

Plasma amino acid profiles at various reproductive stages in female rats

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ABSTRACT. We measured the plasma levels of amino acids at various reproductive stages in female rats, including the estrous cycle, pregnancy and lactation, and compared the resulting amino acid profiles using two- or three-dimensional figures. These figures revealed that the amino acid profiles of pregnant and lactating dams differed considerably from those during the estrous cycle or in male rats. The plasma levels of individual amino acids were almost the same between proestrus, estrus, metestrus and diestrus, and their profiles did not differ significantly. However, the amino acid profiles changed during pregnancy and lactation in dams. The plasma Ser level decreased significantly in mid and late pregnancy, whereas Tyr, Gly and His decreased significantly in the late and end stages of pregnancy, and Trp and Lys significantly decreased and increased at the end of pregnancy, respectively. Much larger changes in amino acid profiles were observed during lactation, when the levels of many amino acids increased significantly, and none showed a significant decrease. Plasma Pro, Ser and Gly levels increased continuously from day 1 until day 15 of lactation, whereas Asn and Met increased significantly from days 1 and 5 respectively until the end of lactation. These results suggest that the profiles of plasma amino acids show characteristic changes according to reproductive stage and that it may be necessary to consider such differences when performing amino acid-based diagnosis.

KEY WORDS: diagnosis, metabolism, reproduction

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The concentrations of individual free amino acids in plasma are precisely maintained through intake of food, proteins or amino acids, and their metabolism in the body. However, pathological conditions, such as liver disease [4], renal disease [6], diabetes [28] and cancer [7], may change amino acid homeostasis. In particular, the progressive stage of cancer can be distinguished by changes in the concentration profiles of individual amino acids in plasma [12]. Therefore, analysis of plasma amino acids levels may be diagnostically useful [15].

It has been reported that there are differences in plasma amino acid concentrations between male and female mammals, suggesting that it may be necessary to consider this sex difference when performing diagnosis based on amino acids [18]. Although one report has examined the relationship between aging and sex difference in terms of plasma amino acid levels [18], there is little information about such differences in female mammals at various reproductive stages, such as during the estrous cycle, pregnancy and lactation. Basal metabolism, protein synthesis, energy consumption and food intake are all considered to be related to changes in amino acid metabolism associated with these reproductive states [3, 9, 11, 22, 26], and therefore, it can easily be assumed that the blood profiles of amino acids will also vary accordingly.

Amino acids are particularly important during lactation,

as they are required for synthesis of milk protein, and the uptake of amino acids into mammary gland tissues would be expected to cause changes in their plasma levels. In addition, as the expression of certain amino acid transporters changes during lactation, this will also have an impact on the plasma levels of amino acids [1]. Similarly, as estrogen and progesterone directly regulate the catabolism of BCAA and proteolysis, respectively [8, 16], changes in the levels of these hormones during the estrous cycle, pregnancy and lactation will affect plasma amino acids.

In the present study, therefore, we examined the plasma levels of various amino acids at different reproductive stages in female rats, including the estrous cycle, pregnancy (early, middle and late periods) and lactation (early, middle and late periods).

MATERIALS AND METHODS

All animals (Charles River, Yokohama, Japan) were housed under controlled temperature ($25 \pm 1^\circ\text{C}$) and 12 hr: 12 hr light: dark conditions (lights on at 07:00 hr) with food and water available *ad libitum*. All of the procedures were performed in accordance with the guidelines for animal care stipulated by the Japanese Physiological Society and approved by the ethics committees of Miyazaki University. All efforts were made to minimize animal pain and suffering, and the number of animals used.

In the first set of experiments, adult female Wistar rats (approximately 10 weeks old) were used. From 1 week prior to sample collection, vaginal smears were taken daily from the rats for determination of the estrous cycle, and accordingly, the animals were subdivided into 4 groups: diestrus (n=9), proestrus (n=7), estrus (n=9) and metestrus (n=16). Blood samples were collected on each day of the estrous

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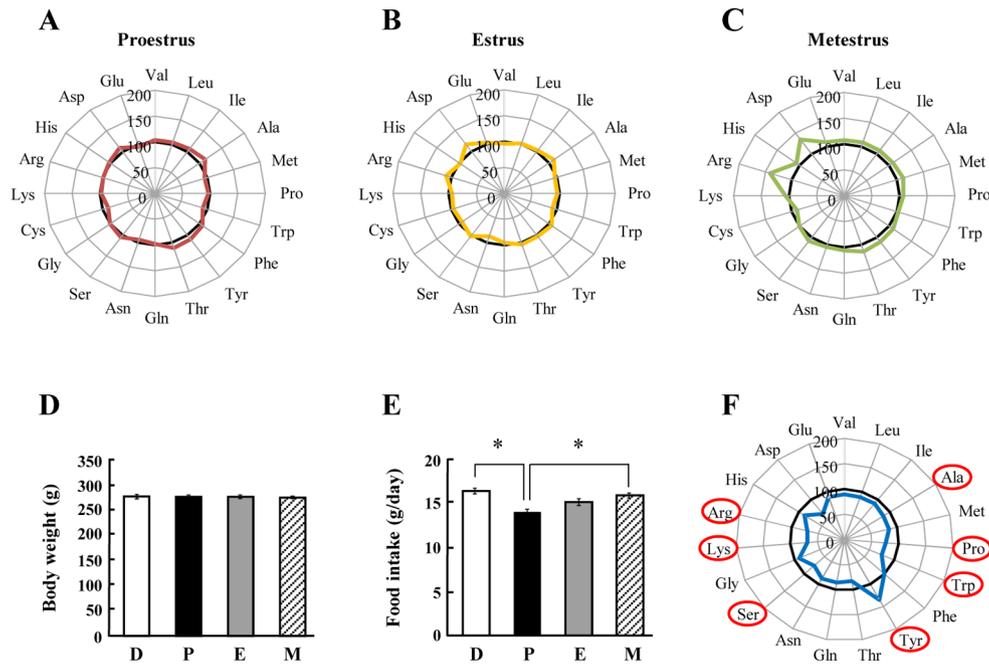


Fig. 1. Alterations in plasma amino acids concentrations (A–C, F), body weight (D) and daily food intake (E) at various stages of the estrous cycle in female rats and also in age-matched male rats. The concentrations of individual plasma amino acids are shown as a percentage ratio relative to the concentrations in diestrus females. A: results for proestrus (red line), B: estrus (yellow line), C: metestrus (green line) and F: age-matched males (blue line). The data for body weight and food intake represent the mean \pm standard error of the mean (SEM). D, diestrus (white bar); P, proestrus (black bar); E, estrus (gray bar); M, metestrus (diagonal bar). Amino acids indicated by red shading and asterisks showed a significant difference vs. diestrus females ($P < 0.05$).

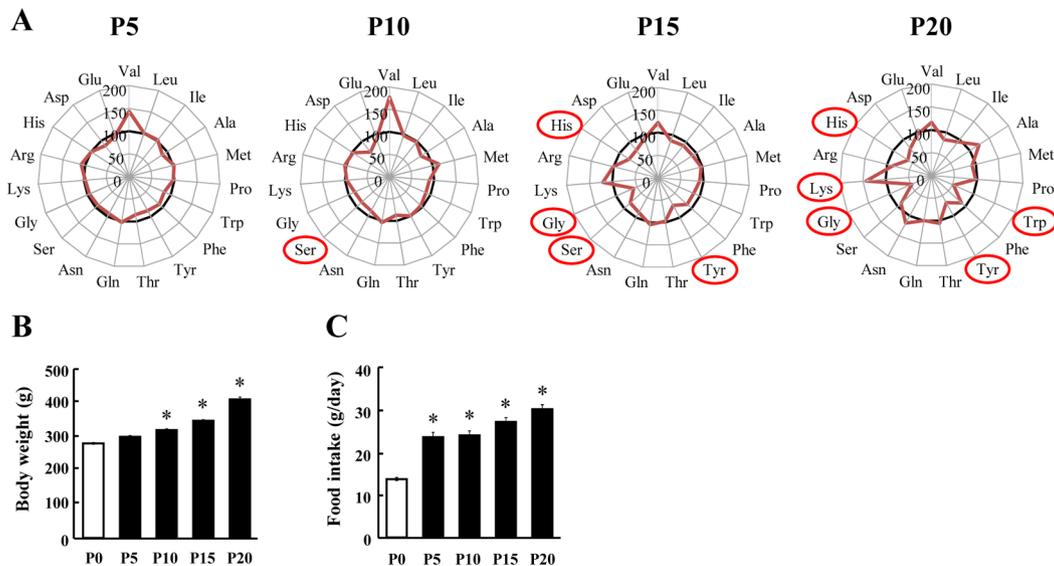


Fig. 2. Temporal alterations in plasma amino acid concentrations (A, red line), body weight (B) and food intake (C) during pregnancy in female rats ($n=4$ /group). The concentrations of individual plasma amino acids are shown as a percent ratio relative to the concentrations in non-pregnant female rats (proestrus; $n=4$, black line). The level of Cys was not detectable. Amino acids indicated by red shading and asterisks showed a significant difference vs. non-pregnant females ($P < 0.05$).

cycle. We showed the plasma amino acid concentration with supplemental table, and the percentage concentration ratios

for the various amino acids were also presented as circular profiles relative to the amino acid concentrations of diestrus.

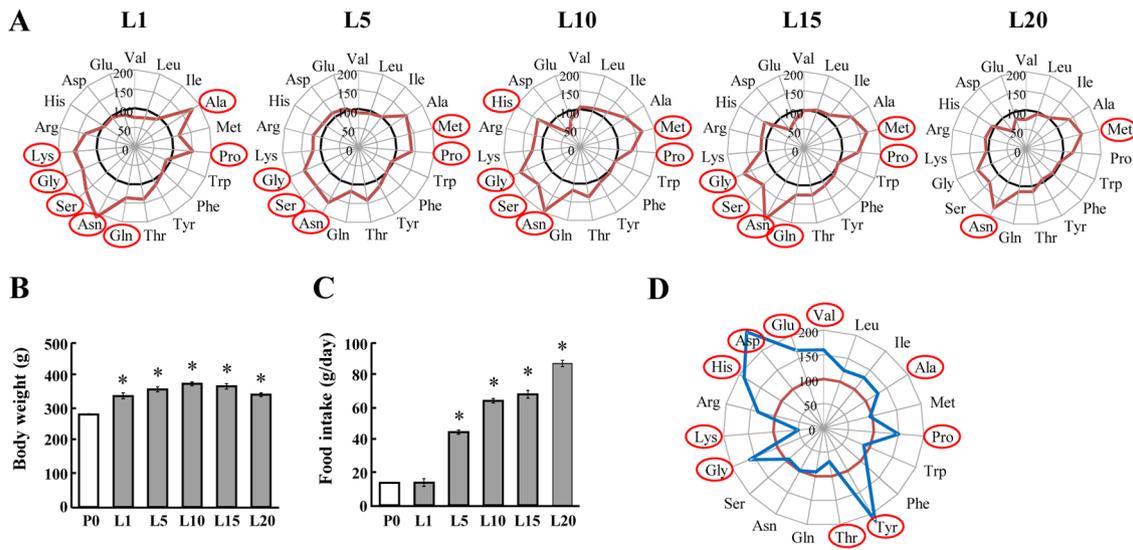


Fig. 3. Temporal alterations in plasma amino acid concentrations (A, red line), body weight (B) and food intake (C) during lactation in female rats (n=4/group). The concentrations of individual plasma amino acids are shown as a percentage ratio relative to the concentrations in non-pregnant females (proestrus; n=4, black line). D shows the results of the pups just after weaning (n=6, blue line) compared with the dams on the day 20 of lactation (n=4, red line). The level of Cys was not detectable. Amino acids indicated by red shading and asterisks showed a significant difference vs. each control group ($P < 0.05$).

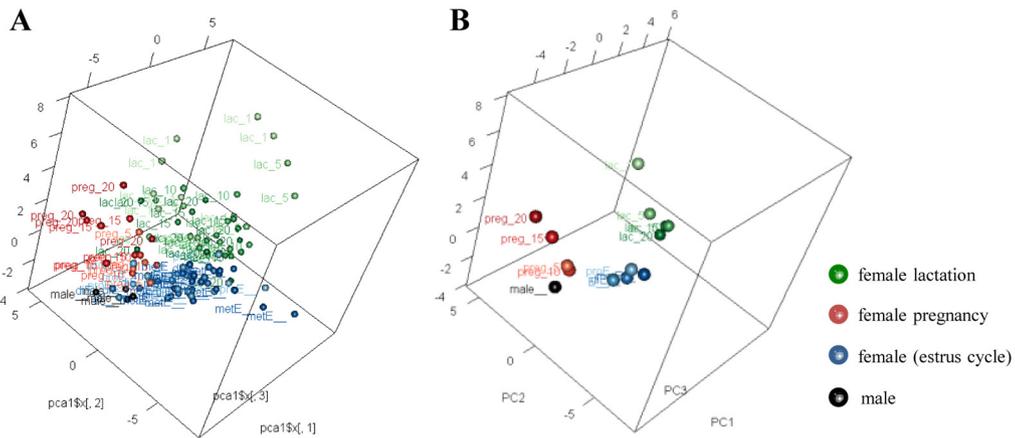


Fig. 4. Principal component analysis (PCA) plots from amino acid concentration data of individuals (A) and median (B) in each experiment. Black plot; male rats age-matched with females in the estrus cycle experiment, blue plot; estrus cycle experiment, red plot; pregnancy period and green plot; lactation period.

In the next experiment, adult female rats were mated on the day of proestrus, and the day after mating was counted as day 1 of pregnancy (P1). Blood samples were taken every 5 days (P5, 10, 15 and 20; n=4). After delivery and during lactation, blood samples were taken from the dams in the same way (L1, 5, 10, 15 and 20; n=4), and we take the samples from the female pups just after weaning (n=6). The percentage concentration ratios for the various amino acids were also presented as circular profiles relative to the concentrations determined just before pregnancy (proestrus).

All blood samples were collected into heparinized capil-

lary tubes by the tail tip incision method, and these were centrifuged immediately at 4°C and 14,000 rpm for 4 min. We think that the possibility of contamination of amino acids derived from erythrocyte by hemolysis is almost none, since we paid scrupulous attention not to hemolyze at the blood sampling and hemolyzed sample was excluded from the assay. The resulting plasma samples were mixed with 2 volumes of 5% (w/w) trichloroacetic acid and centrifuged immediately at 4°C and 10,000 rpm for 20 min to remove the precipitated protein. The supernatant was filtered with an Ultrafree-MC filter (Cat.No. UFC5010BK, Millipore,

Billerica, MA, U.S.A.), and the plasma amino acid concentrations were measured using an automatic amino acid analyzer (L-8800A; Hitachi, Tokyo, Japan). We focused on the 20 amino acids that are the components of proteins: valine (Val), leucine (Leu), isoleucine (Ile), alanine (Ala), methionine (Met), proline (Pro), tryptophan (Trp), phenylalanine (Phe), tyrosine (Tyr), threonine (Thr), glutamine (Gln), asparagine (Asn), serine (Ser), glycine (Gly), cysteine (Cys), lysine (Lys), arginine (Arg), histidine (His), aspartic acid (Asp) and glutamic acid (Glu).

Briefly, amino acids were separated by cation-exchange chromatography and detected spectrophotometrically after post-column reaction with ninhydrin reagent. All sample collections were performed at the same time (11:30–12:30 hr) to minimize any influence of circadian rhythm.

All data were expressed as the mean \pm standard error of the mean (SEM). In the first experiment, differences between groups at the various stages of the estrous cycle were analyzed by one-way ANOVA and *post hoc* Tukey's test. In the other experiment, data for amino acid levels were analyzed by one-way repeated ANOVA, and changes between time points during the experimental period were analyzed by Tukey's test. The sexual difference (Fig. 1F) and the results of the pups (Fig. 3D) were analyzed by Student's *t*-test.

To visualize the results, the concentrations of amino acids among the experimental groups were displayed as 2- or 3-dimensional figures. For the 2-dimensional circular plane figures, we plotted the percentage concentration of each amino acid relative to the control level (at diestrus or proestrus). In the three-dimensional figures, principal components analysis (PCA) was performed to reduce the dimensionality of the data set and to identify new meaningful underlying variables [24]. To obtain a more accurate result in the latter analysis, we used 40 kinds of amino acids and their metabolic intermediates, including taurine, sarcosine, citrulline, ornithine and hydroxyproline, for PCA analysis.

RESULTS

The actual concentrations of the various amino acids in plasma are shown in Tables 1–3. To compare the types of amino acid changes occurring during the estrous cycle, pregnancy or lactation, we displayed the ratios of the respective amino acids at each stage in the form of circular profiles relative to the corresponding values for diestrus or proestrus.

The levels of the individual amino acids in plasma were almost the same among proestrus, estrus, metestrus and diestrus, and their circular profiles showed no significant differences (Fig. 1A–1C, Table 1). On the other hand, the shapes of the circular profiles changed during the course of pregnancy (Fig. 2, Table 2). The plasma Ser level decreased significantly in mid and late (P10 and 15) pregnancy, and Tyr, Gly and His decreased significantly at the late and end stages (P15 and 20) of pregnancy, whereas Trp and Lys significantly decreased and increased at the end of pregnancy, respectively.

Much larger changes in the circular profiles of plasma amino acids were observed during the lactation period

(Fig. 3, Table 3): many amino acids showed significant increases, and none showed a significant decrease. Plasma Pro, Ser and Gly levels increased continuously from day 1 until day 15 of lactation. The levels of Asn and Met increased significantly from days 1 and 5 respectively until the end of lactation. Ala and Lys were increased significantly only on day 1 of lactation, His was increased on day 10, and Gln was increased on days 1 and 15. Fig. 3D shows the results of the pups just after weaning compared with the dams on day 20 of lactation. Many amino acids (Val, Ala, Pro, Tyr, Gly, His, Asp and Glu) showed significant increases, and only 2 amino acids (Thr and Lys) were decreased.

PCA analysis revealed clear differences in the 3D plots among the groups (Fig. 4A and 4B). On the PCA plot, we added the plasma amino acid levels for an age-matched male group ($n=4$) to confirm whether or not a sex difference was evident. The positions of each sample were plotted against the axes of the first three components (PC1, PC2 and PC3) in a 3D space and were colored according to each experimental group (black plot; male rats age-matched with the females used in the estrous cycle experiment, blue plot; estrous cycle experiment, red plot; pregnancy period and green plot; lactation period). PCA analysis allowed visual identification of data patterns and highlighted similarities and differences among the reproductive stages in female rats. Figure 4A shows the PCA results for all individual samples, and Fig. 4B shows the medians for each of the experimental groups. The dispersion of the points assembled in each group.

DISCUSSION

Presentation of the amino acid profiles in a 2-dimensional circular plane, using the levels of amino acids during diestrus or proestrus as controls, demonstrated that the profiles changed during pregnancy and lactation in dams, but not during the estrous cycle. In addition, 3-dimensional PCA analysis revealed a different distribution of plasma amino acid levels among reproductive stages or sex, especially in late pregnancy and early lactation in dams. These observations suggest that the levels of amino acids in female rats differ from those in males and that they change according to reproductive stage.

Generally, it has been considered that protein metabolism differs between males and females, since the sexes differ considerably in muscle mass and energy consumption [2]. Some of these differences may be correlated with sex steroid hormones: testosterone and estrogen stimulate and inhibit protein synthesis, respectively [14, 27]. Also, it has been reported that ovariectomy causes an increase of BCCA and a decrease of Ala in plasma [10].

Changes in the circular amino acid profiles in dams during pregnancy or lactation may result from transitional changes in the plasma levels of steroid. On the other hand, in rats during the estrous cycle, there were no significant differences in the circular profiles, even though plasma steroid hormone levels do vary during the cycle. Although the reason for this unexpected result is unknown, it may be results of short term change of steroid levels in estrus cycle. Another possibility

Table 1. Alterations in plasma amino acid concentrations during the estrous cycle in female rats and age-matched male rats

Amino acids ($\mu\text{mol/l}$)	Diestrus (n=9)	Proestrus (n=7)	Estrus (n=9)	Metestrus (n=16)	age-matched Male (n=4)
Valine	175.8 ± 6.2	182.0 ± 12.1	168.9 ± 5.6	189.6 ± 4.3	157.8 ± 4.8
Leucine	124.4 ± 3.8	129.3 ± 7.5	126.0 ± 4.0	135.2 ± 4.6	110.5 ± 4.2
Isoleucine	72.8 ± 2.0	77.7 ± 5.1	74.7 ± 2.3	78.9 ± 1.4	65.9 ± 3.4
Alanine	408.9 ± 10.7	459.5 ± 27.3	453.2 ± 24.7	444.0 ± 13.4	349.1 ± 31.2*
Methionine	66.6 ± 3.3	63.4 ± 4.9	64.1 ± 1.7	74.5 ± 2.9	56.3 ± 3.4
Proline	223.7 ± 8.9	217.6 ± 17.0	215.1 ± 9.1	238.4 ± 5.9	170.5 ± 6.5*
Tryptophan	141.9 ± 6.8	129.9 ± 7.0	130.1 ± 6.3	145.3 ± 3.6	106.1 ± 7.4*
Phenylalanine	67.0 ± 2.1	71.4 ± 4.1	70.9 ± 2.0	73.0 ± 1.8	66.7 ± 1.8
Tyrosine	67.6 ± 2.5	74.4 ± 5.6	69.3 ± 2.8	74.6 ± 3.0	91.2 ± 4.7*
Threonine	320.4 ± 18.0	357.3 ± 40.1	332.4 ± 25.5	363.9 ± 15.4	265.5 ± 5.0
Glutamine	650.5 ± 30.4	637.5 ± 25.6	619.1 ± 20.5	689.8 ± 18.4	556.3 ± 16.6
Asparagine	51.0 ± 3.2	48.2 ± 4.2	44.4 ± 2.1	53.1 ± 2.2	44.5 ± 2.8
Serine	261.6 ± 11.2	275.4 ± 19.4	267.8 ± 9.8	283.9 ± 9.8	197.5 ± 7.9*
Glycine	281.1 ± 13.1	283.1 ± 17.5	263.8 ± 7.9	282.3 ± 8.8	255.2 ± 3.8
Cysteine	1.5 ± 0.4	1.4 ± 0.4	1.7 ± 0.7	1.4 ± 0.3	ND
Lysine	491.2 ± 19.2	479.6 ± 30.4	461.9 ± 21.9	521.9 ± 20.7	336.8 ± 21.5*
Arginine	178.5 ± 12.6	176.5 ± 4.8	196.5 ± 9.0	251.7 ± 28.1	125.8 ± 14.0*
Histidine	58.3 ± 2.3	59.8 ± 3.1	57.0 ± 1.6	62.3 ± 1.8	51.3 ± 0.6
Aspartate	10.8 ± 1.7	11.8 ± 1.3	12.7 ± 1.7	14.6 ± 1.6	7.0 ± 1.0
Glutamate	91.3 ± 8.2	86.4 ± 7.8	94.3 ± 6.9	99.7 ± 7.6	81.6 ± 13.4

The data represent the mean ± standard error of the mean (SEM, n=7–16). *, $P < 0.05$ vs. diestrus by Student's *t*-test.

Table 2. Alterations in plasma amino acid concentrations during pregnancy

Amino acids ($\mu\text{mol/l}$)	Proestrus (P0)	P5	P10	P15	P20
Valine	172.3 ± 17.9	247.3 ± 83.1	305.8 ± 85.3	211.1 ± 61.1	201.1 ± 64.6
Leucine	123.6 ± 11.0	124.5 ± 6.0	119.6 ± 4.0	107.5 ± 2.6	103.1 ± 9.6
Isoleucine	73.3 ± 7.6	74.9 ± 3.6	71.6 ± 2.2	66.3 ± 1.0	68.5 ± 7.4
Alanine	414.3 ± 26.3	361.6 ± 14.6	348.2 ± 15.7	373.8 ± 49.7	510.6 ± 33.6
Methionine	59.3 ± 4.3	60.3 ± 4.1	67.5 ± 4.4	57.8 ± 5.6	54.0 ± 3.2
Proline	191.6 ± 12.3	186.3 ± 13.0	171.7 ± 8.8	175.2 ± 12.6	184.1 ± 9.1
Tryptophan	119.3 ± 6.2	100.6 ± 1.2	106.2 ± 6.5	101.8 ± 4.3	62.4 ± 5.5*
Phenylalanine	67.8 ± 3.6	61.1 ± 2.3	64.5 ± 3.2	58.8 ± 3.1	59.8 ± 4.9
Tyrosine	74.8 ± 5.2	64.0 ± 3.2	74.4 ± 6.7	51.9 ± 5.8*	50.3 ± 4.9*
Threonine	276.0 ± 16.1	235.1 ± 13.9	240.6 ± 21.0	268.7 ± 13.8	292.1 ± 23.9
Glutamine	601.1 ± 11.4	614.6 ± 20.7	623.2 ± 36.4	631.1 ± 39.5	598.8 ± 24.3
Asparagine	43.1 ± 5.0	40.1 ± 1.6	37.4 ± 1.6	37.7 ± 2.5	50.9 ± 3.2
Serine	246.4 ± 17.6	230.7 ± 6.2	207.5 ± 5.3*	202.8 ± 8.8*	221.8 ± 6.3
Glycine	272.2 ± 21.2	259.5 ± 9.3	232.2 ± 14.0	158.1 ± 7.9*	127.6 ± 3.6*
Cysteine	ND	ND	ND	ND	ND
Lysine	435.8 ± 19.8	422.1 ± 48.3	418.6 ± 31.2	527.4 ± 30.9	623.4 ± 60.3*
Arginine	160.8 ± 20.0	174.6 ± 9.4	166.2 ± 4.4	162.1 ± 24.8	140.4 ± 8.7
Histidine	59.3 ± 4.6	58.9 ± 1.5	57.8 ± 3.1	44.8 ± 3.2*	36.6 ± 2.1*
Aspartate	11.9 ± 2.3	10.3 ± 2.1	8.3 ± 0.8	9.2 ± 1.1	9.0 ± 0.6
Glutamate	87.6 ± 12.5	82.8 ± 15.4	78.1 ± 10.4	80.2 ± 10.1	83.3 ± 10.6

The data represent the mean ± standard error of the mean (SEM, n=4). *, $P < 0.05$ vs. non-pregnant female rats group.

is that pregnant or lactating dams have a higher nutritional demand and food intake in comparison with normal rats [11]. The present study also demonstrated an increase of food intake in pregnant and lactating dams.

In spite of the increase of food intake during pregnancy,

most of the significant changes in plasma amino acid levels were falls, rather than increases. In addition, most of the amino acids that showed reduction during pregnancy were glycogenic amino acids. In pregnant rats, increase of insulin and ketone body, and decrease of glucose are observed in

Table 3. Alterations in plasma amino acid concentrations during lactation in dams and the pups just after weaning

Amino acids ($\mu\text{mol/l}$)	Proestrus (P0)	L1	L5	L10	L15	L20	pups
Valine	172.3 \pm 17.9	130.5 \pm 32.2	154.6 \pm 12.0	182.8 \pm 1.4	168.7 \pm 9.8	129.2 \pm 24.4	206.8 \pm 10.2 [#]
Leucine	123.6 \pm 11.0	97.5 \pm 10.9	115.6 \pm 8.4	133.6 \pm 3.5	129.6 \pm 6.5	117.7 \pm 18.2	146.5 \pm 8.8
Isoleucine	73.3 \pm 7.6	66.1 \pm 4.2	73.6 \pm 5.9	85.5 \pm 1.6	79.8 \pm 4.0	74.2 \pm 11.2	97.0 \pm 3.8
Alanine	414.3 \pm 26.3	739.3 \pm 50.2*	621.8 \pm 41.0	585.1 \pm 35.2	608.9 \pm 61.9	572.1 \pm 44.2	738.9 \pm 33.5 [#]
Methionine	59.3 \pm 4.3	70.0 \pm 6.6	82.5 \pm 3.7*	98.7 \pm 4.2*	99.3 \pm 5.3*	90.5 \pm 6.4*	86.1 \pm 2.7
Proline	191.6 \pm 12.3	288.6 \pm 27.7*	268.7 \pm 18.7*	259.0 \pm 8.9*	268.7 \pm 14.9*	239.4 \pm 15.3	358.2 \pm 39.7 [#]
Tryptophan	119.3 \pm 6.2	106.1 \pm 12.8	113.3 \pm 2.5	118.1 \pm 7.2	115.4 \pm 9.9	113.7 \pm 8.9	99.5 \pm 4.9
Phenylalanine	67.8 \pm 3.6	66.3 \pm 4.8	67.7 \pm 4.7	70.9 \pm 2.1	71.8 \pm 6.4	65.0 \pm 6.4	76.4 \pm 5.6
Tyrosine	74.8 \pm 5.2	85.0 \pm 8.3	87.4 \pm 10.6	81.9 \pm 6.3	84.4 \pm 7.5	69.0 \pm 9.8	148.1 \pm 17.3 [#]
Threonine	276.0 \pm 16.1	386.4 \pm 60.6	390.9 \pm 21.2	377.4 \pm 20.5	347.2 \pm 17.1	316.8 \pm 23.4	219.5 \pm 12.7 [#]
Glutamine	601.1 \pm 11.4	818.9 \pm 40.7*	704.7 \pm 43.1	711.7 \pm 37.1	752.6 \pm 35.3*	688.8 \pm 25.6	626.0 \pm 46.6
Asparagine	43.1 \pm 5.0	90.5 \pm 9*	71.4 \pm 2*	84.3 \pm 3.1*	91.7 \pm 1.9*	76.5 \pm 2.2*	75.7 \pm 12.9
Serine	246.4 \pm 17.6	422.5 \pm 29.7*	372.0 \pm 30.4*	366.3 \pm 12.4*	353.0 \pm 12.1*	321.4 \pm 15.1	300.6 \pm 24.0
Glycine	272.2 \pm 21.2	400.6 \pm 34*	421.6 \pm 41.6*	465.5 \pm 20.6*	461.7 \pm 22.7*	381.3 \pm 14.7	613.2 \pm 25.6 [#]
Cysteine	ND	ND	ND	ND	ND	ND	ND
Lysine	435.8 \pm 19.8	686.5 \pm 100.8*	521.1 \pm 34.9	550.1 \pm 30.8	489.6 \pm 2.6	459.8 \pm 32.2	232.9 \pm 16 [#]
Arginine	160.8 \pm 20	213.8 \pm 20.8	196.5 \pm 17.3	194.9 \pm 8.6	182.5 \pm 5.5	178.7 \pm 13.9	240.2 \pm 26.6
Histidine	59.3 \pm 4.6	56.3 \pm 4.5	66.2 \pm 3.3	78.6 \pm 3.4*	72.3 \pm 3.2	63.2 \pm 6.7	119.4 \pm 7.7 [#]
Aspartate	11.9 \pm 2.3	11.4 \pm 2.0	13.4 \pm 5.8	5.5 \pm 1.4	7.2 \pm 0.7	6.8 \pm 1.2	16.9 \pm 2.2 [#]
Glutamate	87.6 \pm 12.5	76.8 \pm 1.7	93.1 \pm 16.7	66.1 \pm 5.8	65.7 \pm 4.8	71.1 \pm 6.6	119.3 \pm 7.8 [#]

The data represent the mean \pm standard error of the mean (SEM, n=4, 6). *; $P < 0.05$ vs. non-pregnant female rat group, #; $P < 0.05$ vs. dams on day 20 of lactation group.

peripheral blood [5, 13]. Therefore, decrease of glycogenic amino acid during pregnancy may be due to promoting the gluconeogenesis for supply of glucose to the fetus. Another possible reason for the reduction in many plasma amino acid levels may have been an increase in the plasma progesterone level. It has been reported that administration of progesterone to humans causes a marked reduction of many amino acids in plasma [8]. However, if progesterone is the main reason for the decrease of amino acids, this does not explain why many amino acids showed increases in their levels in lactating dams, whose plasma progesterone level is high.

The only amino acid that showed an increased level in plasma at the end of pregnancy was Lys (a ketogenic amino acid). Although the reason for this is unclear, Lys is utilized for acetyl-coenzyme A, some of which is employed for cholesterol synthesis. Furthermore, it has been reported that dietary Lys and Arg are related to plasma cholesterol levels and that a decrease in the ratio of Arg to Lys is associated with an increase in the plasma cholesterol level [23]. Cholesterol may be necessary for the rapid increase in the level of estrogen at the end of pregnancy for initiation of lactation.

In contrast with pregnant dams, all of the amino acids that showed significant changes in lactating dams increased. All of these, except for Lys and Met, were non-essential and glycogenic amino acids. During the lactation in comparison with pregnancy, blood levels of insulin and glucose have returned to normal levels [13]. Therefore, these non-essential and glycogenic amino acids may be for supplying sufficient glucose or amino acid to product the milk in the mammary gland. The increase in the Lys level on the initial day of lactation may have been due to the increase observed at the

end of pregnancy. Ala was significantly increased in early lactation (L1) and showed a tendency to increase as lactation proceeded. An increase of Ala during lactation has been reported previously [25]. It has also been reported that the expression of amino acid transporter (AAT) and lipid synthesis-related genes in the mammary gland is increased more during lactation than during pregnancy in the mouse [20]. In addition, the amino acid concentrations in secreted milk during lactation are different from those of maternal plasma [19]. In the fetus immediately before delivery, amino acid levels are known to be higher than in the mother [17]. On the basis of these observations, we expected that the plasma amino acid levels in rats just after weaning might be higher than in mature rats, and measured the plasma amino acid levels in pups just after weaning. As expected, many kinds of amino acid in plasma showed levels markedly higher than in the dam (Fig. 3D). It might be generally believed that the increase of food intake results in increase of amino acid levels in blood. Certainly, increase of food intake brings about increase of plasma amino acid levels in hyperphagic animals like the ob/ob mouse [21]. However, this correlation between food intake and plasma amino acid levels did not apply to pregnant rats, since main change in amino acid level in pregnant rats was decrease, but not increase, nevertheless, food intake increased. In lactating dam, however, some amino acids concomitant with food intake increased in the present study also. But, the rises of BCAA were not recognized in lactating dam. Therefore, we supposed that the plasma amino acid levels in lactating dam were influenced by not only food intake, but also metabolic change.

In the present study, we showed that imbalance of amino

acid profiles strongly reflects the metabolic changes occurring *in vivo* and that such data would be applicable to various fields, such as diagnosis, treatment with infusion to patient, nutrition for the pregnant women and production of artificial milk.

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