



# Differences in free amino acid concentrations in milk between Wistar and Wistar Kyoto rats

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**ABSTRACT.** Wistar Kyoto (WKY) rats, an animal depression model, display abnormal behaviors such as hypoactivity and depression-like behavior compared with Wistar (WIS) rats as a control. A previous study confirmed a dysfunction of amino acid metabolism in the brain of WKY rats compared with that of WIS rats. At the neonatal stage, free amino acids in milk are important nutrients because they act as immediate nutrients for offspring and may affect later health and behavior of the offspring. Therefore, the present study aimed to investigate free amino acid concentrations in milk and the relationships between free amino acid concentrations in milk and plasma in WIS and WKY rats. The concentrations of ten of the determined free amino acids in milk were significantly higher, but only L-methionine was significantly lower, in WKY rats. Six free amino acids had significantly higher concentrations in colostrum and two free amino acids had higher concentrations in matured milk. Free amino acid concentrations in plasma changed by both genetic background and lactation stage; however, the patterns of change in most free amino acid concentrations except for taurine in plasma were similar between WIS and WKY rats. The transport ratio of free amino acids from plasma to milk was not similar among the free amino acids tested, and each free amino acid was influenced by the genetic background and/or the type of milk.

**KEY WORDS:** colostrum, depression, free amino acid, matured milk, rat

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Wistar Kyoto (WKY) rats are a strain derived from Wistar (WIS) rats and a control strain for spontaneously hypertensive rats (SHR). Additionally, WKY rats are considered as an animal model of major depression. It is well known that WKY rats display abnormal behaviors such as hypoactivity and depression-like behavior compared with WIS rats as a control. Nagasawa *et al.* [20] reported that abnormalities in amino acid as well as monoamine metabolism may induce depression, as the brain amino acid metabolism in WKY rats was considerably different from that in the WIS rats, especially with respect to the lower cystathionine and serine (Ser) levels. Later, to elucidate the mechanism underlying this abnormality, the expression of cystathionine  $\beta$ -synthase and Ser racemase, which are the enzymes involved in the Ser metabolism, was investigated [19]. The lower messenger RNA (mRNA) expression of cystathionine  $\beta$ -synthase was confirmed in WKY rats. On the other hand, the depression-like behavior in WKY rats was attenuated not only by an antidepressant drug [18], but also by nutritional treatments such as supplementation of whole eggs [21] and dietary L-Ser [22].

Amino acids in milk play an important role in the growth and development of offspring. Milk is produced from the blood of dams and it is the only source of nutrition for mammals during early lactation when neonatal animals feed only on milk. Free amino acids in milk are especially important because they act as immediate nutrients. Recent studies reported that nutrition in early infancy may affect later health and behavior of offspring [14, 24]. Concentrations of most individual free amino acids steadily decreased with the progression of lactation [31]. In addition, restraint stress in lactating mice caused changes in the free amino acid metabolism and in the transportation of sulfur-containing amino acids and resulted in higher taurine (Tau) concentration in milk [25]. Considering all these results, it was suggested that free amino acid concentrations in milk can be modified by several factors.

Free amino acid concentration changes in plasma are associated with physical and mental conditions. Previous studies suggested that diabetes and cancer may affect the free amino acid concentrations in plasma [11, 32]. Thus, the levels of several free amino acids in plasma may be useful for the diagnosis of various medical conditions. In addition, the relationships between free amino acid concentrations in plasma and depression or injection of antidepressants were investigated using male WIS and WKY rats [18]. However, free amino acid concentration in the milk could not be predicted by the free amino acid concentration in plasma, since the pattern of free amino acids was mostly different between milk and plasma [17, 25]. This fact suggested that free amino acids in

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plasma are used for milk protein synthesis and/or are metabolized differently for each free amino acid.

Therefore, the present study investigated free amino acid concentrations in both colostrum and mature milk and the relationships between free amino acid concentration in plasma and milk in WIS and WKY rats.

## MATERIALS AND METHODS

### *Animals*

Seven-week-old female WIS and WKY rats were purchased from Charles River Japan, Yokohama, Japan. The rats were housed singly and maintained on a 12 hr light/dark cycle (lights on at 08:00, lights off at 20:00) at a room temperature of  $23 \pm 1^\circ\text{C}$  and humidity 60%. Animals were reared under light conditions of 100 lx. After three weeks of acclimation, each of the seven female WIS and WKY rats were mated 1:1 using another male rat in Plexiglas transparent cages ( $44 \times 20 \times 21$  cm, 1 pair/cage) with wood chips and each male rat was removed ten days after; however, one WIS rat was not impregnated. Thus, six WIS dams and seven WKY dams were used for further experimentation. The day after delivery (PND 1: postnatal day 1), the sex of the offspring was checked, and the number of offspring was standardized to ensure the same conditions among the dams. The number of male and female litters was three each. Water and a standard diet for laboratory rodents (MF, Oriental Yeast, Tokyo, Japan) were available *ad libitum* throughout this experiment. The present study was performed according to the Guideline for Animal Experiments in the Faculty of Agriculture and in the Graduate Course of Kyushu University and conformed to Law No. 105 and Notification No. 6 of the Japanese government.

### *Experimental procedures*

The body weights of dams were measured from experimental day 9 to day 67. Plasma samples were collected on PND 1 (early lactation), PND 14 (mid-term lactation), and PND 28 (late lactation) from the tail vein in 1.5 ml tubes containing heparin. Sampling of blood from the tail vein is considered suitable for repeated blood sampling, but it tends to collect only a small amount of blood. Rats were wrapped with a soft cloth to keep them calm and to restrict movement; and the tail of the rats was locally warmed with  $37^\circ\text{C}$  hot water for 3 min before sampling to expand the veins and promote bleeding. Blood samples were collected under anesthesia with isoflurane (Escaïn®, Mylan, Osaka, Japan) and were centrifuged at  $3,000 \times g$  for 15 min at  $4^\circ\text{C}$  (KUBOTA 3740). Then, the plasma samples were stored at  $-80^\circ\text{C}$  until analysis.

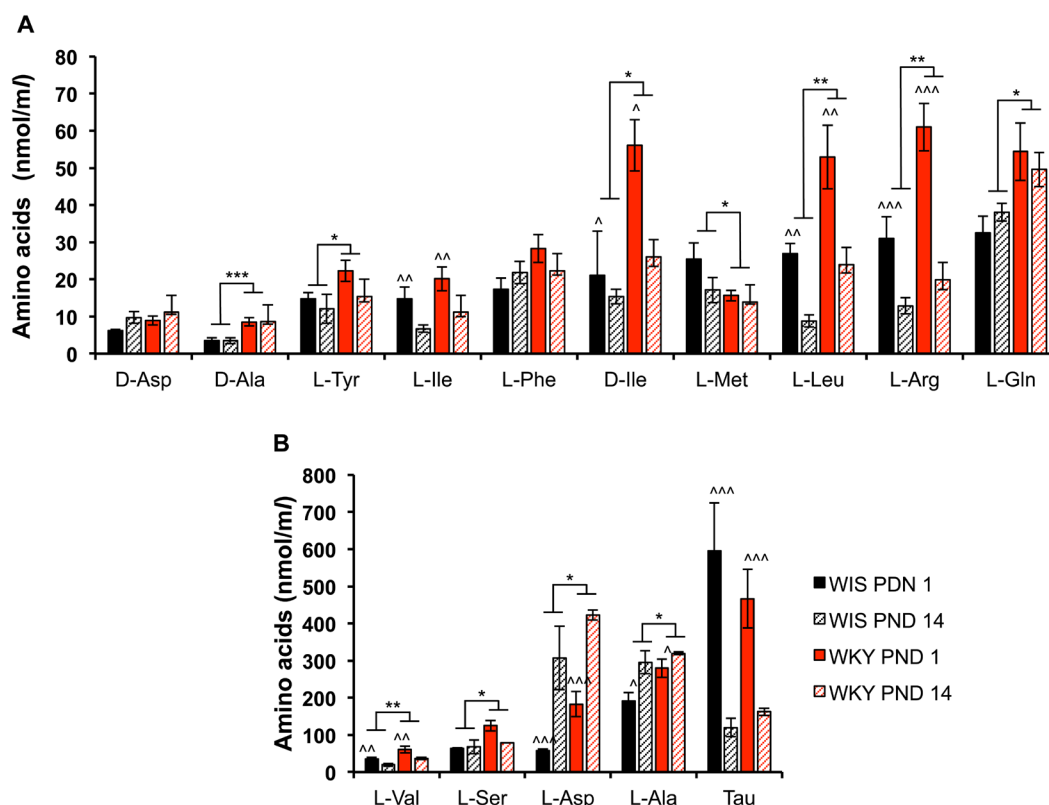
Milk samples collected on PND 1 (colostrum) and PND 14 (mature milk) were used in the analysis of the free amino acid concentrations. Dams and their offspring were separated 5 hr before milking in order to collect enough milk. After 5 hr, dams were anesthetized with isoflurane and were injected subcutaneously with 0.4 ml (4 oxytocin units) of oxytocin (ZENOAQ, Fukushima, Japan) to promote the secretion of milk. Ten min after the injection, they were milked for 10 min using a KN-591 milking machine for mice and rats (Natsume Seisakusho Co., Ltd., Tokyo, Japan) and 1.0–1.3 ml of milk was obtained from each rat. After milking, the rats were put into their home cages. The milk samples were stored at  $-80^\circ\text{C}$  until analysis.

### *Analysis of free amino acid concentrations*

Free D- and L-amino acid levels in plasma and milk were analyzed using the Ultra Performance Liquid Chromatography (UPLC) (Acquity™ UPLC system comprising the Waters Binary Solvent Manager, Waters Sample Manager, and Waters FLR Detector) with an ACCQTAG™ ULTRA C18,  $1.7 \mu\text{m}$ ,  $2.1 \times 100$  mm column (Waters Corp., Milford, MA, U.S.A.). Plasma and milk were prepared by centrifuging at  $14,000 \times g$  for 20 min at  $4^\circ\text{C}$ , and filtered through an ultrafiltration tube (Millipore, Bedford, MA, U.S.A.). Each sample of  $10 \mu\text{l}$  was transferred to a UPLC tube, and  $20 \mu\text{l}$  of the derivatization solution (10 mg of N-acetyl-L-cysteine (NAC) and 8 mg of o-phthalaldehyde (OPA) in 1 ml of methanol) and  $70 \mu\text{l}$  of the 0.4 M borate buffer (pH 10.4) were added. Then, it was left for 2 min in a dark room. The same method was used for the standard solutions containing 16 L-amino acids, 16 D-amino acids, Tau, etc. An aliquot of  $1 \mu\text{l}$  of the derivatized samples was applied to the UPLC system. The excitation and emission wavelengths for fluorescent detection of free amino acids were 350 and 450 nm, respectively. The system was operated with a flow rate of 0.25 ml/min at  $30^\circ\text{C}$ . The UPLC gradient system (A=50 mM sodium acetate (pH 5.9), B=100% methanol) was 10–20% B over 3.2 min, 20% B for 1 min, 20–40% B over 3.6 min, 40% B for 1.2 min, 40–60% B over 3.8 min, 60% B for 1 min, and 60–10% B over 0.01 min. The free amino acid concentrations in the plasma and milk were expressed as nmol/ml. In the present study, we detected L-forms of aspartic acid (Asp), Ser, glutamine (Gln), histidine (His), arginine (Arg), alanine (Ala), methionine (Met), tyrosine (Tyr), valine (Val), phenylalanine (Phe), isoleucine (Ile), and leucine (Leu); D-forms of Asp, Ala, and Ile; and Tau in the plasma. All these free amino acids except for L-His were also detected in the milk.

### *Statistical analysis*

All data are expressed as means  $\pm$  SEM. The results of body weight, concentrations of free amino acids in plasma and milk, and free amino acid concentration ratios (concentration in milk/concentration in plasma) were analyzed by one-way repeated measures analysis of variance (ANOVA). When significant interactions were detected, comparisons between means were carried out using the Tukey-Kramer test. Differences were considered significant at  $P < 0.05$ . All analyses were performed with Stat View (version 5, SAS Institute Cary, NC, U.S.A., 1998). Experimental data were subjected to a Thompson rejection test as described by Kobayashi and Pillai [10] to eliminate outliers ( $P < 0.01$ ) and the remaining data were used for analysis.



**Fig. 1.** Differences in free amino acid concentrations in milk between Wister (WIS) and Wister Kyoto (WKY) rats on PND 1 and PND 14. PND, postnatal day after delivery. (A) low concentration and (B) high concentration amino acids are shown, respectively. Groups are WIS and WKY groups. The number of samples analyzed was 5–7. Values are expressed as means  $\pm$  SEM in nmol/ml. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  vs. strains.  $^{\wedge}P < 0.05$ ,  $^{\wedge\wedge}P < 0.01$ , and  $^{\wedge\wedge\wedge}P < 0.001$  vs. sampling day.

## RESULTS

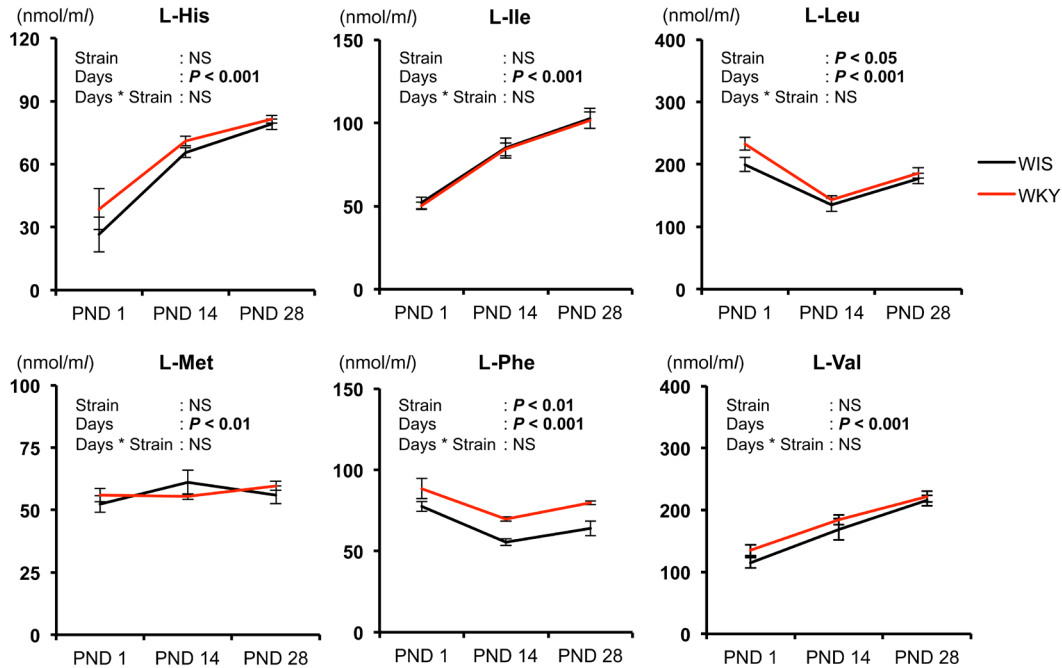
### Free amino acid concentrations in milk and plasma

Differences in free amino acid concentrations in milk between WIS and WKY groups on PND 1 and PND 14 are shown in Fig. 1A and 1B. WKY rats displayed a significantly ( $P < 0.05$ ) lower concentration of L-Met, but significantly ( $P < 0.05$ ,  $P < 0.01$ , or  $P < 0.001$ ) higher concentrations of D-Ala, L-Ala, L-Arg, L-Asp, L-Gln, D-Ile, L-Leu, L-Ser, L-Tyr, and L-Val than in the WIS rats. L-Arg, D-Ile, L-Ile, L-Leu, Tau, and L-Val concentrations on PND 1 were significantly ( $P < 0.05$ ,  $P < 0.01$ , or  $P < 0.001$ ) higher than on PND 14, but the reverse was true for L-Ala and L-Asp. No significant interactions between strains and lactation stages were observed.

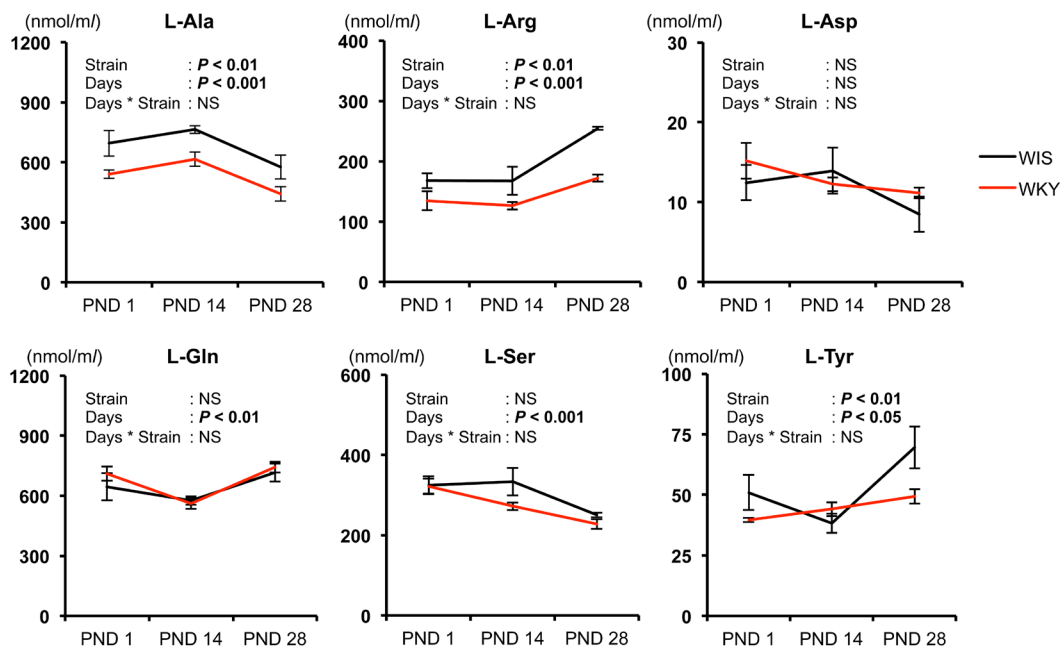
Changes in essential (L-His, L-Ile, L-Leu, L-Met, L-Phe, and L-Val), non-essential (L-Ala, L-Arg, L-Asp, L-Gln, L-Ser, and L-Tyr), and non-proteinogenic (D-Ala, D-Asp, D-Ile, and Tau) free amino acid concentrations in plasma from PND 1 to PND 28 in WIS and WKY rats are shown in Figs. 2–4, respectively. WKY rats displayed significantly ( $P < 0.01$ ) lower concentrations of L-Ala, L-Arg, and L-Tyr, but significantly ( $P < 0.05$  or  $P < 0.01$ ) higher concentrations of D-Ala, L-Leu, and L-Phe than the WIS rats. As the lactation stage progressed, six patterns of change in free amino acid concentrations in the plasma were observed:

1) increasing levels from PND 1 to PND 28 (L-His, L-Ile, and L-Val,  $P < 0.001$ , L-Met,  $P < 0.01$ ); 2) increasing levels from PND 14 to PND 28 (L-Arg, L-Gln, and L-Tyr,  $P < 0.001$ ,  $P < 0.01$ , and  $P < 0.05$ , respectively); 3) decreasing levels from PND 1 to PND 14 and then increasing levels towards PND 28 (L-Leu, L-Phe, and D-Ile,  $P < 0.001$ ); 4) decreasing levels from PND 1 to PND 28 (L-Ser,  $P < 0.001$ ); 5) increasing levels from PND 1 to PND 14 and then decreasing levels towards PND 28 (L-Ala and D-Asp,  $P < 0.001$  and  $P < 0.01$ , respectively); and 6) constant levels (L-Asp, D-Ala, and Tau,  $P > 0.05$ ). A significant ( $P < 0.05$ ) interaction was observed in plasma Tau alone, suggesting elevated levels of Tau in WKY rats compared with WIS rats at PND 1, but the values obtained at both PND 14 and PND 28 were comparable between the two groups.

Table 1 shows changes in the concentration ratios (concentration in milk/concentration in plasma) of free amino acids. The results showed that the ratios of L-Ala, L-Arg, L-Gln, L-Leu, L-Ser, L-Tyr, and L-Val were significantly ( $P < 0.05$  or  $P < 0.01$ ) higher, but those of L-Met and Tau were significantly ( $P < 0.05$  or  $P < 0.01$ ) lower in WKY rats than in WIS rats. The ratios of L-Arg, L-Ile, L-Leu, L-Met, L-Ser, Tau, and L-Val were significantly ( $P < 0.05$ ,  $P < 0.01$  or  $P < 0.001$ ) higher on PND 1 than on PND 14, but the reverse was true for L-Asp, L-Gln, and D-Ile. Significant ( $P < 0.05$  or  $P < 0.01$ ) interactions between strains and lactation stages

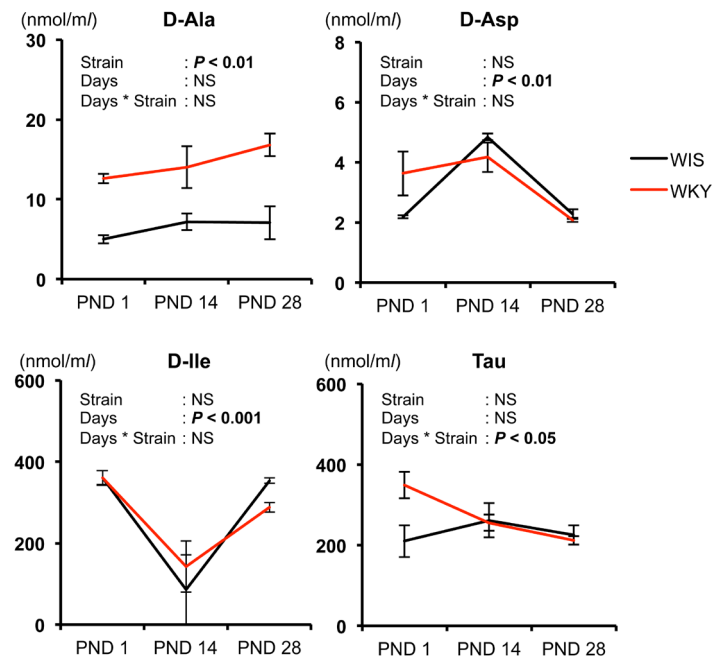


**Fig. 2.** Changes in essential amino acid concentrations in plasma of Wister (WIS) and Wister Kyoto (WKY) rats during lactation. Groups are WIS and WKY groups. The number of samples analyzed was 5–7. Values are expressed as means  $\pm$  SEM in nmol/ml.



**Fig. 3.** Changes in non-essential amino acid concentrations in plasma of Wister (WIS) and Wister Kyoto (WKY) rats. Groups are WIS and WKY groups. The number of samples analyzed was 5–7. Values are expressed as means  $\pm$  SEM in nmol/ml.

were observed in the ratios of L-Arg and Tau. On PND 1, the ratio of L-Arg in the WKY group was higher than in the WIS group and the ratio of Tau was higher in the WIS than in the WKY group, but the differences in the ratios between the WIS and WKY rats disappeared as time progressed.



**Fig. 4.** Changes in non-proteinogenic amino acid concentrations in plasma of Wister (WIS) and Wister Kyoto (WKY) rats. Groups are WIS and WKY groups. The number of samples analyzed was 5–7. Values are expressed as means  $\pm$  SEM in nmol/ml.

**Table 1.** Changes in concentration ratios (concentration in milk/concentration in plasma) of amino acids between Wister (WIS) and Wister Kyoto (WKY) rats during lactation

Strain Days	WIS		WKY		P value		
	PND 1	PND 14	PND 1	PND 14	Strain	Days	Interaction
D-Ala	0.82 $\pm$ 0.22	0.69 $\pm$ 0.25	0.78 $\pm$ 0.01	0.61 $\pm$ 0.07	NS	NS	NS
D-Asp	2.83 $\pm$ 0.20	2.00 $\pm$ 0.30	3.64 $\pm$ 1.17	2.99 $\pm$ 0.47	NS	NS	NS
D-Ile	0.1 $\pm$ 0.0	11.3 $\pm$ 3.2	0.2 $\pm$ 0.0	16.0 $\pm$ 7.3	NS	<0.01	NS
L-Ala	0.38 $\pm$ 0.08	0.36 $\pm$ 0.03	0.53 $\pm$ 0.05	0.54 $\pm$ 0.05	<0.05	NS	NS
L-Arg	0.19 $\pm$ 0.04 <sup>b)</sup>	0.08 $\pm$ 0.02 <sup>b)</sup>	0.63 $\pm$ 0.13 <sup>a)</sup>	0.16 $\pm$ 0.02 <sup>b)</sup>	<0.01	<0.01	<0.05
L-Asp	6.5 $\pm$ 1.1	24.5 $\pm$ 4.2	11.9 $\pm$ 2.7	33.1 $\pm$ 2.1	NS	<0.001	NS
L-Gln	0.05 $\pm$ 0.01	0.07 $\pm$ 0.00	0.08 $\pm$ 0.01	0.11 $\pm$ 0.02	<0.05	<0.05	NS
L-Ile	0.29 $\pm$ 0.06	0.08 $\pm$ 0.01	0.42 $\pm$ 0.08	0.12 $\pm$ 0.01	NS	<0.001	NS
L-Leu	0.15 $\pm$ 0.02	0.09 $\pm$ 0.02	0.25 $\pm$ 0.05	0.16 $\pm$ 0.01	<0.05	<0.05	NS
L-Met	0.49 $\pm$ 0.08	0.28 $\pm$ 0.04	0.28 $\pm$ 0.03	0.24 $\pm$ 0.03	<0.05	<0.05	NS
L-Phe	0.24 $\pm$ 0.04	0.30 $\pm$ 0.02	0.34 $\pm$ 0.04	0.36 $\pm$ 0.04	NS	NS	NS
L-Ser	0.28 $\pm$ 0.07	0.17 $\pm$ 0.01	0.42 $\pm$ 0.05	0.30 $\pm$ 0.02	<0.05	<0.05	NS
Tau	3.28 $\pm$ 0.38 <sup>a)</sup>	0.45 $\pm$ 0.07 <sup>b)</sup>	1.33 $\pm$ 0.28 <sup>b)</sup>	0.65 $\pm$ 0.05 <sup>b)</sup>	<0.01	<0.001	<0.01
L-Tyr	0.35 $\pm$ 0.05	0.32 $\pm$ 0.07	0.64 $\pm$ 0.06	0.42 $\pm$ 0.06	<0.01	NS	NS
L-Val	0.31 $\pm$ 0.04	0.12 $\pm$ 0.02	0.48 $\pm$ 0.08	0.19 $\pm$ 0.02	<0.05	<0.001	NS

The number of samples used was 5–7. The values for the concentration ratios (concentration in milk/concentration in plasma) of each amino acid are expressed as mean  $\pm$  SEM. NS, not significant. Different letters indicate significant difference at  $P < 0.05$ .

### Body weight of dams

Changes in body weights of dams in WIS and WKY rats are shown in Fig. 5. The body weights of dams significantly ( $P < 0.001$ ) increased as time went in both strains, but WKY rats showed significantly ( $P < 0.001$ ) lower body weight than the WIS rats. In addition, a significant ( $P < 0.001$ ) interaction was observed between strains and experimental days, suggesting that difference in body weights between WIS and WKY rats increased in gestation and early lactation periods.

## DISCUSSION

All animals used in the study were kept individually and it was considered that they may be stressed. Stress-induced depression-

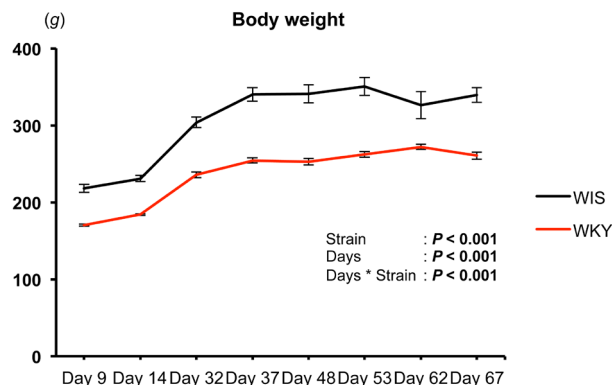


like behavior may be associated with several changes in the stress axis, brain monoamines, and brain amino acid metabolism. These parameters were associated with attenuated depression-like behavior in lactating rats [7]. Accordingly, stress response during lactation in the present study may not so strong.

Milk proteins are synthesized by free amino acids, peptides, proteins and glucose in plasma, and modification of the free amino acid profile in maternal plasma also influences the free amino acid profile in milk [17]. Differences in free amino acid concentrations in milk were observed between WIS and WKY rats; however, there were no significant interactions between the strains and lactation stages. Therefore, the patterns of change in free amino acid concentrations in milk were similar between WIS and WKY rats. The previous study reported that dietary L-Ser decreased body weight of offspring in mice [17]. Dietary L-Ser increased free L-Ser concentration in the milk of dams, but it did not influence free L-Ser in plasma in 13-day-old offspring. However, the concentrations of L-Ser and D-Ser in plasma of 20-day-old offspring (fed both milk and parental diet with L-Ser) were enhanced by dietary L-Ser. In this study, free L-Ser concentration in the milk of WKY rats was higher than that of WIS rats, but the level of increment may not influence the level of L-Ser in plasma of offspring as reported by Nagamachi *et al.*

[17]. Free L-Ser, D-Ile, L-Leu and L-Arg concentration in milk of WKY rats were significantly higher than the WIS rats. However, the effect of these increases in milk on free amino acid levels, development or growth of offspring has been unclear in the present study. Further studies should be done in future. In addition, nutrients other than amino acids may relate the body weights of rats. For example, Teller *et al.* [29] reported that a diet with complex lipid globules decreased accumulated body fat. Moreover, another study suggested that WKY rats have a metabolic changes related lipid metabolism compared with Sprague-Dawley rats [4]. Accordingly, differences in metabolism of nutrients other than amino acids between WIS and WKY rats may induce the difference in the body weights between two strains. L-Arg concentration in milk was higher in WKY rats than in WIS rats. The metabolites of L-Arg such as nitric oxide, L-ornithine, polyamines, L-proline, L-glutamate, creatine, and agmatine have essential functions in both central and peripheral tissues [15], and it is sometimes defined as a semi-essential amino acid. L-Arg administration reduced the inflammatory reaction locally and systemically and attenuated tissue injury in mice [8] and dietary supplementation with Arg also reduced inflammation in the spleen of rats [16]. High L-Arg concentration in milk in WKY rats may contribute to the health of their offspring because of the anti-inflammatory effect. Even though L-Ala and L-Arg in plasma were significantly lower in WKY than in WIS rats, the concentration ratios of these free amino acids were conversely higher in WKY rats. These results suggested that transporters of these amino acids were active in WKY rats and/or these free amino acids were used efficiently for milk protein synthesis in WIS rats. Previous studies reported abundant expression of the mRNA of amino acid transporters, including ASCT2 and CAT1, in the mammary gland in rats [2]. mRNA abundance of ASCT2, prioritized to transport L-Ala, increased at the middle lactation stage compared with early and late lactation stages and decreased towards the weaning stage; and that of CAT1, a transporter of basic amino acids including L-Arg, was higher on days 14 and 16 of lactation than on the first day of lactation. Moreover, it has been reported that the activity and expression of ASCT2 were higher in the kidney of WKY rats than of SHR rats, but this has not been investigated for CAT1 [27]. These results suggest that differences in the activity and expression of amino acid transporters in the mammary gland between strains during lactation may enhance concentration ratios of L-Ala and L-Arg in WKY rats than in WIS rats. However, even though the changes in mRNA abundance of ASCT2 and CAT1 were enhanced as the progress of lactation stage [2], the changes were not completely reflected as an increase in those free amino acid concentrations and concentration ratios. In the present study, L-Ala and L-Arg concentrations in milk were increased and decreased, respectively, and their concentration ratios were constant and decreased, respectively. The levels of L-Ala and L-Arg in milk were not explained by the activity of transporters alone. Milk protein synthesis may associate with free amino acids in milk. In addition, comparison of expression levels of amino acid transporters in the mammary gland between WIS and WKY rats has not yet been investigated. The mechanism influencing differences in the concentration of some free amino acids in milk between WKY and WIS rats remains unclear and further study is needed to investigate the same. However, effects of administration of oxytocin for milking on free amino acid concentrations in milk and plasma were not clear, and peptides, proteins and glucose were not measured here. There is limitation of the study because it is not enough to speculate amino acid metabolism only by free amino acids levels. Further study was needed to clarify the mechanism of differences in free amino acid profiles between WIS and WKY rats.

In the present study, L-Arg, D-Ile, L-Ile, L-Leu, Tau, and L-Val concentrations in milk were higher on PND 1 than on PND 14. This suggested that colostrum is rich in quickly absorbed free amino acids compared to matured milk. The large amount of these free amino acids in milk seems to be derived from the maternal blood via free amino acid transporters and synthesized in the mammary glands [2]. A previous study also reported that Tau is contained in large amounts in milk, especially in the colostrum [28]; and it has been reported that intraventricular injection of Tau stimulated growth hormone (GH) secretion in rats [9]. In



**Fig. 5.** Changes in body weights of dams in Wister (WIS) and Wister Kyoto (WKY) rats. The number of samples analyzed was 5–7. Values are expressed as means  $\pm$  SEM in g. The body weights of dams were measured on day 9; after acclimation, day 14; before pregnancy, day 32 and 37; gestation period, day 53; early lactation (PND 8), and day 62 and 67: late lactation (PND 17 and 22).

addition, L-Arg administration induced GH gene expression in rats [1, 5]. Moreover, L-Val, L-Leu, and L-Ile are classified as branched-chain amino acids (BCAAs) and have an important role in modulating muscle protein metabolism and lead to muscle protein anabolism. Previous studies revealed that BCAAs promote muscle protein synthesis and inhibit muscle protein breakdown in the rat diaphragm [6]. Together, these data may indicate that some free amino acids that aid offspring growth were included at higher levels in colostrum than in matured milk in both WIS and WKY rats.

As with the concentrations of free amino acids in milk, most of the concentration ratios (concentration in milk/concentration in plasma) were also higher on PND 1 than on PND 14. Thus, the high levels of most free amino acid concentrations in milk on PND 1 may be a result of higher transport from maternal plasma to milk.

Significant differences in free amino acid concentrations in plasma between WIS and WKY rats were observed in this study. Nagasawa *et al.* [20] observed differences in the plasma and brain free amino acid profiles of WIS and WKY rats, and suggested that low levels of L-Ser and cystathionine in plasma and some brain sites may cause low motor activity and high depression-like behavior in WKY rats. In this study, a low level of plasma L-Ser was also confirmed in WKY rats.

Changes in plasma free amino acid profiles of WIS rats accompanying the reproductive stage were reported [26], but our results conflict with the previous findings regarding the concentrations of some free amino acids during lactation. Okame *et al.* [26] reported constant plasma concentrations of some free amino acids such as L-Ile, L-Tyr, and L-Val from early lactation to late lactation, but in the present study the concentration of these free amino acids in plasma increased as lactating stages progressed. These differences may be caused by experimental conditions. Further studies are needed to investigate the mechanism underlying changes in levels of free amino acids in plasma during lactation. In addition, interestingly, no significant interactions between strains and lactation stages were observed in most free amino acid concentrations in plasma, suggesting that the changes during lactation were similar between WIS and WKY rats.

Changes in free amino acid concentrations in plasma during lactation may be related to several metabolisms in the maternal body. Previous studies suggested that basal metabolism and food intake were related to changes in amino acid metabolism accompanying the reproductive states [3, 13]. In the present study, the body weights of dams were different between WIS and WKY rats, and the difference in body weights between strains increased in gestation and early lactation periods. This result suggested that WKY may have a different basal metabolism compared with WIS rats in those periods. Moreover, it is well known that changes in sex hormones, such as testosterone and estrogen, are associated with reproductive states. In addition, some hormones, including oxytocin and prolactin, were activated in the peripartum period [12, 23, 30]. Changes in levels of hormones induced changes in protein metabolisms. Moreover, free amino acid concentrations in plasma may change in association with the reproductive states because of protein turnover.

In conclusion, WKY rats have differences in the amounts of some free amino acids in milk that is somewhat associated with free amino acids in plasma. The concentrations of most free amino acids in plasma and milk were changed as lactation progressed, but the patterns of change in free amino acids with the progression of lactation were similar between the two strains. These results suggested that changes in the plasma and milk free amino acid profiles accompanying the various reproductive states were conserved between WKY and WIS rats, even though the amounts of free amino acids appearing in milk were genetically modified.

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**CONFLICT OF INTEREST.** The authors declare that they have no conflicts of interest.

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