Does breastfeeding in the neonatal period influence the cognitive function of very-low-birth-weight infants at 5 years of age?

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Abstract

Aim: Breast milk is rich in docosahexaenoic acid (DHA), which is selectively concentrated in neuronal membranes and is thought to be necessary for optimal neurodevelopment. This study evaluated the relationship between breastfeeding, especially the resultant DHA level in the red blood cell (RBC) membranes of infants, and the cognitive function of very-low-birth-weight infants at 5 years of age. Methods: Eighteen patients were classified into groups that were breastfed or formula-fed or both. We measured the DHA concentration in the RBC membranes of 18 preterm infants at 4 weeks of age. To evaluate cognitive function at the age of 5 years, we asked the children to perform five tests: the Kaufman Assessment Battery for Children, Day–Night Test, Kansas Reflection Impulsivity Scale for Preschoolers (KRISP), Motor Planning Test, and Strengths and Difficulties Questionnaire. Results: The DHA level at 4 weeks after birth was significantly higher in the breastfed infants than in the formula-fed infants. The scores for the Day–Night Test, KRISP, and Motor Planning Test were significantly higher in the breastfed group. There were significant correlations between the scores for the Day–Night Test and for the KRISP and the level of DHA at 4 weeks of age. Conclusion: Breastfeeding in the neonatal periods increases the DHA level in preterm infants and may have an important influence on brain development not only during early infancy but also during the preschool years, especially in terms of cognitive function.

Keywords: Preterm infant; Cognitive function; Breast milk feeding; DHA

1. Introduction

Numerous behavioral and biomedical studies conducted over the past 40 years have established a link between pediatric nutrition and cognitive development, especially intellectual competence. Several studies have shown a positive correlation between breastfeeding and cognitive development in children. A meta-analysis by Anderson et al. concluded that breastfeeding was associated with better cognitive performance, compared with formula-feeding [1–6]. Their findings were adjusted for potential confounding variables such as parental education, socioeconomic status, gestational week, and birth-weight. These findings suggest that breast milk provides nutrients required for the development of the brain and that polyunsaturated fatty acids (PUFAs) are especially beneficial. Many studies suggest a relationship between PUFAs and intellectual development. Others have found that giving pregnant women docosahexaenoic acid (DHA) supplements influences their children’s development [7–10].

The long-term effects of breastfeeding and DHA on cognitive function have not been investigated adequately, especially in terms of the executive function of preterm children. Some studies have reported a low level of executive function in preterm infants compared with term infants, and this has been linked to low levels of DHA, an essential nutrient for infant development.
This study evaluates the relationship between breastfeeding, with a focus on the resultant DHA level in red blood cell (RBC) membranes in early infancy, and the cognitive function of very-low-birth-weight infants at 5 years of age.

2. Methods

2.1. Subjects

Thirty-eight infants were enrolled between 1999 and 2000 at Juntendo University Hospital in Tokyo, Japan. We measured the DHA concentration in the RBC membranes of 30 preterm infants at 4 weeks of age. We identified 38 premature infants using our enrollment criteria. Of these, 26 subjects were invited to participate in development tests. Twelve subjects were excluded: 4 had moderate or severe cerebral palsy, 4 were missing DHA data for the neonatal period, 2 had very severe chronic lung disease, requiring oxygen during infancy, 2 had minor anomalies, and 1 had a hearing problem. We invited these 26 subjects to return for another examination at 5 years, and 18 responded. Eighteen cases in this trial to participate in intelligence testing using the Kaufman Assessment Battery for Children (KABC) and other tests of prefrontal cortex function. Of the 26 invited children, 18 were assessed at 5 years of age, using the KABC and other cognitive function tests.

The guardians of our subjects all gave written informed consent, and the protocol was approved by the regional ethics committee. Data on the mothers were collected from pregnancy records, and data on the infants were collected from birth records. This study was approved by our institutional review board and was performed only after obtaining informed consent from the parents of the participating patients.

2.2. Assessing long-chain polyunsaturated fatty acids (LC-PUFA)

Blood samples were collected from each infant by venipuncture at 4 weeks of age. After the erythrocyte pellet was collected by centrifugation, it was washed twice in 0.9% NaCl. Erythrocyte lipids were extracted with chloroform–methanol and were fractionated into phospholipids and nonpolar lipids using thin-layer chromatography. Phospholipid fatty acids were transesterified in sodium methoxide–methanol. Methyl esters were extracted in petroleum spirits and identified using gas chromatography. The composition of each fatty acid was expressed as a percentage of the total fatty acids.

2.3. Assessing cognitive function

To evaluate cognitive function in the 5-year-old children, we administered the following five assessments: the KABC, the Day–Night Test, the Kansas Reflection Impulsivity Scale for Preschoolers (KRISP), the Motor Planning Test, and the Strengths and Difficulties Questionnaire (SDQ).

2.4. Kaufman Assessment Battery for Children

The KABC is designed for children aged 2.5 years through 12.5 years. Two scales were used in this study, the sequential processing, and simultaneous processing scales. These scales are combined into a Mental Processing Composite, which is a global measure of intellectual functioning that correlates well with other IQ measures. Each scale produces a standardized score with a mean of 100 and a SD of 15. Helland et al. [11] used the KABC to evaluate the effect of supplementing the maternal diet with cod-liver oil during pregnancy and the first 3 months after delivery. Children whose mothers had received the cod-liver oil supplement had significantly higher Mental Processing Composite scores than children whose mothers received corn oil.

2.5. Day–Night Test

The Day–Night Test measures a child’s ability to ignore distracting information while attempting to give a correct answer. Children are first taught to say “day” when shown a card with a picture of the moon and “night” when shown a card with a picture of the sun. The task is demanding because the picture suggests a different answer. However, even young children quickly learn to give the correct answers. Children are then tested on a sequence of 16 cards presented in random order. Those with a lower ability to ignore distracting information give fewer correct answers. For example, they would tend to say “night” for the moon card and “day” for the sun card. Performance on this test improves steadily between 3 and 6 years of age [12]. The Day–Night task tests a child’s ability to keep two rules in mind and to inhibit the normal response to the image on the card, responding instead in a novel, opposite manner [13]. The child’s first response is recorded for each trial, including the practice trials. A “0” is recorded for an incorrect response, and a “1” is recorded for a correct response in the “Correct 0 or 1” section. The measure is the total number of correct responses (range, 0–16).

2.6. KRISP

The KRISP measures the speed of processing and impulsivity. We used a four-choice version of the test, which is an alternative version suitable for younger children (3–6 years). The measure is the total number of correct responses (range, 0–20).
2.7. Motor planning test

This test assesses the ability to organize a rapid sequence of finger movements within a time limit. Harvey et al. [14] reported lower motor planning scores in extremely-low-birth-weight children (at 4 years of age) compared with normal controls. In this test, the child touches each of four fingers to the thumb in sequence (forefinger to little finger) without missing a finger (impersistence) or striking any finger twice (perseveration). The measure is an efficiency score defined as the number of correct four-finger sequences completed within 60 s, averaged across both hands. The clock begins with the verbal command to start, and the number of correctly completed sequences in 60 s is recorded. No count is made for sequences in which the child fails to touch all fingers, does not maintain the correct sequence, does not contact the thumb, or makes contact below the first joint of the finger. The task is then repeated on the contralateral hand.

2.8. Strengths and Difficulties Questionnaire (SDQ)

The Strengths and Difficulties Questionnaire (SDQ) is a brief behavioral screening instrument that identifies emotional symptoms, conduct problems, hyperactivity, peer problems, and prosocial behavior within the borderline/clinical range. The measure is the total score of each category (range, 0–150) [15]. For the SDQ score, a lower score is better.

2.9. Statistical analysis

The results are expressed as means ± standard deviation (SD). Proportions were compared using a $\chi^2$ test with the continuity correction. Means were compared using a Mann–Whitney $U$ test for independent samples. A $P$ value of less than 0.05 was considered statistically significant.

3. Results

3.1. Study population

Table 1 presents the birth data and other characteristics of the study population. The 18 patients were classified into either the breastfed group (BM; $n = 10$) or the group fed formula with or without breast milk (formula; $n = 8$). The breastfeeding group was defined as the group fed milk consisting of more than 80% breast milk during the first month of life. The infants fed less than 80% breast milk were classified as the control group. Based on your comments, we added this to the text. And the duration of breastfeeding was not significantly difference.

For the BM group, the mean gestational age was 28.7 ± 3.2 weeks; the mean birth-weight, 1016.4 ± 302.2 g; the mean body weight at 5 years, 16.2 ± 1.8 kg; the mean body length at 5 years, 104.4 ± 1.8 cm; and the mean head circumference, 51.8 ± 2.0 cm. For the formula-fed group, the respective values were 30.7 ± 1.6 weeks, 1188.0 ± 296 g, 16.2 ± 1.9 kg, 106.9 ± 1.82 cm.

Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Breast milk group $(n = 10)$</th>
<th>Formula group $(n = 8)$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (week)*</td>
<td>28.7 ± 3.2</td>
<td>30.7 ± 1.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Birth-weight (g)*</td>
<td>1016.4 ± 302.2</td>
<td>1188.0 ± 296.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sex (M:F)**</td>
<td>5:5</td>
<td>4:4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Chronic lung disease $(n)**</td>
<td>0</td>
<td>3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Perventricular leukomalacia $(n)**</td>
<td>0</td>
<td>0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Retinopathy of prematurity $(n)**</td>
<td>2</td>
<td>1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Intraventricular hemorrhage $(n)**</td>
<td>0</td>
<td>0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Necrotizing enterocolitis $(n)**</td>
<td>0</td>
<td>0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Intrauterine growth retardation $(n)**</td>
<td>4</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td>(Number of symmetrical IUGR)</td>
<td>(1)</td>
<td>(1)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Maternal age (year)*</td>
<td>31.2 ± 3.2</td>
<td>30.3 ± 2.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Maternal education level (under 12 years)</td>
<td>0</td>
<td>0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Duration of breastfeeding $(d)$</td>
<td>72.2 ± 45.2</td>
<td>59.2 ± 32.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>% DHA at 4 weeks of age*</td>
<td>4.13 ± 0.62</td>
<td>2.7 ± 0.62</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>% Arachidonic acid at 4 weeks of age*</td>
<td>12.52 ± 0.47</td>
<td>12.28 ± 1.24</td>
<td>n.s.</td>
</tr>
<tr>
<td>Body length at 5 years (cm)*</td>
<td>104.4 ± 4.5</td>
<td>106.9 ± 1.82</td>
<td>n.s.</td>
</tr>
<tr>
<td>Body weight at 5 years (kg)*</td>
<td>16.2 ± 1.8</td>
<td>16.2 ± 1.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>Head circumference at 5 years (cm)*</td>
<td>51.8 ± 2.0</td>
<td>50.0 ± 1.3</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Data are means ± SD or numbers.

n.s., not significant.

*Significance of difference tested with by Mann–Whitney $U$ test.

**Significance of difference tested with $\chi^2$ test.
and 50.0 ± 1.3 cm. Only the head circumference at 5 years was significantly different between the two groups. And there was no significant difference in maternal age or maternal education level between the two groups.

3.2. Fatty acid analysis

The DHA concentration in the RBC membranes at 4 weeks after birth was significantly higher in the BM group than in the formula-fed group (Table 1), but the amino acid levels did not differ between the two groups.

3.3. Cognitive tests

The BM group had a significantly higher score than the formula-fed group for sequential processing on the KABC test; the scores for simultaneous processing and mental processing did not differ significantly between the groups (Table 2). The scores on the Day–Night Test, KRISP, and Motor Planning Test were significantly higher in the BM group compared with the formula-fed group, while the hyperactive and emotional total scores on the SDQ were significantly better in the formula-fed group (Table 3).

Regression analysis revealed a significant correlation of the DHA level at 4 weeks of age with the Day–Night Test score ($P = 0.002$; Fig. 1) and with the KRISP score ($P = 0.002$; Fig. 2). The subjects in the present study who has higher DHA level at 4 weeks of age, the better score of Day–Night Test and KRSP. The DHA level at 4 weeks was not significantly correlated with the sequential or simultaneous scores on the KABC, the Motor Planning Test score, or any SDQ score (data not shown).

4. Discussion

This is the first study to examine the relationship between breastfeeding in the neonatal periods and cognitive function of preterm children at 5 years of age in Japan. Our findings indicate that breastfeeding in neonatal periods improves cognitive function, especially executive function, at 5 years of age. In addition, breastfeeding increased the DHA level in preterm infants at 1 month of age. Our findings indicate that the DHA supplied by breastfeeding has a positive effect on the development of brain function, especially the development of executive function, at 5 years of age following preterm births.

Previous studies have reported a low level of executive function in preterm infants compared with term infants, which has been linked to low levels of DHA, an essential nutrient for infant development. We found a positive correlation between the DHA level at 4 weeks of age and the scores of the KRISP and Day–Night Test, which evaluate executive function, including short span memory (working memory) and attention span. Colombo et al. [16] reported a positive correlation between high scores on a sustained attention test in infancy and feeding with DHA-rich milk in early infancy. They postulated that the DHA levels in infancy affect the development of the attention span. Our findings also suggest a link between DHA and attention in later childhood. However, our small sample population did not allow us to correlate nutritional effects at specific time points, although it is intriguing to consider the value of DHA for both infant and early childhood development.

The survival of preterm infants has increased considerably in recent decades. Although developmental outcomes vary with the age of the child, follow-up studies across longer intervals show that preterm infants are at even greater risk of developmental problems, including poor cognitive function and behavioral problems such as attention deficit hyperactivity disorder (ADHD) and learning disorders [17]. Breastfeeding, especially if prolonged, may be associated with improved developmental achievement in the preterm infant [18,19]. We found a positive correlation between breastfeeding in the neonatal periods and head circumference, which reflects brain volume, at 5 years of age. Moreover,

### Table 2

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Mental processing</th>
<th>Simultaneous processing</th>
<th>Sequential processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast milk (10)</td>
<td>100.9 ± 14.6</td>
<td>99.3 ± 13.8</td>
<td>106.7 ± 14.5</td>
</tr>
<tr>
<td>Formula (8)</td>
<td>94.5 ± 11.8</td>
<td>94.6 ± 15.9</td>
<td>94.7 ± 11.6</td>
</tr>
</tbody>
</table>

Data are means ± SD. *Scores are significantly different between groups; $P < 0.05$, Mann–Whitney U test.

### Table 3

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Day–Night Test</th>
<th>KRISP</th>
<th>Motor planning test</th>
<th>Strengths and difficulties questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast milk (10)</td>
<td>14.1 ± 1.4</td>
<td>17.2 ± 0.8</td>
<td>18.8 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>Formula (8)</td>
<td>11.1 ± 0.9</td>
<td>15.0 ± 1.4</td>
<td>12.0 ± 3.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD. Differences between groups are significant at *$P < 0.05$ or **$P < 0.01$, Mann–Whitney U test. The lower score is better in the SDQ test.
Breast milk is reportedly related to improved maternal mood and interactive behaviors, which indirectly contribute to infant development, but adjustments for confounding factors such as nutrition and maternal characteristics must be made in these analyses. Agostoni reported the importance of two nutritional components of human milk: a balanced content of amino acids and the presence of LC-PUFAs, especially DHA [18]. In our study, DHA in breast milk appeared to have a positive influence on the growing brain of very-low-birth-weight infants. However, we need more data to adjust for confounding factors in order to clarify the effect of breastfeeding on the development of executive function.

Previously, in most of the previous comparative studies on breast and formula-feeding, the investigators had meticulously studied various confounders that could affect child development as maternal education or intelligence, since it has been shown that parental intelligence influence cognitive development of children.

Several studies using a variety approaches, including tests of visual acuity, problem solving, and general neurological development, have shown a positive correlation between breastfeeding and cognitive development during infancy [1–6]. Some studies have reported that breast milk is necessary in early infancy [7,8]. The long-term effects of breast milk are especially important in preterm children because of the risks of low-level executive function, which may result in ADHD or other psychiatric diseases in later life.

In our study, the serum DHA status of breastfed preterm infants was taken to reflect the n-3 LC-PUFA content of the breast milk; however, the PUFA content is greatly influenced by maternal diet and metabolism during lactation [21].

Many previous studies have proved that longer duration of breastfeeding had more beneficial effects. However, that was not significant different, in this study. So we suppose the breast feeding in the neonatal periods is more important than that of later infancy to the brain development. However, we need additional dietary data to confirm this relationship. We also must clarify whether the serum DHA level is significantly higher in breastfed preterm infants than in those fed formula or both formula and breast milk.

In addition, we had to examine the DHA status at different points, not only in the neonatal period so that we should explain more closely about the possible association between high DHA levels at newborn period and later cognitive development.

The choice of cognitive tests used to evaluate the developing brain during childhood is the most important affecting the study results. The methods we used, KRISP and the Day–Night Test, have been used to examine appropriate executive functioning in preschool children and have been found useful for identifying differences in the development of executive function.

Our findings are based on a small number of very-low-birth-weight infants, and further studies are needed to establish a firm link between breastfeeding, especially DHA status, and the development of instrumental cognitive skills such as executive function.

In conclusion, our findings suggest that breastfeeding in the neonatal periods might be promote brain development in very-low birth infants during infancy which could be influence to cognitive development in later childhood.

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References