Journal of Food and Nutrition Research, 2019, Vol. 7, No. 6, 415-426
Available online at http://pubs.sciepub.com/jfnr/7/6/2
Published by Science and Education Publishing
DOI:10.12691/jfnr-7-6-2

Dietary Intake of Gangliosides and Correlation with Serum Ganglioside Concentration: Cross-Sectional Study among Chinese Toddlers Aged 24-48 Months

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Received March 05, 2019; Revised April 19, 2019; Accepted May 27, 2019

Abstract Gangliosides are a group of sialic-acid-containing sphingolipids, found in brain and neural tissues. The various biological roles of gangliosides have been well reported and include calcium homeostasis, regulation of neurons, neural repair, immune system functions, and cellular functions. Furthermore, dietary gangliosides have been shown to improve cognitive development in the early postnatal period and to increase brain neuroplasticity. However, the dietary intake status of gangliosides among Chinese toddlers is unknown. The aim of this study was to determine the dietary intake and food source of gangliosides among Chinese toddlers and to probe its correlation with serum ganglioside concentration. Total of 213 Chinese toddlers aged 24-48 months were enrolled in this cross-sectional study in Beijing and Xuchang City, China. A food frequency questionnaire and 24-h dietary recall methods were used to collect both long- and short-term dietary information. Food items selected from dietary records and blood serum samples collected from 197 toddlers were analyzed for ganglioside composition using high performance liquid chromatography-mass spectrometry. The average dietary total ganglioside intake among the Chinese toddlers was 4.21 mg/day. Dairy products, meats, and growing-up milk powders were the predominant food sources of dietary gangliosides with relative proportions of 38, 31, and 29% of the daily dietary total ganglioside intake, respectively. The average serum total ganglioside concentration was 14.86 μg/mL, with GM3 making up 96%. No significant correlation was found between the dietary total ganglioside intake and the serum total ganglioside concentration. As this correlation was inconclusive, further investigation is required to understand ganglioside metabolism and the mechanisms contributing to the role of gangliosides in the growth and cognitive development of toddlers.

Keywords: *ganglioside, dietary intake, serum ganglioside, Chinese toddlers*

Cite This Article: Shengjie Tan, Ai Zhao, Bertram Fong, Meichen Wang, Sophie Gallier, and Yumei Zhang, "Dietary Intake of Gangliosides and Correlation with Serum Ganglioside Concentration: Cross-Sectional Study among Chinese Toddlers Aged 24-48 Months." *Journal of Food and Nutrition Research*, vol. 7, no. 6 (2019): 415-426. doi: 10.12691/jfnr-7-6-2.

1. Introduction

Gangliosides (GAs) have sialic acid residues in the carbohydrate moiety [1]. The different GA classes are characterized by the number and position of sialic acids [2]. GAs are widely distributed in body tissues. They are found predominantly in the nervous system, constitute 6-10% of total lipids in the brain [3], and are essential components of plasma membranes [4].

The various biological roles of GAs have been well reported and include calcium homeostasis, regulation of neurons [5], neural repair [6], immune system functions [7], and cellular functions [8]. GAs are found to be abundant in the fetal and infant hippocampal region of the brain and their concentration increases by three-fold up to 5 years of age [9]. This indicates that GAs play a crucial role in neuronal and brain development [10]. Studies have revealed that GAs could influence brain neuroplasticity and the ability to undergo morphological and functional remodeling as the basis of learning and memory [11,12,13]. Therefore, natural and synthetic GAs may be used as a therapeutic agent for treating a variety of neurological diseases and for treating younger children with neurological impairment [3,14,15].

Miklavcic et al. [16] demonstrated that increasing the dietary GA intake could increase the plasma GA concentration in adults. This effect was also observed in infants [17]. In addition, increased dietary GA intake was associated with improved cognitive outcomes; infants fed a GA-enriched infant formula had higher intelligence quotient (IQ) scores and better hand-eye coordination than infants fed a standard infant formula [17]. Furthermore, maternal dietary GA intake throughout pregnancy and lactation has been recognized to have effects on the offspring's learning and behavior [10,14,18].

Importantly, the dietary GA intake should be studied to understand whether a normal diet provides sufficient levels of GAs and what minimum intake level is required to prevent deficiency and associated negative effects on health. Unfortunately, data on dietary GA intake are scarce because of the diversity of this sphingolipid family and the lack of robust methods to measure the GAs accurately across various food matrices. Although, there are limited studies reporting GA concentrations in foods, it is currently not possible to estimate the dietary total GA (TGA) intake in the Chinese population based on the common and diverse food categories that are consumed in China [19,20]. To the best of our knowledge, GA status and dietary GA intake in Chinese toddlers have never been reported. As optimizing the daily dietary TGA intake in early life is likely to be important for the healthy growth and brain development of toddlers and may have long term health effects, the daily dietary TGA intake in toddlers should be determined and recommendations about food consumption to optimize this intake should be made. Therefore, this study was undertaken: (1) to investigate the daily dietary TGA intake and serum GA concentration among Chinese toddlers; (2) to explore the associations between the daily dietary TGA intake and the serum TGA concentration.

2. Materials and Methods

2.1. Study Design and Participants

This cross-sectional study was conducted between August and October 2016 in Xuchang City and Beijing, China. Toddlers were recruited and examined either at the Maternal and Child Health Hospital in Xuchang City or at an early learning center in Beijing. The inclusion criteria were that the toddlers should be healthy and aged between 24 and 48 months. The exclusion criteria included (1) any physical disabilities, (2) any infectious diseases, and (3) any mental health problems.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by the Peking University Institutional Review Board (IRB00001052-14081). Written informed consents were obtained from all legal guardians of participants before the study.

It was computed that 182 was the minimal sample size that would be required to estimate the serum GAs concentration with a power of 90% and a 5% level of confidence. The calculation was based on the serum GD3 + GM3 concentration of 12.2 μg/mL [standard deviation (SD) 2.9 μg/mL] in breast-fed infants aged 6 months [17]. Two hundred and sixty healthy toddlers and their caregivers were invited to participate; 213 participants were enrolled in the study and completed the demographics and food questionnaires, and anthropometry. Neurodevelopment assessments were conducted on 110 toddlers. Caregivers of 197 participants agreed to the collection of blood samples.

2.2. Data Collection

A validated structured questionnaire was used to collect background information including each toddler's birth date and gender, the parental heights, weights, and education backgrounds, and the family income. Other informations, such as the toddler's and parents' allergies and the toddler's eating behavior, were recorded.

Each toddler's height and weight were measured by a trained investigator to the nearest 0.1 cm or 0.1 kg, respectively. Physical developmental status was classified based on the z-scores calculated with the software WHO Anthro (version 3.2.2, January 2011). A toddler was considered to be overweight if the weight-for-height z-score was above +2 and to be underdeveloped if one of the three z-scores (weight-for-age, height-for-age, weight-for-height) was below -2.

2.3. Dietary Survey

Short term dietary food intake information was collected using a 24-hr dietary recall. The food record included all foods and drinks consumed by the toddler on the day prior to the examination visit. The daily dietary TGA intake was calculated based on the food intake in the 24-hr dietary recall record and the GA contents of these foods. When the milk powder brand was not given, its GA content was assumed to be the average GA content of the milk powders investigated in this survey. All foods were grouped into five-categories: growing-up milk powders (GUMPs), dairy products, meats (including eggs), fish and fish products and pastry.

A food frequency questionnaire (FFQ; considered to be long term dietary information) containing 15 food categories was used to evaluate the dietary food habits based on the type of food, their intake frequencies, and their average intakes in the last month. Among the food categories, meats, seafood, freshwater products, eggs, dairy products, and GUMPs were thought to contain GAs. They were classified into three groups (meats, fish and fish products, dairy), consistent with the 24-h dietary data, and the average intakes were calculated. Meats included meats and eggs. Fish and fish products included seafood and freshwater products. Dairy products included all the dairy products and GUMPs because GUMPs could not be separated from the dairy products in the FFQ datasets. As pastry was not commonly consumed by Chinese toddlers, it was not included the FFQ.

The 24-hr dietary recall and FFQ forms were completed by caregivers with the help of a trained investigator.

2.4. Determination of GAs in Foods

Animal-based foods and processed food products in the 24-hr dietary recall record were analyzed for GAs and the data were used for the daily dietary TGA intake calculations. Foods of plant origin were excluded as they do not contain GAs [14]. Sixty-four food items, grouped into five different food categories, were tested. The GA testing was carried out at the Fonterra Research and Development Centre, New Zealand, using high performance liquid chromatography-mass spectrometry (Acquity I Class system, Waters, Milford, MA, USA), as previously described [21]. Nine classes of GAs in these food samples were tested: GM1, GM2, GM3, GM4, GD1a, GD1b, GD3, GT1b, and GQ1b.

2.5. Serum Sample Collection and Determination of GAs

Fasting blood was collected from 197 toddlers for the determination of serum GA concentrations using ultra-high performance liquid chromatography-mass spectrometry, based on a previously published method with some modifications [22]. Briefly, GAs were extracted from 200 µL of plasma using methanol/chloroform (2: 1). A 5 µL aliquot of the final extract was injected on to the chromatography system (Acquity I Class system, Waters, Milford, MA, USA), which was coupled to a XEVO G2-S QTOF mass spectrometer (Waters, Milford, MA, USA). The autosampler was maintained at 4°C. GA separation was achieved on a BEH HILIC column (2.1 mm x 100 mm, 1.7 µm) with a Van Guard BEH HILIC 1.7 µm guard column (Waters, Milford, MA, USA) at 45°C. Mobile phase A was 95% acetonitrile with 10 mM ammonium acetate and 0.1% formic acid; mobile phase B was 50% acetonitrile with the same concentrations of ammonium acetate and formic acid as mobile phase A. The linear gradient was: from 0 to 2 min, 1% B; from 2 to 12.5 min, 1-95% B; from 12.5 to 13 min, 95% B; from 13 to 13.5 min, 95-1% B; from 13.5 to 18 min, 1% B. The flow rate was 0.4 mL/min. The mass spectrometer was operated in negative ion electrospray ionization mode with the capillary voltage set at -3000 V and the ion source temperature set at 120 °C. The cone gas and the desolvation gas were set to 50 and 800 L/h, respectively. The scan range of the mass spectrometer was from 500 to 1700 m/z with a data scan rate of 0.4 s. The GA standards used in this study were purchased from Matreya Lipids and Biochemicals (State College, PA, USA) and were dissolved in 90% acetonitrile to produce eight-point calibration curves (from 0.78 to 10 µg/mL). GA concentrations were calculated based on peak areas of the standards and the test samples, using accurate mass extracts for each of the individual GAs [22].

2.6. Statistical Analysis

Data analysis was conducted using SAS 9.4 statistical software (SAS Institute, Cary, NC, USA). Categorical variables were described as frequency and percentage. Continuous variables were described as mean ± SD, or median and quartiles depending on the normality test. The Wilcoxon or Kolmogorov-Smirnov test was used to compare the differences in GA intake among toddlers with different characteristics, including gender, delivery mode, weight group, parental education, family income, picky eating behavior, and food allergies of participants and their parents. Partial correlation based on Pearson correlation analysis was used to explore the correlation between the dietary intake and the serum concentration for each GA and the correlation between food group intake and serum GA concentration after adjusting for sex, age, height, and weight

3. Results

3.1. Characteristics of the Participants

A total of 213 toddlers aged 24-48 months who met the recruitment criteria were included in this study. Boys represented 52.1% of the toddler cohort. The average age of all participants was 39.9 ± 5.2 months. All other demographic information is provided in Table 1.

3.2. GA Content of Selected Foods

Five food categories (comprising 64 food items of animal origin or containing animal-based ingredients) reported in the 24-h recall records were analyzed for GAs. The GA contents of the food categories are summarized in Table 2. Those of the individual food items are provided in the supplementary material (Table S1). GUMPs and dairy products had high average GD3 concentrations, at 5.91 and 1.28 mg/100 g, respectively (Table 2). Meats contained all measured GA classes and were rich in GM3 (Table 2), whereas fish and fish products, and pastry had low TGA contents, with less than 1 mg/100 g food (Table 2).

3.3. Dietary GA Intake from 24-h Recall Records

The GA contents of the animal-based foods and the foods containing animal-based ingredients, and the 24-h recall records were used to calculate the dietary TGA intake of each participant (Table 3). The average daily dietary TGA intake for the Chinese toddler was 4.21 mg/day, with GD3 making up 70% (2.96 mg/day) and GM3 making up 22% (0.92 mg/day) of the intake. The remaining GA classes (GM1, GM2, GM4, GD1a, GD1b, GT1b, and GQ1b) made up approximately 8% (0.33 mg/day) of the daily dietary TGA intake. Only one participant did not consume any animal-based food on the day prior to blood collection. The energy-adjusted daily dietary TGA intakes were also calculated and are presented in the supplementary material (Table S2).

Table 3 shows that dairy products (38%) were the predominant source of GAs in the toddlers' diets, followed by meats (31%) and GUMPs (29%). Fish and fish products and pastry contributed less than 2% of the GAs in the diet. Dairy products were the main source of GD3 (53.5%), followed by GUMPs (41.6%). Meats were the main source of GM3 (97%).

3.4. Long Term Food Intake Frequency from FFQ

The average intake of animal-based food over a month was calculated using the FFQ data (Table S3). Meats (including meats and eggs) were consumed at an average of 70 g/day and fish and fish products were consumed at an average of only 8 g/day. However, dairy products (including GUMPs) were consumed at an average of 180 g/day, i.e., much more than for the other two food categories.

Table 1. Principle information of participants and their parents

Characteristics	*n*	%	*Mean*	*SD*
Participants (n = 213)				
Gender (boys)	111	52.1		
Age (months)			39.9	5.2
Height (cm)			99.6	4.9
Weight (kg)			15.5	2.4
Cesarean delivery	133	62.4		
Weight status’				
Overweight	7	3.3		
Normal	203	95.8		
Underdeveloped	2	0.9		
City				
Beijing	103	48.4		
Xuchang	110	51.6		
Mother				
BMI (kg/m^2)			22.2	3.4
Education†				
Middle school and below	38	18.0		
High school and technical secondary school	60	28.4		
Diploma	63	29.9		
Bachelor degree or above	50	23.7		
Father				
BMI (kg/m^2)			24.2	3.2
Education†				
Middle school and below	35	16.6		
High school and technical secondary school	54	25.6		
Diploma	68	32.2		
Bachelor degree or above	54	25.6		
Family				
Average monthly individual income (yuan, RMB) †				
Less than 2000	59	28.1		
2000-	87	41.4		
4000-	48	22.9		
8000-	16	7.6		
Factors related to dietary behavior				
Picky eater	87	41.4		
Food allergy of participants	16	7.5		
Food allergy of parents	19	9		

BMI, body mass index; RMB, renminbi (Chinese currency); SD, standard deviation.
† Missing values: weight status (n = 1); mother education (n = 2); father education (n = 2); average monthly individual income (n = 3).

Table 2. Summary of ganglioside concentrations (mg/100 g) in five food categories consumed by Chinese toddlers

Food Category	GM1	GM2	GM3	GM4	GD1a	GD1b	GD3	GT1b	GQ1b	TGA
GUMP							5.91			5.91
Dairy			0.00				1.28			1.28
Meats	0.00	0.00	1.30	0.00	0.03	0.00	0.11	0.00	0.00	2.33
Fish and fish products		0.00	0.13		0.00		0.03			0.21
Pastry			0.01	0.00			0.29			0.42

TGA, total gangliosides; GUMP, growing-up milk powder.
Data are presented as median. The ganglioside concentrations of the individual food items are provided as supplementary material (Table S1).

Table 3. Dietary ganglioside intake among Chinese toddlers aged 24-48 months.

Dietary GA	Average Intake (mg/day) and Proportion (%)			Average Intake (mg/day) and Proportion (%) from Food Categories[†]									
				GUMP		Dairy		Meats		Fish and Fish Products		Pastry	
	Mean	SD	%	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
GM1	0.001	0.00	0.1					0.001	100.0				
GM2	0.04	0.30	1.0					0.04	99.9	0.00[‡]	0.1		
GM3	0.92	0.94	21.9			0.003	0.3	0.89	96.8	0.02	2.1	0.01	0.8
GM4	0.20	0.36	4.8					0.20	99.6			0.001	0.4
GD1a	0.06	0.10	1.4					0.06	99.9	0.00[‡]	0.1		
GD1b	0.01	0.02	0.2					0.01	100.0				
GD3	2.96	3.20	70.3	1.23	41.6	1.59	53.5	0.08	2.8	0.002	0.1	0.06	2.0
GT1b	0.008	0.00	0.1					0.002	100.0				
GQ1b	0.01	0.02	0.2					0.01	100.0				
TGA	4.21	3.44	100.0	1.23	29.3	1.59	37.7	1.30	30.9	0.02	0.5	0.07	1.6

GA, ganglioside; GUMP, growing-up milk powder; TGA, total gangliosides.
[†] The GUMP category included only growing-up milk powder. The dairy category included all other dairy products. The meats category included meat and poultry eggs. The fish and fish products category included all fish, shrimp, shellfish, and processed fish, such as fish balls etc., from sea and river. The pastry category included all bakery and biscuit items.
[‡] These values are < 0.001 mg/day.

3.5. Serum GA Concentrations in the Toddlers

The serum TGA concentrations varied from 5.65 to 43.8 µg/mL, with an average of 14.86 µg/mL. The major serum GA was GM3, with an average concentration of 14.27 µg/mL. The average GD3 concentration was 0.5 µg/mL. The concentrations of all other serum GAs (GD1a, GD1b, GM1, GM2, GM4, GQ1b, and GT1b) were below 0.05 µg/mL (Table 4).

3.6. Correlations between Serum GA Concentration and Dietary GA Intake from 24-h Recall

The correlations between dietary GA intake and serum GA concentration are shown in Table 5 for each GA class. The dietary GM1 intake was found to be weakly inversely correlated with the serum GD3 concentration. The dietary GM3 intake was found to be weakly inversely correlated with the serum GM3 and TGA concentrations. The dietary GM4 intake was found to be weakly inversely correlated with the serum GM1 concentration. The dietary GT1b intake was found to be weakly inversely correlated with the serum GM1 and GD3 concentrations. However. the absolute values of the correlation coefficients were all less than 0.2.

Table 4. Serum ganglioside† concentrations in Chinese toddlers aged 24-48 months (µg/mL)

Variable	Mean	SD	Median
GD1a	0.02	0.00	0.02
GD1b	0.02	0.00	0.02
GD3	0.5	0.07	0.49
GM1	0.01	0.00	0.01
GM2	0.04	0.00	0.04
GM3	14.27	5.18	13.43
GT1b	0.01	0.00	0.01
TGA	14.86	5.23	14.07

TGA, total gangliosides.
[†] GM4 and GQ1b are not measured in serum.

3.7. Correlations between Serum GA Concentration and Food Intakes from the FFQ

No significant correlations between the average long-term food intakes [for the different food categories – meat, fish and fish products, and dairy products (including GUMPs)] and the serum GA concentrations were found (Table S4).

4. Discussion

GAs are complex bioactive lipids that are difficult to measure in food because of the diversity of their class and molecular species and their presence in low quantity [2,22]. Even though some studies have reported the GA contents of certain foods, not all the foods consumed by the toddlers have been analyzed previously and some foods may have been analyzed with different analytical methods with variable accuracy [22-28]. In addition, as GAs are not classed as essential nutrients, they are not commonly analyzed or are not included in food composition tables. The present study enrolled 213 Chinese toddlers and collected their dietary intake information. Animal-based and processed products were tested for their GA content using the same analytical method as previously reported in another study conducted in Malaysia [19]. This allowed comparison of the dietary intakes. To the best of our knowledge, the present study is the first to report GA consumptions and serum GA concentrations in Chinese toddlers.

4.1. GAs in Foods

GAs have been isolated from animal-derived foods including milk, other dairy products, meat, and eggs [23,24,26,28]. Plant-based foods are not a source of GAs because plants cannot synthesize sialic acid [14]. Concentrations and class distributions of GAs vary across different animal species, and even across tissue types within the same animal [27]. In the present study, 64 common foods consumed by Chinese toddlers were analyzed and their average GA contents in nine classes and their TGA contents were reported. A previous study reported that egg yolks contain GM3, GM4, and GD3 [28]. However, only GM3 and GM4 were predominantly detected in eggs in this study. GD3 was the major GA class that was measured in the dairy foods in this study, and previous studies have reported GD3 as the predominant GA in cow's milk with minor quantities of GM3 and GT3 [25,29].

4.2. Dietary GA Intake from 24-h Recall Records and its Influence Factors

There are limited studies reporting the dietary GA intake in general or specific populations, and particularly in infants and toddlers. Pham et al. [20] investigated that the food consumptions of 19 healthy adults in Canada and the GA (measured as lipid-bound sialic acid) contents of six specific foods. The average daily intake of GAs was as low as < 100 μg N-acetylneuraminic acid/1000 kcal. Furthermore, Vesper et al. [30] reviewed that the dietary sphingolipid intake among the US population and estimated that the GA intake was 0.3-0.4 g/day. However, GAs constitute only a small proportion of the sphingolipids found in foods [14]. Only one previous study has looked at the GA intake in infants and toddlers; Khor et al. [19] investigated that the GA consumption in urban Malaysian toddlers aged 12-24.5 months. The toddlers consumed an average TGA amount of 5.86 ± 0.56 mg/day[19], which is much higher than the intake reported in the present study (4.21 ± 3.44 mg/day). The difference can be explained by the different age groups of the participants and the associated different consumption patterns of GUMPs and other foods. In the Malaysian study, 75-85% of the toddlers were receiving GUMP, which provided 5.05 ± 0.56 mg/day of TGAs, whereas the toddlers in our study were older on average and had started to consume more diverse foods and less GUMP. In the present study, the main GA-rich food sources in the diets of the Chinese toddlers were dairy products, meats, and GUMPs, representing 38, 31, and 29% of the diet, respectively.

As reviewed by Palmano et al. [10], available evidence suggests that dietary GAs may positively affect cognitive functions, particularly in the early postnatal period. Some studies have shown that the neonatal brain GA concentration can be influenced by diet [31]. In addition, animal experiments and human clinical trials have provided evidence to support that supplementary dietary GAs could improve cognitive functions [10,13,14,32]. In the critical period of fast growth and development of the brain in the first 2-3 years of life, nutrition deficiency or suboptimal nutrition results in impaired development, fewer neuronal connections, impaired synaptic connectivity, and irreversible consequences for cognitive functions throughout life [33]. Exogenous GAs can exhibit neurotrophic properties, which play a role in neuroplasticity and regeneration [34]. Hence, it is valuable to understand which foods can provide sufficient levels of GAs.

A previous nutritional survey among preschool children indicated that paternal education was a determinant of toddlers' nutritional status [35]. Although, this study also looked at the association between the daily dietary TGA intake and the characteristics of the participants, parental income or education status did not have any marked influence on the dietary TGA intake (Table S5). Furthermore, the results indicated that the population that participated in this survey was relatively homogeneous.

Table 5. Correlations† between 24-h dietary intake of each ganglioside and serum ganglioside concentration

Dietary GA		Serum GA							
		GD1a	GD1b	GD3	GM1	GM2	GM3	GT1b	TGA
GD1a	Corr	0.017	–0.030	0.051	0.085	–0.018	–0.031	–0.070	–0.030
	P	0.818	0.682	0.480	0.239	0.806	0.670	0.335	0.679
GD1b	Corr	0.019	–0.001	0.072	0.099	–0.002	–0.012	–0.046	–0.011
	P	0.789	0.985	0.324	0.172	0.984	0.872	0.522	0.883
GD3	Corr	0.026	0.021	0.004	0.083	–0.050	–0.065	0.073	–0.064
	P	0.717	0.772	0.958	0.254	0.494	0.370	0.312	0.376
GM1	Corr	–0.062	–0.080	–0.144	–0.133	0.035	–0.052	–0.065	–0.053
	P	0.394	0.268	0.047	0.066	0.628	0.477	0.370	0.465
GM2	Corr	–0.123	–0.040	–0.051	–0.112	–0.059	–0.026	–0.040	–0.026
	P	0.089	0.578	0.484	0.121	0.415	0.722	0.579	0.716
GM3	Corr	–0.028	–0.121	–0.087	0.003	–0.084	–0.148	–0.112	–0.147
	P	0.703	0.094	0.232	0.968	0.248	0.041	0.122	0.041
GM4	Corr	0.039	–0.056	–0.135	–0.151	–0.051	–0.074	–0.029	–0.076
	P	0.593	0.437	0.061	0.037	0.482	0.305	0.690	0.298
GQ1b	Corr	0.035	0.027	0.086	0.075	0.017	0.014	–0.025	0.015
	P	0.634	0.711	0.237	0.303	0.811	0.843	0.735	0.832
GT1b	Corr	–0.022	–0.075	–0.170	–0.178	0.075	–0.037	–0.040	–0.039
	P	0.761	0.303	0.019	0.013	0.302	0.613	0.583	0.595
TGA	Corr	0.011	–0.024	–0.037	0.056	–0.080	–0.112	0.028	–0.112
	P	0.880	0.740	0.613	0.443	0.269	0.121	0.697	0.123

GA, ganglioside; TGA, total gangliosides.
† Adjusted with sex, month age, height, and weight.

4.3. Food Intake from FFQ

According to the Chinese dietary guidelines for toddlers aged 2-3 years, the recommendation for meats, eggs, and aquatic products in total is 50-70 g/day [36]. The toddlers in this study consumed on average about 78 g/day (total of meats, seafood, freshwater products, and eggs from the FFQ), which fulfils the Chinese dietary guideline requirements. The Chinese dietary guidelines for children 2-3 years old also recommend a dairy product consumption of 500 g/day [36], with no distinction between dairy products and GUMPs. Unfortunately, the consumption of dairy products (and GUMPs) by the toddlers in this study was measured to be 180 g/day (liquid equivalent, Table S3), well below the recommended 500 g/day, despite the large proportion of the daily dietary GA intake (38.1%) that was provided by this food category. Although, the intake of dairy products is increasing in the overall Chinese population, the lower than recommended daily intake for these toddlers may have been because dairy is traditionally not part of the Chinese toddler's diet. A lower dairy intake among Chinese toddlers was reported in our previous study [37].

4.4. Serum GA Concentrations and Correlation with Dietary GA Intake

As reported in previous studies, GM3 is the predominant class of GAs in the serum [38]. This was also confirmed in our study, in which the average serum TGA concentration was 14.86 µg/mL and the average serum GM3 concentration was 14.27 µg/mL. For some nutrients (e.g., carbohydrates, amino acids), dietary intake can be correlated with levels in the blood [39,40]; however, GA metabolism may be more complex. The consumption of dietary GAs was hypothesized to lead to an increase in serum GAs [41]. An 8-week GA-supplementation intervention in adults showed a significant increase (35%) in serum GD3 at the end of the supplementation period [16]. These researchers used a high dose of GAs (43 mg/day), i.e., about ten-fold higher than in the present study. However, in the present study, there was no correlation between the 24-h dietary TGA intake and the serum TGA concentration or at the individual GA class levels, except for some weak inverse correlations between a few GA classes (Table 5). These weak inverse correlations between some GA classes could not be explained, even when superimposed against the GA biosynthetic pathway [42], where GM3 is the precursor for other GA classes (a, b, and c series). Likewise, several animal feeding studies measured the GA levels in target organs and also found conflicting results. Reis et al. [43] conducted an animal experiment and found that, when piglets were administered a GD3-fortified formula (treatment) or a standard formula (control), the brain GM1 level increased in the control group but not in the treatment group and the brain GD3 level did not differ between groups. Another animal study showed that rats supplemented with a complex milk lipid ingredient, as a source of GA, from early age to young adulthood had improved cognitive measures of novelty recognition and spatial memory; however, the brain GA levels were not different from those in the control group (no supplementation) [44]. These inconsistent findings highlight the complex metabolic fate of GAs. McJarrow et al. [14] speculated that dietary GAs may be remodeled in the enterocyte when absorbed in the intestine. Degraded GAs could be used for the de novo biosynthesis of new GAs. Because we found no correlation between the dietary TGA intake and the serum TGA concentration, we also speculate that dietary GAs are directly stored in other tissues such as cell membranes. Further studies are needed to understand the metabolic fate and mechanisms of action of dietary GAs.

In the long term food study (FFQ data), there was no correlation between the TGA intake from the three different food categories and the serum TGA concentration or at the individual GA class levels. Further longer term nutrition intervention studies investigating food consumption, dietary TGA intake, and functional and physiological outcomes, including measuring changes in serum GA metabolites, are needed to determine the optimal dietary TGA intake.

4.5. Strengths and Limitations

There are some strengths in the present study. The number of participants recruited was sufficient to fulfil the basic statistical requirement. All the toddlers were from China and had lived in fixed places for years; no immigrant was enrolled. The toddlers had stable food consumption habits, which helped to weaken the bias of the dietary survey.

A general limitation was the use of the FFQ and the dietary recall to assess dietary intake; as not all records were precise, some assumptions had to be made. A duplicate portion sampling method would provide more precise data [45]. The present study used a 24-h dietary recall on one day to estimate the daily intake of GAs. Several days, including a weekend, should be included in the dietary data survey.

This was an observational study in which the dietary GA consumption varied and was not controlled. The levels consumed by these toddlers were low compared with those in intervention studies, in which high levels (supplementation doses) were consumed and a difference in serum GAs was observed [16,17].

Furthermore, there is very little understanding of the possible natural diurnal variation in serum GAs, if any, and whether it may have an impact on the blood sampling time.

5. Conclusions

The average dietary GA consumption among Chinese toddlers aged 24-48 months was 4.21 mg/day. GD3 and GM3 constituted the main dietary GA intake. Dairy products, meats, and GUMPs were the predominant food sources of GAs. The correlation between the dietary GA intake and the serum GA concentration was inconclusive and further studies are warranted to understand the metabolic fate of GAs and to determine optimal dietary levels.

Ganglioside Nomenclature

GM1: βDGalp(1-3)βDGalNAc[αNeu5Ac(2-3)]βDGalp(1-4)βDGlcp(1-1)Cer
GM2: βDGalpNAc(1-4)[αNeu5Ac(2-3)]βDGalp(1-4)βDGlcp(1-1)Cer
GM3: αNeu5Ac(2-3)βDGalp(1-4)βDGlcp(1-1)Cer
GM4: NeuAcα2,3Gal-Cer
GD3: αNeu5Ac(2-8)αNeu5Ac(2-3)βDGalp(1-4)βDGlcp(1-1)Cer
GD1a: αNeu5Ac(2-3)βDGalp(1-3)βDGalNAc(1-4)[αNeu5Ac(2-3)]βDGalp(1-4)βDGlcp(1-1)Cer
GD1b: βDGalp(1-3)βDGalNAc(1-4)[αNeu5Ac(2-8)αNeu5Ac(2-3)]βDGalp(1-4)βDGlcp(1-1)Cer
GT1b: αNeu5Ac(2-3)βDGalp(1-3)βDGalNAc(1-4)[αNeu5Ac(2-8)αNeu5Ac(2-3)]βDGalp(1-4)βDGlcp(1-1)Cer
GQ1b: αNeu5Ac(2-8)αNeu5Ac(2-3)βDGalp(1-3)βDGalNAc(1-4)[αNeu5Ac(2-8)αNeu5Ac(2-)]βDGalp(1-4)βDGlcp(1-1)Cer
where
αNeu5Ac = 5-acetyl-α-neuraminic acid is the sialic acid
βDGalp = beta-D-galactopyranose
βDGlcp = beta-D-glucopyranose
βDGalNAc = N-acetyl-beta-D-galactopyranose
Cer = ceramide (general N-acylated sphingoid)

Acknowledgements

Blood serum GA analyses were conducted by Chen Chang (Department of Obstetrics and Gynecology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China).

Statement of Competing Interests

The authors declare that they have no competing interests.

List of Abbreviations

FFQ: food frequency questionnaire; GA: ganglioside; GUMP, growing up milk powder; RMB, renminbi; SD, standard deviation; TGA, total ganglioside.

Supplementary Materials

Supplementary materials are available online at …Table S1: Total ganglioside concentrations (mg/100 g) of products consumed by participants. Table S2: Daily dietary intake of gangliosides among Chinese toddlers aged 24-48 months (mg/1000 kcal). Table S3: Intake frequency and amount of food containing gangliosides among Chinese toddlers aged 24-48 months based on the food frequency questionnaire [median (25^{th}-75th)]. Table S4: Correlation between long term average food intake and serum ganglioside concentration. Table S5: Major dietary ganglioside intake (mg/day) among toddlers with different characteristics.

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Supplementary Materials

Table S1. Total ganglioside concentrations (mg/100 g) of products consumed by participants.

Food Category	Food Item	mg/100 g product									Total Gangliosides
		GM1	GM2	GM3	GM4	GD1a	GD1b	GD3	GT1b	GQ1b	
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	9.780	0.000	0.000	9.780
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	4.811	0.000	0.000	4.811
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	5.320	0.000	0.000	5.320
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	6.064	0.000	0.000	6.064
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	5.558	0.000	0.000	5.558
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	8.250	0.000	0.000	8.250
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	3.865	0.000	0.000	3.865
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	2.056	0.000	0.000	2.056
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	3.960	0.000	0.000	3.960
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	12.220	0.000	0.000	12.220
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	7.635	0.000	0.000	7.635
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	4.500	0.000	0.000	4.500
GUMP	Growing up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	5.915	0.000	0.000	5.915
GUMP	Growing-up milk powder	0.000	0.000	0.000	0.000	0.000	0.000	6.780	0.000	0.000	6.780
GUMP	Growing-up milk powder (average)	0.000	0.000	0.000	0.000	0.000	0.000	6.194	0.000	0.000	6.194
Dairy	Fresh cow’s milk	0.000	0.000	0.000	0.000	0.000	0.000	1.730	0.000	0.000	1.730
Dairy	Fresh cow’s milk	0.000	0.000	0.000	0.000	0.000	0.000	1.284	0.000	0.000	1.284
Dairy	Fresh cow’s milk	0.000	0.000	0.000	0.000	0.000	0.000	1.620	0.000	0.000	1.620

Food Category	Food Item	mg/100 g product									Total Gangliosides
		GM1	GM2	GM3	GM4	GD1a	GD1b	GD3	GT1b	GQ1b	
Dairy	Fresh cow's milk (chocolate)	0.000	0.000	0.000	0.000	0.000	0.000	1.138	0.000	0.000	1.138
Dairy	UHT milk	0.000	0.000	0.000	0.000	0.000	0.000	1.340	0.000	0.000	1.340
Dairy	Yoghurt (plain)	0.000	0.000	0.005	0.000	0.000	0.000	0.825	0.000	0.000	0.830
Dairy	Yoghurt	0.000	0.000	0.004	0.000	0.000	0.000	0.790	0.000	0.000	0.794
Dairy	Whole milk powder (standard)	0.000	0.000	0.000	0.000	0.000	0.000	11.200	0.000	0.000	11.200
Dairy	Cheddar cheese	0.000	0.000	0.015	0.000	0.000	0.000	0.810	0.000	0.000	0.825
Dairy	Ice cream	0.000	0.000	0.000	0.000	0.000	0.000	1.660	0.000	0.000	1.660
Dairy	Flavored yoghurt milk	0.000	0.000	0.000	0.000	0.000	0.000	0.298	0.000	0.000	0.298
Fish	Carp - common (*Cyprinus carpio*)	0.000	0.000	0.131	0.000	0.000	0.000	0.065	0.000	0.000	0.196
Fish	Promfret (Bramidae)	0.000	0.000	0.058	0.000	0.000	0.000	0.034	0.000	0.000	0.091
Fish	Ribbon fish	0.000	0.050	0.121	0.000	0.124	0.000	0.035	0.000	0.000	0.328
Fish	Snapper (*Chrysophrys auratus*)	0.000	0.000	0.073	0.000	0.000	0.000	0.018	0.000	0.000	0.091
Fish	Prawn/shrimp (Vannamei))	0.000	0.000	0.005	0.000	0.000	0.000	0.010	0.000	0.000	0.015
Fish	Squid (tubes)	0.000	0.000	0.735	0.000	0.000	0.000	0.018	0.000	0.000	0.753
Fish	Fishball	0.000	0.000	0.231	0.000	0.000	0.000	0.000	0.000	0.000	0.231
Fish	Cat fish (Basa - *Pangasius bocourti*)	0.000	0.000	2.184	0.000	0.000	0.000	0.058	0.000	0.000	2.242
Meats	Chicken feet	0.000	2.083	0.000	0.362	0.011	0.000	0.027	0.000	0.000	2.483
Meats	Chicken gizzard	0.000	0.000	3.737	0.000	0.031	0.000	0.167	0.000	0.000	3.935
Meats	Chicken wing	0.036	0.000	2.196	0.000	0.058	0.008	0.144	0.000	0.000	2.441
Meats	Duck breast	0.000	1.018	0.645	0.000	0.239	0.102	0.268	0.000	0.000	2.272
Meats	Duck leg	0.000	1.058	1.296	0.000	0.179	0.057	0.313	0.000	0.000	2.903
Meats	Beef blade steak (lean)	0.000	0.000	0.823	0.000	0.000	0.000	0.083	0.000	0.000	0.905
Meats	Beef rump (with fat)	0.000	2.016	0.000	0.113	0.006	0.000	0.118	0.000	0.000	2.254
Meats	Pork belly (streaky pork)	0.000	0.000	1.788	0.000	0.306	0.076	0.091	0.000	0.073	2.334
Meats	Pork rib	0.000	0.000	1.849	0.000	0.345	0.054	0.114	0.000	0.053	2.416
Meats	Pig trotters	0.000	0.000	1.781	0.000	0.268	0.097	0.090	0.000	0.101	2.337
Meats	Chinese pork sausage	0.000	0.000	0.837	0.000	0.137	0.049	0.295	0.000	0.018	1.336
Meats	Pork skin (crackling)	0.000	0.000	0.247	0.000	0.189	0.141	0.256	0.000	0.149	0.981
Meats	Pig tongue	0.265	0.000	3.253	0.000	0.497	0.043	0.041	0.040	0.000	4.139
Meats	Luncheon	0.011	0.000	0.278	0.000	0.015	0.002	0.035	0.000	0.000	0.341
Meats	Lamb rump steak	0.000	0.000	4.848	0.000	0.232	0.000	0.097	0.000	0.000	5.177
Meats	Chicken nugget	0.000	0.000	1.434	0.000	0.000	0.000	0.108	0.000	0.000	1.541
Meats	Minced beef meat (lean)	0.000	0.000	0.458	0.000	0.000	0.000	0.020	0.000	0.000	0.478
Meats	Chicken heart	0.000	0.000	2.913	0.000	0.026	0.000	0.630	0.000	0.000	3.569
Meats	Chicken thigh (with skin)	0.000	0.000	1.082	0.000	0.000	0.000	0.284	0.000	0.000	1.366
Meats	Whole chicken egg (free range)	0.000	0.000	0.057	1.018	0.000	0.000	0.001	0.000	0.000	1.076
Meats	Whole chicken egg (caged)	0.000	0.000	0.940	0.460	0.000	0.000	0.090	0.000	0.000	1.490
Meats	Chicken egg yolk (caged)	0.000	0.000	3.297	3.400	0.000	0.000	0.313	0.000	0.000	7.010
Meats	Whole quail egg	0.416	0.630	1.450	0.420	0.200	0.000	0.366	0.000	0.000	3.481
Pastry	Sponge cake	0.000	0.000	0.066	0.002	0.000	0.000	0.095	0.000	0.000	0.162
Pastry	Chiffon cake (Massimo brand)	0.000	0.000	0.570	0.147	0.000	0.000	0.285	0.000	0.000	1.003
Pastry	Pancake (milk, cream, egg) NZ	0.000	0.000	0.041	0.000	0.000	0.000	3.371	0.000	0.000	3.412
Pastry	Bread (butter scotch)	0.000	0.000	0.009	0.000	0.000	0.000	0.290	0.000	0.000	0.299
Pastry	Baby/milk biscuit	0.000	0.000	0.000	0.000	0.000	0.000	0.551	0.000	0.000	0.551
Pastry	Cream-filled biscuit	0.000	0.000	0.000	0.000	0.000	0.000	0.117	0.000	0.000	0.117
Pastry	Standard biscuit	0.000	0.000	0.014	0.000	0.000	0.000	1.054	0.000	0.000	1.069
Pastry	Water/wheat biscuit	0.000	0.000	0.000	0.000	0.000	0.000	0.207	0.000	0.000	0.207

GUMP, growing-up milk powder; UHT, ultra-high-temperature treated.

Table S2. Daily dietary intake of gangliosides among Chinese toddlers aged 24-48 months (mg/1000 kcal)

Dietary GA	Mean	SD	Maximum	Minimum	P25†	P50†	P75†
GM1	0.000	0.004	0.055	0.000	0.000	0.000	0.000
GM2	0.028	0.233	2.499	0.000	0.000	0.000	0.000
GM3	0.717	0.634	3.146	0.000	0.253	0.594	0.994
GM4	0.163	0.283	2.232	0.000	0.000	0.075	0.229
GD1a	0.047	0.061	0.398	0.000	0.000	0.027	0.071
GD1b	0.011	0.015	0.099	0.000	0.000	0.005	0.017
GD3	2.366	2.336	13.221	0.000	0.365	1.806	3.597
GT1b	0.000	0.001	0.008	0.000	0.000	0.000	0.000
GQ1b	0.009	0.013	0.095	0.000	0.000	0.004	0.014
TGA	3.341	2.408	13.221	0.000	1.670	2.889	4.480

GA, ganglioside; TGA, total gangliosides, SD, standard deviation.
† P25, P50 and P75 represents the percentiles of the dietary GA intake.

Table S3. Intake frequency and amount of food containing gangliosides among Chinese toddlers aged 24–48 months based on the food frequency questionnaire

Food Category†	Average Intake (g/day)	
	Median	IQR
Meats	70	47.14–100.00
Fish and fish products	8.17	4.00–17.14
Dairy	180	85.71–250.00

IQR, interquartile range.
† The dairy category included all dairy products and growing-up milk powders. The meats category included meat and poultry eggs. The fish and fish products category included all fish, shrimp, shellfish, and processed fish, such as fish balls etc., from sea and river.

Table S4. Correlation† between long term average food intake‡ and serum ganglioside§ concentration

Serum GA	Food Intake from FFQ					
	Meats		Fish and Fish Products		Dairy Products	
	Corr	*P*	Corr	*P*	Corr	*P*
GD1a	0.004	0.953	0.041	0.582	0.020	0.781
GD1b	0.049	0.509	–0.036	0.621	–0.039	0.597
GD3	0.023	0.755	–0.056	0.445	0.012	0.872
GM1	0.032	0.664	–0.005	0.947	0.046	0.528
GM2	0.026	0.721	–0.094	0.200	–0.058	0.430
GM3	0.021	0.776	–0.047	0.523	–0.026	0.725
GT1b	0.059	0.425	–0.033	0.656	–0.047	0.525
TGA	0.021	0.774	–0.047	0.520	–0.026	0.728

GA, ganglioside; TGA, total gangliosides; FFQ, food frequency questionnaire.
† Pearson correlation, adjusted for sex, month age, height, and weight.
‡ Average food intake was calculated as g/day from FFQ data.
§ GM4 and GQ1b are not measured in serum.

Table S5. Major dietary ganglioside intake (mg/day) among toddlers with different characteristics

Characteristics of Participants		TGA		GD3		GM3	
		Median (25th-75th)	*P*†	Median (25th-75th)	*P*†	Median (25th-75th)	*P*†
Gender	Boys	3.46 (1.36–5.80)	0.301	1.73 (0.19–4.18)	0.145	0.59 (0.32–1.28)	0.926
	Girls	3.70 (2.01–5.99)		2.64 (0.88–4.52)		0.66 (0.30–1.27)	
Delivery mode	Vaginal	3.13 (1.20–5.64)	0.205	1.73 (0.16–3.64)	0.095	0.66 (0.33–1.37)	0.701
	Cesarean	3.74 (1.77–5.99)		2.33 (0.87–4.52)		0.66 (0.30–1.20)	
Weight category	Underdevelopment	2.37 (0.65–4.10)	0.575	0.07 (0.06–0.08)	0.118	0.34 (0.24–0.44)	0.341
	Normal	3.55 (1.59–5.84)		2.00 (0.44–4.33)		0.66 (0.33–1.30)	
	Overweight	5.80 (2.21–6.93)		4.49 (1.23–6.91)		0.53 (0.10–0.95)	
Mother education	Middle school and below	3.08 (1.13–5.18)	0.515	1.48 (0.06–4.13)	0.311	0.54 (0.28–0.91)	0.611
	High school and technical secondary school	3.75 (1.38–6.81)		2.27 (0.25–5.20)		0.62 (0.33–1.39)	
	Diploma	3.42 (1.66–5.80)		1.85 (0.51–3.42)		0.75 (0.26–1.43)	
	Bachelor degree or above	4.05 (2.27–5.68)		2.96 (1.04–4.50)		0.78 (0.37–1.31)	

Characteristics of Participants		TGA		GD3		GM3	
		Median (25th-75th)	$P^{\dagger}$	Median (25th-75th)	$P^{\dagger}$	Median (25th-75th)	$P^{\dagger}$
Father education	Middle school and below	2.17 (0.76–6.93)	0.517	1.58 (0.06–5.37)	0.299	0.44 (0.14–1.00)	0.224
	High school and technical secondary school	3.64 (1.70–5.61)		1.74 (0.28–3.72)		0.76 (0.36–1.62)	
	Diploma	3.54 (1.47–5.99)		2.08 (0.29–4.55)		0.69 (0.37–1.10)	
	Bachelor degree or above	3.80 (2.27–5.89)		2.78 (1.40–4.52)		0.64 (0.25–1.18)	
Average monthly income	Less than 2000	3.32 (1.19–5.64)	0.255	1.63 (0.16–3.33)	0.153	0.54 (0.33–1.37)	0.455
	2000–	3.35 (1.55–5.80)		1.94 (0.70–4.30)		0.57 (0.25–1.28)	
	4000–	4.01 (2.10–7.98)		3.13 (0.55–5.37)		0.78 (0.43–1.33)	
	8000–	4.77 (2.21–5.72)		3.43 (1.22–4.47)		0.90 (0.66–1.18)	
Picky eater	Yes	3.92 (1.59–5.99)	0.461	2.42 (0.38–4.87)	0.400	0.54 (0.18–1.24)	0.068
	No	3.32 (1.66–5.80)		1.85 (0.66–4.13)		0.67 (0.38–1.31)	
Food allergy of participants	Yes	3.67 (1.59–6.36)	0.817	1.50 (0.44–5.12)	0.960	0.72 (0.27–1.36)	0.833
	No	3.56 (1.66–5.83)		2.14 (0.66–4.30)		0.65 (0.32–1.24)	
Food allergy of parents	Yes	3.90 (0.77–5.99)	0.640	1.58 (0.05–4.39)	0.394	0.73 (0.18–1.70)	0.440
	No	3.55 (1.66–5.83)		2.10 (0.66–4.33)		0.65 (0.32–1.20)	

TGA, total gangliosides.
† *P* was calculated by the Wilcoxon or Kolmogorov-Smirnov test among subgroup.