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Association of complex lipids containing gangliosides with cognitive development of 6-month-old infants

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ABSTRACT

Background: Human breastmilk contains gangliosides which may play an important role in infant neurodevelopment.
Aim: A pilot study was conducted to assess the impact of infant formula supplemented with gangliosides from complex milk lipid on cognitive functions of normal healthy infants.
Study design: The study was a double-blind, randomized, controlled, parallel group clinical trial in which infants received the treatment or control product from 2 to 8 weeks of age until 24 weeks of age. The control group (n = 30) received standard infant formula and the treatment group (n = 29) received the same formula supplemented with complex milk lipid to increase the ganglioside content to approximately 11 to 12 μg/ml. A reference group (n = 32) consisted of normal healthy exclusively breast-fed infants.
Outcome measures: Cognitive development using the Griffith Scales and serum gangliosides was measured before (2–8 weeks of age) and after intervention (24 weeks of age).
Results: Ganglioside supplementation using complex milk lipids significantly increased ganglioside serum levels (control group vs treatment group, P = 0.002) and resulted in increased scores for Hand and Eye coordination IQ (P < 0.006), Performance IQ (P < 0.001) and General IQ (P = 0.041). Cognitive development scores and serum ganglioside levels for the treatment group did not differ from the reference group.
Conclusions: Supplementation of infant formula with complex milk lipid to enhance ganglioside content appears to have beneficial effects on cognitive development in healthy infants aged 0–6 months, which may be related to increased serum ganglioside levels.

1. Introduction

Gangliosides are complex glycosphingolipids which make up approximately 10% of the total mass of brain lipids and contain negatively charged sialic acids [1]. Gangliosides play a role in the formation of synapses between neural cells and also their functioning during the process of neural transmission by facilitating transmitter molecule binding to synaptic membranes [2]. They also contribute to neural growth, modulate neural functions and are involved in neuritogenesis, information storage and the process of memory formation. Gangliosides are also presumed to act as substrates for neural layer formation which generates higher cognitive functions in the brain [3]. Brain growth and maturation is associated with an increase in ganglioside levels, with accretion being highest in the pre-natal and early post-natal periods [1].

Guidelines for infant feeding in Indonesia follow UNICEF/WHO recommendations which endorse exclusive breastfeeding until the age of 6 months and where this is not possible, the use of standard infant formula. Exclusive breastfeeding until the age of 6 months is thought to result in a child's optimum cognitive development [4–7]. It has been presumed that this is due to optimal mother–child interactions, and perhaps higher docosahexanoic acid (DHA) levels in breastmilk. However, other nutrients present in breastmilk may also contribute to cognitive development, in particular milk fat membrane lipid components such as gangliosides. Human breastmilk contains gangliosides at significantly higher levels than cow's milk based infant formula products [1]. Further, it has been shown that the brains of infants who were breastfed contained higher levels of gangliosides than the brains of babies who were fed standard infant formula [8]. Given the role of gangliosides in brain development and function, this raises the possibility that ganglioside intake may contribute to an infant's cognitive development and memory. To date, studies investigating the correlation between the level of dietary ganglioside intake and cognitive functions have only been undertaken in animal models [9,10]. Only one study in humans has investigated the benefits of ganglioside-supplemented feeding but this was with

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premature infants and only to identify the microbe composition in feces [11]. Therefore, the aim of the current study was to investigate the effects of ganglioside supplementation into infant formula using a complex milk lipid ingredient, on the cognitive development functions of infants aged 0–6 months old, which is a critical period of brain growth and development. Supplementation was designed to bring the ganglioside concentration in the infant formula into line with the average value for breastmilk. Although the content and distribution of gangliosides in human breastmilk vary with lactation, the predominant ganglioside in early lactation is GD3 (a disialylated ganglioside) while GM3 becomes more predominant in later lactation. GD3 is also the predominant species in cow's milk [1].

2. Materials and methods

2.1. Study design

The study was a double-blind, randomized, controlled, parallel group clinical trial with the primary objective of determining the impact of infant formula supplemented with gangliosides from complex milk lipid on cognitive functions of normal healthy infants who received the product from 2 to 8 weeks of age until 24 weeks of age. A secondary measure was to determine whether supplementation influenced serum ganglioside levels. A reference group of exclusively breast-fed infants was also included in the study [12]. The trial was carried out from May 2008 through February 2009 in two different areas in Bandung, West Java, Indonesia, and centralized at two different public health centers: Garuda public health center, in the western part of Bandung, and Bojong Soang public health center, in the South East. Ethical approval was obtained from the Ethics Committee for Health Research at the Faculty of Medicine, Padjadjaran University/Hasan Sadikin Hospital, Bandung. Signed informed consent was required from parents.

2.2. Study subjects

Infants were screened from birth up until 8 weeks of age from the areas of Garuda and Bojong Soang public health centers, Bandung, West Java, Indonesia. Subjects were selected using consecutive admission; all were apparently healthy term babies, singleton births, with birth weight ≥2.5 kg, and had no asphyxia. Infants were excluded if at screening they were identified to have a congenital anomaly such as congenital heart disease, problems with hearing and eyesight, and if they had not been exclusively breastfed. Infants were dropped out of the trial if they were subsequently found to have received non-compliant feeding during intervention, or to have moved to an unidentified address.

At the time when the mothers independently decided to cease breastfeeding and introduce infant formula, the infants were randomly assigned to receive either a standard infant formula (control group) or the supplemented infant formula (treatment group) containing added complex milk lipids to enhance the levels of milk gangliosides (true experimental study). A reference group was formed of infants who continued to be exclusively breastfed (non-randomized). Randomization was by use of random permutation blocks stratified for each site.

$$n = \frac{2\sigma^2(Z\alpha+Z\beta)^2}{\Delta^2}$$

n = Subject number
Δ = Difference of means
σ = Pooled standard deviation
$Z\alpha$ and $Z\beta$derived from standard normal distribution, for confidence intervals (CI) 95% ($Z\alpha = 1, 96$) and Power of test 80% ($Z\beta = 0, 84$).

2.3. Study interventions

The control group received a standard infant formula while the treatment group received the same infant formula with added complex milk lipid to increase the ganglioside GD3 content by approximately 2–3 mg/100 g (AnmumInfacare, Fonterra Co-operative Group Auckland, New Zealand). The complex milk lipid ingredient is a natural milk derived component that is acceptable for use in infant formula, and can be added at a level to give total ganglioside content within the range of ganglioside content in human breastmilk. Both study products were similar in composition (Table 1) except for increased arachidonic acid, phospholipid and ganglioside contents in the Complex Milk Lipid-supplemented formula. The amount of formula feeding was measured through daily feeding records, which were verified and collected weekly by trained field assistants. Data collection for breastmilk consumption was conducted twice a week by the field assistants, using a *24-Hour Dietary Recall method*. These data were later verified by a nutritionist and analyzed to estimate the amount of nutrients consumed by the infants during the research using *Nutri Survey for Window*, designed by Universitas Indonesia Seameo-Tropmed in 2005. The two product samples were tested using standardized nutrient composition testing by accredited laboratories (Analytical Services Group Laboratory, Fonterra Research Centre, Palmerston North, New Zealand; Asure Quality, Auckland, New Zealand).

2.4. Data collection

Information was collected for each subject (birth weight, length and head circumference, gestation in completed weeks, APGAR score, number of live-birth infants in pregnancy, birth order of child, race, feeding history at study entry-breast-fed or formula-fed, duration of exclusive human milk or formula feeding, type of formula used), and also for the parent's characteristics (education, occupation) and socioeconomic status.

2.5. Physical examination

A physical examination was performed on the subjects. Infant weight, length and head circumference were measured at baseline (age 2–8 weeks) and at monthly intervals throughout the intervention period (until the age of 6 months). These measurements were used to calculate indices of height-for-age, weight-for age, weight-for-height,

Table 1
Typical composition of infant formula study products (per 100 g)[a].

	Complex lipid-supplemented formula	Standard formula
Energy (kJ)	2130	2130
Energy (kcal)	510	510
Protein (g)	13.1	13.1
Whey protein (g)	8	8
Carbohydrate (g)	55.1	54.8
Sialic acid (mg)	261	261
Fat (g)	26.3	26.3
Linoleic acid (g)	3.0 (12%FA)[b]	3.0 (12%FA)[b]
α-Linolenic acid (g)	0.3 (1.2%FA)[b]	0.3 (1.2%FA)[b]
Docosahexaenoic acid (g)	0.04 (0.2%FA)[b]	0.05 (0.2%FA)[b]
Iron (mg)	8.4	8.2
Choline (mg)	130	128
Arachidonic Acid (g)	0.06 (0.24% FA)[b]	0.005 (0.02%FA)[b]
Phospholipids (mg)	235	220
Gangliosides[c] (mg)	9	6

Note.
[a] Variation between replicates ± 5–10%
[b] FA = total fatty acids
[c] Measured as GD3.

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head circumference and plotted into reference Z-score tables based on WHO Child Growth standard using *WHO Anthro 2007 Version*. Head circumference was measured using *Feca 212 baby band* and plotted on the new *CDC 2000 percentile curves* for the head circumference.

2.6. Growth status

Nutritional status was assessed using anthropometric of height-for-age, weight-for age and weight-for-height indices; Z-scores were calculated with the EPINUT program in Epi Info (version 3.3, 2004). Stunted was defined as a height-for-age<-2 (Z-score); underweight is defined as a weight-for age<-2 (Z-score); and wasted was defined as a weight-for-height<-2 (Z-score), on the basis of *WHO Child Growth Standards 2007 Version*. We defined microcephaly as a head circumference <3 percentile and macrocephaly as a head circumference >97 percentile.

2.7. Biochemical analyses

Hemoglobin (Hb), Ferritin, and Total Iron Binding Capacity (TIBC) were analyzed at baseline (age 2–8 weeks) by the Clinical Diagnostic Laboratory, Hasan Sadikin Hospital, Bandung, Indonesia. Gangliosides GD3 and GM3 in the serum were analyzed at Fonterra Research Centre, Palmerson North, New Zealand, using liquid chromatography-high resolution mass spectrometry [13]. Arachidonic acid in serum was determined in the upper phase of the extracted serums[13] measured by Asure Quality in Auckland, New Zealand using Gas Chromatography [14].

2.8. Cognitive assessment

The Griffiths Mental Development Scale (GMDS) was used to assess cognitive development before (at baseline, mean age 3.79± 1.85 weeks, range 2–8 weeks) and after intervention (at 24 weeks), and was administered by a pediatric neurologist certified to administer the Griffith assessment in Santosa Bandung Hospital International. The neurologist was blinded to study group. The GMDS covers five different scales: Locomotor, Personal–Social, Hearing and Speech, Eye and Hand Coordination and Performance which appear in a form of numeric scales. A General IQ (Total Scale) is produced from these five scores. This is a published and validated screening tool for assessing multiple aspects of cognitive development.

2.9. Study size

Determination of sample size was based on a study by Novita [15] in 2007 on comparison of values of the five Griffith scales between infants receiving exclusive breastfeeding and those receiving non-exclusive breastfeeding. Based on this data the pooled within subject standard deviation for the different scales of the Griffith Mental Development test was estimated to be between 3.5 and 15. Novita observed differences of 11 to 14; hence to detect a difference of 11 with a standard deviation of 14.5, a power of 80% and a significance of 5%, 28 subjects per group were required. The study size was increased to 35 subjects per group to allow for a drop-out rate of up to 20%.

2.10. Statistical analysis

Statistical analysis included all subjects in the control and treatment groups. Results for the breast-fed group are included as a reference group only. Ganglioside data and baseline cognitive scores were analyzed using 2-sample *t*-test. Cognitive scores at 6 months of age were analyzed for the effect of treatment after adjusting for age, Hb, and TIBC at baseline, as well as socioeconomic factors such as Family Size, Mother's Education and Occupation, and Father's Occupation using analysis of variance (SAS 6.1). Data was transformed, if required, to achieve homogeneity of variance. Results are presented as least-squares means and 95% confidence intervals. Categorical data was analyzed using Pearson's chi-squared test (Minitab 15.1) and results are presented as counts and percentages. Statistical significance is declared if $P<0.05$.

3. Results

From 133 infants registered, 110 met the inclusion criteria and were included in the study (Fig. 1). Infants whose mothers chose not to provide breast milk were randomly assigned to either the control group (standard infant formula, n=35) or the treatment group (ganglioside-supplemented infant formula, n=35). Forty infants who continued to be exclusively breastfed constituted a reference group. A total of 19 babies were dropped out during the trial. These included five babies in the control group: one infant was withdrawn due to consuming complementary feeding (weaning solids) (months 3–4), two infants were withdrawn due to consuming different infant formula while being hospitalized for suffering from acute upper-respiratory-tract infection (URTI-A) (months 4–5), and two infants were withdrawn due to consuming different infant formula and complementary feeding (months 5–6); six babies in the treatment group: one infant was withdrawn due to consuming different infant formula while being hospitalized for suffering from URTI-A (months 3–4), three infants were withdrawn due to consuming complementary feeding (months 4–5), and two infants were withdrawn due to consuming different infant formula and complementary feeding (months 5–6); eight babies in the reference group: one infant was withdrawn due to consuming infant formula (months 3–4), five infants were withdrawn due to consuming infant formula and complementary feeding (months 4–5), and two infants were withdrawn due to consuming infant formula and complementary feeding while being hospitalized for suffering from URTI-A (months 5–6) (see also Fig. 1).

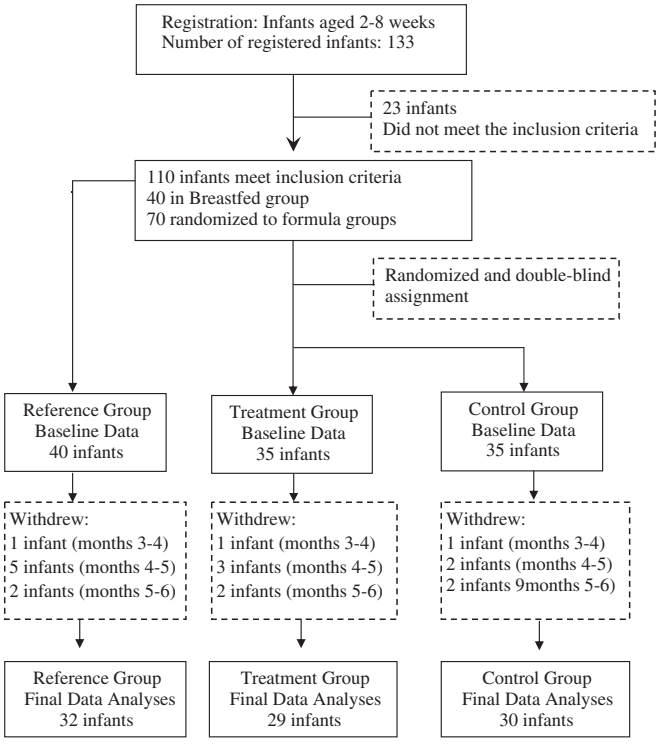

Fig. 1. Flow chart of participant registration, randomization and follow-up.

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There were no significant differences between the control and supplemented intervention groups in baseline anthropometric measures, iron status (Hb, ferritin and TIBC) and familial socioeconomic parameters (Table 2). Similarly, the levels of the major classes of serum gangliosides, GM3 and GD3, were not significantly different between control and the treatment groups at baseline. There were no significant differences between the control and the treatment groups in individual cognitive development scores and total score (General IQ) at baseline (i.e. age 2–8 weeks of age) (Table 3). The number of infants with reported minor illness such as fever and cough did not differ significantly between the control and the treatment groups throughout the trial (data not shown) and there were no instances of diarrhea, allergy, vomiting or colic in either of the groups. Infant growth was measured monthly up to the age of six months using anthropometric assessment with length-for-age, weight-for-age, weight-for-length indices, and head circumference. There were no significant differences between the control and supplemented group with respect to length-for-age (Z-score)/ZSc-L/A, weight-for-age (Z-Score)/ZSc-W/A, weight-for-length (Z-Score)/ZSc-W/L, and head circumference measures ($P > 0.05$) (Fig. 2).

Cognitive development scores at 6 months of age for the control and treatment groups and additionally the reference group are shown in Table 4. To account for any potentially confounding variable such as socioeconomic factors, data for the control and treatment groups were adjusted accordingly using analysis of variance. No significant difference was found between the control and treatment groups for the Griffith Locomotor, Personal–Social, and Hearing and Speech scores. However, there was a significant increase in scores for Hand and Eye Coordination and Performance and also for Total Score (General IQ) in the treatment group. Notably, all scores in this group were similar to those of the reference group ($P > 0.05$; 2 sample *t*-test using both adjusted and unadjusted scores for the treatment group).

There was a significant impact of the supplemented infant formula on serum ganglioside levels at 6 months (Table 5). The levels of serum GM3, GD3 and total ganglioside (GM3 + GD3) were all significantly increased in the treatment compared to the control group ($P < 0.01$). Additionally, the serum ganglioside levels in the treatment group were not significantly different from the reference group at 6 months of age. Interestingly, the ratio of GM3 to GD3 was increased over baseline in all groups and there was no significant difference between the control and treatment groups either at baseline or at 6 months of age.

The amount of arachidonic acid in the extracted serum samples was not significantly different between the 2 formula-fed groups (data not shown).

4. Discussion

Cognitive development of infants in this study was measured with Griffith instruments, which test individual components of development.

Table 2
Baseline characteristics of Infants in treatment group, control group and reference group. Values for categorical data are counts and percentages; all other values are means and 95% confidence intervals.

	P-value	Supplemented formula (treatment group) (n = 35)	Standard formula (control group) (n = 35)	Breast-fed (reference group) (40)
Birth weight (kg)	0.529	3.09 (2.96–3.22)	3.14 (3.02–3.26)	3.11 (3.00–3.22)
Weight-for-age (Z-score) (ZSc-WA) at birth	0.605	−0.471 (−0.753–0.190)	−0.373 (−0.637–0.109)	−0.389 (−0.643–0.135)
Weight-for-length (Z-score) (ZSc-WL) at baseline (2–8 weeks)	0.602	−0.242 (−0.355–0.129)	−0.281 (−0.382–0.180)	−0.252 (−0.311–0.192)
Birth sequence, n (%)				
First child	0.348	10 (28.6)	14 (40)	13 (32.5)
Second child		14 (40.0)	16 (45.7)	16 (40.0)
Third child		6 (17.1)	2 (5.7)	5 (12.5)
>Third child		5 (14.3)	3 (8.6)	6 (15)
Biochemistry				
Hb (g/dL)	0.304	11.7 (11.2–12.1)	12.0 (11.5–12.4)	11.9 (11.5–12.4)
Ferritin (ng/mL)	0.363	368.9 (297.3–440.4)	328.5 (256.9–400.1)	371.0 (325.4–416.7)
TIBC (μg/dL)	0.885	276.5 (259.1–293.8)	280.2 (252.3–308.1)	273.9 (250.5–297.2)
GM3 (μg/mL)	0.463	8.106 (7.335–8.877)	7.703 (6.932–8.474)	7.996 (7.355–8.637)
GD3 (μg/mL)	0.518	2.531 (2.140–2.922)	2.351 (1.960–2.742)	2.477 (2.076–2.878)
GM3 + GD3 (μg/mL)	0.405	10.637 (9.655–11.619)	10.054 (9.072–11.036)	10.473 (9.538–11.408)
GM3/GD3	0.619	3.545 (3.124–3.967)	3.695 (3.273–4.117)	3.658 (3.220–4.096)
Socioeconomic factors, n (%)				
Father education (length of education)				
≤9 years (junior high school)	0.630[a]	20 (57.1)	18 (51.4)	20 (50.0)
>9 years (senior high school and scholar)		15 (42.9)	17 (48.6)	20 (50.0)
Mother education (length of education)				
≤9 years (junior high school)	0.803[a]	22 (62.9)	23 (65.7)	0 (50.0)
>9 years (senior high school and scholar)		13 (37.1)	12 (34.3)	20 (50.0)
Father's occupation				
Working	0.303[a]	32 (91.4)	34 (97.1)	38 (95.0)
Unemployed		3 (8.6)	1 (2.9)	2 (5.0)
Mother's occupation				
Working	0.629[a]	19 (54.3)	21 (60)	0 (0.0)
Unemployed		16 (45.7)	14 (40)	40 (100.0)
Number of family members				
≤4	0.806[a]	14 (40)	13 (37.1)	19 (47.5)
>4		21 (60)	22 (62.9)	21 (52.5)
Parent's salary/month				
Low	0.398[a]	13 (37.1)	11 (31.4)	21 (52.5)
Medium		16 (45.7)	21 (60.0)	16 (40.0)
High		6 (17.1)	3 (8.6)	3 (7.5)

[a] Pearson chi-square test; all others are *t*-test.

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Table 3
Baseline Griffith scales of infants in treatment group, control group and reference group. Values are means and 95% confidence intervals; analysis by 2-sample *t*-test.

Griffith scale	P-value	Supplemented formula (treatment group) (n = 35)	Standard formula (control group) (n = 35)	Breast-fed (reference group) (n = 40)
Locomotor IQ	0.219	99.3 (96.3–102.3)	96.7 (93.6–99.7)	102.1 (98.9–105.3)
Personal–social IQ	0.409	107.0 (103.7–110.4)	105.1 (101.7–108.4)	114.8 (110.1–119.4)
Hearing and speech IQ	0.485	108.2 (105.1–111.3)	106.6 (103.5–109.7)	108.4 (105.0–111.7)
Hand and eye coordination IQ	0.866	115.5 (111.5–119.6)	115.0 (111.0–119.1)	111.7 (108.4–115.0)
Performance IQ	0.204	123.5 119.2–127.8	119.6 (115.3–123.9)	121.0 (116.9–125.1)
General IQ	0.388	111.2 (107.8–114.6)	109.1 (105.8–112.5)	112.4 (108.9–115.9)

The benefit of using this tool is that it is appropriate for use in very young children, and can be standardized for age to reduce the impact of a range of starting ages due to mothers own decision to formula feed. The five cognition scores produce a total development score. The GMDS has been used in clinical and research settings and the outcome measures have been described in a number of ways including — Developmental Quotient [16], General Quotient of Cognitive Development [17], Intelligence Quotient or IQ Score [18]. This study, which involved 30 normal healthy term infants receiving standard infant formula (control), and 29 receiving a ganglioside-supplemented infant formula (treatment) revealed that there were no significant differences in scores for Locomotor, Social Interaction, and Hearing and Speech scales between the control and treatment groups. However, there were significant increases in Hand-Eye Coordination and Performance scores and also Total score in the treatment group. Overall results for the ganglioside-supplemented group were comparable to those for a reference breast-fed group. This suggests that ganglioside supplementation to more closely match the intake of breast-fed infants may provide some advantages in cognitive development, particularly those aspects related to motor skills. The level of gangliosides (measured as GD3 only) in the supplemented infant formula was around 11–12 μg/mL. This level is within the range of total ganglioside levels in human milk, 3.4–16.2 μg/mL [19–21].

Illness and iron-deficiency anemia can affect infant cognitive development and are therefore potential trial confounders. In Indonesia, 12–18-month-old babies suffering from iron-deficiency anemia have lower IQ scores determined using Bayley Infant Scale of Development-II (BSID-II) than those without anemia [22]. Although in the current study there was no difference between the 2 formula-fed groups for iron status at baseline, cognitive endpoints were, however, adjusted for baseline iron status. Since both formulations were balanced for iron, and formula consumption over the period of study was comparable, iron status is not likely to have contributed to difference in cognitive scores. Reported illness during the trial showed no significant differences in morbidities (fever and cough) between the two groups and is also unlikely to have accounted for cognitive differences between groups.

All Griffith scores in this study were on the high side of normal compared to international scales of IQ assessment [23]. While there are currently no population-based data for infant IQ (0–6 months) in Indonesia to act as a standard of comparison, it may be that the small number of study subjects and a recruitment demographic which may not have been representative of a wider population have skewed the scores towards the higher end. It may be that in a wider study with baseline IQ more in line with expected distribution around the norm, even greater differences associated with ganglioside supplementation may be noted.

Sources of pure gangliosides are not available to fortify infant formula, and therefore the complex milk lipid ingredient used as a food-acceptable source of gangliosides also contains other components such as phospholipids and fatty acids which might potentially contribute to cognitive development. There was no difference in DHA levels in the control and supplemented formula and total phospholipid values were similar. However, the level of arachidonic acid in the complex milk lipid ganglioside-supplemented infant formula was 0.3% of total fatty acids compared with 0.02% in the standard infant formula. Such levels for arachidonic acid are below the typical values reported for human breastmilk of 0.44% of fatty acids [24]. Several studies have reported no effect on infant cognitive development and growth of much higher levels of arachidonic acid

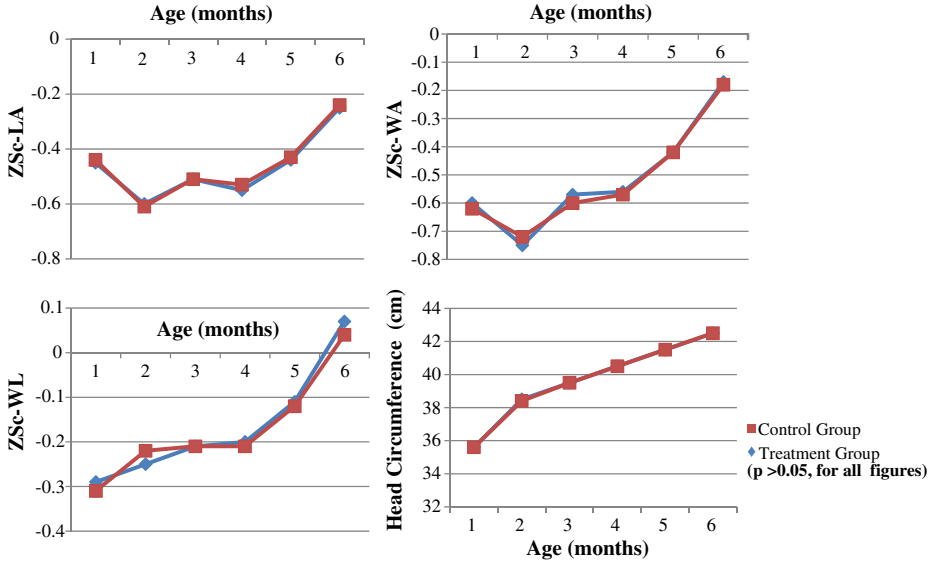

Fig. 2. Growth chart. Length-for-age (Z-Score)/ZSc-LA, Weight-for-age (Z-Score)/ZSc-WA, Weight-for-length (Z-Score)/ZSc-WL and Head Circumference.

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Table 4
Griffith Scale outputs of 6 month-old infants in treatment group, control group and reference group. Data are presented as means of raw scores and 95% confidence intervals.

Griffith Scale	P-value	Supplemented formula[a] (treatment group) (n = 29)	Standard formula[a] (control group) (n = 30)	Breast-fed[b] (reference group) (n = 32)
Locomotor IQ	0.225	120.0 (114.3–123.2)	117.2 (111.1–123.2)	113.7 (110.9–116.5)
Personal–social IQ	0.368	121.2 (115.1–127.4)	119.0 (112.5–125.5)	115.4 (112.0–118.8)
Hearing and speech IQ	0.114	120.3 (114.7–126.0)	116.7 (110.7–122.7)	115.1 (112.1–118.1)
Hand and eye coordination IQ	0.006	129.5 (123.0–136.0)	122.0 (115.1–128.9)	123.9 (120.3–127.6)
Performance IQ	<0.001	131.1 (125.7–136.5)	123.2 (117.5–128.9)	127.8 (124.9–130.8)
General IQ	0.041	125.4 (119.7–131.1)	120.6 (114.6–126.7)	120.0 (116.8–123.2)

[a] Results based on analysis of variance for the effect of treatment group after adjustment for age, Hb, and TIBC at baseline, as well as socioeconomic factors such as family size, mother's education and occupation, and father's occupation.
[b] Raw means.

intake [4–7]. Only a few studies have looked at the benefits of DHA alone or in combination with arachidonic acid, but these have reported no differences suggesting arachidonic acid has no additional benefit on visual acuity or growth [25,26]. The lack of effect on arachidonic acid levels in the serum of the two formula fed groups in the current study further suggests that dietary intake of arachidonic acid did not play a role in the effects observed. Overall, this suggests that the increases in cognitive scores observed in this study were contributed by gangliosides and that gangliosides may contribute to cognitive developmental functions by increasing hand-eye coordination and performance IQ. These functions rely on the impulse transmission process which is facilitated by gangliosides in the synaptic membrane.

The observed differences in serum ganglioside levels may be directly related to the differences in ganglioside intake. Others have reported a difference in ganglioside status (an increase in serum levels, and content in the brain and other tissues) in animal studies when the diets were supplemented with complex milk lipid gangliosides [9]. This is the first study to report human infant serum ganglioside levels after dietary supplementation and indicates that supplementation results in serum ganglioside levels more closely resembling those of breast-fed infants. Interestingly, although complex lipid supplementation contributed predominantly the GD3 form of ganglioside, the disposition of GM3 and GD3 species as assessed by their ratio remained similar in both formula groups, and in fact closely mapped that of the breast-fed group. GM3 becomes the predominant ganglioside in breast milk after the first week of lactation [1]. This suggests that it is the dietary ganglioside content rather than the specific form of ganglioside that influences serum ganglioside status.

The results from this study agree with earlier work published in young animals showing increased intake of gangliosides from complex milk lipids increased ganglioside status [27] and when ganglioside supplementation was extended beyond weaning, there was a significant impact on cognitive development seen with a faster rate of learning [9]. The unadjusted data from the current study show that there were differences in some scores of cognitive development between breast-fed infants and those receiving standard infant formula (control group, data not shown). This result is in line with other studies in breast-fed infants [28–30]. Horwood et al. [30] reported that performance IQ of exclusively breastfed infants was 6.2 points higher than non-breastfed infants, and verbal IQ was 10.2 points higher. This supports the view that standard infant formula may not optimize the nutrition required for infants to reach their developmental potential.

Since there were no reports of allergy, diarrhea, vomiting or colic during the trial and no significant differences in reported minor illness between the two formula groups throughout the trial, it is reasonable to conclude that infant formula supplemented with complex milk lipids to enhance the ganglioside levels supports normal growth, is well tolerated and is safe to be consumed by infants.

Overall, this is the first study in healthy normal infants who were not able to be breastfed, to investigate the cognitive benefits of increasing the intake of gangliosides via a complex milk lipid-fortified infant formula. While it is difficult to attribute the observed cognitive development benefits in the present study to increases in gangliosides alone, the impact of ganglioside supplementation on serum levels supports the hypothesis that the level of ganglioside intake is important. Although the small number of trial participants is an obvious limitation of the trial and conclusions there from, the findings from this pilot study are sufficiently compelling to warrant further investigation and confirmation in larger studies.

Conflict of interest

Angela Rowan is employed by Fonterra Co-operative Group Ltd. Dr. Dida Gurnida received a study grant and commercial product from Fonterra Brands Ltd (AME) to support a PhD program.

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Table 5
Levels of serum gangliosides GM3 and GD3 in treatment group, control group and reference group at 6 months of age. Data are presented as means and 95% confidence intervals.

	P-value	Supplemented formula (treatment group) (n = 29)	Standard formula (control group) (n = 30)	Breast-fed (reference group) (n = 32)
GM3 (μg/mL)	0.003	9.035 (8.215–9.854)	7.266 (6.461–8.072)	9.910 (9.011–10.810)
GD3 (μg/mL)	0.007	2.228 (1.967–2.489)	1.712 (1.456–1.969)	2.288 (2.044–2.531)
GM3 + GD3 (μg/mL)	0.002	11.262 (10.244–12.281)	8.978 (7.977–9.979)	12.198 (11.168–13.228)
GM3/GD3	0.532	4.323 (3.806–4.840)	4.551 (4.042–5.059)	4.627 (4.059–5.196)

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