Original Article

Effect of single essential amino acid excess during pregnancy on dietary nitrogen utilization and fetal growth in rats

Masahiro Mori[1] PhD, Yoshiaki Yamashita[1] PhD, Yuzo Hiroi[2] PhD, Sumie Shinjo[2] MS, Ryu Asato[3] MS, Kazuko Hirai[4] PhD, Kazuhiko Suzuki[5] PhD and Shigeru Yamamoto[6] PhD

[1]*Department of Nutritional Science, Kinran College, Suita, Osaka, Japan*
[2]*Department of Nutritional Chemistry and Nutrition Education, Nakamura, Gakuen University, Fukuoka, Japan*
[3]*Department of Nutrition, Faculty of Medicine, University of the Ryukyus, Nishihara, Okinawa, Japan*
[4]*Department of Nutritional Biochemistry, Faculty of Human Life Science, Osaka City University, Sumiyoshi, Osaka, Japan*
[5]*Department of Nutritional Science, Faculty of Health and Welfare Science, Okayama Prefectural University, Sohjya, Okayama, Japan*
[6]*Department of Nutrition, School of Medicine, University of Tokushima, Kuramoto, Tokushima, Japan*

The effect on pregnant rats of individual amino acids added excessively to the diet or intermittently administered in excess directly with a stomach tube was examined. When methionine was excessively added to the diet at a 5% level, amino acid imbalance in plasma was induced and food intake decreased remarkably to approximately one-fifth of that of control rats. However, when administered directly into the stomach, food intake remained almost normal, except for excess of methionine, tryptophan or threonine, and an adverse effect of excess amino acid was not observed. However, in the case of a decrease in food intake, various adverse effects were observed. This was especially so for the group that was administered methionine and whose food intake was far below that of the control group: decrease in maternal body weight, delayed growth of products of conception, and further, significant decrease in brain and liver cells of the fetus were observed. In addition, the changes in nitrogen balance were well correlated with the changes in bodyweight in all groups. In the methionine group, in particular, nitrogen balance was negative throughout the period because of a decrease in food intake, and utilization of dietary nitrogen was inferior. The majority of the various lesions observed with excess administration of individual amino acids were not due to a direct effect of excess amino acid but were mainly caused by the remarkable decrease in food intake.

Key words: amino acids, methionine, fetal brain, food intake.

Introduction

It is well known that the nutritive value of poor quality proteins is improved by the addition of the limiting amino acids. However, if safe and effective supplementation of amino acids is not conducted, amino acid imbalance may occur, even with the use of a relatively small quantity, and various undesirable effects may appear. Harper *et al.* classified lesions by imbalanced amino acid into three types; imbalance, antagonism and toxicity.[1] Of these, lesions due to a diet containing excess amino acids are generally termed toxicity, and it is known that a decrease in food intake, growth suppression and various pathological changes are induced by excess amino acids.[1–10] However, there are many discrepancies among researchers in this field and consistent results have not been obtained.

The reasons for this inconsistency are that source and level of dietary protein, excess levels of amino acids, and differences in strain, sex, age of animals and experimental period etc. have an important relation to their manifestation.[2,3] For example, Muramatsu *et al.* described that when individual amino acids were excessively added to a 10% casein diet at a 5% level for male weanling rats, methionine (Met) caused the most severe growth suppression, followed in order by phenylalanine (Phe), tryptophan (Trp), threonine (Thr), valine (Val), lysine (Lys), isoleucine (Ile) and leucine (Leu).[2–4] However, Matsueda and Niiyama reported that when individual amino acids were added in excess to a 6% casein diet at a 5% level, the most severe growth suppression of pregnant rats was still observed in Met, but for others it was, in order, Leu, Trp, Val, Lys, Ile, Thr and Phe.[5]

Although the results are different depending on the difference in experimental conditions, the elucidation of various toxicities induced by excess amino acids existing in plasma and tissues is considered useful not only for the accumulation of basic data for study on inborn errors of amino acid metab-

Correspondence address: Dr Masahiro Mori, Department of Nutritional Science, Kinran College, 5-25-1 Fujishirodai, Suita, Osaka, 565–0873, Japan.
Tel: 81 6 6872 0673 (ext.181); Fax: 81 6 6872 7306
Email: m-mori@kinran.ac.jp
Accepted 7 April 1999

olism but also for the accumulation of important and useful basic data for further progress of amino acid nutrition in the future, such as safe and effective amino acid supplements to food, preparation of amino acid infusion mixtures etc.

For the above reasons, the effect of excess amino acid in different cases must be examined in detail systematically, and at the same time measures for toxicity alleviation must be studied.[4,11–16] However, not only are there few systematic studies in this field but also nearly no systematic studies undertaken on pregnancy.[5,17–21]

We have already shown that various toxicities occurred when a diet containing excess Met was given to pregnant rats and we assumed that the majority of these toxicities were caused by the remarkable decrease in food intake.[22] On this occasion, therefore, we administered Val, Lys, Ile, Thr, Leu, Phe, Trp and Met individually to pregnant rats through a stomach tube for consecutive days in order to minimize the decrease in food intake. We then examined the influences of excess administration of individual amino acids on the growth of products of conception, especially on the fetal brain and liver.

Materials and methods

Female Sprague-Dawley rats, weighing approximately 150 g, were given a 20% casein diet (20% CA) until they weighed approximately 190 g. Then they were mated with male rats of the same strain. Smear tests were performed every morning and the day when sperm existed was termed as day 1 of pregnancy. We used cow's milk casein (Oriental Yeast, Tokyo, Japan) as the protein source of 20% CA. The other compositions were 44.3% α-corn starch, 22% sucrose, 5% corn oil, 5% mineral mixture (composition (in mg/kg): $CaHPO_4 2H_2O$, 7280; KH_2PO_4, 12 860; NaH_2PO_4, 4680; NaCl, 2330; Ca-lactate, 17 550; Fe-citrate, 1590; $MgSO_4$, 3590; $ZnCO_3$, 55; $MnSO_4 4–6H_2O$, 60; $CuSO_4 5H_2O$, 15; KI, 5) (Oriental Yeast), 1% vitamin mixture (composition (in mg/kg): thiaminHCl, 12; riboflavin, 40; pyridoxineHCl,8; vitamin B-12, 0.005; ascorbic acid, 300; D-biotin, 0.2; folic acid, 2; calcium pantothenate, 50; *P*-aminobenzoic acid, 50; niacin, 60; inositol, 60; choline chloride, 2000; tocopheryl acetate, 50; menadione, 52 and (in IU) retinyl acetate, 6000; ergocalciferol, 1000. The cellulose was used to increase the bulk of the vitamin mixture.) (Oriental Yeast), 2% cellulose powder, 0.3% L-methionine and 0.4% choline-chloride, by weight.

Experiment 1

A 20% casein diet was given *ad libitum* or restricted to 5 g/day together with 250 or 450 mg/day of Met from day 1 of pregnancy (Fig. 1). Met was maintained at 45°C in an incubator after it had been heated and melted, and administered in 3 cc portions six times per day, 250 or 450 mg/day in all, once every 2 h starting from 0700. It was administered directly into the stomach using a stomach tube. The relationship of excess Met and food intake against maintenance of pregnancy was studied. For the control group, 0.9% physiological saline was administered in the same manner.

Experiment 2

During days 1–11 of pregnancy, a 20% casein diet was given *ad libitum*. The animals were divided into two groups on day 12 of pregnancy. One group was given a 20% casein diet to which 5% Met was added excessively. To the other group, besides a 20% casein diet, 210 mg/day Met was administered directly into the stomach during days 12–22 of pregnancy in the same manner as Experiment 1. For the control group, a 20% casein diet was given through the whole period. In this way, it was possible to examine whether or not the decrease in food intake caused by excess Met intake was due to an amino acid imbalance in the plasma.

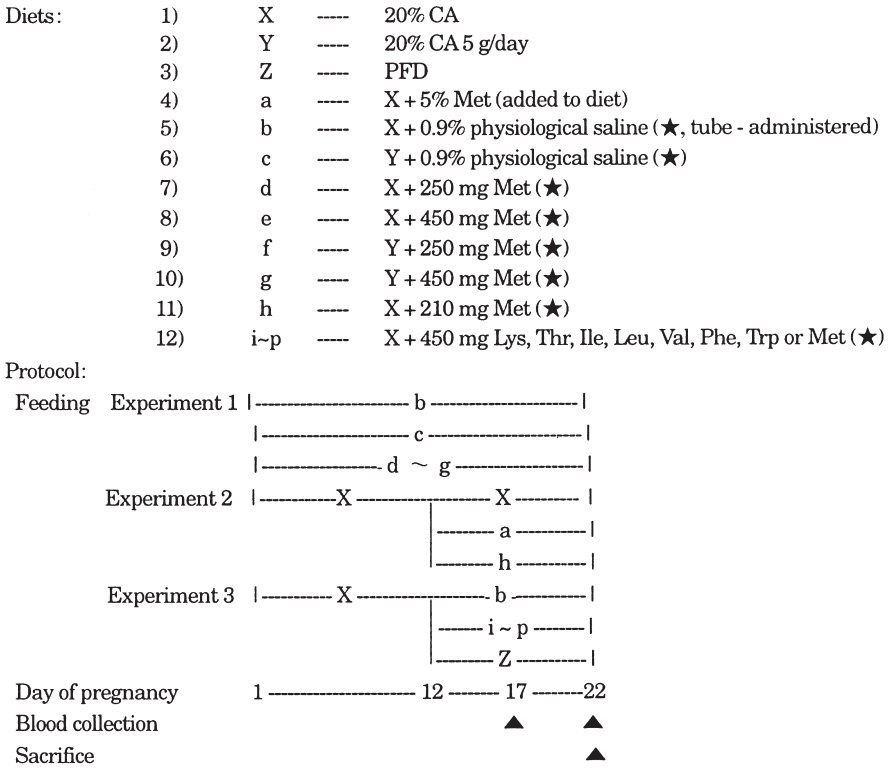

Figure 1. Experimental design.

Table 1. Effects of excess methionine (Met) on maintenance of pregnancy and food intake during pregnancy

Groups*	Food intake (g/21 days)	Protein intake (g/day)	Energy intake (kJ/day)	Met (% food intake)	No. pregnancies maintained (*n*/total)	Maintenance (%)
20% CA restricted						
Control (5)†	105	1.00	75.6	0.8	1/5	20
+ 250 mg Met (7)	105	1.00	75.6	5.8	0/7	0
+ 450 mg Met (6)	105	1.00	75.6	9.8	0/6	0
20% CA *ad libitum*						
Control (5)	347 ± 12‡	3.29 ± 0.13	247.8 ± 4.2	0.8	5/5	100
+ 250 mg Met (9)	264 ± 43§	2.50 ± 0.39**	189 ± 29.4**	2.8 ± 0.5	3/9	33
+ 450 mg Met (4)	231 ± 42**	2.20 ± 0.40**	163.8 ± 29.4**	4.9 ± 0.9	1/4	25

*Excess Met was administered with a stomach tube; †numbers in parentheses represent the numbers of rats; ‡values are means ± SD; §,**significantly different from values for control group at levels of 1 and 0.1%, respectively.

Experiment 3

A 20% casein diet was given *ad libitum* from day 1 of pregnancy. From day 12 of pregnancy, 450 mg/day of Val, Lys, Ile, Thr, Leu, Phe, Trp or Met was administered in the same manner as Experiment 1. Furthermore, a protein free diet (PFD) was given to another group from day 12 of pregnancy to calculate net protein utilization (NPU).[3] The PFD was made by replacing 20% casein with 13% α-corn starch and 7% sucrose. The animals were housed in metabolic cages from day 12 of pregnancy and observed for nitrogen balance during the amino acid administration period. On day 22 of pregnancy the rats were killed and fetal growth, especially protein and nucleic acid contents in the brain and liver, was examined. For the control group, 0.9% physiological saline was administered in the same manner.

Protein content in tissues was measured using the Lowry method.[23] For other tissues, protein content was determined by multiplying nitrogen by 6.25 after determination by a semimicro Kjeldahl method.[24] RNA and DNA in the fetal brain and liver were measured spectrophotometrically.[25]

Statistical analysis

Results are expressed as mean ± SD. Significance of difference was calculated by Student's *t*-test. Values were considered significant when $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Results

Table 1 includes data for maintenance of pregnancy and food intake during pregnancy (Experiment 1). Total food intakes of the unrestricted groups were 264 g, 231 g and 347 g for the 250 mg, 450 mg and control groups, respectively. Food intake decreased as levels of Met increased. The maintenance of pregnancy also decreased from 100% in the control group to 33 and 25% for the 250 mg and 450 mg groups, respectively. However, because food intake of the excess Met group was sufficient to maintain pregnancy when considering results of other experiments, it seems that this decrease in

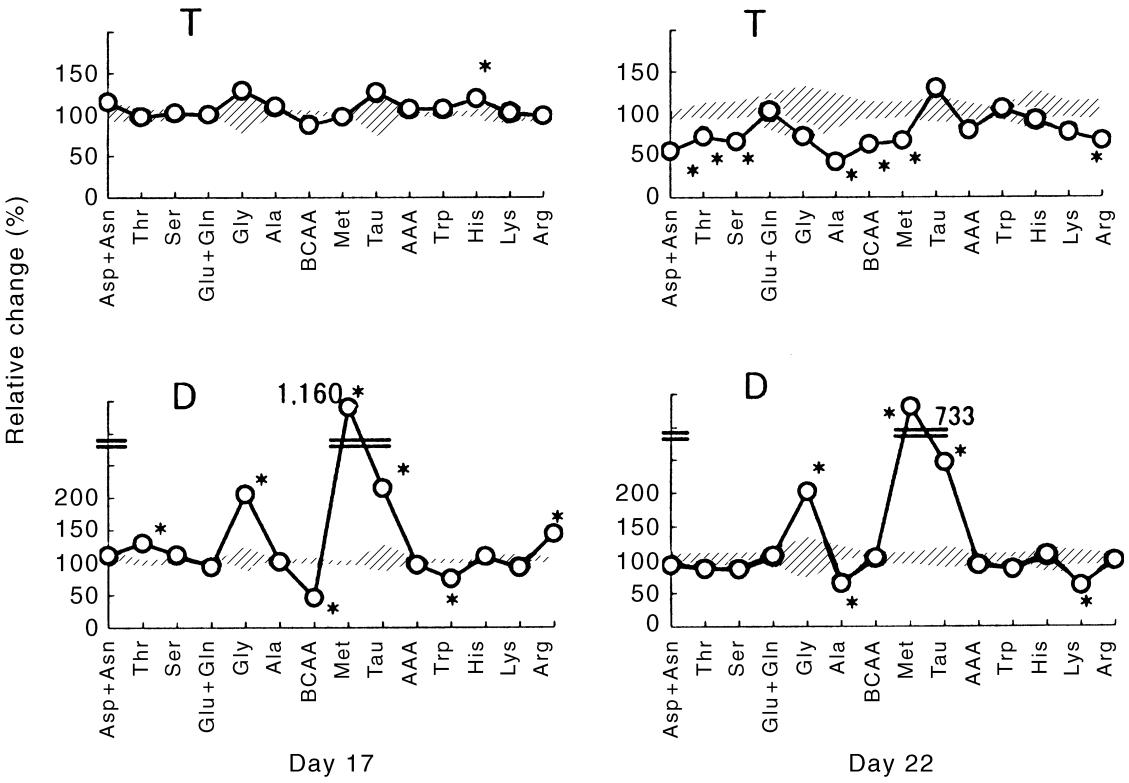

Figure 2. Effects of excess methionine (Met) on amino acid pattern in plasma. T, 20% CA + 21 Omg Met (tube-administered). D, 20% CA + 5% Met (added to diet). =, SD area in control group. *$P < 0.05$.

maintenance is due to the toxicity of Met. On the other hand, maintenance of pregnancy in the restricted group was 20% in the control group and 0% both in the 250 and 450 mg groups. The levels of excess Met at that time were 0.8, 5.8 and 9.8%, respectively. Therefore, these results show that maintenance of pregnancy is highly affected by maternal deprivation of both protein and energy; furthermore, when Met is in excess of more than 5%, maintenance of pregnancy becomes completely impossible due to the toxicity of Met.

Table 2 and Figure 2 shows the food intake and amino acid pattern in plasma when excess Met was added to diet and when it was administered with a stomach tube directly during days 12–22 of pregnancy (Experiment 2). In order to establish the cause of decrease in food intake as a result of excess Met intake, the amino acid pattern in plasma was examined when excess Met was added to the diet and when it was administered with a stomach tube directly. In the former case, food intake decreased to 20% of that of the control group, while in the latter case food intake was 60% of that of the control group. With regard to light periods and dark periods, food intake of the tube-administered group and the control group in dark periods was twice as much as that in light periods, while food intake of the added-to-diet group in dark periods was only 1.3 times as much as that in light periods. As for the amino acid pattern in plasma at this time, in the tube-administered group the pattern was similar to that of the control group. However, in the added-to-diet group Met, glycine (Gly) and taurine (Tau) were significantly high and an imbalance of the amino acid pattern in plasma was observed.

Table 3 shows food intake during days 12–22 of pregnancy (Experiment 3). A remarkable decrease in food intake appeared when individual amino acids were added to the diet excessively.[2,5–7,14] However, in the case of direct intragastric administration of individual amino acids, the decrease in food intake was almost completely prevented, except for Thr, Trp and Met. In addition, in the Thr, Trp and Met groups, the rats ingested 90, 88 and 44%, respectively, of the amounts of the control group, and significant improvement in food intake were observed when compared with the cases in which excess amino acid was added to the diet.

Table 4 shows bodyweight gain and protein utilization (Experiment 3). The toxicity of excess amino acid during pregnancy was examined by maternal growth and the most severe growth suppression was observed in the excess Met group. The effect then became smaller, in order, in the Trp, Phe, Leu and Thr groups and slight growth suppression was observed. No growth suppression was observed in the Ile, Lys and Val groups. Secondly, the influence of excess amino acid upon protein utilization was examined. In the Met, Trp and Leu groups, NPU was significantly small when compared with 57 of the control group. As for the Met group, NPU decreased remarkably to 12 and an adverse effect to protein synthesis was suggested.

Table 5 shows reproductive performances and fetal growth (Experiment 3). Concerning products of conception, the Met group weighed 39 g and the Phe group weighed 63 g, while the control group weighed 75 g. Thus, in both groups a significant decrease was observed but other groups were nearly equivalent to the control group. Secondly, with regard to the weight of the fetus and fetal brain and liver, significant decrease in the brain weight due to administration of excess amino acid was observed in all groups; in the Met group in particular, it was only 60% of normal weight.

Table 6 shows nucleic acid and the protein contents in fetal liver and brain (Experiment 3). Commonly in all groups,

Table 2. Effects of excess methionine (Met) on food intake and change in bodyweight during days 12–22 of pregnancy

Groups	Food intake			Met intake	Bodyweight			Body-
	Light period (g/10 days)	Dark period (g/10 days)	Total (g/10 days)	(mg/10 days)	Day 1 (g)	Day 12 (g)	Day 22 (g)	weight gain (g/10 days)
Control (20% CA) (6)*	61	122	183	1530	199	257	338	+81
+ 5% Met† (6)	16§	21§	37§	2062	196	260	225	–35§
+ 210 mg Met‡ (10)	34§**	76§**	110§**	3013§**	195	244§**	254§**	+10§**

*Numbers in parentheses represent the numbers of rats; †excess Met was added to diet; ‡excess Met was administered with a stomach tube; §significantly different from values for control group at the level of 5%; **significantly different from values for 20% CA + 5% Met group at the level of 5%.

Table 3. Effects of individual essential amino acid excess on food intake during days 12–22 of pregnancy

Groups*	No. rats	Food intake (g/10 days)	Protein intake (g/day)	Energy intake (kJ/day)	Amino acid intake		
					Food (mg/10 days)	Tube (mg/10 days)	Total (%)
Control (20% CA)	9	197 ± 9†	3.9 ± 0.1	298.2 ± 12.6		0	
+ 450 mg Val	6	203 ± 19	4.1 ± 0.4	306.6 ± 29.4	2324 ± 217	4500	3.4 ± 0.2
+ 450 mg Lys	5	189 ± 12	3.8 ± 0.2	285.6 ± 16.8	2606 ± 168	4500	3.8 ± 0.1
+ 450 mg Ile	4	203 ± 20	4.1 ± 0.4	306.6 ± 29.4	1862 ± 181	4500	3.2 ± 0.2
+ 450 mg Thr	7	178 ± 12§	3.6 ± 0.2§	268.8 ± 16.8§	1406 ± 90	4500	3.3 ± 0.2
+ 450 mg Leu	5	195 ± 44	3.9 ± 0.9	294 ± 67.2	3150 ± 716	4500	4.1 ± 0.8
+ 450 mg Phe	6	171 ± 36	3.4 ± 0.8	260.4 ± 50.4	1519 ± 288	4500	3.5 ± 0.6
+ 450 mg Trp	5	175 ± 19‡	3.5 ± 0.5‡	264.6 ± 29.4‡	467 ± 46	4500	2.8 ± 0.2
+ 450 mg Met	8	86 ± 43**	1.7 ± 0.9**	130.2 ± 63**	665 ± 330	4500	6.0 ± 2.9

*Excess amino acids were administered with a stomach tube; †values are means ± SD; ‡,§,**significantly different from values for control group at levels of 5, 1 and 0.1%, respectively. Val, valine; Lys, lysine; Ile, isoleucine; Thr, threonine; Leu, leucine; Phe, phenylalanine; Trp, tryptophan; Met, methionine.

Table 4. Effects of individual amino acid excess on bodyweight gain and protein utilization

Groups*	Bodyweight Day 12 (g)	Bodyweight Day 22 (g)	Bodyweight gain (g/10 days)	Protein efficiency ratio	Net protein utilization
Control	254 ± 20†	347 ± 27	+ 93	2.38 ± 0.25	57 ± 9
+ 450 mg Val	257 ± 11	352 ± 12	+ 95	2.32 ± 0.25	49 ± 7
+ 450 mg Lys	256 ± 10	349 ± 14	+ 93	2.45 ± 0.06	51 ± 8
+ 450 mg Ile	257 ± 4	349 ± 8	+ 92	2.24 ± 0.28	50 ± 6
+ 450 mg Thr	251 ± 17	336 ± 18	+ 85	2.36 ± 0.18	54 ± 3
+ 450 mg Leu	248 ± 9	330 ± 23	+ 82	2.10 ± 0.27	46 ± 7‡
+ 450 mg Phe	242 ± 16	317 ± 26‡	+ 75	2.21 ± 0.39	49 ± 9
+ 450 mg Trp	249 ± 17	325 ± 2**	+ 72	2.06 ± 0.27‡	46 ± 3§
+ 450 mg Met	251 ± 4	235 ± 36**	– 16	–0.94 ± 0.41**	12 ± 7**

*Excess amino acids were administered with a stomach tube; †values are means ± SD; ‡,§,**significantly different from values for control group at levels of 5, 1 and 0.1%, respectively. Val, valine; Lys, lysine; Ile, isoleucine; Thr, threonine; Leu, leucine; Phe, phenylalanine; Trp, tryptophan; Met, methionine.

Table 5. Effects of individual amino acid excess on reproductive performance and fetal growth

Groups*	Products of† conception (g)	Uterus (g)	Placenta (mg)	Litter size	One fetus (g)	Fetus brain‡ (mg)	Fetus liver (mg)
Control	76 ± 9§	3.29 ± 0.29	440 ± 26	11 ± 1	5.4 ± 0.4	181 ± 7	412 ± 34
+ 450 mg Val	74 ± 6	3.12 ± 0.20	422 ± 42	11 ± 1	5.2 ± 0.1	167 ± 3‡‡	398 ± 17
+ 450 mg Lys	80 ± 7	3.28 ± 0.29	419 ± 38	12 ± 1	5.3 ± 0.3	168 ± 11**	404 ± 38
+ 450 mg Ile	76 ± 16	3.19 ± 0.56	434 ± 62	13 ± 1	5.3 ± 0.3	170 ± 2††	410 ± 41
+ 450 mg Thr	67 ± 12	3.43 ± 0.78	442 ± 78	11 ± 2	4.7 ± 0.3††	166 ± 6‡‡	373 ± 37**
+ 450 mg Leu	64 ± 13	2.97 ± 0.28**	481 ± 61	9 ± 2	5.1 ± 0.4	164 ± 5‡‡	424 ± 43
+ 450 mg Phe	63 ± 9**	3.33 ± 0.72	417 ± 50	11 ± 1	4.4 ± 0.8††	149 ± 16‡‡	352 ± 85
+ 450 mg Trp	69 ± 10	3.25 ± 0.55	422 ± 39	11 ± 2	4.9 ± 0.4**	162 ± 5‡‡	404 ± 38
+ 450 mg Met	39 ± 5‡‡	2.36 ± 0.36‡‡	246 ± 54‡‡	11 ± 2	2.6 ± 0.1‡‡	110 ± 3‡‡	193 ± 70‡‡

*Excess amino acids were administered with a stomach tube; †products of conception consist of the uterus, placenta, fetus and amniotic fluid; ‡whole brain including cerebrum, brain stem and cerebellum; §values are means ± SD; **,††,‡‡significantly different from values for control group at levels of 5, 1 and 0.1%, respectively.

Table 6. Effects of individual amino acid excess on nucleic acid and protein contents in fetal liver and brain

Groups*	DNA Conc.† (mg/g)	DNA Total (mg)	RNA Conc. (mg/g)	RNA Total (mg)	Protein Conc. (mg/g)	Protein Total (mg)	RNA/ DNA
Liver							
Control	7.2 ± 1.6‡	2.96 ± 0.17	10.7 ± 0.9	4.41 ± 0.63	119 ± 5	49 ± 4	1.49 ± 0.23
+ 450 mg Val	6.6 ± 0.5	2.63 ± 0.12††	10.8 ± 0.5	4.68 ± 0.15	125 ± 5§	50 ± 3	1.78 ± 0.05**
+ 450 mg Lys	6.4 ± 0.2	2.58 ± 0.30**	10.7 ± 0.4	4.33 ± 0.51	121 ± 10	48 ± 6	1.68 ± 0.08§
+ 450 mg Ile	6.4 ± 0.4	2.62 ± 0.39§	11.0 ± 0.5	4.49 ± 0.54	126 ± 7§	51 ± 4	1.71 ± 0.06§
+ 450 mg Thr	7.3 ± 1.7	2.73 ± 0.70	10.4 ± 1.1	3.88 ± 0.40	120 ± 15	45 ± 8	1.42 ± 0.24
+ 450 mg Leu	6.5 ± 0.6	2.78 ± 0.44	11.0 ± 0.3	4.66 ± 0.51	126 ± 5§	54 ± 6	1.68 ± 0.12
+ 450 mg Phe	8.0 ± 1.2	2.83 ± 0.93	10.9 ± 0.9	3.85 ± 0.84	120 ± 8	42 ± 12	1.36 ± 0.23
+ 450 mg Trp	7.5 ± 2.5	3.03 ± 0.82	10.9 ± 1.1	4.40 ± 0.23	122 ± 13	49 ± 5	1.45 ± 0.33
+ 450 mg Met	7.5 ± 1.3	1.45 ± 0.29	12.0 ± 0.2**	2.31 ± 0.08††	126 ± 6§	24 ± 1††	1.59 ± 0.25
Brain							
Control	6.0 ± 0.6	1.08 ± 0.10	4.9 ± 0.2	0.88 ± 0.06	90 ± 16	16 ± 2	0.81 ± 0.08
+ 450 mg Val	5.8 ± 0.4	0.96 ± 0.04**	4.6 ± 0.1**	0.77 ± 0.03††	90 ± 6	15 ± 1	0.80 ± 0.04
+ 450 mg Lys	6.0 ± 0.2	1.01 ± 0.12	4.7 ± 0.3	0.79 ± 0.08§	92 ± 2	15 ± 1	0.78 ± 0.06
+ 450 mg Ile	6.0 ± 0.4	1.02 ± 0.04	4.9 ± 0.2	0.84 ± 0.04	86 ± 8	15 ± 1	0.83 ± 0.04
+ 450 mg Thr	5.7 ± 0.5	0.95 ± 0.09§	4.5 ± 0.5	0.75 ± 0.08**	79 ± 7	13 ± 1**	0.79 ± 0.06
+ 450 mg Leu	6.2 ± 0.5	1.04 ± 0.10	5.0 ± 0.3	0.83 ± 0.05	95 ± 3	16 ± 1	0.79 ± 0.03
+ 450 mg Phe	5.7 ± 0.3	0.84 ± 0.07††	4.8 ± 0.7	0.71 ± 0.16§	78 ± 6	12 ± 2**	0.85 ± 0.15
+ 450 mg Trp	6.2 ± 0.4	1.01 ± 0.08	4.7 ± 0.3	0.76 ± 0.06**	79 ± 9	13 ± 2§	0.75 ± 0.07
+ 450 mg Met	6.5 ± 0.5	0.71 ± 0.02††	4.9 ± 0.2	0.54 ± 0.04††	86 ± 3	9 ± 1††	0.76 ± 0.04

*Excess amino acids were administered with a stomach tube; †concentration is indicated by mg/g fresh tissue; ‡values are means ±SD; §, **,†† significantly different from values for control group at levels of 5, 1 and 0.1%, respectively. Conc., concentration.

Table 7. Effects of individual amino acid excess on nitrogen balance during days 12–22 pregnancy

Groups*	Intake *n* (mg/10 days)	Urinary *n* (mg/10 days)	Fecal *n* (mg/10 days)	Nitrogen balance (mg/10 days)
Control	5289 ± 233†	2838 ± 252	438 ± 62	2013 ± 448
+ 450 mg Val	5991 ± 490§	3402 ± 673‡	420 ± 45	2169 ± 271
+ 450 mg Lys	5924 ± 332§	3066 ± 401	436 ± 68	2422 ± 545
+ 450 mg Ile	5920± 517§	3808 ± 444**	440 ± 25	1672 ± 375
+ 450 mg Thr	5306 ± 330	3154 ± 235‡	360 ± 34§	1792 ± 205
+ 450 mg Leu	5721 ± 1160	3784 ± 664§	414 ± 89	1523 ± 619
+ 450 mg Phe	5104 ± 986	3010 ± 388	380 ± 51	1714 ± 822
+ 450 mg Trp	5317 ± 504	3278 ± 360‡	440 ± 14	1599 ± 262‡
+ 450 mg Met	2735 ± 1150**	3226 ± 571	180 ±13**	–671 ± 1590**

*Excess amino acids were administered with a stomach tube; †values are means ±SD; ‡, §, ** significantly different from values for control group at levels of 5, 1 and 0.1%, respectively.

a decrease in nucleic acid concentrations in fetal liver due to administration of excess amino acid was not observed and protein concentration in the Val, Ile, Leu and Met groups was higher than that of the control group, although in other groups there was no difference. Next, DNA content decreased in the Val, Lys, Ile and Met groups. The effect was remarkable in the Met group and both protein and nucleic acid contents were about one-half of those of the control group. However, an influence on the RNA : DNA ratio and the protein : DNA ratio from excess amino acid administration was not observed in all groups. The influences of excessively administered amino acid on protein and nucleic acid concentrations in the brain were hardly seen in all groups but as for the total contents of protein and nucleic acid, the significant decrease in protein content was observed in the Thr, Phe, Trp and Met groups. Secondly, DNA content decreased significantly to 0.96, 0.95, 0.84 and 0.71 mg in the Val, Thr, Leu and Met groups, respectively, against 1.08 mg for the control group. However, there was no influence from excess amino acid administration on the RNA : DNA ratio or the protein : NA ratio.

Table 7 shows nitrogen balance during days 12–22 of pregnancy (Experiment 3). Nitrogen intake was significantly low in the Met group but in other groups it was almost the same as the control group or increased by approximately 400–700 mg. However, for tube-administered groups, 500–900 mg of nitrogen had already been contained in excess amino acid administered through a stomach tube; thus, real intake of nitrogen from the diet was equivalent to that of the control group or approximately 600 mg less. With regard to nitrogen excretions, more urinary nitrogen was excreted in all groups than in the control group by approximately 200–1000 mg, while fecal nitrogen decreased in the Met and Thr groups, with no difference being noted in other groups. Although nitrogen balance throughout the total period decreased significantly to –671 and 1599 mg in the Met and Trp groups against 2013 mg for the control group, a significant difference was not seen in other groups.

Discussion

It is known that various lesions are caused by the effect of individual amino acids that are added to the diet excessively.[1–10] However, it seems that most of these lesions are not caused by the direct effect of excess amino acid but mainly due to the remarkable decrease in food intake.[6] In order to examine a direct adverse effect by excess amino acid, therefore, it is preferable to carry out the experiments minimizing the decrease in food intake. Therefore, as we presumed that the decrease in food intake would be controlled to some extent if excess amino acid was administered directly with a stomach tube, we administered 210, 250 or 450 mg/day of essential amino acids individually, through a stomach tube. Consequently, except in the Met, Trp and Thr groups, there was no significant decrease in food intake, which was observed when excess amino acid was added to the diet.

Food intake of the Trp and Thr groups was 88 and 90% of that of the control group, respectively, and fair improvement was observed. However, the Met group ingested only 44% of the amount of the control group, which was a slight improvement as compared with the remarkable decrease to approximately 20–30% of the amount of the control group when added to the diet.[2,5] When the effect of excess individual amino acids was examined under these conditions, the significant decrease in protein efficiency ratio (PER) and NPU was observed with severity in order of Met and Trp. It is also reflected in the growth of rats and the growth rate during the administration period of Met was –6%. Furthermore, nitrogen balances were significantly low in the Met and Trp groups. This was the case in the Met group, in particular, which showed a negative nitrogen balance of –671 mg; this corresponded to the change in bodyweight.

From the above results it might be predicted that, in the respective groups of Met and Trp, impediments to the utilization of dietary protein in the body and, further, acceleration of body protein decomposition occur simultaneously with the remarkable decrease in food intake.[22,26]

In the experiment examining the cause of the decrease in food intake, food intake when excess Met was administered with a stomach tube was three times as much as that when it was added to the diet, while in the dark periods it was 3.6 times as much. At this time the amino acid pattern in the plasma of the tube-administered group showed the same pattern as that of the control group but when excess Met was added to the diet, the values of Met, Gly and Tau were significantly high and an imbalance of the amino acid pattern in the plasma was seen.

Accordingly, the following can be presumed. Administering excess Met with a stomach tube causes, temporarily, something abnormal in the amino acid pattern in plasma but

gradually it becomes normalized and food intake recovers. However, administering excess Met by adding to the diet always leads to abnormality of amino acid in plasma and suppresses food intake.

As to the relation of excess Met and food intake against maintenance of pregnancy in rats, food intake decreased as the levels of excess Met became high and at the same time the maintenance rate of pregnancy got low. Judging from these results and the report by Niiyama that approximately 80% of pregnant rats abort when energy intake is restricted to around one-third of that of the control group, it is thought that excess Met severely affects maintenance of pregnancy; when 450 mg/day Met is administered, energy intake is 66% of that of the control group.[27] However, 75% of pregnant rats abort. Excess Met seems to cause the decrease of the maintenance rate of pregnancy, nearly equivalent to the case that energy intake is restricted to approximately one-third of that of the control group.

With regard to the growth of products of conception, the first notable fact was that excess amino acid administration resulted in a significant decrease in the fetal brain weight in all groups, and especially in the Met group where it decreased to approximately 60% of normal weight. For other tissues, the growth was almost normal except in the Met group: in that group, though, both products of conception and the fetal liver were approximately 50% of those of the control group. We assume that such growth suppression of products of conception was mainly caused by the decrease in food intake rather than the direct toxic effect of Met.[6] However, when considering the findings that the maternal protein/energy malnutrition affects various fetal tissues through different pathways and that the fetal liver weight decreases correlatively with the fetal bodyweight while the fetal brain is protected during maternal protein/energy restriction,[28] as well as the fact that the weight and protein contents of the fetal brain significantly decreased in the Met group being, it is also possible that Met or its derivative directly inhibits protein synthesis in the fetal brain.[5]

The size and number of cells in these tissues were checked with the protein : DNA ratio and the total DNA. The effect of excess amino acid on cell size was hardly seen, but as for the cell number, the significant decrease caused by excess Met administration was observed in both fetal liver and brain. Consequently, we presume that the decrease in cell numbers in the fetal liver and brain is mainly due to the state of protein/energy deficit induced by the decrease in food intake. This takes into consideration not only the report by Niiyama, which shows that the decrease in cell number occurs because DNA synthesis through both *de novo* and salvage pathways is inhibited if protein deficiency becomes severe, but also the data of our studies in which the direct effect of excess Met was observed, excluding the decrease in cell number due to the decrease in food intake by Met administration.[27]

References

1. Harper AE, Benevenga NJ, Wohlhueter RM. Effects of ingestion of disproportionate amounts of amino acids. Physiol Rev 1970; 50: 428–557.
2. Muramatsu K, Odagiri H, Morishita S, Takeuchi H. Effect of excess levels of individual amino acids on growth of rats fed casein diets. J Nutr 1971; 101: 1117–1126.
3. Muramatsu K, Takeuchi H, Funaki Y, Chisuwa A. Influence of protein source on growth-depressing effect of excess amino acids in young rats. Agr Biol Chem 1972; 36: 1269–1276.
4. Muramatsu K. Amino acid toxicity and its nutritional control in the rat. J Jpn Soc Nutr Food Sci 1984; 37: 399–418.
5. Matsueda S, Niiyama Y. The effects of excess amino acids on maintenance of pregnancy and fetal growth in rats. J Nutr Sci Vitaminol 1982; 28: 557–573.
6. Cieslak DG, Benevenga NJ. The effect of amino acid excess on utilization by the rat of the limiting amino acid – lysine and threonine at equalized food intakes. J Nutr 1984; 114: 1878–1883.
7. Benevenga NJ, Steele RD. Adverse effects of excessive consumption of amino acids. Ann Rev Nutr 1984; 4: 157–181.
8. Nagaoka S, Aoyama Y, Yoshida A. Effect of tyrosine and some other amino acids on serum level of cholesterol in rats. Nutr Rep Inter 1985; 31: 1137–1148.
9. Katayama T, Hayashi J, Kishida M, Kato N. Effect of dietary excess amino acids on the concentrations of cholesterol, α-tocopherol, ascorbic acid, and copper in serum and tissues of rats. J Nutr Sci Vitaminol 1990; 36: 485–495.
10. Shibata K, Taniguchi I, Onodera M. Effect of adding branched-chain amino acids to a nicotinic acid-free, low-protein diet on the conversion ratio of tryptophan to nicotinamide in rats. Biosci Biotech Biochem 1994; 58: 970–971.
11. Benevenga NJ. Toxicities of methionine and other amino acids. J Agric Food Chem 1974; 22: 2–9.
12. Katz RS, Baker DH. Methionine toxicity in the chick: Nutritional and metabolic implications. J Nutr 1975; 105: 1168–1175.
13. Harter JM, Baker DH. Factors affecting methionine toxicity and its alleviation in the chick. J Nutr 1978; 108: 1061–1070.
14. Fico ME, Hassan AS, Milner JA. The influence of excess lysine on urea cycle operation and pyrimidine biosynthesis. J Nutr 1982; 112: 1854–1861.
15. Finkelstein JD, Martin JJ. Methionine metabolism in mammals. J Biol Chem 1986; 261: 1582–1587.
16. Sugiyama K, Kushima Y, Muramatsu K. Effect of dietary glycine on methionine metabolism in rats fed a high-methionine diet. J Nutr Sci Vitaminol 1987; 33: 195–205.
17. Boggs DE, Waisman HA. Influence of excess dietary phenylalanine on pregnant rats and their fetuses. Proc Soc Exp Biol Med 1964; 115: 407–410.
18. Kerr GR, Chamove AS, Harry MA, Harlow HF, Waisman HA. The effect of maternal hyperphenylalaninemia durring pregnancy in the rhesus monkey. Pediatrics 1968; 42: 27–36.
19. Vian AT, Leathem JH, Wannemacher RW Jr. Pregnancy and excess dietary methionine in rats. Fed Proc 1969; 28: 638.
20. Knipfel JE, Elliot JE. Gestational dietary methionine levels as related to growth and ^{35}S–distribution in maternal and fetal rats. Nur Rep Int 1974; 10: 103–112.
21. Suyama I, Tani M, Matsumura M, Isshiki G, Okano Y, Oura T, Nishimura K. Fetal heart malformations in experimental hyperphenylalaninemia in pregnant rats. Cong Anom 1989; 29: 15–29.
22. Mori M, Yamagami I. Effect of excess methionine during pregnancy on fetal growth in rats. J Jpn Soc Nutr Food Sci 1978; 31: 235.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193: 265–275.
24. Oser BL. Total nitrogen. In: Oser BL, ed. Hawk's physiological chemistry. New York: McGraw-Hill, 1965.
25. Wannemacher RW Jr, Banks WL Jr, Wunner WH. Use of a single tissue extract to determine cellular protein and nucleic acid concentrations and rate of amino acid incorporation. Anal Biochem 1965; 11: 320–326.
26. Daniel RG, Waisman HA. The effects of excess amino acids on the growth of the young rat. Growth 1968; 32: 255–265.
27. Niiyama Y. Protein nutrition in pregnancy. J Jpn Soc Nutr Food Sci 1978; 31: 117–127.
28. Endo S, Niiyama Y, Kamori K, Inoue G. Effect of protein deprivation during pregnancy on nucleic acid and protein syntheses in fetal rat brain and liver. Nutr Rep Int 1974; 10: 209–219.