0.7 ± 0.04	0.7 ± 0.1	0.7 ± 0.1
Tau[2]	7.9 ± 0.6	9.9 ± 0.7	5.6 ± 0.6	5.0 ± 0.6	12 ± 1.1	13 ± 1.8
NEAA[3]						
Ala	5.4 ± 0.4	6.7 ± 0.6	3.3 ± 0.5	3.5 ± 0.2	4.3 ± 0.4	4.3 ± 0.6
Asn	0.6 ± 0.06	0.7 ± 0.06	0.5 ± 0.08	0.4 ± 0.03	0.9 ± 0.1	0.7 ± 0.09
Asp	4.0 ± 0.4	4.3 ± 0.3	0.7 ± 0.1	0.8 ± 0.1	2.9 ± 0.6	4.9 ± 0.5*
Cit	0.1 ± 0.01	0.1 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
Gln	1.5 ± 0.2	1.0 ± 0.1*	3.3 ± 0.4	4.3 ± 0.4	9.2 ± 1.4	8.8 ± 1.2
Glu	9.1 ± 0.9	8.0 ± 0.8	2.0 ± 0.1	2.1 ± 0.1	9.4 ± 0.8	9.2 ± 1.2
Gly	5.0 ± 0.3	5.8 ± 0.5	1.7 ± 0.3	1.8 ± 0.3	1.9 ± 0.2	1.8 ± 0.3
Orn	0.9 ± 0.1	1.2 ± 0.4	0.7 ± 0.2	0.5 ± 0.09	0.3 ± 0.1	0.4 ± 0.1
Pro	1.3 ± 0.1	1.9 ± 0.2*	0.7 ± 0.1	1.3 ± 0.01*	0.7 ± 0.1	0.8 ± 0.1
Ser	2.1 ± 0.09	2.6 ± 0.2*	1.2 ± 0.2	0.8 ± 0.1	1.9 ± 0.2	1.4 ± 0.2
Tyr	0.7 ± 0.04	1.0 ± 0.1*	0.4 ± 0.05	0.4 ± 0.02	0.5 ± 0.05	0.5 ± 0.1

Values are mean ± SEM of 6 animals

* $p < 0.01$ compared with LPD group

** $P < 0.001$ compared with LPD group

1) Essential amino acid

2) Taurine is an essential amino acid in feline species, and its conditional essentiality has been proposed in human infants.

3) Non-essential amino acid

in kidneys of cats fed the high-protein diet(Table 4).

Homeostasis of tissue free amino acid concentrations has been generally well maintained in the skeletal muscle, heart, brain and spleen of cats adapted to the low- and high-protein diets for 5 weeks (Table 4 and 5). Proline and threonine were the two amino acids for which the adaptive mechanisms favoring homeostasis were either overloaded or not functioning properly in cat tissues, and thereby accumulated consistantly across the various tissues by the high-protein diet(Table 4 and 5). Sharp contrast to above tissues, which were resistant to large fluctuations in the dietary level of protein, free amino acid concentrations in jejunum were considerably elevated by the high-protein diet($p < 0.01$ or $p < 0.001$) fed ad libitum, most probably due to the digestion of large amounts of protein and the subsequent absorption of free amino acids from the lumen into the epithelium of small intestine. The concentration of taurine, a free amino acid not involved in protein

Table 5. Free amino acid concentrations in spleen, brain and jejunum of cats fed the low-protein diet(LPD) and the high-protein diet(HPD)

	Brain		Spleen		Jejunum	
	LPD	HPD	LPD	HPD	LPD	HPD
	nmole/ml		μmole/g wet tissue			
EAA[1]						
Arg	0.46 ± 0.04	0.55 ± 0.04	2.0 ± 0.3	1.7 ± 0.3	10 ± 1.2	21 ± 1.7**
His	0.37 ± 0.03	0.30 ± 0.05	1.1 ± 0.3	0.9 ± 0.06	3.2 ± 0.4	7.2 ± 0.7**
Ile	0.14 ± 0.01	0.20 ± 0.05	0.8 ± 0.1	0.9 ± 0.05	5.3 ± 0.8	14 ± 1.9**
Leu	0.33 ± 0.03	0.45 ± 0.1	2.0 ± 0.3	2.2 ± 0.1	11 ± 1.5	28 ± 3.2**
Lys	0.47 ± 0.05	0.55 ± 0.1	2.0 ± 0.2	2.0 ± 0.1	13 ± 1.6	28 ± 2.5**
Met	0.09 ± 0.01	0.12 ± 0.03	0.34 ± 0.1	0.4 ± 0.1	3.8 ± 0.5	8.2 ± 0.9**
Phe	0.22 ± 0.01	0.25 ± 0.04	1.0 ± 0.1	1.1 ± 0.06	4.6 ± 0.6	11 ± 1.2**
Thr	0.47 ± 0.03	0.75 ± 0.1*	2.1 ± 0.2	1.4 ± 0.3	8.2 ± 1.0	17 ± 1.9**
Trp	-	-	-	-	0.46 ± 0.1	1.0 ± 0.2**
Val	0.3 ± 0.04	0.32 ± 0.07	1.3 ±0.2	1.4 ± 0.3	7.5 ± 1.1	18 ± 1.9**
Tau[2]	2.7 ± 0.3	2.0 ± 0.4	8.1 ± 0.7	10 ± 0.7	8.2 ± 0.5	7.5 ± 0.5
NEAA[3]						
Ala	1.4 ± 0.03	1.4 ± 0.2	3.8 ± 0.4	4.0 ± 0.3	16 ± 1.5	30 ± 3.5**
Asn	0.1 ± 0.01	0.2 ± 0.04*	1.0 ± 0.1	1.1 ± 0.06	3.9 ± 0.6	8.4 ± 0.9**
Asp	5.6 ± 0.5	5.8 ± 1.0	4.4 ± 0.4	5.5 ± 0.3*	7.9 ± 0.9	16 ± 1.9**
Cit	0.1 ± 0.01	0.1 ± 0.01	0.05 ± 0.005	0.05 ± 0.01	-	-
Gln	3.6 ± 0.4	2.8 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	5.6 ± 0.5	11 ± 1.2**
Glu	7.4 ± 0.8	5.7 ± 0.3	10.8 ± 0.8	13 ± 0.7*	17 ± 1.0	33 ± 3.6**
Gly	1.8 ± 0.05	1.7 ± 0.1	5.2 ± 0.4	5.5 ± 0.4	13 ± 1.0	20 ± 2.3**
Orn	0.1 ± 0.02	0.11 ± 0.01	0.51 ± 0.1	0.4 ± 0.2	0.9 ± 0.1	3.6 ± 1.5
Pro	0.3 ± 0.01	0.4 ± 0.01*	1.9 ± 0.2	2.1 ± 0.2	9.8 ± 1.1	17 ± 1.4**
Ser	1.1 ± 0.05	1.0 ± 0.2	3.4 ± 0.3	3.3 ± 0.3	13 ± 1.1	23 ± 2.5**
Tyr	0.17 ± 0.02	0.21 ± 0.03	1.3 ± 0.1	1.4 ± 0.1	4.5 ± 0.6	10 ± 1.0**

Values are mean ± SEM of 6 animals

* $p < 0.01$ compared with LPD group

** $P < 0.001$ compared with LPD group

1) Essential amino acid

2) Taurine is an essential amino acid in feline species, and its conditional essentiality has been proposed in human infants.

3) Non-essential amino acid

synthesis, remained unchanged in the jejunum of animals fed the high-protein diet(Table 5).

Discussion

Feline species require higher dietary levels of protein for growth and maintenance than do other mammals[8]. Protein requirement for growing kittens is known to be 18~20% when the diets exceed all the essential amino acid requirements[9]. Apparently, a diet containing 10% casein plus 10% soyprotein used in the present study did not provide an adequate level of all the essential amino acids to sustain growth(Fig. 1). Different organs in the body appear to respond differently to the dietary level of protein in terms of growth. The high-protein diet affected kidney and liver positively, both on organ weights and their relative ratios to body weight. However, the sizes of brain and heart did not respond to the dietary level of protein(Table 2).

It is now well documented that taurine is an essential amino acid in feline species because of their limited capacity to synthesize taurine[10] and high demand for some biological functions, such as bile acid conjugation[11]. In 1981, Committee of the National Research Council[9] estimated the taurine requirement for cats between 250mg and 1,000mg/kg dry diet, when the sulfur amino acid requirement is just met. In the present study, 1,500mg taurine/kg diet was added to the experimental diets to establish normal levels of blood and tissue taurine in growing cats. It has been repeatedly observed in our laboratory that high-protein diets significantly reduce plasma taurine concentration but elevate whole blood taurine concentration($p < 0.001$). Further investigations are needed to provide an explanation for this phenomenon.

Diurnal changes in free amino acid concentrations in the plasma and muscle have been reported to be 10% of the means in rats[15]. In this study, kittens were killed from 09:00 to 11:00 and any diurnal effect present over this time period presumably would be quite small.

Concentrations and proportions of each free amino acids in the plasma pool of a subject consuming adequate level of protein appear to be kept within a narrow range across the species[12-14]. Our findings that the long term feeding of the high-protein diet elevated essential amino acid concentrations, but reduced the levels of non-essential amino acid in the plasma of cats is in accordance with the observations made by other investigators. According to the study by Tew et al.[16] on cats fed 20% protein vs 57% protein diets, the high-protein diet resulted in 110~120% increases in branched-chain amino acid concentrations, 160% increase in threonine level, and 20~46% increases in the concentrations of other essential amino acids in plasma. Glycine concentration was low in the plasma and was also clearly depressed in the kidney of cats fed the high-protein diet[16]. Similar effects of high-protein diet on plasma free amino acid concentrations were observed in species other than cats ; high-protein diets significantly decreased plasma concentrations of alanine, serine, glycine, glutamine and threonine, and increased plasma concentrations of branched-chain amino acids along with other essential amino acids in rats[17][18]. Significantly elevated concentrations of branched-chain amino acids and threonine have also been reported to be the characteristics of amino acid pattern in the plasma of human infants fed formulas containing protein above the levels found in human milk[19].

Alanine is an important inter-organ nitrogen carrier. Theoretically, alanine production is enhanced with high-protein diets through the metabolism of glutamine and acidic amino acids by intestine, and from the transamination of branched-chain amino acids in muscle[20]. It is speculated that in animals fed high-protein diets, hepatic catabolism and utilization of alanine for gluconeogenesis exceeds the digestive and peripheral release of alanine, evoking a noticeable hypoalaninemina.

Among the essential amino acids increased by the long-term feeding of high-protein diets, the elevations in branched-chain amino acids were most dramatic in blood of human infants[19], rats[17][18] and cats[16], which was again proved by the present study. This phenomenon could possibly be explained by the fact that unlike most other amino acid catabolic enzyme systems, the aminotransferase responsible for the initial step in the degradation of branched-chain amino acids is not concentrated in liver[21], and does not increase in activity by the protein intake at the levels above the requirement[22][23].

The physiological significance of this strong correlation between protein intake and the concentration of branched-chain amino acids in plasma has not been established.

Despite the significant increase in blood branched-chain amino acid concentrations by the high-protein diet, branched-chain amino acid concentrations in most tissues(except kidney and jejunum) remained unchanged by the dietary level of protein in the present study. Limited reports on branched-chain amino acid concentrations in rat tissues demonstrated that the concentrations of these amino acids in the brain was significantly elevated by the high-protein diet, but the presence of positive effect of high-protein diet on branched-chain amino acid concentrations in liver and mammary gland depended on the quality of protein (wheat vs casein) added in the diet[17][18].

High-protein diets consistently induced a fall in the concentrations of threonine, serine and glycine in plasma, liver and muscle of rats[4][17], which is one of the major contradicts to the phenomenon observed in cats, and also in humans[19]. Threonine, serine and glycine share some common metabolic pathways such as serine hydroxymethyltransferase and threonine- serie dehydratase. It is now well documented that high-protein diets promote the induction of amino acid catabolizing enzymes in the rat liver, and hepatic threonine-serine dehydratase activity has been shown to be the most responsive enzyme[4][17]. The absence of similar enzyme induction in cats[7] is reflected in the increased concentrations of threonine in the plasma and various tissues of animals fed the high-protein diet. The physiological significance of this elevated threonine concentration in various tissue of cats awaits further investigtion.

In conclusion, despite the concept that there in an adaptive reponse of the metabolism to maintain homeostasis of free amino acid concentrations in the body, high-protein diets alter the concentrations of several circulating amino acids. Homeostasis of free amino acid concentrations in the body could be achieved by the combination of following adaptive responses ; 1) intestinal absorption, 2) renal reabsorption, and/or 3) metabolism, most possibly via enzymatic regulation. It is speculated that the transport and/or metabolism of certain amino acids such as branched-chain amino acids, threonine and proline appears to be either less tightly regulated or easily overloaded in cats, and possibly in humans by the large consumption of dietary protein, and their concentrations are substantially elevated in blood and tissues. Whereas the catabolism and utilization of several non-essential amino acids appear to be greatly enhanced by high-protein diets, which would be supported by the significant reductions in the circulating levels of such amino acids.

Since the present study did not include normal protein diet group, the statistical significance of the data was compared between HPD and LPD groups. The blood and tissue free amino acid concentrations in animals fed 'normal' protein diet would presumably be somewhere in the middle of two extreme values, as described in the study by Peters and Harpers[2], where plasma dispensible amino acid and branched-chain amino acid concentrations changed gradually as the protein content in the diet varied from 5 to 75% gradually in increment of 5%. The characteristics of metabolic adaptation to the changes in dietary level of protein need to be further investigated employing studies on the activities of gluconeogenic, ureogenic and transaminating enzymes in cat tissues, and closely monitoring the progressive changes in free amino acid concentrations in blood and tissues as the protein deficiency or surplus proceeds.

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=국 문 초 록=

단백질 섭취 수준에 따른 고양이의 혈액 및 조직의 유리 아미노산 농도의 변화

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저단백 식이(20% 단백질)와 고단백 식이(60% 단백질)를 5주간 섭취한 고양이에 있어서 혈액과 조직의 유리 아미노산 농도의 변화를 살펴 봄으로써 단백질 섭취 수준에 따른 대사 적응의 효율성을 평가하고자 하였다. 실험 식이로 5주간 사육한 결과, 고단백 식이군은 436±43g의 누적 체중 증가를 보인 반면, 저단백 식이군은 128±40g의 체중 감소를 나타냈다. 고단백 식이를 섭취한 고양이의 경우 간과 신장의 크기가 저단백 식이군에 비해 유의적으로 증가하였다($p < 0.001$). 고단백 식이군에 있어서 혈장의 필수 아미노산(branched-chain amino acids, threonine, tryptophan, phenylalanine과 methionine)의 농도는 유의적으로 증가한 반면, 불필수 아미노산(alanine, asparagine, glycine, glutamine과 serine)의 농도는 유의적으로 감소하였으며($p < 0.01$ 또는 $p < 0.001$), 단백질 섭취 수준에 따른 전혈의 유리 아미노산 농도의 변화 역시 혈장에서와 비슷한 추이를 나타냈다. 대부분의 조직에서 유리 아미노산의 농도는 장기간의 단백질 섭취 수준의 큰 변화에도 불구하고 평형(homeostasis)을 대체로 유지하는 반면, proline과 threonine의 농도는 고단백 식이를 섭취한 고양이의 혈액은 물론 간, 신장, 골격근육 및 뇌 등의 조직에서 모두 유의적으로 높게 나타나고 있다($p < 0.01$ 또는 $p < 0.001$). 타우린과 ornithine을 제외한 소장내의 모든 유리 아미노산의 농도는 고단백 식이군에서 유의적으로 증가하였는데, 이것은 고단백 식이의 섭취 후 소화되어 유리된 아미노산이 소장 점막을 통해 빠르게 흡수되어 세포내에 머물고 있기 때문인 것으로 사료된다.