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Original Article

The dynamic changes of gangliosides in breast milk and the intake of gangliosides in maternal and infant diet in three cities of China

Shengjie Tan[1], Chang Chen[2], Ai Zhao[3], Meichen Wang[1], Wenzhi Zhao[1], Jian Zhang[1], Hao Li[1], Yumei Zhang[1]

[1]Department of Nutrition and Food Hygiene, School of Public Health, Peking University, Xueyuan Road 38, Haidian District, Beijing, China; [2]Department of Obstetrics and Gynaecology, The First Affiliated Hospital of Chongqing Medical University, Youyi Road 1, Yuzhong District, Chongqing, China; [3]Department of Social Medicine and Health Education, School of Public Health, Peking University, Xueyuan Road 38, Haidian District, Beijing, China

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Abstract: Objective: To study the ganglioside intake of lactating mothers and its effect on the breast milk and infants. Methods: The related information of mothers and infants was obtained by questionnaire survey, including the recipe, family information, and so on. The content of gangliosides in the mothers' food and breast milk was tested by HPLC-MS. The intake of gangliosides for infants was recorded and calculated. Then the dynamic changes of the content of gangliosides in breast milk and the impact on the development of infants were evaluated. Results: GD3 was rich in milk and dairy products. The average intake of gangliosides for lactating mothers was 6.33 mg/day, of which GM3 was 3.02 mg/day and GD3 was 1.51 mg/day. The main food sources of gangliosides were meat (46.6%), eggs (26.6%), and dairy products (18.9%). The average content of gangliosides in breast milk was 9.58 mg/L. The content in 0-7 days after delivery (15.95 mg/L) was the highest, and then gradually decreased with time, getting the lowest in 6 months after delivery (6.47 mg/L). GM3 and GD3 were the two main types in breast milk. The average milk intake of infants under 6 months gradually increased from 570 mL to 1367 mL, and the daily intake of gangliosides was relatively stable, with a median of 6.4 mg. There was no significant relationship between the intake of gangliosides and physical development in infants. Conclusion: This study is the first to report the dietary ganglioside intake of Chinese city mothers. This study is also the first to indirectly infer the demand of infant ganglioside by detecting the components of breast milk. It will accumulate basic data for improving the diet of Chinese mothers and the recommended amount of infant nutrients.

Keywords: Gangliosides, breast milk, lactating mother, infants

Introduction

The timeframe from pregnancy to early postnatal is usually considered to be a critical stage for the brain growth and development of an embryo or newborn. During that period, both the brain and nervous system develop extremely fast, with rapid increase in volume and mass [1]. Compared with the embryonic period, the growth rate of the brain decreases gradually after delivery, and the brain structure of babies is close to that of adults by the age of 2 years old [2]. It is widely recognized as 1000 days of early life from the beginning of pregnancy to the baby becoming 2 years old, which indicates the importance of maternal and infant health for this stage [3]. This special stage is the key construction period for brain development, and it needs lots of nutrients as basic raw materials or to provide energy. Therefore, early life is also considered to be a critical window for nutrition which affects current and long-term health.

Ganglioside is an important component of cell membrane structure, as well as a substance with high content of nerve cells. Most importantly, it is one of the key nutrients in the early growth and development of infant brain [4]. We collected the breast milk of Chinese mothers at different postpartum times in previous studies, and detected the content of gangliosides in breast milk by HPLC-MS. The results showed that the content of gangliosides in breast milk was extremely high in the early postpartum period, and then decreased with time gradually [5, 6]. It was suggested that the demand for gangliosides was large in the early postnatal period and decreases with age. The trend of ganglioside content was consistent with that of brain development.

Many studies have focused on the fish intake of lactating mothers or pregnant women, especially the intake of deep-sea fish. It is believed that deep-sea fish can provide pregnant women or mothers with rich long-chain polyunsaturated fatty acids (LC PUFAs). The intake of LC PUFAs by pregnant women or lactating mothers is beneficial to the neurodevelopment of offspring [7, 8]. Some scholars speculate that mothers' intake of gangliosides as functional lipids will benefit infants [9]. Thus, we speculated that the gangliosides in the diet may promote the increase of gangliosides and other bioactive substances in infants by lactating mothers' intake and breast milk, which is beneficial to the health of infants.

However, there are few studies on ganglioside intake of mothers or infants. Gangliosides are difficult to detect for their complex structure, and there are no data of gangliosides content in the food composition table, which makes it difficult to estimate the intake of gangliosides. The content of gangliosides in human milk and food samples was detected by our group under the guidance of Dr. Bertram Fong, the founder of HPLC-MS.

Methods and material

Criteria for inclusion

The data of the breastfeeding mother infant cohort were collected in The MCH hospital or community hospital in Beijing, Suzhou, and Xuchang from December 2014 to December 2016. The lactating mothers were included if they were 20-40 years old and healthy, did not drink and smoke, and had lived in residence for more than 3 years. The exclusion criteria were Type I or type II diabetes, hypertension, infectious diseases (tuberculosis, viral hepatitis, and HIV infection), mental disorders and memory disorders, major diseases such as tumor, liver and kidney failure, and those who could not answer questions. The inclusion criteria of infants were healthy newborns who were born naturally or by cesarean section in accordance with the inclusion/exclusion criteria of mothers, with a gestational age of 37-42 weeks, a birth weight of ≥ 2500 g, and a body length of ≥ 47 cm. The exclusion criteria were premature infants (<37 weeks), low birth weight infants (<2500 g), sick or birth defects, and deformed infants.

Basic information collection

Basic information was collected, which contained information about lactating mothers, babies, and families by self-designed questionnaire. All the questionnaires were completed in the social health service center or the home of the respondents with the assistance of the investigators. This study was in full compliance with the provisions of the declaration of Helsinki and had obtained the examination qualification of the biomedical ethics committee of Peking University with the ethical approval number RB00001052-16038.

Dietary questionnaire survey

The specific dietary intake of lactating mothers was collected by 24-hour dietary records. The dietary survey was carried out with each questionnaire. At the beginning of this study, the investigators would have handed the follow-up schedule materials to the mothers. At each follow-up visit, the investigator would issue a questionnaire at the next time point and ask the mothers to record the whole day's diet on the specified date, including all the food and drink consumed. We also collected the average intake and frequency of various food categories in the month before the survey date.

Body measurement and infants milk intake

The height, weight, and waistline of lactating mothers, and the length, weight, and head circumference of infants were measured. The milk intake of infants aged 1 month, 4 months and 6 months was measured and collected by xa-16001 (Mettler Toledo, Zurich, Switzerland). The specific method was to measure the weight change of a baby before and after each lactation on the whole survey day and record the sum as the milk intake of the baby on that day. A total of 36 milk samples were collected from 1-month-old infants, 6 from 4-month-old infants and 10 from 6-month-old infants.

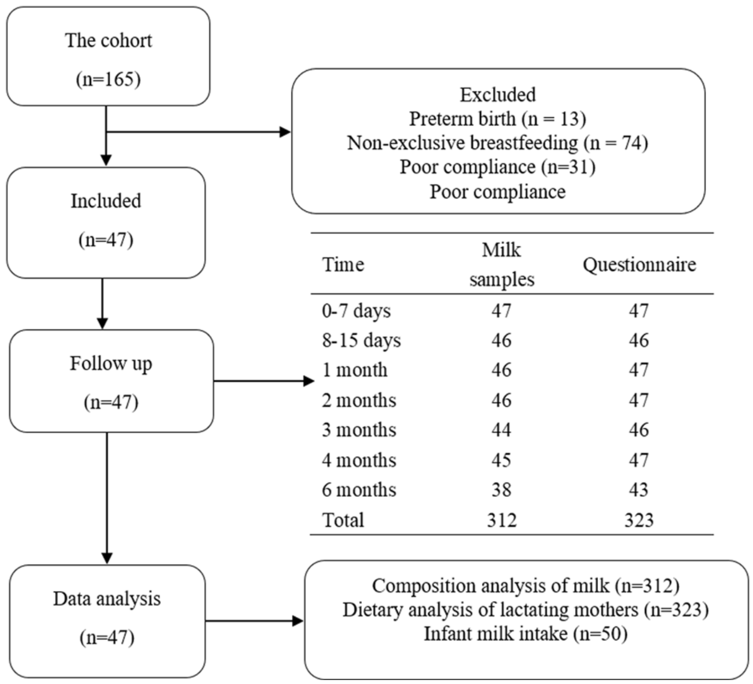

Figure 1. The flow chart of mother infant cohort in 3 cities of China.

Table 1. Schedule of follow-up survey of mother infant cohort

Time	Mother			Infant	
	Milk sample	Body measure	Mother questionnaire	Infant questionnaire	Body measure
0-7 day	√	√	√	√	
8-15 day	√		√		
1 month	√	√	√	√	√
2 months	√	√	√	√	√
3 months	√	√	√	√	√
4 months	√	√	√	√	√
6 months	√	√	√	√	√

Food composition analysis

According to the entries of animal food and processed food in the 24-hour dietary records of lactating mothers, a list of samples to be tested was compiled. Then, the same or similar food samples were purchased at the local market, which were used to detect the gangliosides content. A total of 5 categories and 43 kinds of food were selected and the content of ganglioside was detected. There were 9 kinds of gangliosides detected in each food. The gangliosides consisted of GM1, GM2, GM3, GM4, GD1a, GD1b, gt1b, GQ1b, and GD3. The testing work was completed by the R & D Center of Fonterra group in New Zealand by HPLC-MS method [10]. The intake of gangliosides of lactating mothers was calculated by 24-hour dietary record. The content of gangliosides in food, and macronutrient intake was calculated by 24-hour dietary records and Chinese food composition table.

Breast milk collection and component testing

Breast milk was collected according to strict and regular procedures. The macronutrients were tested by using the Miris HMA breast milk composition analyzer (Miris Holding, Uppsala, Sweden). The breast milk was taken out from the refrigerator at -80°C and thawed at room temperature before each test. After all the milk melted, it was fully mixed with an oscillator and an ultrasonic cell lyser, and then tested on the machine. The results included total energy (kcal/100 mL), fat (g/100 mL), carbohydrate (g/100 mL), crude protein (g/100 mL), true protein (g/100 mL), and dry matter (g/100 mL). The quantitative analysis method of gangliosides in breast milk was modified according to the published LC-MS method[11].

Estimation of the intake of gangliosides in infant diet

According to the standard of Reference Intake of Dietary Nutrients for Chinese Residents, the energy requirement (EER) of infants aged 6 months and below is 90 kcal/(kg·day). The energy density in breast milk was used to calculate the amount of milk needed by each infant every day. Then, the amount of ganglioside in breast milk was used to calculate the daily intake of ganglioside.

$$TGA\_intake = \frac{90}{Energy\_hm} \times TGA\_hm \times Bodyweight$$

Among them, TGA_intake was the estimated intake of ganglioside. The unit was mg/day.

Table 2. Basic characteristics and relevant information of the lactating mothers

Characteristics	Beijing	Suzhou	Xuchang	Total
Number of respondents	27	13	7	47
Age of pregnancy	31.4±3.4	27.0±3.6	31.0±2.6	30.2±3.8
Nationality				
Han	25 (92.5)	13 (100)	6 (85.7)	44 (95.7)
Others	2 (7.5)	0	1 (14.2)	3 (4.3)
Occupation				
Government and institutions	6 (22.2)	2 (16.7)	3 (50.0)	11 (24.4)
Public utilities	5 (18.5)	0	4 (50.0)	9 (17.8)
Mass media industry	2 (7.4)	0	0	2 (4.4)
Commercial trade	2 (7.4)	0	0	2 (4.4)
Telecommunications	2 (7.4)	0	0	2 (4.4)
Manufacturing	0	1 (8.3)	0	1 (2.2)
Hotel and tourism	1 (3.7)	1 (8.3)	0	2 (4.4)
Retail	0	1 (8.3)	0	1 (2.2)
Transportation	0	1 (8.3)	0	1 (2.2)
Other occupations	9 (33.3)	7 (50.0)	0	16 (33.3)
Education background				
Junior middle school	0	1 (8.3)	0	1 (2.2)
High school	1 (3.7)	4 (33.3)	0	5 (10.9)
Junior college	5 (18.5)	3 (25.0)	2 (28.6)	10 (21.7)
Bachelor's degree	11 (40.7)	5 (33.3)	4 (57.1)	20 (41.3)
Master and above	10 (37.0)	0	1 (14.3)	11 (23.9)
Weight before pregnancy (kg)	57.2±10.8	55.3±10.4	54.9±5.8	56.4±9.9
Weight gain during pregnancy (kg)	14.1±4.9	16.0±6.1	14.8±3.9	14.7±5.0
Number of deliveries				
1	23 (85.2)	10 (75.0)	5 (71.4)	38 (80.4)
2	4 (14.8)	3 (25.0)	2 (28.6)	9 (19.6)
Blood type				
A	8 (29.6)	3 (23.1)	2 (28.6)	13 (27.66)
B	8 (29.6)	1 (7.7)	3 (42.9)	12 (25.53)
O	4 (14.8)	6 (46.2)	0	10 (21.28)
AB	2 (7.4)	2 (15.4)	0	4 (8.51)
Unknown	5 (18.5)	1 (7.7)	2 (28.6)	8 (17.02)
Number of pregnancies				
1	17 (63.0)	9 (66.7)	2 (28.6)	28 (58.70)
2	6 (22.2)	2 (16.7)	2 (28.6)	10 (21.74)
3	4 (14.8)	2 (16.7)	2 (28.6)	8 (17.39)
4	0	0	1 (14.3)	1 (2.17)
Allergy	2 (7.4)	2 (16.6)	0	4 (8.70)
Food allergy	1 (3.7)	1 (8.3)	0	2 (4.35)
Number of taking dietary supplements	21 (84)	12 (92.3)	4 (66.6)	37 (88.1)
Passive smoking				
None	23 (88.0)	8 (58.3)	7 (100)	38 (81.8)
1-2 days per week	3 (8.0)	5 (33.3)	0	8 (13.6)
Everyday	1 (4.0)	0	0	1 (2.3)

Results were shown as frequency (%) or mean ± SD.

Table 3. Basic characteristics and relevant information of infants

Characteristics	Beijing	Suzhou	Xuchang	Total
Gender				
Boy	17 (63.0)	8 (58.3)	4 (57.1)	29 (60.9)
Girl	10 (37.0)	5 (41.7)	3 (42.9)	18 (39.1)
Birth length (CM)	50.3±1.9	49.2±1.0	51.9±1.6	50.7±1.8
Birth weight (kg)	3.5±0.5	3.1±0.3	3.7±0.4	3.5±0.5
Nationality				
Han	24 (88.9)	13 (100.0)	7 (100.0)	44 (93.5)
Others	3 (11.1)	0	0	3 (6.5)
Gestational weeks	39.1±1.1	39.3±1.1	39.9±0.9	39.3±1.1
Mode of delivery				
Spontaneous	11 (40.7)	7 (53.8)	3 (42.9)	27 (56.5)
Cesarean	16 (59.3)	6 (46.2)	4 (57.1)	20 (43.5)

Results were shown as frequency (%) or mean ± SD.

Energy_hm was the energy density in breast milk. The unit was kcal/ml. TGA_hm was the content of ganglioside in breast milk. The unit was mg/L. Bodyweight was the infant weight. The unit was kg.

Quality control

Quality control of the scheme design including index selection, investigation method, and corresponding detection method were strictly demonstrated to ensure scientific and rigorous investigation design. Quality control of the investigation process contained investigator training, organization and management of field investigation, and project progress supervision. The staff of measurement quality link were well trained. Data verification was conducted. Epidata3.1 Software and parallel double input was used to ensure the quality of the data.

Data analysis

All data analysis was done by SAS 9.4 Statistical software (SAS Institute: Cary, NC, USA), according to normal distribution. Classification variables were described by frequency and percentage. Continuous variables were described by mean ± standard deviation or median and quartile. Significance was set at $P<0.05$.

Results

Basic information description of respondents

There were 47 pairs of mother and infant included in the study. All the subjects were followed up from 0-7 days to 6 months after delivery. The specific follow-up and sampling was revealed (**Figure 1**). The follow-up schedule of the questionnaire was shown (**Table 1**). Basic information, information related to pregnancy and childbirth, health status during pregnancy, dietary behavior during lactation, and physical activity of lactating mothers was collected. There were further sub items for each item (**Table 2**). The basic information of babies included gender, length and weight at birth, nationality, gestational week, and delivery mode (**Table 3**). The basic information of the family included the age, occupation, education level, height and weight of fathers, the number of permanent residents, monthly income per capita, monthly expenditure per capita, and monthly food consumption of the family (**Table 4**).

Macronutrients and gangliosides intake of lactating mothers

The intake of macronutrients and gangliosides in lactating mothers within 6 months after delivery was summarized (**Table 5**). The average intake of energy was 1907.51 kcal/day, which was basically stable during lactation. The average intake of protein was 84.54 g/day, which increased first and then decreased. The average intake of fat was 75.18 g/day. It suddenly decreased to 67.39 g/day in 8-15 days postnatal, but the rest time was basically stable. The average intake of carbohydrate was 233.91 g/day. The intake trend was the same as that of protein. The average intake of dietary gangliosides was 6.33 mg/day. The intake was relatively stable at the beginning, but began to decline after 3 months of postpartum. GM3 and GD3 were the two main types of dietary gangliosides. The average intake of the whole postpartum 6 months was 3.61 mg/day and 1.76 mg/day. GM4 and GD1a were the second. The average intakes of the entire postpartum 6 months were respectively 0.47 mg/day and 0.31 mg/day. The average intakes of the other five were lower than 0.1 mg/day. GM1 and GT1b was 0.

Gangliosides were mainly supplied by animal foods. Meat, egg, and milk were the major sources (**Table 6**). The average monthly food

Table 4. Basic information of father and family

Characteristics	Beijing	Suzhou	Xuchang	Total
Age of pregnancy	32.6±5.6	29.1±4.0	31.5±2.1	31.5±5.0
Occupation				
Government and institutions	3 (11.1)	0	2 (28.6)	5 (10.9)
Public utilities	7 (25.9)	0	3 (42.9)	10 (21.7)
Mass media industry	1 (3.7)	1 (8.3)	0	2 (4.4)
Commercial trade	4 (14.8)	0	0	4 (8.7)
Telecommunications	5 (18.5)	0	0	5 (10.9)
Manufacturing	1 (3.7)	5 (33.3)	0	6 (10.9)
Hotel and tourism	0	1 (8.3)	0	1 (2.2)
Special service industry	1 (3.7)	0	0	1 (2.2)
Retail	0	3 (25.0)	0	3 (6.5)
Transportation	0	1 (8.3)	0	1 (2.2)
Other occupations	5 (18.5)	2 (16.7)	2 (28.6)	9 (19.6)
Education background				
High school	2 (7.4)	9 (66.7)	1 (14.3)	12 (23.9)
Bachelor's degree	16 (59.3)	4 (33.3)	6 (85.7)	26 (56.5)
Master and above	9 (33.3)	0	0	9 (19.6)
BMI (kg/m^2)	26.0±4.4	22.5±2.9	24.2±1.2	24.8±4.0
Household population				
2	2 (7.4)	3 (23.1)	1 (14.3)	6 (12.8)
3	9 (33.3)	4 (30.8)	2 (28.6)	15 (31.9)
4	7 (25.9)	2 (15.4)	3 (42.9)	12 (25.5)
5	4 (14.8)	1 (7.7)	1 (14.3)	6 (12.8)
6 and above	5 (18.5)	3 (23.1)	0 (0.0)	8 (17.0)
Monthly income (¥)				
1501-2000	1 (3.7)	0 (0.0)	1 (14.3)	2 (4.3)
2001-3000	1 (3.7)	1 (7.7)	3 (42.9)	5 (10.6)
3001-4000	0 (0.0)	6 (46.2)	3 (42.9)	9 (19.1)
4001-6000	5 (18.5)	1 (7.7)	0 (0.0)	6 (12.8)
6001-8000	8 (29.6)	2 (15.4)	0 (0.0)	10 (21.3)
Above 8000	12 (44.4)	3 (23.1)	0 (0.0)	15 (31.9)
Monthly outcome (¥)				
501-1500	0 (0.0)	1 (7.7)	4 (57.1)	5 (10.6)
1501-2000	3 (11.1)	0 (0.0)	3 (42.9)	6 (12.8)
2001-3000	5 (18.5)	3 (23.1)	0 (0.0)	8 (17.0)
3001-4000	11 (40.7)	5 (38.5)	0 (0.0)	16 (34.0)
4001-6000	3 (11.1)	1 (7.7)	0 (0.0)	4 (8.5)
6001-8000	3 (11.1)	1 (7.7)	0 (0.0)	4 (8.5)
Above 8000	2 (7.4)	2 (15.4)	0 (0.0)	4 (8.5)
Family monthly food consumption (¥)	2307.4±866.4	1866.7±795.8	1385.7±817.4	2052±891

Results were shown as frequency (%) or mean ± SD.

intake of lactating mothers within 6 months after delivery was investigated by FFQ (**Table 7**).

The relationship between the average intake of different kinds of food and the intake of gangliosides was analyzed (**Table 8**). The intake of green leafy vegetables (β=-0.0037, P=0.001), milk and dairy products (β=0.0032, P=0.034), and nuts (β=-0.023, P=0.017) was related to the intake of gangliosides. The distribution of dietary gangliosides intake in different groups

Table 5. Average intake of dietary nutrients of lactating mothers (mean ± SD)

Sampling time	0-7 days	8-15 days	1 month	2 months	3 months	4 months	6 months	Total average
Energy (kcal)	1845.89±901.68	1790.75±624.59	1934.60±694.59	2152.82±789.05	1928.78±755.47	1942.31±609.38	1743.52±635.30	1907.51±727.13
Protein (g)	80.89±37.17	83.20±34.06	87.48±38.43	97.61±48.76	84.61±37.31	83.40±35.88	73.39±26.54	84.54±37.76
Fat (g)	75.78±54.82	67.39±27.80	76.25±29.26	79.43±35.90	75.60±37.61	78.04±37.22	73.90±40.32	75.18±38.20
Carbohydrate (g)	218.75±107.40	222.59±105.01	231.78±116.12	275.35±126.69	241.22±109.83	238.26±100.85	207.13±85.89	233.91±109.19
GM1 (mg)	0.00±0.00	0.00±0.00	0.01±0.04	0.01±0.04	0.00±0.01	0.00±0.01	0.00±0.01	0.00±0.02
GM2 (mg)	0.03±0.15	0.04±0.16	0.00±0.01	0.00±0.01	0.04±0.18	0.13±0.68	0.09±0.49	0.05±0.33
GM3 (mg)	3.68±2.25	3.71±1.76	4.02±2.43	3.73±2.79	3.45±2.44	3.59±2.56	3.02±2.11	3.61±2.35
GM4 (mg)	0.67±0.51	0.60±0.39	0.48±0.43	0.42±0.35	0.35±0.42	0.41±0.44	0.33±0.29	0.47±0.42
GD1a (mg)	0.25±0.34	0.29±0.24	0.35±0.38	0.34±0.36	0.37±0.39	0.36±0.38	0.24±0.28	0.31±0.34
GD1b (mg)	0.05±0.08	0.06±0.05	0.07±0.08	0.07±0.07	0.07±0.07	0.08±0.09	0.06±0.07	0.07±0.08
GD3 (mg)	1.64±1.98	1.79±2.14	1.83±1.82	2.01±2.00	1.41±1.91	2.12±2.07	1.51±1.71	1.76±1.95
GT1b (mg)	0.00±0.00	0.00±0.00	0.00±0.01	0.00±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
GQ1b (mg)	0.05±0.08	0.06±0.05	0.07±0.08	0.07±0.07	0.07±0.07	0.08±0.09	0.05±0.05	0.06±0.07
TGA (mg)	6.36±3.47	6.54±3.12	6.83±3.52	6.65±3.57	5.77±3.67	6.77±3.75	5.28±2.99	6.33±3.46

TGA: total gangliosides.

Table 6. Analysis of the source and contribution rate (%) of gangliosides in the diet of lactating mothers

	Sampling time		Pastry	Dairy products	Egg	Fish	Meat
TGA	0-7 days	Average	0.12±0.26	1.20±1.86	2.07±1.69	0.53±1.06	2.17±2.63
		Contribution	2.6	15.9	36.4	11.3	33.8
	8-15 days	Average	0.10±0.23	1.41±2.04	1.89±1.28	0.44±1.03	2.56±2.01
		Contribution	1.6	17.1	33.6	6.5	41.2
	1 month	Average	0.05±0.24	1.44±1.83	1.44±1.37	0.26±0.76	3.34±2.74
		Contribution	0.9	19.8	23.8	2.8	52.8
	2 months	Average	0.14±0.30	1.56±2.00	1.28±1.13	0.22±0.85	3.18±2.97
		Contribution	2.1	23.2	24.6	2.8	47.3
	3 months	Average	0.06±0.19	1.03±1.78	0.98±1.30	0.18±0.41	3.03±3.01
		Contribution	1.4	15	22.5	7	54
	4 months	Average	0.09±0.19	1.65±2.06	0.99±1.03	0.29±0.64	3.32±3.24
		Contribution	2.2	23.3	21.5	6	47
	6 months	Average	0.16±0.40	0.92±1.56	0.88±0.89	0.09±0.26	2.44±2.63
		Contribution	4.5	17.6	23.1	3.6	51.3
	Total	Average	0.10±0.27	1.32±1.88	1.36±1.33	0.29±0.77	2.86±2.78
		Contribution	2.1	18.9	26.6	5.7	46.6
GD3	0-7 days	Average	0.12±0.25	1.20±1.86	0.13±0.10	0.02±0.04	0.11±0.15
		Contribution	11.1	31.2	29.9	7	20.7
	8-15 days	Average	0.09±0.22	1.41±2.04	0.11±0.08	0.02±0.04	0.13±0.13
		Contribution	5.5	34.1	28.6	3.2	28.6
	1 month	Average	0.05±0.24	1.44±1.83	0.09±0.08	0.01±0.03	0.16±0.11
		Contribution	4.8	41	18.7	0.9	34.6
	2 months	Average	0.13±0.29	1.56±2.00	0.08±0.07	0.01±0.03	0.15±0.15
		Contribution	7.8	43.6	17.4	1.5	29.7
	3 months	Average	0.06±0.18	1.03±1.78	0.06±0.08	0.01±0.02	0.14±0.14
		Contribution	5	30.6	18.7	6.7	38.9
	4 months	Average	0.08±0.19	1.65±2.06	0.06±0.06	0.01±0.02	0.19±0.23
		Contribution	9.7	44.1	14.1	2.7	29.4
	6 months	Average	0.15±0.40	0.92±1.56	0.05±0.05	0.00±0.01	0.15±0.20
		Contribution	9.6	31.2	16.6	2.4	40.1
	Total	Average	0.10±0.26	1.31±1.88	0.08±0.08	0.01±0.03	0.15±0.16
		Contribution	7.6	36.7	20.7	3.5	31.5
GM3	0-7 days	Average	0.00±0.01	0.00±0.00	1.31±1.07	0.50±1.00	1.71±2.05
		Contribution	0.1	0	40.5	14.6	44.6
	8-15 days	Average	0.01±0.04	0.00±0.00	1.19±0.81	0.42±0.98	2.01±1.59
		Contribution	0.3	0	37.1	9.8	52.7
	1 month	Average	0.00±0.00	0.00±0.00	0.89±0.87	0.24±0.72	2.71±2.15
		Contribution	0	0	25.4	4.7	69.9
	2 months	Average	0.01±0.01	0.00±0.00	0.79±0.72	0.21±0.81	2.56±2.39
		Contribution	0.2	0.1	30.1	5.8	63.8
	3 months	Average	0.00±0.02	0.00±0.00	0.60±0.82	0.16±0.39	2.39±2.40
		Contribution	0.5	0	21.5	9.5	68.5
	4 months	Average	0.00±0.01	0.00±0.00	0.59±0.62	0.27±0.61	2.49±2.53
		Contribution	0.3	0.1	25.7	8.5	65.4
	6 months	Average	0.01±0.01	0.00±0.00	0.55±0.55	0.09±0.25	1.92±2.13

	Contribution	0.4	0.1	30.8	7.3	61.4
Total	Average	0.00±0.02	0.00±0.00	0.85±0.84	0.27±0.73	2.26±2.20
	Contribution	0.3	0	30.3	8.7	60.8

The analysis data were from 24-hour dietary records. The mean value was the average daily intake (mg) contributed by each food group. The contribution rate was the percentage of this group of food in the total food.

and different time after parturition was analyzed and the comparison between groups was conducted. There was no significant difference in the average dietary gangliosides' intake within 6 months. However, there was a significant difference among mothers with different education levels at 4 months after childbirth, mothers with different baby gender at 6 months after childbirth, mothers with different family per capita expenditure at 3 months after childbirth, and mothers with different gestational age at 3 months after childbirth, but there was no significant trend (**Table 9**).

Dynamic changes of gangliosides in breast milk and influencing factors

The energy of breast milk remained about 60 kcal/100 mL within 6 months after delivery. The content of fat and carbohydrate remained stable, but the protein content gradually decreased with time. The crude protein content gradually decreased from 2.47 g/100 mL in 0-7 days after delivery to 1.00 g/100 mL, and the true protein content gradually decreased from 1.97 g/100 mL to 0.79 g/100 mL (**Table 10**).

The average content of gangliosides in breast milk within 6 months after delivery was 9.58 mg/L, which was mainly composed of GM3 and GD3. The average content was 2.16 mg/L and 7.42 mg/L, respectively. The highest content of ganglioside was 15.95 mg/L at 0-7 days postnatal, and then gradually decreased to the lowest 6.47 mg/L at 6 months postnatal. The content of GD3 was 13.66 mg/L in 0-7 days postnatal, and then decreased gradually, reaching a minimum of 4.40 mg/L in June postnatal. The content of GM3 was stable, but also slightly decreased from 2.29 mg/L to 2.08 mg/L (**Table 10**).

The distribution of gangliosides in breast milk under different characteristic groups was analyzed and compared among groups. The results showed that there was no significant difference in the content of ganglioside among different cities, infant gender, delivery mode, pregnancy age, and blood group. There was no significant difference between the groups at different time points (**Table 11**).

The content of total gangliosides in breast milk was used as the dependent variable, and the average intake of food in the past month in FFQ was used as the independent variable for mixed linear model analysis. The results showed that there was a significant negative correlation between the content of ganglioside and the change of time (β=-1.3613, P<0.001). However, there was no correlation between the average intake of various foods and the content of gangliosides in breast milk (**Table 12**). While the total ganglioside in breast milk was used as the dependent variable, the macronutrients and the intake of ganglioside were used as the independent variables for the mixed linear model analysis. The results showed that there was a negative correlation between the content of gangliosides in human milk and postpartum time (β=-1.2932, P<0.001). At the same time, it was found that there was a positive correlation between the intake of GM4 and the content of total gangliosides in human milk. (β= 2.0056, P=0.001) (**Table 13**).

Intake and health effects of gangliosides in infants

A total of 50 24-hour infants' milk intake data were collected, including 34 1-month-old infants' milk intake data, with an average milk intake of 627 g, 6 4-month-old infants' milk intake data, with an average milk intake of 1235 g and 10 6-month-old infants' milk intake data, with an average milk intake of 1196 g (**Table 14**).

Because we failed to measure the actual milk intake of all infants at all time points, we calculated that the milk intake of infants was about 150-160 mL/kg based on the energy demand of infants (90 kcal/kg·day) in the Reference Intake of Dietary Nutrients for Chinese Residents (2013 Edition) and the energy density of human milk measured in this study, and

Table 7. Average daily intake of different types of food during lactation

Sampling time	0-7 days	1 month	2 months	3 months	4 months	6 months	Total
Grain products (g)	225.0 (150.0-300.0)	200.0 (180.0-300.0)	200.0 (200.0-300.0)	240.0 (180.0-300.0)	225.0 (150.0-300.0)	200.0 (155.0-300.0)	217.5 (150.0-300.0)
Tubers (g)	28.6 (14.3-57.1)	28.6 (14.3-30.0)	28.6 (6.7-42.9)	22.9 (14.3-57.1)	28.6 (14.3-42.9)	17.9 (7.7-42.9)	28.6 (10.0-42.9)
Tuber vegetables (g)	50.0 (21.4-100.0)	28.6 (13.3-100.0)	28.6 (3.3-100.0)	42.9 (14.3-100.0)	42.9 (21.4-100.0)	42.9 (11.4-92.9)	42.9 (14.3-100.0)
Green vegetables (g)	175.0 (100.0-200.0)	200.0 (100.0-200.0)	150.0 (100.0-200.0)	150.0 (100.0-300.0)	142.9 (100.0-200.0)	125.0 (70.0-300.0)	150.0 (100.0-200.0)
Fruit (g)	250.0 (128.6-400.0)	150.0 (100.0-300.0)	200.0 (120.0-300.0)	200.0 (130.0-360.0)	200.0 (100.0-300.0)	189.3 (100.0-220.0)	200.0 (100.0-300.0)
Livestock meat (g)	100.0 (50.0-200.0)	100.0 (50.0-142.9)	100.0 (30.0-200.0)	100.0 (50.0-150.0)	85.7 (50.0-100.0)	82.9 (41.4-132.1)	100.0 (47.9-150.0)
Fish and shrimp (g)	14.3 (5.7-42.9)	25.7 (0.0-42.9)	21.4 (3.3-46.9)	21.4 (0.0-42.9)	14.3 (1.7-57.1)	14.3 (3.3-28.6)	14.3 (2.9-42.9)
Egg (g)	60.0 (50.0-120.0)	100.0 (60.0-180.0)	60.0 (50.0-120.0)	60.0 (42.9-100.0)	50.0 (32.1-85.7)	50.0 (34.3-80.0)	60.0 (50.0-120.0)
Milk and milk products (mL)	220.0 (114.3-250.0)	200.0 (35.7-250.0)	142.9 (15.5-250.0)	114.3 (0.0-250.0)	178.6 (71.4-250.0)	142.9 (71.4-250.0)	160.7 (71.4-250.0)
Soybean (g)	14.3 (10.0-28.6)	14.3 (0.0-28.6)	19.3 (1.7-42.9)	17.1 (0.0-32.1)	14.3 (2.9-28.6)	15.7 (4.5-42.9)	14.3 (2.9-32.1)
Soymilk (mL)	0.0 (0.0-28.6)	0.0 (0.0-16.7)	0.0 (0.0-34.5)	0.0 (0.0-35.7)	0.0 (0.0-28.6)	0.0 (0.0-28.6)	0.0 (0.0-28.6)
Nuts (g)	12.9 (3.6-30.0)	6.7 (0.0-21.4)	4.1 (0.0-14.3)	6.7 (0.0-20.0)	3.3 (0.0-12.9)	3.6 (0.0-14.3)	5.7 (0.0-20.0)
Oil (mL)	20.0 (10.0-21.5)	20.0 (10.0-20.0)	20.0 (10.0-20.0)	20.0 (10.0-25.0)	18.0 (9.0-21.0)	20.0 (10.0-24.0)	20.0 (9.5-30.0)
Salt (g)	6.0 (6.0-10.0)	6.0 (4.0-9.0)	6.0 (6.0-10.0)	6.0 (6.0-9.0)	6.0 (6.0-10.5)	6.0 (6.0-10.0)	6.0 (5.9-10.0)
Water (mL)	1200.0 (600.0-1750.0)	1200.0 (800.0-1500.0)	1250.0 (600.0-2000.0)	1000.0 (675.0-1500.0)	1000.0 (600.0-1600.0)	1200.0 (600.0-1750.0)	1200.0 (600.0-1800.0)
Beverage (mL)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.3)
Brown sugar (g)	0.0 (0.0-0.3)	0.0 (0.0-0.3)	0.0 (0.0-5.7)	0.0 (0.0-1.5)	0.0 (0.0-1.4)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Rice wine (mL)	0.0 (0.0-0.0)	0.0 (0.0-3.3)	0.0 (0.0-3.3)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Millet porridge (g)	57.1 (0.0-150.0)	46.4 (2.7-107.1)	80.0 (6.7-200.0)	92.9 (8.6-200.0)	57.1 (0.0-142.9)	40.0 (0.0-114.3)	20.8 (0.0-142.9)
Crucian carp soup (mL)	28.6 (0.0-71.4)	28.6 (3.3-85.7)	42.9 (6.7-107.1)	28.6 (0.0-85.7)	10.0 (0.0-71.4)	20.0 (0.0-71.4)	0.0 (0.0-57.1)
Broth and bone soup (mL)	57.1 (0.1-121.4)	57.1 (15.0-85.7)	71.4 (26.7-171.4)	57.1 (12.5-135.7)	35.7 (0.0-85.7)	57.1 (0.3-128.6)	13.8 (0.0-74.3)

The data were from FFQ, which reviewed the average food intake in the past month and shown as median p25 to p75.

Table 8. The relationship between the average intake of food and the intake of ganglioside in lactating mothers

Types of food	Estimated regression coefficient	F	*P*
Cereal crop	-0.0020	0.95	0.332
Tubers	-0.0033	0.21	0.648
Tuber vegetables	-0.0003	0.02	0.897
Green vegetables	-0.0037	6.60	0.001
Fruits	-0.0006	0.24	0.622
Livestock meat	0.0035	3.58	0.061
Fish and shrimp	-0.0021	0.18	0.669
Eggs	0.0055	3.70	0.056
Milk and dairy products	0.0032	4.55	0.034
Soybean	0.0008	0.01	0.921
Soybean Milk	0.0001	0.00	0.951
Nuts	-0.0230	5.81	0.017
Grease	-0.0063	0.56	0.457
Salt	0.0717	3.57	0.061
water	0.0001	0.92	0.339
Beverage	-0.0020	0.04	0.849
Brown sugar	-0.0033	0.30	0.583
Rice Wine	-0.0025	0.30	0.587
Millet Congee	0.0011	0.49	0.483
Crucian carp soup	0.0057	3.36	0.069
Broth and bone soup	0.0021	1.34	0.250

The mixed linear model was used. The dependent variable was the intake of gangliosides. The independent variable was the intake of various foods.

calculate the milk intake data of each infant according to the measured body weight of the infant (**Table 15**). The estimated value was close to the average milk intake of some infants measured in this study.

Combined with the content of gangliosides in breast milk measured in this study, the intake of gangliosides in infant diet was calculated (**Table 16**). According to the ganglioside intake per kilogram of body weight, the highest intake was 0-7 days after parturition. The median was 2133.6 μg, and then gradually decreased with time. The intake of gangliosides per kilogram of body weight was 863.4 μg in 6 months postnatal. Similarly, the intake of GD3 and GM3 decreased with time.

The average length at birth of the investigated infant was 50.7 cm, and the average length at the age of 6 months was 68.8 cm. The average birth weight was 3.5 kg, and the average weight at the age of 6 months was 8.4 kg. The head circumference gradually increased from 37.2 cm at the age of 1 month to 43.1 cm at the age of 6 months (**Figure 2**). Through the mixed effect model analysis, it was not found that the average intake of gangliosides within 6 months was related to the growth and development of infant's body length and head circumference. However, it was found that the body weight development of infants was related to the average intake of total gangliosides and GD3 within 6 months. However, after adjusting for possible confounding factors, the association between body weight and ganglioside intake at 6 months of age also disappeared. The growth of infants' body weight was significantly correlated with the intake of carbohydrate at 6 months old (β=0.024, P=0.0009) (**Table 17**).

Discussion

Ganglioside is a kind of bioactive lipid with complex structure, which is difficult to detect. Before Bertram Fong et. al. improved the HPLC-MS method in 2009 [11], most of the detection work was mainly based on TLC [12]. However, the estimation results were often inaccurate for its limitations. In the article published with HPLC-MS method, Bertram et. al. detected the content of 8 kinds of gangliosides in brain samples of rats. This method overcame the disadvantages of TLC, and the author showed that this method was not limited to the detection of these 8 kinds of gangliosides. Subsequently, the team reported the content of gangliosides in dairy products in 2011 [13], and the content of gangliosides in pork, beef, chicken, and fish in 2016 [10]. At the same time, many other studies began to use this method to report the content of gangliosides in various biological samples or foods, indicating that this method has gradually matured and has been widely recognized [5, 6, 14]. In recent years, there have been many reports on the content of gangliosides in various foods or ingredients, but it is still not enough to cover the common food types and cannot be used to estimate the intake of gangliosides in human diet. In this study, we first collected the common animal food or processed food (5 categories and 43 foods in total) in the dietary records

Table 9. Distribution of dietary ganglioside intake of lactating mothers under different characteristic groups

Characteristics		0-7 days	8-15 days	1 month	2 months	3 months	4 months	6 months	Total
City	Beijing	5.88 (4.59~8.51)	6.43 (4.59~9.22)	6.03 (4.50~7.72)	6.26 (4.25~8.19)	4.97 (3.42~8.23)	6.21 (3.41~7.40)	4.98 (3.38~6.32)	5.69 (4.25~8.10)
	Suzhou	5.68 (4.28~7.22)	5.66 (3.73~6.42)	5.00 (3.53~8.73)	5.28 (4.56~7.15)	3.46 (2.83~10.68)	5.42 (3.23~10.15)	4.10 (2.81~7.67)	5.28 (3.39~7.42)
	Xuchang	5.88 (4.16~7.94)	5.41 (4.29~7.50)	8.31 (6.22~12.19)	7.40 (3.75~8.84)	4.81 (4.32~6.57)	7.50 (5.65~11.29)	4.28 (2.38~4.83)	5.98 (4.33~8.29)
	P[1]	0.868	0.378	0.133	0.849	0.97	0.463	0.515	0.408
Education background	Junior college and below	6.30 (4.28~7.42)	5.66 (4.78~6.75)	5.64 (3.93~6.94)	5.49 (4.54~8.07)	4.77 (3.40~6.59)	7.26 (4.63~11.29)	5.06 (3.60~8.52)	5.68 (4.28~7.50)
	Bachelor's degree	6.76 (4.59~8.05)	6.53 (4.98~7.68)	5.94 (3.53~9.23)	6.87 (5.08~8.84)	4.63 (2.33~9.36)	3.93 (2.44~6.04)	4.28 (3.05~5.60)	5.58 (3.41~8.05)
	Master and above	5.16 (2.49~5.88)	5.12 (4.29~10.87)	6.26 (5.00~10.60)	4.26 (3.75~6.90)	6.09 (4.25~8.87)	7.00 (6.03~11.73)	4.83 (2.66~6.30)	5.51 (4.25~8.45)
	P[1]	0.297	0.827	0.47	0.144	0.792	0.007	0.478	0.762
Infant gender	Boy	5.51 (3.50~8.51)	6.37 (4.56~7.44)	6.26 (4.50~7.72)	5.69 (4.26~8.84)	4.97 (3.42~9.36)	6.22 (3.96~11.64)	5.60 (3.81~7.63)	5.79 (4.25~8.45)
	Girl	6.66 (4.85~7.57)	5.75 (4.29~9.22)	5.80 (3.53~10.33)	5.73 (3.75~7.25)	4.35 (2.15~6.59)	6.20 (2.66~8.77)	3.48 (2.63~4.87)	5.35 (3.45~7.45)
	P[1]	0.354	0.857	0.853	0.379	0.159	0.417	0.041	0.162
Mode of delivery	Cesarean section	5.78 (3.89~8.00)	6.03 (4.83~8.14)	6.24 (4.52~7.29)	6.51 (5.21~9.32)	4.66 (2.86~9.49)	5.65 (2.44~10.03)	4.61 (3.36~6.30)	5.67 (3.53~8.05)
	Spontaneous labo	5.88 (4.59~7.22)	6.04 (4.37~7.50)	5.32 (4.50~8.72)	5.07 (4.00~7.45)	4.97 (3.29~8.23)	6.92 (4.52~9.69)	4.91 (2.94~8.45)	5.54 (4.06~8.00)
	P[1]	0.837	0.825	0.732	0.185	0.942	0.205	0.715	0.624
Per-capita income (¥)	Below 6000	6.09 (4.83~7.61)	5.67 (4.98~6.45)	5.06 (3.64~7.45)	6.48 (4.56~8.73)	5.44 (3.46~9.36)	6.96 (5.01~10.78)	4.74 (2.94~6.13)	5.68 (4.31~7.94)
	6000-8000	5.33 (4.59~6.61)	6.61 (4.06~7.05)	7.61 (6.23~9.23)	5.05 (4.26~6.85)	4.78 (3.29~7.25)	4.38 (2.66~10.45)	3.87 (2.57~5.60)	5.51 (3.57~7.64)
	Above 8000	5.88 (3.71~8.75)	6.53 (4.37~11.09)	5.38 (4.65~10.33)	5.29 (3.77~7.25)	3.82 (2.33~8.87)	4.79 (2.53~7.40)	5.06 (3.38~6.82)	5.28 (3.45~8.77)
	P[1]	0.885	0.589	0.248	0.67	0.395	0.201	0.54	0.78
Per-capita outcome (¥)	Below 3000	5.69 (4.16~7.79)	5.59 (4.31~7.39)	5.07 (4.10~7.89)	6.44 (4.39~8.84)	6.05 (4.74~9.36)	6.84 (5.47~11.64)	4.79 (3.81~7.41)	5.77 (4.36~8.26)
	3000-4000	5.88 (5.07~7.42)	5.90 (4.59~7.05)	6.51 (5.00~9.23)	5.69 (4.26~7.45)	3.82 (2.24~4.95)	7.00 (3.35~10.03)	4.98 (3.60~6.13)	5.65 (4.19~7.26)
	Above 4000	5.73 (4.15~9.02)	6.95 (4.18~10.16)	5.79 (3.83~8.98)	5.18 (3.58~7.54)	6.69 (2.81~10.19)	4.20 (2.42~6.72)	3.37 (2.68~6.82)	5.10 (3.23~9.06)
	P[1]	0.941	0.507	0.566	0.729	0.046	0.059	0.512	0.447
Age of pregnancy	20-30	6.47 (4.22~8.15)	5.59 (4.98~6.87)	5.06 (3.93~8.12)	5.64 (4.04~8.00)	4.97 (3.45~9.36)	6.04 (4.24~10.15)	3.82 (2.68~5.63)	5.36 (3.77~8.13)
	Above 30	5.59 (4.46~7.76)	6.39 (4.33~9.98)	6.47 (5.04~8.41)	6.17 (4.25~8.19)	4.52 (2.33~6.09)	6.75 (3.35~8.77)	5.55 (3.47~7.39)	5.90 (4.25~8.05)
	P[1]	0.941	0.507	0.566	0.729	0.046	0.059	0.512	0.568
Number of permanent residents	3 and below	5.50 (3.75~7.86)	5.67 (4.06~7.20)	5.16 (3.93~9.35)	5.10 (3.58~6.87)	5.44 (3.42~9.36)	6.70 (4.20~9.52)	4.61 (2.68~6.30)	5.29 (3.71~7.68)
	4	6.13 (4.31~8.35)	5.91 (4.30~10.50)	7.29 (5.82~9.59)	6.68 (4.33~8.52)	4.25 (1.85~7.25)	6.39 (3.41~9.00)	4.68 (1.89~6.32)	5.84 (4.26~8.68)
	5 and above	6.33 (5.49~7.57)	6.39 (5.65~7.39)	5.06 (2.89~7.64)	7.45 (5.64~11.83)	6.05 (2.33~10.11)	6.04 (2.66~10.45)	4.98 (3.56~7.30)	6.15 (4.04~9.23)
	P[1]	0.606	0.458	0.132	0.135	0.402	0.951	0.71	0.451

The results were shown as mg/day and quartile median.

Table 10. Mean content of macronutrients and gangliosides in human milk. The results were shown as mean ± SD

Sampling time	Energy (kcal/100 mL)	Fat (g/100 mL)	Carbohydrate (g/100 mL)	Crude protein (g/100 mL)	True protein (g/100 mL)	Dry matter (g/100 mL)	GM3 (mg/L)	GD3 (mg/L)	TGA (mg/L)
0-7 days	56.22±8.87	2.06±0.89	6.59±0.61	2.47±0.49	1.97±0.41	11.33±1.05	2.29±0.53	13.66±5.72	15.95±5.94
8-15 days	60.41±12.98	2.72±1.24	6.63±0.50	1.93±0.39	1.56±0.31	11.51±1.67	2.13±0.30	10.58±4.39	12.71±4.48
1 month	65.75±20.59	3.41±2.00	6.81±1.06	1.58±0.51	1.26±0.39	12.02±2.62	2.25±1.15	7.21±3.22	9.46±3.10
2 months	60.24±12.12	2.88±1.30	6.98±0.53	1.29±0.25	1.02±0.21	11.35±1.39	2.17±0.80	5.22±2.07	7.39±2.17
3 months	61.77±12.05	3.14±1.39	6.94±0.53	1.11±0.32	0.87±0.25	11.40±1.30	2.11±0.65	5.35±2.32	7.46±2.27
4 months	59.72±16.49	2.98±1.54	6.90±1.09	1.04±0.28	0.82±0.22	11.13±2.24	2.07±0.65	4.90±2.45	6.96±2.48
6 months	57.65±12.80	2.79±1.35	6.90±0.66	1.00±0.30	0.79±0.25	10.90±1.49	2.08±0.82	4.40±1.89	6.47±1.97

Table 11. Distribution of gangliosides in breast milk under different characteristic groups

Characteristics		0-7 days	8-15 days	1 month	2 months	3 months	4 months	6 months	Total
City	Beijing	14.70±4.96	12.47±4.31	9.46±2.71	7.19±2.11	7.38±2.46	6.82±2.52	9.30±4.39	6.20±2.01
	Suzhou	17.17±7.72	13.56±4.55	8.80±3.45	6.96±2.34	7.07±2.07	6.82±2.09	9.64±5.40	7.17±2.21
	Xuchang	18.69±5.45	12.20±5.46	10.68±3.92	8.94±1.59	8.41±1.89	7.76±3.13	10.59±5.17	6.16±0.90
	P^1	0.206	0.748	0.440	0.116	0.456	0.660	0.261	0.394
Infant gender	Boy	15.01±5.42	13.13±5.27	9.55±3.46	7.56±2.53	7.44±2.42	7.18±2.66	9.57±4.77	6.27±2.09
	Girl	17.46±6.67	12.30±3.02	9.41±2.59	7.17±1.59	7.56±2.14	6.66±2.33	9.69±4.97	6.70±1.91
	P^1	0.184	0.549	0.889	0.563	0.866	0.505	0.827	0.522
Mode of delivery	Cesarean section	17.50±7.40	12.38±4.59	9.96±3.45	7.83±2.11	7.57±2.23	7.16±2.34	10.01±5.31	6.96±2.24
	Spontaneous labor	14.78±4.39	13.14±4.47	9.14±2.86	7.10±2.24	7.41±2.40	6.80±2.68	9.30±4.43	6.07±1.75
	P^1	0.184	0.549	0.889	0.563	0.866	0.505	0.203	0.522
Age of pregnancy	20-30	17.74±6.69	13.31±4.55	9.19±2.96	7.34±2.26	7.38±2.17	6.85±1.95	9.84±5.31	6.41±1.80
	Above 30	14.75±5.14	12.50±4.55	9.90±3.27	7.57±2.16	7.66±2.46	7.14±2.93	9.54±4.47	6.55±2.22
	P^1	0.184	0.549	0.889	0.563	0.866	0.505	0.596	0.522
Type of blood	A	15.54±4.05	12.97±4.40	9.89±3.48	7.73±2.21	7.87±2.93	7.56±2.98	9.87±4.41	7.11±2.41
	B	15.05±6.71	12.81±5.02	10.36±3.57	7.66±2.58	8.49±2.32	7.26±2.75	9.88±4.93	6.10±2.02
	O	16.64±8.47	13.09±3.68	9.11±2.37	7.06±2.29	6.75±1.67	6.35±1.23	9.33±5.14	6.44±1.54
	AB	13.26±2.92	12.15±3.73	7.29±1.75	6.01±1.41	5.96±0.98	5.16±0.50	8.05±3.75	4.96±0.41
	P^1	0.184	0.549	0.889	0.563	0.866	0.505	0.486	0.522

The results were shown as mg/L and mean ± SD.

Table 12. The relationship between the average intake of food and the content of gangliosides in breast milk

Independent variable	Estimated review coefficient	t value	*P* value
Postpartum time	-1.3613	-10.13	<0.001
Cereal crop	-0.0014	-0.57	0.571
Tubers	0.0008	0.1	0.924
Tuber vegetables	0.0020	0.77	0.442
Green vegetables	-0.0025	-1.37	0.172
Fruits	0.0018	1.2	0.233
Livestock meat	0.0027	1.21	0.227
Fish and shrimp	-0.0087	-1.42	0.159
Eggs	-0.0022	-0.65	0.520
Milk and dairy products	0.0018	1.14	0.255
Soybean	-0.0051	-0.53	0.598
Soybean Milk	0.0009	0.27	0.784
Nuts	0.0146	1.26	0.210
Grease	0.0047	0.5	0.617
Salt	-0.0462	-1.03	0.304
Water	0.0002	1.68	0.096
Beverage	-0.0021	-0.18	0.855
Brown sugar	-0.0083	-1.14	0.256
Rice Wine	-0.0018	-0.29	0.771
Millet Congee	0.0001	0.02	0.981
Crucian carp soup	-0.0045	-1.21	0.229
Broth and bone soup	-0.0030	-1.41	0.160

The mixed linear model was used. The dependent variable was the content of ganglioside in breast milk. The independent variable was the average intake of various foods.

of the subjects through 24-hour dietary survey. Then, we collected these food samples from the market to detect the content of ganglioside and established the food composition table of the dietary ganglioside of the mothers and children by ourselves, instead of the double meal method.

The composition of human milk is affected by the nutritional health of the mother. Compared with normal weight mothers, overweight and obese mothers secrete more fatty acids (especially saturated fatty acids). A study in South Korea showed that the content of various fatty acids in breast milk was positively correlated with the intake of corresponding fatty acids in the diet of mothers [15]. Agnieszka et. al. found that the variation of the fat content in human milk was significantly related to the BMI of mothers [16]. As a kind of lipid, gangliosides may also be affected by the dietary intake and body composition of lactating mothers. Although there have been research reports on the content of gangliosides in food since 1978, for the detection technology is not mature enough. It costs a lot of manpower and material resources to detect a small number of samples or 1-2 kinds of gangliosides [17]. With the development of technology and economy, reports on the content of gangliosides in various foods have been published gradually. However, there is no breakthrough in the detection method. Gangliosides are based on LBSA, which is not the result of absolute content. In 2011, Canadian scholars analyzed the ganglioside intake of 19 adults by first recording the diet and then collecting food samples for detection [18]. The result was <100 μg LBSA/4187 kJ. In 2016, Khor et. al. collected the dietary data of 12-24.5-month-old children in Malaysia by double meal method, detected and calculated the content of ganglioside in the food, and the result was that the dietary ganglioside intake of children in this stage was 5.86 mg/day [19]. This study is the first to report the absolute intake of dietary gangliosides. In addition, the analysis of the food source of the daily dietary nutrients is helpful to optimize the dietary structure and take in enough nutrients.

The content of gangliosides in breast milk was reported from 1995. The content of gangliosides in human milk of Spanish mothers was 1.6-23.8 mg/L within 11-150 days of postpartum [20] and for Japanese mothers it was 11.1-21.8 mg/L within 21-49 days of postpartum [21]. The two detection methods above were based on the traditional TLC method, so the results were calculated indirectly based on LBSA. After 2009, the improved HPLC-MS method was used to detect gangliosides in animal food, and the absolute content was obtained directly, which promoted the development of gangliosides detection. In 2013, this method was used to report that the content of ganglioside in breast milk of Singapore mothers within 30-120 days after delivery was 4.6-5.6 mg/L [22]. In 2014, Giuffrida et. al. tested the content of breast milk in China, which was 9.1-10.7 mg/L within 30-120 days after delivery and was also the cross-sectional

Table 13. The relationship between the intake of dietary nutrients and the content of gangliosides in breast milk

Independent variable	Estimated review coefficient	t value	*P* value
Postpartum time	-1.2932	-11.63	<0.001
Energy	-0.0009	-0.17	0.868
Protein	-0.0325	-1.34	0.181
Fat	0.0161	0.32	0.752
carbohydrate	0.0037	0.17	0.866
GM1	-22.8045	-0.78	0.434
GM2	-0.2250	-0.29	0.769
GM3	0.3371	1.56	0.121
GM4	2.0056	3.27	0.001
GD1a	-4.6721	-1.85	0.065
GD1b	12.6497	0.99	0.326
GD3	-0.0043	-0.04	0.969
GT1b	137.4600	0.68	0.497
GQ1b	2.1592	0.16	0.873

The mixed linear model was used. The dependent variable was the content of ganglioside in breast milk. The independent variable was the intake of dietary nutrients.

Table 14. The milk intake of partial infants

Age of infants (month)	Mean (g)	SD	maximum	minimum
1	627.1	299.8	1594.5	253.0
4	1235.4	1269.9	3638.3	362.1
6	1196.7	1295.9	3464.5	372.5

survey of breast milk in the early stage of our research group[23]. In 2015, Ma et. al. tested the content of breast milk in South China within 8 months after delivery, which was 13.1-20.9 mg/L [6]. In this study, the content of gangliosides in breast milk of Chinese mothers was 6.5-15.9 mg/L within 6 months after delivery. Although there are differences in various research methods and results, the total content is basically in the range of 0-20 mg/L.

The content of total gangliosides in breast milk is basically the same, but there are differences in the proportion of different types of gangliosides. Ma et. al. found that GM3 was the main ganglioside in breast milk, followed by GD3 in the study from South China in 2015. Moreover, the proportion of GM3 was higher than GD3, which did not change in 1-8 months after delivery, but the difference was growing [6]. Pan et. al. found that GD3 was higher than GM3 in the early postpartum period. GM3 gradually exceeded GD3 as the main component over time. In this study, GD3 was always higher than GM3, and the concentration gap between the two decreased with time [21]. In 2015, Ma et. al. studied the content of ganglioside in Malaysian breast milk, which was highly consistent with this study. GD3 was higher in the early postnatal period, and then gradually decreased with time. GM3 was basically stable in the second month postnatal period, but the total content of ganglioside was gradually decreased [24]. The variation of gangliosides in different studies may be caused by various factors, among which dietary factors may be important. Because the dietary habits of different countries are quite different, especially the custom of "sitting on the moon" in China within one month after birth, the postpartum diet is greatly affected. Although there have been previous studies on the content and changes of breast milk ganglioside, there has been a lack of research on the dynamic changes of breast milk ganglioside in China, and no analysis on the influencing factors of breast milk ganglioside content is conducted. In this study, the correlation between the characteristics of mothers' milk and the level of gangliosides in human milk was studied, but no significant correlation factors were found, suggesting that large sample size in-depth study may be needed in the future.

The dietary gangliosides of exclusively breastfed infants are all derived from breast milk. Gangliosides in breast milk have many benefits to the health of infants, mainly including promoting nerve development, improving immune function, and intestinal health. However, there are few studies on the intake and demand of gangliosides in infants. In this study, the recommended intake of energy and the energy density of breast milk were used to indirectly estimate the intake of gangliosides. At the same time, this study also verified the estimated value through the measured data of some infants' milk intake. Priyanka et. al. studied the milk intake of Sri Lanka infants and found that the average milk intake of 3-month-old infants was 772 g/day, and that of 6-month-old infants was 800 g/day [25]. The survey of

Table 15. The intake estimates of included infants

Postpartum time	mL/kg				mL per infant			
	Mean	SD	maximum	minimum	Mean	SD	maximum	minimum
0-7 days	163.5	25.7	130.4	195.7	570.0	122.9	404.3	716.6
8-15 days	154.9	32.1	108.4	206.4				
1 month	155.4	82.5	67.7	518.4	731.0	467.4	243.6	2695.9
2 months	154.6	27.6	102.3	202.2	887.1	197.5	537.9	1233.7
3 months	151.5	31.9	109.8	230.8	1011.1	290.4	658.5	1892.3
4 months	169.8	86.4	102.3	526.3	1199.8	510.5	646.5	3210.5
6 months	163.8	37.8	103.4	243.2	1367.2	316.8	837.9	2091.9

Table 16. The estimates of average daily intake of gangliosides in infants

Postpartum time	TGA	GD3	GM3
Intake per kilogram of body weight (μg)			
0-7 days	2133.6 (1728.6~4003.2)	1839.1 (1318.0~3578.8)	423.9 (330.7~474.3)
8-15 days	1465.3 (1338.0~1804.5)	1180.9 (1043.7~1427.9)	310.2 (282.4~388.3)
1 month	1357.0 (1003.5~1740.5)	987.8 (709.4~1412.7)	296.6 (257.8~338.4)
2 months	1087.8 (908.1~1187.0)	705.3 (583.8~865.3)	313.6 (253.9~362.1)
3 months	923.5 (781.9~1231.0)	702.3 (525.2~898.9)	279.3 (239.6~310.2)
4 months	927.5 (720.5~1305.8)	653.5 (486.4~917.8)	291.2 (255.5~355.5)
6 months	863.4 (718.5~1270.2)	582.1 (443.6~873.9)	274.3 (247.3~348.6)
Intake per infant (mg)			
0-7 days	7.43 (6.19~12.41)	5.75 (4.67~11.09)	1.32 (1.15~1.68)
8-15 days	6.10 (4.38~7.18)	4.54 (3.07~6.25)	1.29 (1.15~1.62)
1 month	6.14 (4.68~7.42)	4.23 (3.09~5.05)	1.82 (1.44~2.00)
2 months	5.48 (5.08~7.56)	4.21 (3.53~5.36)	1.77 (1.54~1.98)
3 months	6.80 (5.25~8.58)	4.69 (3.85~6.69)	2.15 (1.82~2.48)
4 months	7.38 (6.17~12.33)	4.92 (3.68~8.53)	2.47 (2.01~2.72)

European and American countries showed that the average milk intake of 4-month-old infants is 700-800 g/day, but the actual range of each infant was exceptionally large, about 450-1200 g/day [26]. The estimated milk intake of infants in this study was about 1000 ml/day at the age of 3 months and 1300 ml/day at the age of 6 months. The difference of infant milk intake between different countries and regions in the world also increased the diversity of ganglioside intake. In this study, the body length and weight data of infants in 0-6 months and the estimated intake of ganglioside were collected and analyzed. It was not found that the physical development of infants was related to the intake of ganglioside in 6 months. This was probably due to the fact that babies were healthy and exclusively breastfeeding in this study. Although the intake of gangliosides was different, they were sufficient to meet the normal developmental needs of infants. It also indicates that the ganglioside intake of infants in this study can serve as a data base for nutrient intake and provide recommendations for the consumption of gangliosides in formula fed infants or infants in other disease states.

Overall, this study is the first to report the intake of dietary gangliosides of urban dairy mothers in China, and the design of cohort follow-up observation is used to report the dynamic changes of intake within 6 months after delivery. This study is also the first to report the dynamic changes of gangliosides in human milk by cohort follow-up and infer the demand of infant ganglioside by detecting the components of human milk (macronutrients, gangliosides), so as to accumulate basic data for improving the recommended amount of infant nutrients. There are some limitations in the process of trying to solve the problem. First,

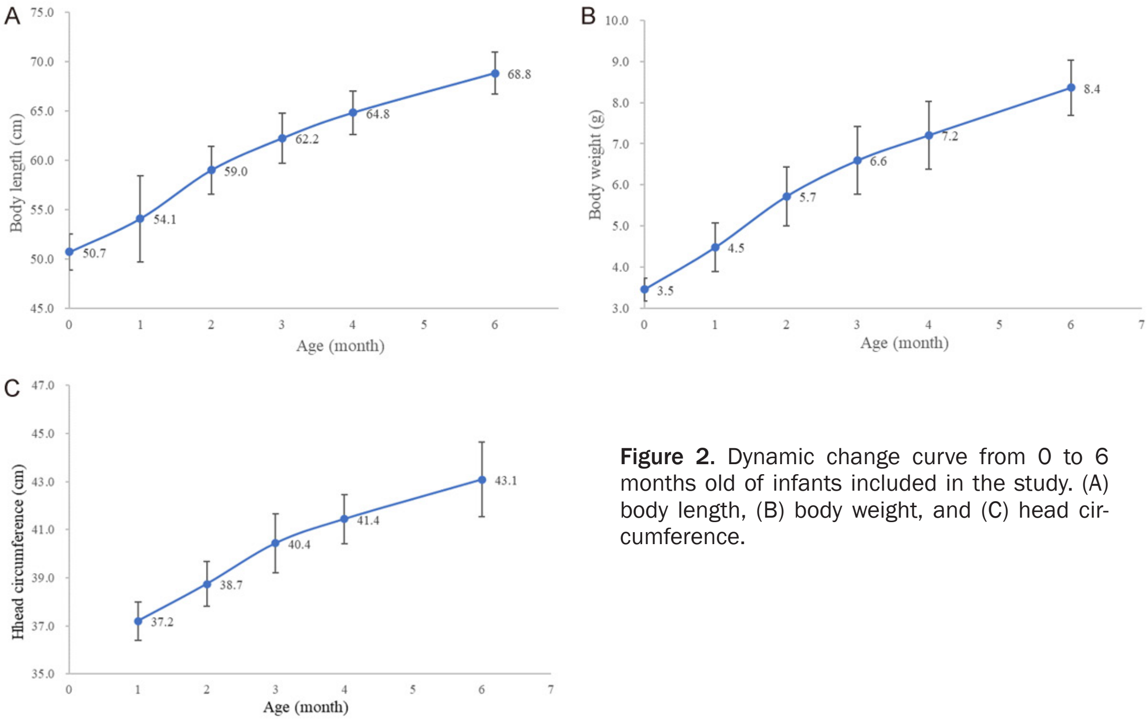

Figure 2. Dynamic change curve from 0 to 6 months old of infants included in the study. (A) body length, (B) body weight, and (C) head circumference.

Table 17. The relationship between the average intake of gangliosides and physical development in infants

Dependent variable	Model	Independent variable	Estimated regression coefficient	t value	*P* value
Body length	Model 1	TGA	-0.001	-0.010	0.996
		GD3	0.001	0.060	0.956
		GM3	-0.180	-0.270	0.789
	Model 2	TGA	-0.040	-0.320	0.749
		GD3	-0.030	-0.190	0.847
		GM3	-0.500	-0.700	0.487
Body weight	Model 1	TGA	0.070	2.170	0.035
		GD3	0.075	2.090	0.042
		GM3	0.306	1.720	0.092
	Model 2	TGA	0.034	1.120	0.268
		GD3	0.040	1.270	0.210
		GM3	-0.040	-0.270	0.789
Head circumference	Model 1	TGA	-0.020	-0.330	0.742
		GD3	-0.020	-0.370	0.715
		GM3	-0.009	-0.030	0.977
	Model 2	TGA	-0.060	-0.690	0.491
		GD3	-0.060	-0.690	0.496
		GM3	-0.150	-0.350	0.725

Factors include baseline measurements and energy intake were adjusted in Model 1. Baseline measurements, energy intake, gender, mode of delivery, and macronutrient mean intakes (fat, protein, and carbohydrate) were adjusted in Model 2.

only 47 pairs of mothers and infants were included in this study. In view of funding constraints, more samples were not included to detect ganglioside content, and there was a lack of sample representation. In this study, however, the repeated measurement data of the same survey object at 7 time points were obtained by using the follow-up observation in the queue, which had advantages in reflecting individual changes. Secondly, the indirect calculation method was used in this study. The content of gangliosides in food was measured first, and then the food intake was obtained through 24-hour dietary survey. The intake was estimated by combining the two methods. The more accurate way is the double meal method, which can directly measure the ganglioside content of the mother's diet. However, due to the cost pressure and the difficulty of detection, the same widely recognized 24-hour dietary record was used in this study. Moreover, this study was a 24-hour record of non-continuous 7-day diet, which is better in data stability.

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Disclosure of conflict of interest

None.

Address correspondence to: Yumei Zhang, Department of Nutrition and Food Hygiene, School of Public Health, Peking University, Xueyuan Road 38, Haidian District, Beijing 100191, China. E-mail: yumeizhang20@163.com

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