DOI: 10.20705/jjnhs.20.1_14

看護科学研究 vol. 20, 14-24 (2022)

原著

Habitual dietary intake and glutamic acid in durable milk at one month postpartum

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Received 19 October 2021; Accepted 1 February 2022

Abstract

Glutamic acid (Glu), an umami component in human milk, has been suggested to play an important role in the prevention of allergies and infections in infants, but the relationship between mothers' habitual diet and Glu in human milk is not clear. Therefore, we aimed to clarify the relationship between maternal dietary habits and Glu concentrations in durable milk during the first month postpartum in 41 lactating women using a cross-sectional prospective study. Metrics included habitual dietary intake at 1 month postpartum (Self-Reported Dietary History Questionnaire), Glu concentration in durable milk whey, and plasma at 1 month postpartum. There was no correlation between Glu concentration in plasma and durable milk Glu concentration at 1 month postpartum. Food items that were correlated with plasma and milk Glu concentrations were sparse in Glu or did not contain Glu; therefore, we could not determine the relationship between Glu concentrations in durable milk and maternal dietary intake. In addition, Glu concentration in durable milk was significantly higher among multipara than primipara and among mothers with a relatively high degree of physical activity. Although the study did not show that mothers' habitual dietary intake affected milk Glu, Glu concentrations in durable milk were approximately 30 times higher than those found in plasma. Further research is needed to clarify the relationship between individual factors and milk or plasma Glu.

Key words

durable milk, glutamic acid, habitual dietary intake, plasma, umami

1. Introduction

It is widely known that human milk has many benefits for mothers and children; infants benefit from its high antimicrobial protein content and mother-infant interaction is promoted through the act of breastfeeding. Human milk is synthesized and produced from the mother's blood, and it has been shown that one of the factors that affects this production is the mother's diet during breastfeeding. Breastfeeding mothers tend to believe that their diet influences the quality and composition of their milk and therefore pay attention to their diets during lactation to provide better milk for their children. However, there is a lack of research regarding the relationship between diet and quality of milk.

In Japan, Japanese food containing umami, which enhances the taste of ingredients, has long been a recommended diet for lactation. Glutamic acid (Glu) is the most abundant amino acid in human milk. It has been suggested that Glu plays a role in appetite regulation, salt reduction, cognitive development, and protection against allergies and infections in infants through intestinal immune function (van Sadelhoff et al 2020). In addition, Glu plays an important role in the metabolism of amino acids and proteins in the body and imparts the umami taste sensation to protein-containing foods, thereby enhancing the perception of "deliciousness" and influencing food preference (San Gabriel et al 2018, Uneyama 2015). It is thought that the flavor of food ingested by the mother changes the taste of human milk (Kawamura 1985, Spahn et al 2019) and exposure to a variety of tastes during lactation leads to infants liking healthier foods (Beauchamp and Mennella 2009, Forestell 2017, Harris 2008, Schwartz et al 2013, Ventura and Worobey 2013).

It has been reported that the longer the duration of breastfeeding, the higher the preference for the umami taste in human infants (Schwartz et al 2013). Similarly, increasing the amount of free glutamate in conditioned milk can bring the infant's intake of conditioned milk closer to the ideal level by creating a moderate sense of satiety (Berthold 2018, Ventura et al 2012). Thus, exposure to Glu during lactation is expected to have many benefits in terms of taste learning and health promotion in infants.

Based on the benefits of Glu described above, this study was conducted to clarify the scientific basis for the benefits of consuming human milk rich in Glu, an umami-presenting amino acid, in infants. The purpose of this study was to clarify the relationship between maternal eating behavior during the first month postpartum and the concentration of Glu in durable milk secreted at one month postpartum. As midwives and nurses are the healthcare professionals who are closest to lactating mothers, this study aimed to make mothers aware of how the umami component of breast milk is beneficial to their infants and support them in maximizing these benefits.

2. Methods

2. 1 Research design

This association validation study employed a prospective design. Metrics included habitual dietary intake at one month postpartum (using the Self-Reported Dietary History Questionnaire; DHQ), whey in durable milk, and plasma at one month postpartum. The DHQ is a structured, self-administered questionnaire that assesses the consumption frequency and portion size of selected foods commonly consumed in Japan, as well as general dietary behavior and usual cooking methods (Kobayashi et al 2011a, Sasaki et al 1998). It also includes a self-report survey item on one's physical activity level (Ishikawa-Takata et al 2008).

Blood samples were obtained by collecting a second sample at the one-month postpartum checkups of the participants. Privacy was ensured when the participants were pumping human milk. This study was approved by the ethics committee of our university.

2. 2 Study model

Given that the flavor of food ingested by a mother alters the taste of the human milk she expresses, our basic concept is that their eating behaviors affect the content of their milk (Figure 1). Therefore, our goal was for mothers to engage in eating behavior that utilizes "umami," exposing their infants to Glu via human milk, thereby

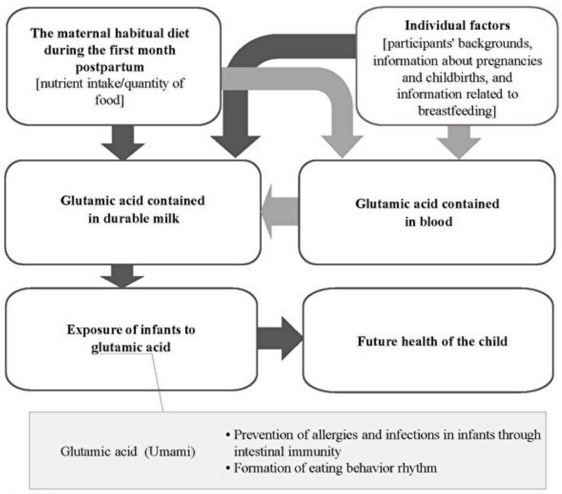

Figure 1. Schematic diagram of the study

contributing to establishing healthy eating habits for children in the future, as they develop a heightened preference for umami.

2. 3 Subject criteria

The participants were 66 women between the ages of 20 and 39 years at one month postpartum. Each participant provided informed consent. Participants were recruited during antenatal checkups at a maternity hospital [blinded for review]. The study was conducted between July 2017 and February 2019. Criteria for inclusion were having had a normal pregnancy and parturition, currently undergoing puerperium, and having a body mass index of 18.5–25 when not pregnant. The exclusion criteria were the following: mothers who were not lactating; people who smoke tobacco; people who drink alcohol; patients with a history of or ongoing treatment for otorhinolaryngologic, psychiatric, and neurological diseases; or patients with gestational hypertension syndrome, gestational diabetes, or breast problems. During the participants' outpatient visits in the third trimester (after 26 weeks of gestation), written information and verbal explanation of the purpose, method, and ethical considerations of the study were provided and written consent to participate in the study was obtained from each individual.

To increase the reliability of the data, participants who received maternal transport during the study period and those whose DHQ reporting errors were ±30 or higher were excluded. Therefore, after the exclusions, 41 participants remained. The attributes of interest are listed in Table 1. The participants were compensated with a 1000-yen tradeable coupon. The required sample size was calculated using an automatic sample size calculator, assuming a standard error of 2.5%, a confidence level of 95%, and a standard deviation of 10. As there is a lack of similar previous studies and we expected large individual differences in glutamic acid data, this sample was appropriate.

Table 1. Target attributes

	Mean ± SD	Range
Age (years)	30.8 ± 3.6	21–39
Height (cm)	159.0 ± 4.8	150–172
Body weight when not pregnant (kg)	52.2 ± 4.9	43.0–63.0
BMI when not pregnant (kg per m^2)	20.6 ± 1.5	18.5–24.3
Body weight at delivery (kg)	62.5 ± 6.2	50–78
Gestational week at parturition (wk)	38.9 ± 1.3	36–41
Blood loss (g)	369.2 ± 302.3	50–1345
Infant height (cm)	48.9 ± 1.7	45.0–52.0
Infant weight (g)	3112.8 ± 334.8	2368–3892

Note: N=41

2. 4 Sampling and data collection

Pregnant and puerperal versions of the coded self-administered DHQ were distributed to consenting pregnant women and collected at their one-month postpartum checkup. During the blood tests conducted at one-month postpartum, additional blood samples were collected using 5 mL collection tubes. The collected blood was separated into clotting and plasma layers using a centrifuge at 3000 rpm for 15 min at 4 ℃. The upper plasma layer was removed using a micropipette, transferring 200 to 600 μL into a microtube, which was kept at -80℃ in a deep freezer. Durable milk was collected manually into a disposable polypropylene measurement container. Durable milk was collected from the women during the day (from 8:00 to 20:00), which provided a sample of an amount of milk that did not affect breastfeeding. After cooling the sampled human milk to 4℃, it was separated into milk fat and whey layers using a centrifuge at 15000 rpm for 15 min at 4℃. A micropipette was used to extract 200–600 μL of the whey layer. This was transferred to a microtube and kept at -80℃ in a deep freezer. Deep-frozen plasma and whey were submitted to SRL Co., Ltd., which analyzed the amino acids using liquid chromatography-mass spectrometry (LC-MS).

Using a questionnaire, information was obtained regarding participants' attributes and information on their gestation and parturition periods (delivery date, delivery mode, and volume of blood loss) and breastfeeding (frequency of feeding and the shape of the teats and breasts postpartum). Furthermore, we obtained information about the proportions of Japanese, Western, and Chinese cuisines consumed during each period, based on a total of 10 dishes. Information on the participants' eating preferences was obtained via interviews before, during, and after pregnancy, as well as examination of entries made in participants' medical charts and maternal and child health handbooks.

2. 5 Data analysis

Data were analyzed using IBM SPSS Statistics version 27 for Windows, with significance defined as $p < 0.05$. After calculating descriptive statistics and checking for normality of the data using the Shapiro-Wilk test, we assessed correlation coefficients (Pearson, Spearman), unpaired t-tests, and one-way analysis of variance.

3. Results

3. 1 Attributes of interest and information on the gestational and parturition periods and breastfeeding

All data are presented as mean ± standard deviation. As seen in Table 1, 41 participants were included in the analyses, of whom 22 (53.7%) were primipara and 19 (46.3%) were multipara. The number of weeks at delivery was 38.9 ± 1.3, and the delivery time for vaginal delivery, excluding Cesarean section (six participants), was 676 ± 1009 minutes. The amount of blood loss was 369 ± 302 mL, and 11 women (26.8%) had abnormal bleeding (blood loss of 500 mL or more within 2 h postpartum).

Forty participants, namely all but one, had chosen to breastfeed. Breast type was type I in 3 mothers (7.3%), type IIa in 14 (34.1%), type IIb in 19 (46.3%), and type III in 5 (12.2%); the nipple shape was normal in 35 (85.4%), flat in 5 (12.2%), and depressed in 1 (2.4%). Of the 19 multipara mothers, 13 (68.4%) had breastfed their previous child, and 6 (31.6%) used mixed feeding. At the time of the 1-month checkup, 26 infants (63.4%) were fully breastfed and 15 (36.6%) had been mixed-fed; the average approximate incidence of direct breastfeeding was 9.7 ± 1.7 times per day.

3. 2 Glutamic acid concentration

The concentration of Glu in whey of durable milk was 1223.8 ± 401.1 μmol/L. Glu was the most abundant amino acid contained in whey (Figure 2). Approximately 31.0 ± 11.3 days after delivery, 4.5 ± 1.9 mL of milk was collected at the 1-month checkup, at 13:00. The plasma Glu concentration at one month postpartum was 73.5 ± 19.1 μmol/L (Figure 3).

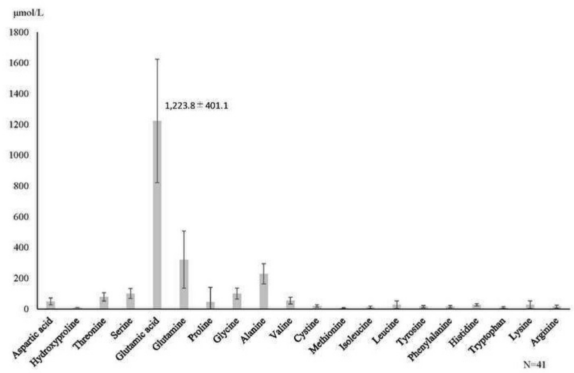

Figure 2. Concentrations of major amino acids in whey of durable milk

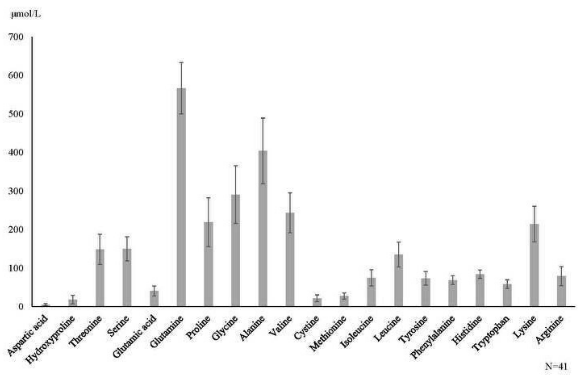

Figure 3. Concentrations of major amino acids in plasma

3. 3 Eating behaviors and food preferences

Data from 151 food items, including 32 nutrients, 23 food groups, and beverages and seasonings obtained by the DHQ, were analyzed by converting them to intake per 1000 kilocalories. Based on previous validation results, it is not recommended to calculate amino acid (as well as isoflavone, pantothenic acid, vitamin K manganese, heptanoic acid, etc.) intake using the DHQ (Sasaki 2012). The estimated amino acid intake was a tentative calculation and was, therefore, excluded from the analysis.

To understand food preferences, a questionnaire was used to survey the most favorable tastes among sweet, umami, salty, astringent, and sour tastes. The favorite tastes of the study participants were sweet 22 (53.7%), umami 16 (39.0%), salty 4 (4.9%), and sour 1 (2.4%).

3. 4 Relationship between plasma Glu concentration and milk Glu concentration

There was no significant correlation found between plasma Glu concentration and durable milk Glu concentration at one month postpartum ($r = 0.21$, $p > 0.05$).

3. 5 Relationship between diet and Glu concentration in durable milk during lactation

Positive correlations between eating behavior during lactation and durable milk Glu concentration were found for seven food items and Retinol ($r = 0.36$, $p < 0.05$), C20:4 (n-6) ($r = 0.34$, $p < 0.05$). Furthermore, negative correlations were found for four food items and C22:0 ($r = -0.42$, $p < 0.01$) (Table 2).

Table 2. Correlation between food group, food quantity, and glutamic acid concentrations in durable milk

Food classification	Names of foods	Glu content (mg) per 100 g	Correlation coefficient (r)
Cereals	Chinese noodles	1400–3500	$.492^{**b}$
Dairy	Cottage cheese	3100	$.441^{**a}$
Cereals	Instant noodles	4300	$.437^{**a}$
Sugar and sweeteners	Sugar (coffee and tea)	0	$.371^{*a}$
Dairy	Cheese	1700–9900	$.314^{*b}$
Meats	Liver	1800–2600	$.312^{*b}$
Eggs	Chicken eggs	1700	$.309^{*b}$
Fruits	Watermelon	79	$-.374^{*b}$
Beverage	Green tea oolong	0	$-.342^{*a}$
Fish/Seafood	Other shellfish	1100–2800	$-.340^{*a}$
Fish/Seafood	Tsukudani	4400（1700）	$-.339^{*b}$

Note: *p < 0.05, **p < 0.01
a = Pearson's product-factor correlation coefficient
b = Spearman's rank correlation coefficient

3. 6 Relationship between diet during lactation and plasma Glu concentration

Although no food group showed a correlation with plasma Glu concentration during lactation, positive correlations were found for seven food items and cholesterol ($r = 0.31$, $p < 0.05$), C18:3 (n-3) ($r = 0.33$, $p < 0.05$), and negative correlations were found for three food items and Cu ($r = -0.31$, $p < 0.05$) (Table 3).

Table 3. Correlation between food group, food quantity, and glutamic acid concentrations in plasma at 1 month postpartum

Food classification	Names of foods	Glu content (mg) per 100 g	Correlation coefficient (r)
Beans	Tofu products	1100–1400	$.372^{*b}$
Meat	Ham and sausage	2500–2900	$.367^{*a}$
Fruits	Strawberries	150	$.362^{*b}$
Cereals	Instant noodles	4300	$.344^{*b}$
Fats and oils	Dressings	170–850	$.329^{*b}$
Meats	Ground beef and pork	2500–2700	$.317^{*b}$
Vegetables	Chinese cabbage	180–200	$.311^{*b}$
Vegetables	Vegetable juice	290–400	$-.434^{**b}$
Fruits	Juice 50	42–200	$-.382^{*b}$
Beverages	Tea	0	$-.370^{*b}$

Note: *p < 0.05, **p < 0.01
a = Pearson's product-factor correlation coefficient
b = Spearman's rank correlation coefficient

3. 7 Relationship with food preference

There was no significant difference in the concentration of Glu in milk and plasma according to favorite foods.

3. 8 Association with other individual factors

There was a significant difference in Glu concentration in whey between primipara and multipara (primipara: 1083.3 ± 303.4 μmol/L; multipara: 1386.4 ± 445.2 μmol/L; independent-samples test, $p < 0.05$). There were no other significant differences between groups. There were no differences in Glu concentrations according to the age of the participants, mode of delivery, shape of the teats and breasts, or method of infant nutrition. There was no correlation between plasma concentrations of amino acids required for Glu synthesis (glutamine, arginine, proline, and histidine) and milk Glu concentrations.

In contrast, plasma Glu concentration was positively correlated with non-pregnant weight (r

= 0.39, p < 0.05) and maternal weight immediately after delivery (r = 0.44, p < 0.01).

Sixteen individuals, who were initially excluded due to measurement errors in the DHQ, were included to examine associations with individual factors other than diet. The results showed a significant difference in Glu concentration in whey of durable milk depending on whether the physical activity level was light or moderate (light: 1134.0 ± 355.0 μmol/L; moderate: 1500 ± 495.8 μmol/L; independent-samples t-test, p < 0.05; Figure 4). Furthermore, a positive correlation was found with the number of pregnancies (r = 0.29, p < 0.05) and a significant difference was found between primipara and multipara (primipara: 1036.1 ± 331.0 μmol/L; multipara: 1290.5 ± 399.1 μmol/L; independent samples t-test, p<0.05; Figure 5).

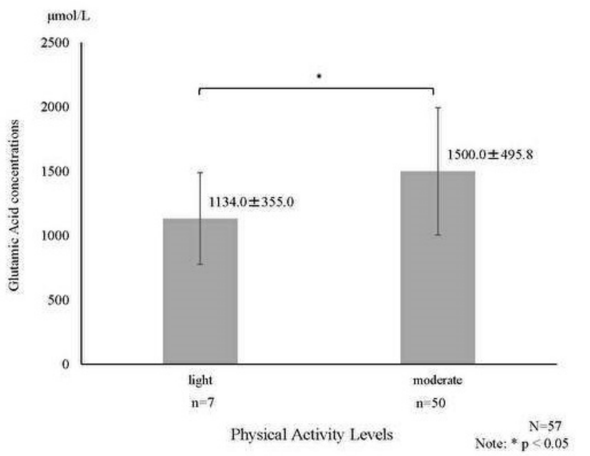

Figure 4. Glutamic acid concentrations in durable milk by physical activity levels

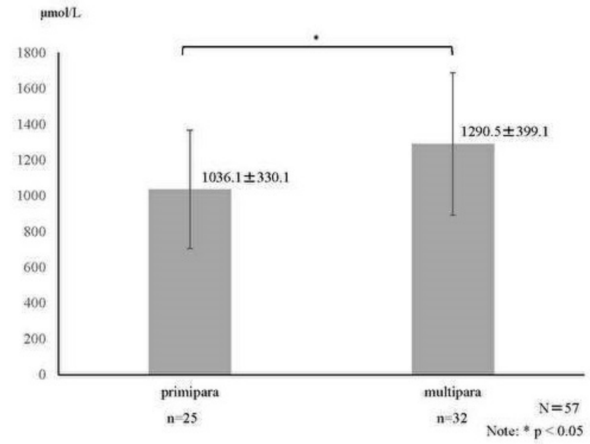

Figure 5. Glutamic acid concentrations in durable milk by primipara and multipara

4. Discussion

4. 1 Glu concentrations in milk whey

In this study, the durable milk samples were not collected throughout a 24 h period, but were rather collected at one point in time. A previous report found that free amino acid content in milk was significantly lower during the night (20:00 to 8:00) versus day (Kobayashi et al 2011b). Therefore, it is possible that the content of free amino acids varies depending on the time of collection. To minimize the effect of diurnal variation in this study, milk was collected during participants' one-month postpartum checkups at the same facility. In a systematic review of amino acid concentrations in the human milk of 4,747 women, the concentration of Glu in durable milk (21–60 days postpartum) was 1175.0 μmol/L (Zhang et al 2013). Therefore, glutamate concentrations in whey obtained in this study are likely reasonable.

4. 2 Relationship between Glu concentrations in plasma and milk whey

As human milk is synthesized and produced from blood in mammary gland cells, it is assumed that the same is true for Glu; therefore, we examined the relationship between plasma Glu concentration and whey Glu concentration. However, no relationship was found, consistent with the result of a study conducted by Nakai et al(2021), who studied 16 colostrum specimens.

In a study by Stegink et al(1972), plasma and human milk-free amino acid concentrations were measured after fasting mothers consumed 6 g of monosodium glutamate (MSG) in an aqueous solution and a solution dissolved in a liquid, called slender; the authors also concluded that the transfer of plasma Glu to human milk was negative. Additionally, a study in which adult males were given 0.3 g per kg of body weight per day of Glu showed no significant change in blood Glu concentration, therefore suggesting that it was utilized by the epithelial cells in the small intestine (Kurihara et al 2000). The metabolism of amino acids is affected by a variety of factors, and it is difficult to correctly assess the metabolic rate of glutamate (Kurihara et al 2000). In this study, we examined the relationship between plasma concentrations of glutamine, arginine, proline, and histidine, the raw materials for glutamate synthesis, and milk Glu concentrations; no significant relationships were found.

In this study, the concentrations of Glu in milk were approximately 30 times that in plasma, which is consistent with the results of a study on colostrum by Nakai et al(2021). Human umami receptors have high specificity for Glu (Matsumoto et al 2014), and it has been shown that the concentrations of Glu in human milk are higher than those in cows and other animals (Sarwar et al 1998). The results of this study suggest that Glu content in milk is enriched and produced by an as yet unknown mechanism. However, it may be significant that human milk is particularly rich in glutamate.

4. 3 Diet and Glu concentration in whey and plasma during lactation

Table 2 shows that Chinese noodles ($r = 0.49$, $p < 0.01$), cottage cheese ($r = 0.44$, $p < 0.01$), and instant noodles ($r = 0.44$, $p < 0.01$) were the food items that showed particularly strong positive correlations with Glu concentrations in durable milk. Among the foods associated with plasma Glu concentrations, instant noodles (whey: $r = 0.44$, $p < 0.01$; plasma: $r = 0.34$, $p < 0.05$) were also associated with durable milk Glu concentrations. Instant noodles were the only food with this commonality in this study. These noodles contain a relatively high amount of glutamate, with a maximum of 780–4300 mg per 100 g, and correspond to foods with names including "instant noodles" in the Food Composition Database of the Ministry of Education, Culture, Sports, Science, and Technology (n.d.).

In addition, Chinese noodles, which were related to the concentration of Glu in durable milk, are used in Chinese dishes that are considered to utilize a lot of Glu. Similarly, cottage cheese is known as food with notable umami.

The Glu content per 100 g of food with the name "Chinese noodles" was 1900 to 4300 mg, and that of "Cottage cheese" was 3100 mg, both of which are relatively high in Glu. Conversely, "tsukudani" (preservable food boiled down in soy sauce; $r = -0.39$, $p < 0.05$), which was negatively correlated with Glu concentration in durable milk, contained 4400 mg of Glu per 100 g. As can be seen from Tables 3 and 4, there were no clear differences in Glu content between the food items with a positive correlation and those with a negative correlation. Furthermore, there was no relationship between the strength of the correlation and Glu content among foods with positive as opposed to negative correlations.

Therefore, it is clear from the present study that the habitual oral intake of foods associated with DHQ scores is not directly related to an increase in Glu concentrations in plasma and durable milk. In addition, the results of the present study show that there was no correlation between the Glu concentrations in durable milk and plasma, indicating that there was no apparent relationship between the intake of food that increases the Glu concentration in plasma and the Glu concentration in durable milk. Since there are no reports that the micronutrients and fatty acids that were found to be associated in this study are related to the mechanism of human milk Glu synthesis in the body, there is no scientific basis for this association.

4. 4 Association with other individual factors

In this study, the concentration of Glu in durable milk was significantly higher in multiparas than in primiparas. However, a previous study that examined the relationship between various perinatal factors and total protein in human milk reported that the protein concentration in milk secreted by first-time mothers was significantly higher, that protein concentration was negatively correlated with the amount of human milk secreted, that women in their thirties secreted less milk than women in their twenties, and that there was an inverse correlation between maternal age and the enlargement and hypertrophy of mammary tissue during pregnancy (Yoneyama and Nagata 1990). The present study found no statistically significant differences in Glu concentration according to age or breast shape. This suggests that Glu concentration in human milk may not be related to protein concentration.

Daily milk secretion could not be measured in this study because of the burden on the mothers during lactation. In addition, it has been reported that the mode of delivery can cause significant differences in sugars, triglycerides, and protein concentrations in colostrum, while durable milk showed almost the same values (Herai et al 1992). Similar to the results of this study, Glu was not significantly related to delivery mode in durable milk in a study by Herai et al (1992). Glu concentration in colostrum whey tended to be negatively correlated with hemorrhages, which suggests a relationship between Glu concentration in colostrum and risk of hemorrhages (Nakai et al 2021). The results of this study showed that Glu concentration in durable milk at one month was not related to hemorrhages at calving, even though several days had passed since calving.

Although there are no previous studies on the relationship between maternal exercise and levels of amino acids in human milk, the effect of increasing 3'-sialyl lactose, an oligosaccharide with infection-fighting properties, has recently been reported in humans (Harris et al 2020).

Based on the results of this study, we believe that there may be a relationship between exercise and amino acid concentration in human milk. Protein concentration in human milk has been reported to be significantly higher in primipara women and negatively correlated with milk secretion (Yoneyama and Nagata 1990). Therefore, it has been inferred that Glu concentration is lower in mothers with more frequent deliveries; however, the opposite was true in this study. Although there is no scientific evidence to verify the relationship between the number of parturitions and milk composition, it was suggested that the number of deliveries may indirectly affect the process of glutamic acid synthesis in the mammary gland. These results suggest that there is a need for further research on the relationship between Glu concentration in milk and maternal individual factors. Furthermore, it may be possible to identify other care methods that benefit infants with a rich source of Glu through human milk rather than dietary choice.

4. 5 Limitations

The limitations of this study are as follows: (1) Because we could not measure the amount of Glu actually consumed by the participants, we could not determine whether the oral intake of the umami substance Glu itself, or the amount consumed, was related to the concentration of Glu in human milk. (2) Recall bias may have existed due to the use of the DHQ, which requires participants to write down their dietary history in retrospect over a period of one month. The DHQ may have affected ascertaining dietary intake due to its use characteristics, especially that women tend to underestimate their own dietary intake. (3) Because the DHQ was not used to analyze participants with measurement error of more than ±30%, it is possible that important associations were missed due to the small sample size. (4) Since it is not known whether ingested amounts of Glu are reflected in levels of Glu in human milk after ingestion, it is possible that the diet immediately before sample collection influenced the results, rather than eating habits during the month. (5) The metabolic timing of the increase in Glu in human milk after Glu ingestion is unknown. (6) The metabolic mechanisms of Glu in the mammary glands have not been elucidated. These limitations should be addressed in future studies.

5. Conclusion

This study examined the relationship between eating behavior during the mother's first month postpartum and the concentration of Glu in durable milk at one month postpartum. It was expected that infants would benefit from Glu through milk if the dietary recommendations for mothers to produce Glu-rich milk were clarified; however, in this study, no relationship between habitual eating behaviors, as calculated by the DHQ, and Glu concentration in human milk was found. Moreover, since the existence of a relationship between Glu concentration in human milk and individual factors was suggested, there is a need to clarify this relationship. As midwives and nurses working with mothers and babies during the lactation period, we will continue our research to find scientific evidence that fosters the health of mothers and babies and to find ways to support them.

The authors declare that there are no conflicts of interest.

Funding

This work was supported by the Japan Society for the Promotion of Science KAKENHI (grant number 18K1038300).

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgment

We are deeply grateful to the participants and staff for their cooperation with this study.

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This study was approved by the Shiga University of Medical Science Ethics Committee (Approval No. 28-220).

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