Original Article

Toddler neurodevelopment is associated with ganglioside intake but not serum ganglioside

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Background and Objectives: Gangliosides (GAs) are important components of neural tissue and cell membrane. This study aims to investigate the association between toddlers' neurodevelopment, dietary GA intake, and serum GA concentration. **Methods and Study Design:** A cross-sectional study was conducted in Beijing and Xuchang, Henan Province in China. 110 eligible healthy toddlers aged 24–48 months were recruited. Food frequency questionnaire (FFQ) and 24-h dietary recall were used to collect dietary information. Blood serum samples obtained from participants were used to perform GA composition analysis with high-performance liquid chromatography-mass spectrometry (HPLC–MS). The neurodevelopment level was assessed with the Gesell Developmental Scale (GDS). **Results:** Dietary ganglioside GD3, total GA, and seafood intake were identified to be associated with the gross motor developmental quotient (DQ). An inverse association was revealed between the fine motor DQ and fruit intake. No correlation was detected between serum GA concentration and DQ. **Conclusions:** Dietary GA intake but not serum GA concentration is associated with neurodevelopment. Further prospective studies are needed to probe the relationships between the recommended dietary GA intake and toddlers.

Key Words: dietary gangliosides, neurodevelopment, serum gangliosides, food intake, HPLC-MS

INTRODUCTION

Neurodevelopment including cognition, emotion, behavior, social adaption, was defined as sustaining developing processes of the brain and nervous system.[1] The function of neurodevelopment is particularly important for school performance, educational qualification, and labor quality of adolescent.[2,3] Children with impaired neurodevelopment may show signs of being unhealthy, such as poor academic function, mental illness, or lack of economic productivity.[4] Rapid growth and development occur in the brain and nervous tissue. Therefore, we should focus on various influencing factors of neurodevelopment, such as environmental, nutritional, or behavioral exposure.[5] Previous studies demonstrated that neurodevelopment could be affected if children do not get adequate nutrition.[6]

Gangliosides (GAs) are a subclass of glycosphingolipids that have sialic acid residues in the carbohydrate moiety.[7] They were discovered in brain tissue in 1884 by German physician Johannes L. W. Thudichum.[8] GAs as components of the cell membrane is found in all kinds of tissue throughout the body.[9] However, they are more concentrated in the nervous system and brain.[10] GAs are located in the outer leaflets of the cell membrane and are connected with glycan structures, which are useful to the communication function between cells.[11] Change of GA expression in the brain is highly specific to the region[12] and strongly correlated with neurodevelopmental processes such as neural tube formation, neuritogenesis, synaptogenesis, axonogenesis, and myelination.[13-15] Moreover, GAs are also proved to be abundant in the fetal and infant hippocampal region of the brain.[16]

Previous animal experiments have indicated the critical role of GAs in neurodevelopment. Mice acquired different levels of neurodevelopmental defects when selective GA synthase genes were knocked out. For example, neuronal degeneration, loss of sensory and motor functions, demyelination, axonal deterioration, behavioral abnormalities, and learning and memory loss.[17–20] Neonatal piglets' spatial learning and brain composition can be improved by GA-fortified formula compared with a con-

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trol group (normal diet with no added GA).[21] Up to now, few human trials had been reported to assess the impact of orally administered GAs on cognitive or neural development. In a large-scale human trial, 2230 children suffering from cerebral palsy were distributed randomly into two groups.[22] Infants fed a GA-enriched infant formula had higher IQ and improved hand-eye coordination than infants fed a standard infant formula.[22] Although the importance of GAs has been demonstrated, the efficacy and recommended intake have not been well studied in a normal diet. Moreover, a previous study demonstrated that increasing dietary GA intake could increase plasma GA concentration among adult.[23] Whereas another study showed no difference of GA concentration in serum or piglet brain tissue between intervention (fortified with ganglioside GD3 (Dihexose disialic acid ganglioside)) or control group (no added GD3).[24] Interestingly, rats supplemented with GA-containing milk showed improved cognitive and spatial function. However, brain GA concentration was not increased compared with a control group.[25] Based on the evidence mentioned above, we speculate that serum GA concentration as a biomarker to determine whether dietary GA intake is sufficient.

Based on the evidence mentioned above, GAs should be studied to understand its role in neurodevelopment among toddlers. Due to the limited data on dietary GA intake and serum GA concentration in infants and toddlers, there is still poor evidence about the association between neurodevelopment, dietary GA intake, and serum GA concentration. This study was conducted to investigate the association between dietary GA intake, serum GA concentration, and neurodevelopment in toddlers aged 24–48 months.

METHODS

Study design and participants

The cross-sectional study was conducted in August 2016 in Xuchang, Henan province, and October 2016in Beijing, China. All children were considered and recruited consecutively. Toddlers were recruited and examined at the Maternal and Child Health Hospital in Xuchang

(during their routine health examination) and the early learning center in Beijing. The inclusion criteria were that toddlers should be healthy and aged 24-48 months. The exclusion criteria were listed as follows: 1) any physical disabilities; 2) any infectious diseases; 3) any mental health problems; 4) any metabolic disorders. A total of 110 participants were enrolled in the study and completed the demographics/food questionnaires and the anthropometry/neurodevelopment assessment. Caregivers of all participants had agreed to the collection of blood samples. 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 consent was obtained from all legal guardians of the participants before the study.

Data collection

A validated structured questionnaire was used to collect background information, including toddler's birth date, gender, preterm, delivery mode, parental height, weight, educational background, and family income. Other information such as toddlers' and parents' food allergy and toddlers' eating behavior, including picky eater, feeding mode in the first 6 months, and initiation time for introducing supplementary food were recorded.

Toddlers' height and weight were measured by a trained investigator. Physical developmental status was classified based on z-scores calculated with WHO Anthro software (version 3.2.2, January 2011). A toddler was considered as overweight when the weight-for-height z-score was above +2. Meanwhile, A toddler was considered as underdeveloped when the three z-score (either weight-for-age, height-for-age, weight-for-height) was below -2.

Neurodevelopment evaluation

The Chinese version of the Gesell Developmental Scale (GDS) was used to assess the neurodevelopment of the participants.[26,27] The purpose of the GDS is to examine children's general developmental progress (Children younger than 6 years of age). The GDS is an individualized face-to-face test administered by individuals experienced in psychometrics. Assessment work in Xuchang was conducted by a trained doctor at the Xuchang Maternal and Child Health Hospital. Meanwhile, a trained doctor complete the assessment work at the Peking University Sixth Hospital in Beijing. The GDS is used to assess development from five perspectives: gross motor, fine motor, language, adaptive, and personal–social functions. The Developmental quotient (DQ) = (the estimated developmental age (DA) * chronological age) / 100. Children were considered to have neurodevelopmental retardation when their DQ in any specific domain was 75 or below.[27,28]

Dietary survey

Nutrient intake, such as GAs, total energy, fat, was collected using 24-h dietary recall. The food record included all food and drink consumed by the toddler on the day before the examination visit. Nutrient intake was calculated based on the food intake from the 24-h dietary recall record and the Chinese Food Composition Table (version 2009).[29] Due to a lack of GA content in the food composition table, GA testing in 64 kinds of food was carried out at Fonterra Research and Development Centre, New Zealand, using HPLC–MS as previously described.[30] A food frequency questionnaire (FFQ) was used to evaluate the dietary food habits based on 12 types of food intake frequencies and their calculated average intakes in the last month. The 24-h dietary recall and FFQ forms were completed by caregivers with the help of a trained investigator.

Serum sample collection and determination of GAs

Fasting blood was collected from 110 toddlers. serum GA levels were determined by using UPLC–MS based on a previously published method with some modifications.[30] Briefly, GAs were extracted from 200 μL of plasma using methanol/chloroform (2:1). A total of 5 μL of the final extract was injected onto the UPLC system (Acquity I Class system, Water, Milford, MA) coupled to a XEVO G2-S QTOF mass spectrometer (Water, Milford, MA) The autosampler was maintained at 4°C. Separation of

GAs 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 (Water, Milford, MA) 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 gradients were: 0–2 min, 1% B; 2–12.5 min, 1–95% B; 12.5–13 min, 95% B; 13–13.5 min, 1–95% B; 13.5–18 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 an ion source temperature set at 120°C. The cone gas and desolvation gas were set to 50and 800 l/h, respectively. The scan range of the mass spectrometer was 500-1700 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 (PA, USA) and dissolved in 90% acetonitrile to produce eight-point calibration curves (0.78-10 μg/ml). GA concentrations were calculated based on peak areas of the standards and the test samples, using the accurate mass extract for each of the individual GAs.[30]

Statistical analysis

Data analysis was conducted using SAS 9.4 statistical software (SAS Institute; Cary, NC, USA). Categorical variables were presented as frequencies and percentages. Continuous variables were presented as means ± standard deviation, or medians and quartiles depending on the normality test used.

Student's t-test or ANOVA were used to compare differences in the DQ among toddlers with different characteristics: gender, age, weight status, preterm or not, delivery mode, feeding mode in the first six months, initiation time for introducing supplementary food, parental education, food allergy of participants and their parents, and picky eating behavior. Spearman correlation analysis was used to explore the correlation between the DQ, dietary GA intake, food category intake, serum GA concentration after adjusting for gender, parental education, initiation time for introducing supplementary food, energy intake, fat intake, age, delivery mode, the preterm, and feeding mode in the first 6 months. A p value below 0.05 is considered to be statistically significant.

RESULTS

Participants' characteristics and neurodevelopment level

Demographics information for participants and their parents are provided in Table 1. The average DQs of participants in the adaptive function, gross motor, fine motor, language, and personal-social function domains were 91.2±9.7, 98.2±13.7, 100.5±10.0, 95.4±17.1, and 94.9±13.3, respectively. Based on the standard of 75, there was one toddler considered to have a gross motor developmental concern. Six toddlers were considered to have language issues. One toddler was considered to lack personal-social function.

Table 1. Background characteristics of subjects and their parents (n=110)

Characteristics	*n* (%) / mean±SD
Gender (%boys)	54 (49.1)
Age (months)	39.8±4.5
Weight status†	
Underdeveloped	2 (1.8)
Normal	103 (94.5)
Overweight	4 (3.7)
Preterm	5 (4.6)
Caesarean section	69 (62.3)
Feeding style in first 6 months	
Exclusive breast-feeding	65 (59.1)
Mixed feeding	30 (27.3)
Exclusive formula-feeding	15 (13.6)
Initiation time for introducing supplementary food (month)	6.6±1.9
Mother	
BMI	22.4±3.1
Education	
Middle school and below	20 (18.1)
High school and technical secondary school	28 (25.4)
Diploma	31 (28.1)
Bachelor degree or above	31 (28.1)
Father	
BMI	24.6±3.1
Education†	
Middle school and below	19 (17.43)
High school and technical secondary school	26 (23.85)
Diploma	36 (33.03)
Bachelor degree or above	28 (25.69)
Family	
Average monthly individual income† (RMB, yuan)	

BMI: body mass index; RMB: renminbi (Chinese currency).
†Missing value: weight status (n=109), father's education (n=109), average monthly income (n=109).

Related factors of neurodevelopment level

Table 2 shows the association between the DQ and the characteristics of the participants. Girls had a higher fine motor DQ than boys in the present survey ($p<0.05$). Toddlers who were given supplementary food after 6 months had a higher language DQ compared with those before 6 months. Higher parental education was also correlated to the higher DQ for the toddlers. The details of the associations are presented in Table 2.

Association between neurodevelopment and dietary intake

The consumption of dietary individual GAs was very low, except for GD3 (2.02±3.21 mg/day), GM3 (0.94±0.85 mg/day), and total GA intake (4.35±3.53 mg/day) (Supplementary Table S1). Associations between the DQ, dietary GD3, GM3, and total GA intake are analyzed (Table 3). Positive associations could be retrieved between the gross motor DQ and dietary GD3 and total GA intake.

The average intakes for food categories, associations between the DQ, and the food category intakes are summarized in Table 4. An inverse association was calculated between the fine motor DQ and fruit intake. However, a positive association was found between the gross motor DQ and seafood intake.

Association between neurodevelopment and serum GA concentration

The average total serum GA concentration was 14.49±5.23μg/L. GD3 (0.50±0.07 μg/L) and GM3 (13.90±5.1 μg/L) were the two predominant individual GAs (Supplementary Table S2 and Supplementary Table

Table 2. DQ among toddlers with different characteristics

Characteristics	Adaptive	Gross motor	Fine motor	Language	Personal–social
Gender					
Boy	89.5±9.5	96.4±11.0	98.2±9.7*	93.4±16.6	92.7±10.8
Girl	92.9±9.7	100.0±15.8	102.7±9.7*	97.3±17.4	97.0±15.0
Age (months)					
>24	89.0±6.4	99.5±10.8	99.6±7.9	90.8±10.5	92.4±9.5
>36	91.6±10.2	98.0±14.2	100.7±10.3	96.2±17.9	95.3±13.8
Weight status					
Underdeveloped	86.5±4.9	87.0±1.4	103.0±9.9	81.5±7.8	85.5±0.7
Normal	91.5±9.9	98.4±14.1	100.7±10.0	95.7±17.5	95.1±13.6
Overweight	87.0±4.2	98.5±4.1	98.3±7.7	95.0±6.9	93.3±2.2
Preterm					
Yes	89.2±8.5	90.6±9.0	98.8±14.3	88.0±7.8	87.2±4.7
No	91.3±9.8	98.6±13.8	100.6±9.8	95.7±17.3	95.2±13.4
Delivery mode					
Vaginal	91.5±8.9	99.0±11.8	102.0±10.5	96.2±17.5	95.1±12.3
Caesarean	91.1±10.2	97.8±14.8	99.6±9.6	94.9±16.9	94.7±13.9
Feeding mode					
Exclusive breast-feeding	91.6±8.5	98.6±14.5	102.2±9.6	95.1±15.0	94.4±13.4
Mixed feeding	90.7±12.0	100.1±14.1	99.0±10.3	97.6±22.2	96.6±14.7
Exclusive formula-feeding	90.6±10.3	92.9±7.6	96.3±9.5	92.2±14.2	93.7±9.4
Initiation time for introducing supplementary food (months)					
>4	88.9±8.4	95.9±9.1	99.3±9.3	89.9±9.8*	90.9±9.2
>6	91.7±10.0	98.7±14.6	100.8±10.2	96.6±18.2*	95.7±13.9
Mother's education					
Middle school and below	86.3±5.1*	92.7±4.1	98.7±8.9	85.4±6.8*	88.4±4.1*
High school and technical secondary school	90.3±8.9*	96.3±9.6	99.1±9.2	91.4±14.1*	91.9±8.7*
Diploma	92.1±11.2*	102.6±21.2	99.5±9.6	98.2±20.2*	98.8±18.4*
Bachelor degree or above	94.4±10.2*	99.2±9.9	104.0±11.1	102.7±17.3*	97.9±12.6*
Father's education					
Middle school and below	86.6±5.8	91.9±4.5*	98.5±9.6	85.8±9.0*	87.9±4.7
High school and technical secondary school	90.0±9.6	96.3±9.9*	100.0±9.1	93.3±16.4*	95.3±11.8
Diploma	92.3±10.3	103.1±19.4*	101.5±10.2	98.8±19.1*	97.6±17.7
Bachelor degree or above	94.2±10.5	98.2±10.2*	100.9±11.0	99.8±17.1*	95.8±10.6
Picky eater					
Yes	91.2±10.2	96.6±13.1	100.5±10.8	94.1±18.9	92.5±11.6
No	91.4±9.5	99.5±14.2	100.4±9.4	96.7±15.6	96.8±14.2
Food allergy					
Yes	91.8±12.7	100.2±15.0	98.8±11.4	101.8±20.0	95.2±11.7
No	91.2±9.3	98.0±13.6	100.8±9.8	94.5±16.6	94.8±13.5
Parental food allergy					
Yes	95.2±13.9	96.0±19.0	101.2±12.2	98.5±25.9	93.1±15.8
No	90.8±9.1	98.5±13.1	100.5±9.7	95.1±16.0	95.1±13.0

DQ: Developmental quotient.

*$p<0.05$ with Student's t-test or ANOVA.

Table 3. Association between major dietary ganglioside intake (mg/day) and DQ among participants†

Dietary GA	Adaptive	Gross motor	Fine motor	Language	Personal-social
GD3					
Corr	0.025	0.280	0.031	0.036	0.077
p	0.814	0.007	0.768	0.733	0.463
GM3					
Corr	0.109	0.039	0.027	0.020	–0.014
p	0.299	0.713	0.795	0.849	0.897
TGA					
Corr	0.071	0.231	–0.036	–0.027	–0.034
p	0.497	0.026	0.731	0.799	0.745

DQ: Developmental quotient; GA: ganglioside; GD3: Dihexose disialic acid ganglioside; Corr: correlation coefficient; TGA: total ganglioside.†Spearman's correlation, adjusted for gender, parents' education, initiation time for introducing supplementary food (months), energy intake (kcal/day), fat intake (g/day), age (months), delivery mode, preterm, feeding mode in first 6 months.

Table 4. Association between food category intake (g/day) and developmental quotient (DQ) among subjects†

Food category	Average intake		Adaptive	Gross motor	Fine motor	Language	Personal–social
Grain	134.2±65.0	Corr	0.025	–0.062	0.010	0.109	–0.125
		p	0.819	0.563	0.924	0.306	0.241
Starchy food	16.7±22.1	Corr	0.032	0.096	0.140	0.132	0.152
		p	0.768	0.369	0.188	0.214	0.154
Vegetable	77.2±87.3	Corr	–0.046	0.124	–0.161	0.121	0.192
		p	0.665	0.246	0.131	0.256	0.070
Fruit	171.8±166.8	Corr	–0.149	–0.086	–0.232	–0.058	0.016
		p	0.161	0.418	0.028	0.589	0.883
Meat	35.1±52.7	Corr	0.105	–0.021	0.088	0.070	–0.029
		p	0.323	0.843	0.411	0.515	0.785
Seafood	8.0±12.3	Corr	0.185	0.210	0.030	0.135	0.061
		p	0.080	0.047	0.781	0.205	0.568
Freshwater products	6.9±12.9	Corr	0.040	0.127	0.103	0.003	0.060
		p	0.705	0.231	0.336	0.978	0.572
Eggs	45.2±27.0	Corr	–0.006	–0.057	–0.174	–0.105	–0.065
		p	0.957	0.591	0.102	0.324	0.544
Dairy products	208.8±206.0	Corr	0.040	0.107	0.011	–0.066	–0.118
		p	0.706	0.317	0.917	0.539	0.268
Soybean products	22.1±37.8	Corr	–0.060	0.072	0.121	–0.068	–0.063
		p	0.573	0.502	0.256	0.524	0.555
Nuts	6.7±11.3	Corr	–0.173	–0.080	–0.174	–0.132	–0.058
		p	0.104	0.453	0.101	0.216	0.587
Beverages	47.2±86.8	Corr	0.081	–0.131	0.081	0.057	0.047
		p	0.449	0.217	0.447	0.592	0.660

DQ: Developmental quotient;Corr: correlation coefficient.
†Spearman's correlation, adjusted for gender, parents' education, initiation time for introducing supplementary food (months), energy intake (kcal/day), fat intake (g/day), age (months), delivery mode, preterm, feeding mode in first 6 months.

S3). Associations between the DQ and serum GD3, GM3, and total GA concentration are analyzed (Table 5). No association could be calculated between the DQ and serum GA concentration.

DISCUSSION

Neurodevelopment is an important part of toddlers' development and has critical effects on labor performance in the future.[31] The long-term process of neurodevelopment from conception to adulthood is influenced by many factors including genetics and environment.[32] Up to now, nutrition is coming to be known as the primary concern because it is the material basis of growth and development.[33] In this study, our results suggested that dietary GD3, total GA and seafood intakes are positively associated with the gross motor DQ. No association was calculated between serum GA concentration and DQ.

GAs are important components of neuronal cells and play a critical role in the brain and neurodevelopment, especially in early life.[13,17,18] Previous studies focus on putative compositional physiology, animal experiments, and human intervention trials. Such studies explore the functions of GA functions or mechanisms with limited population relevance. Due to the diversity and the quantitative method development of the sphingolipid family,[17,34-35] there have been few large-scale population surveys on dietary GA intake and their effects on health outcomes. To the best of our knowledge, the present study is the first study to assess the association between dietary GA intake and neurodevelopment among toddlers aged 24-48 months. The previous study has suggested that the toddlers started weaning at the age of 24-48 months.[36,37] The transition of infant nutrition in the weaning period was marked by the food intake and its composition.[38] The

Table 5. Association serum GA concentration (μg/ml) and DQ among subjects †

Dietary GA	Adaptive	Gross motor	Fine motor	Language	Personal-social
GD3					
Corr	0.025	0.280	0.031	0.036	0.077
p	0.814	0.007	0.768	0.733	0.463
GM3					
Corr	0.109	0.039	0.027	0.020	–0.014
p	0.299	0.713	0.795	0.849	0.897
TGA					
Corr	0.071	0.231	–0.036	–0.027	–0.034
p	0.497	0.026	0.731	0.799	0.745

GA: ganglioside; DQ: Developmental quotient; Corr: correlation coefficient; TGA: total ganglioside.
† Spearman's correlation, adjusted for gender, parents' education, initiation time for introducing supplementary food (months), energy intake (kcal/day), fat intake (g/day), age (months), delivery mode, preterm, feeding mode in first 6 months.

nutrient pattern was changed from a high-fat to a high-carbohydrate diet.[39] Meanwhile, lipid metabolism was changed from relying heavily on breast milk to a combination of diet source and synthesis by the infant.[38] However, there was still no recommended GA intake for toddlers. Therefore, whether a low dietary GA intake is associated with delay of neurodevelopment is still unknown. In this study, positive correlations could be calculated between gross motor DQ score, dietary GD3, and total dietary GA intake, indicating that the dietary GA intake after weaning might contribute to neurodevelopment. Prospective studies on the effects of daily dietary GA intake on toddlers' neurodevelopment are needed to ascertain the appropriate daily intake amount. Moreover, previous studies have suggested that fish consumption was beneficial to neurodevelopment.[40-42] They had focused on the seafood intake of pregnant women, their offspring's neurodevelopment, and their relationships.[41,43,44] Most of those studies suggested that an improvement in children's neurodevelopment with higher maternal fish intake but not other seafood.[42,45] For example, Schmiedel et al revealed that fish consumption could explain 4% of the variance by regression analysis.[40] Fish is frequently recommended for toddlers because it is an important source of long-chain fatty acids, which are thought to be beneficial to the neurodevelopment.[46] It is worth noting that fish is also an important source of dietary GAs.30 In the present study, a positive correlation was detected between seafood intake and the gross motor DQ in 24–48-month-old toddlers. Moreover, a negative correlation was identified between fruit intake and fine motor DQ. The mechanism and metabolic pathway for the association between dietary GA intake and early neurodevelopment are still not clear. Serum concentration level has been used as the nutrition indicator to determine whether they are sufficient to supply to the body.[47-49] In a pilot study, the researchers speculated the correlation between cognitive development and serum GA concentration.[50] They suggested that serum GA concentration was not associated with the DQ. Moreover, serum concentration may not be a favorable indicator of GA storage in the body. Dietary GAs was speculated to be absorbed and broken down into smaller molecules used to synthesize new GAs in the neural tissue or membrane structure.[51] Based on the evidence obtained in the current and previous studies, we speculated a conceptual diagram that conveys the relevant nutritional biology (Figure 1). To the best of our knowledge, this study is the first to explore the relationship between serum GA concentration and neurodevelopment among toddlers in China.

There are some strengths in our study: this is the first study to explore the relationship between neurodevelopment, dietary GA intake, and serum GA concentration in the toddler population. The results obtained in the present

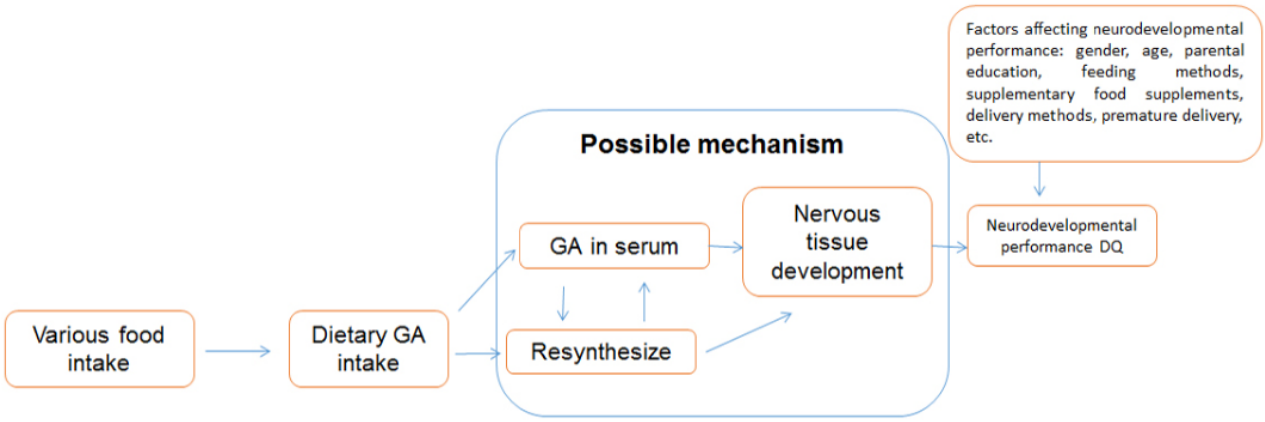

Figure 1. The intake of food in the diet determines the intake of dietary GA. 2. This study found that dietary GA intake is associated with neurodevelopmental performance DQ, but the specific mechanism is unclear. 3. Serum GA content has not been found to be related to neurodevelopmental performance and may be affected by various factors. For example: GA may be directly absorbed into blood, or it may be directly supplied to nerve tissue through decomposition and re-synthesis. The specific mechanism needs to be studied. 4. There are many factors that affect the realization of neurodevelopment. This study collected a large number of factors and controlled them in the statistical analysis. GA: Ganglioside; DQ: Developmental quotient.

study provides the basic data to develop recommended dietary GA intakes. However, there are several general limitations in this study: 1) We had only provided a snapshot of the dietary intake and hints at possible causal associations. 2) We only used 24-h dietary recall to estimate the daily GA intake. A seven-day dietary recall is more appropriate to reflect daily GA intake status. 3) docosahexaenoic acid (DHA) as a potential confounder cannot be calculated based on the current Chinese Food Composition Table.

Conclusion

Dietary GD3, total GA and seafood intakes are positively associated with the gross motor DQ. No association was calculated between serum GA concentration and DQ. Further prospective studies are needed to study the effect of the dietary GA intake on neurodevelopment in toddlers. Meanwhile, randomized controlled trials are needed to verify causality between GA intake on neurodevelopment in toddlers.

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AUTHOR DISCLOSURES

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Supplementary table 1. Average intake of dietary gangliosides (mg/day) among participants

Dietary gangliosides†	Mean±SD	Median (P25–P75)‡
GM1	0.00±0.00	0.00 (0.00–0.00)
GM2	0.04±0.31	0.00 (0.00–0.00)
GM3	0.94±0.85	0.72 (0.37–1.29)
GM4	0.24±0.44	0.09 (0.00–0.32)
GD1a	0.06±0.07	0.03 (0.00–0.09)
GD1b	0.01±0.02	0.01 (0.00–0.02)
GD3	2.02±3.21	0.43 (0.06–2.92)
GT1b	0.00±0.00	0.00 (0.00~0.00)
GQ1b	0.01±0.01	0.01 (0.00–0.02)
TGA	4.35±3.53	3.37 (1.74–5.99)

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
†TGA: total ganglioside.
‡P25 and P75 represent percentiles of dietary ganglioside intake.

Supplementary table 2. Average concentration (μg/ml) of serum gangliosides among participants

Serum gangliosides†	Mean±SD	Median (P25–P75)‡
GD1a	0.02±0.00	0.02 (0.02–0.02)
GD1b	0.02±0.01	0.02 (0.01–0.02)
GD3	0.50±0.07	0.49 (0.45–0.52)
GM1	0.01±0.00	0.01 (0.01–0.01)
GM2	0.04±0.00	0.04 (0.04–0.04)
GM3	13.90±5.17	13.35 (10.88–15.26)
GT1b	0.01±0.00	0.01 (0.01–0.01)
TGA	14.49±5.23	13.93 (11.42–15.86)

†TGA: total ganglioside.
‡P25 and P75 represent percentiles of serum ganglioside concentration.