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Long-term effect of high-dose supplementation with DHA on visual function at school age in children born at <33 wk gestational age: results from a follow-up of a randomized controlled trial[1]

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ABSTRACT

Background: Children born preterm are at risk of visual-processing impairments. Several lines of evidence have contributed to the rationale that docosahexaenoic acid (DHA) supplementation of preterm infants may improve outcomes in visual processing.

Objective: The aim was to determine whether at 7 y of age children who were born very preterm and who received a high-DHA diet have better visual-processing outcomes than do infants fed a standard-DHA diet.

Design: This was a follow-up study in a subgroup of children from a randomized controlled trial. Infants were randomly assigned to milk containing a higher concentration of DHA (1% of total fatty acids; high-DHA group) or a standard amount of DHA (0.2–0.3% of total fatty acids as DHA; control group). The randomization schedule was stratified by sex and birth weights of <1250 or ≥1250 g. A total of 104 (49 in the high-DHA group and 55 in the standard-DHA group) children aged 7 y were assessed on a range of visual-processing measures, including visual acuity, contrast sensitivity, vernier acuity, binocular stereopsis, and visual perception.

Results: There was no evidence of differences between the high-DHA and standard-DHA groups in any of the visual-processing measures. In the majority (12 of 13) of variables assessed, the direction of effect favored the control group. The study was large enough to detect a moderate treatment effect, if one truly existed.

Conclusion: Supplementing human milk with DHA at a dose of ~1% of total fatty acids given in the first months of life to very preterm infants does not appear to confer any long-term benefit for visual processing at school age. This trial was registered at anzctr.org/au as ACTRN12606000327583. *Am J Clin Nutr* 2016;103:268–75.

Keywords: docosahexaenoic acid, DHA, preterm infant, very preterm, visual processing

INTRODUCTION

Preterm children are at risk of a number of visual impairments, including difficulties with visual acuity (VA)[9] (1–3), contrast sensitivity (CS) (4, 5), stereopsis (3, 4), and visual perception (1, 6, 7), all skills that are important for a range of more complex and adaptive visual tasks such as classroom learning and overall school performance (8, 9). Brain injury (10–12), retinopathy of prematurity (13–16), and oxygen therapy during the neonatal period (17) have been reported to be associated with visual impairments, but other factors such as specific nutrients and agents that might optimize ocular and cortical development require further exploration.

DHA (22:6n–3), an n–3 long-chain PUFA, is found in high concentrations in both the cerebral cortex and the retina and is important for visual processing, such as photo-transduction (18, 19), regeneration of rhodopsin (20, 21), and maturation of the cortical visual pathway (22–24). During the last trimester of pregnancy a substantial amount of DHA is transferred from mother to fetus; thus, infants born early (e.g., very preterm infants; <33 wk gestational age) are denied an adequate intrauterine supply of DHA at a stage when brain growth is at its greatest (25, 26). Several trials have aimed to supplement preterm infant formulas with DHA in an attempt to achieve a dietary exposure to DHA that is comparable to the breast milk of women consuming a Western-style diet (~20 mg/kg per day or

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[9] Abbreviations used: AA, arachidonic acid; CS, contrast sensitivity; DINO, DHA for the Improvement of Neurodevelopmental Outcome in Preterm Infants; FrACT, Freiburg Visual Acuity Test; logMAR, log of the minimum angle of resolution; RWH, Melbourne Royal Women's Hospital; TVPS-3, Test of Visual Perceptual Skills, Third Edition; VA, visual acuity; VEP, visual evoked potential.

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0.2–0.3% of total fatty acids). These first clinical trials reported positive short-term effects for visual and retinal outcomes in formula-fed preterm infants after supplementation with DHA compared with unsupplemented feeding (27–29) and provided the basis for supplementation of preterm infant formula. However, the proportion of total fatty acids in these studies was substantially lower than the fetus would accrue in the uterus during the last trimester, which is equivalent to a dietary DHA content of ~60 mg/kg per day, >1% of total fatty acids (26, 30). Another issue with these studies was the use of supplemented formula feedings, because breast milk is crucial to the clinical management of preterm infants (e.g., 31–34). The randomized controlled trials that meet the physiologic requirements of DHA and provided human milk showed some short-term benefits of DHA supplementation (35–37).

In acknowledgment of these limitations, we evaluated a range of visual-processing functions in children at 7 y of age who were born very preterm and entered into a randomized controlled trial in which they were either fed breast milk (or formula when required) supplemented with 1% DHA (an amount of DHA designed to match the fetal accretion rate) or they were fed according to current practice (~0.3% of total dietary fatty acids). It was hypothesized that very preterm children who received high-dose DHA would show better performance across a range of visual-processing measures compared with very preterm children who received standard-dose DHA. It was expected that CS, binocular stereopsis, and spatial relation tasks would have the largest benefits given the preferential effects of DHA.

METHODS

Participants

The participants for this study were recruited as part of a follow-up for a multicenter randomized controlled trial that assessed the long-term outcomes of very preterm infants supplemented with high-dose DHA. The DINO (DHA for the Improvement of Neurodevelopmental Outcome in Preterm Infants) cohort was initially recruited between 2001 and 2005 from 5 collaborating centers [Women's and Children's Hospital, Adelaide; Flinders Medical Centre, Adelaide; Royal Women's Hospital, Melbourne (RWH); King Edward Memorial Hospital, Perth; and Royal Brisbane and Women's Hospital, Brisbane]. Infants born at <33 wk gestational age were eligible. Infants with major congenital or chromosomal abnormalities were excluded as were infants from a multiple birth when not all live-born infants were eligible or who were in other trials of fatty acid supplementation. This trial was registered at the Australian New Zealand Clinical Trials Registry as ACTRN12606000327583.

The procedure for allocating participants to treatment or placebo was previously described in detail (36). In summary, infants were randomly assigned to milk containing a higher concentration of DHA (1% of total fatty acids; high-DHA group) or a standard amount of DHA (0.2–0.3% of total fatty acids as DHA; control group). The randomization schedule was stratified by sex and birth weights of <1250 or ≥1250 g. All families, researchers, and clinicians were unaware of group allocation. Infants were fed their assigned diets from enrollment (within 5 d of commencing enteral feeds) until term. During the intervention period, lactating women were asked to consume 6 × 0.5-g capsules/d. Women in the high-DHA group were given capsules supplying a total DHA content of 900 mg/d as triglyceride from DHA-rich tuna oil, which increased breast-milk DHA concentrations to ~1% of total fatty acids (36) without altering the naturally occurring concentration of arachidonic acid (AA; 20:4n−6) in breast milk (38, 39). Women in the placebo group were given soy-oil capsules, which contains no DHA and does not alter the DHA content of breast milk (36, 38). A formula matching the higher-DHA or standard-DHA content was provided if formula was required. The concentration of AA was the same for both groups.

To date, neurodevelopment at 18 mo (36), 3–5 y (40), and 7 y (41) and early respiratory and allergy outcomes (42) have been reported from the trial. For the current report, the sample consisted of only children recruited from the RWH site. The RWH cohort originally comprised 124 participants; 8 died and 2 had withdrawn from the original DINO trial leaving 114 eligible to participate in this study. All procedures were conducted in accordance with the trial protocol, which was approved by the Human Research Ethics Committee of the RWH. Participants were assessed at 7 y of age, corrected for prematurity to avoid bias in cognitive test scores (43); for consistency, we adopted the same approach for the visual-processing measures.

Visual assessments

The Freiburg Visual Acuity Test

The Freiburg Visual Acuity Test (FrACT) is a computerized test used to assess VA, CS, and vernier acuity (44). It is available for download (http://www.michaelbach.de/fract.html) and was developed primarily for clinical research studies (45). The FrACT has high correspondence with other commonly used measures and high test-retest reliability (46–48). The participant uses a directional keypad to indicate the orientation of the "gap" (among 4 possible directions: up, down, left, right) of the Landolt target. The participant, while sitting at a fixed distance from the computer screen, is instructed to identify the position of the gap by selecting 1 of 4 positions on a keypad. Each of the tests uses Best PEST (parameter estimation by sequential testing) adaptive threshold estimation to adjust the difficulty of the task across trials according to the participant's performance.

Participants were given several practice items for each task to ensure comprehension of task demands. We also used child-friendly stickers on the monitor and keypad that corresponded to the orientation of the gap to limit cognitive demand. Children with motor difficulties were able to make verbal responses. VA was assessed monocularly, and the untested eye was patched with a translucent eye patch through which form perception was not possible. The eyelid was open underneath the eye patch, which allowed a small amount of light to reach the covered eye in an attempt to minimize the effects of binocular inhibitory interactions such as pupil size differences and binocular rivalry. VA was measured with habitual correction (e.g., glasses) where applicable. The eye with the best VA was used for subsequent tests.

VA

The FrACT VA test adjusts the size of the Landolt-C, which is presented as a black symbol on a lighter background. The test

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adjusts the size of the target (and hence the width of the gap) across trials over the full range of VA from ~0.01 to ~3.0 by using a logarithmic scale. If the participant responds correctly, the test item decreases in size for the next trial, whereas an incorrect response causes the test item to increase in size; there are a total of 30 trials, which yields a test-retest comparable to the Early Treatment Diabetic Retinopathy Study procedure (46). Decimal acuity scores were converted to log of the minimum angle of resolution (logMAR) scores for data analysis in which a value of 0.0 represents "average" 6/6 vision and smaller and negative scores are indicative of better acuity. As is commonly used, logMAR equivalent scores <0.20 were considered normal (4, 5, 49, 50) and participants with logMAR scores ≥0.20 were determined to be clinically impaired.

CS

The FrACT CS test estimates the contrast threshold similarly; however, in this task the diameter of the target is kept constant and its contrast is adjusted across trials by modulating the symbol luminance level. When the participant responds correctly, contrast is reduced in the next trial by increasing the symbol luminance. When the participant responds incorrectly, contrast is increased in the next trial by decreasing the symbol luminance. At the end of each 30-trial test run, the program records the subject's Weber contrast, which was then converted into its logarithm of CS score. In this experiment, the CS target diameter was 30 min of arc contrast. Lower scores indicate poorer performance. Previous studies of the Pelli-Robson test reported mean scores in normally sighted participants between 1.70 and 1.88 and logarithm of CS scores ≤1.50 were considered impaired (51, 52).

Vernier acuity

Vernier acuity, also referred to as hyperacuity, is defined as the ability to perceive a difference in relative spatial localization of ≥2 visual stimuli. In the Vernier Acuity Test, the observer is asked to indicate the direction of the line offset from a target line; the displacement between the lines varies across trials. If the participant responds correctly, the line offset decreases for the next trial, whereas an incorrect response causes the line offset to increase; there are a total of 36 trials. Stimulus line total height is 0.5°, 0.5 min of arc bar sigma, and 0.2 min of arc gap height.

Testing conditions

In each of the FrACT tests, children were allowed to respond to each trial at their own pace. Stimuli were displayed on a 22-inch ViewSonic LED color monitor driven by a Hewlett-Packard ProBook 6550b. The luminance and contrast from this screen were measured and verified before each participant was tested, and assessments were carried out in the same assessment room. The monitor was placed 170 cm from participants, and head positioning was closely monitored by the assessor.

Randot Preschool Stereoacuity Test

The Randot Preschool Stereoacuity Test evaluates the perception of binocular stereopsis at disparities ranging from 40 to 800 s of arc. The test consists of a set of 3 test booklets. Each test booklet contains 2 sets of 4 random dot patterns for identifying or, for the low-functioning children, matching with 4 two-dimensional black-and-white illustrations presented in a different order displayed on the opposite page of the book. Book 1 contains intermediate disparities (200 and 100 s of arc), book 2 contains fine disparities (60 and 40 s of arc), and book 3 contains coarse disparities (800 and 400 s of arc). Testing begins with book 1 or book 3. To determine the testability of the child, the child is first asked to identify the two-dimensional pictures that he or she will be required to identify or to match during the test. After the successful identification of the 2-dimensional pictures, testing proceeds by asking the child to identify or to match the corresponding 3-dimensional random dot pictures on the opposite page, which are visible only through the polarized glasses. At each level of disparity, the child must match or identify at least 2 of the 3 random dot figures correctly to be considered as passing and to proceed to the next level of the test with a smaller binocular disparity. Resolutions ≤70 s of arc were considered to be normal (53).

Judgment of line orientation

The judgment of line orientation is a motor-free task designed to measure deficits in the perception of line orientation in children and adults (54). For each of the 30 black-and-white test items, participants were asked to visually match 2 target lines, on the basis of their orientation, to 2 lines within a multiple-choice array of 11 differently oriented lines drawn at 18° intervals and arranged in a semicircle. The participant was shown each item on a stimulus card and asked to point or give a verbal response indicating their answer; this task was untimed. Participants were shown all items regardless of errors made. Correct responses were assigned 1 point each and summed. With the use of mean (±SD) age for the summed raw scores calculated separately for boys and girls, raw scores were transformed into z scores. Impairment scores were also calculated for each participant, in which z scores that fell below −1.5 (indicating that they were ≥1.5 SD below the mean) were considered to be "impaired."

Test of Visual Perceptual Skills, Third Edition

Visual perception was assessed with 5 subtests from the Test of Visual Perceptual Skills, Third Edition (TVPS-3) (55), including visual discrimination, spatial relations, form constancy, figure ground, and visual closure. Each subtest consists of 16 items; stimuli are black-and-white designs, and items are arranged in a sequence of increasing difficulty. Each subtest required the child to select 1 item from 4 or 5 choices; a predetermined ceiling was 3 consecutive incorrect items when the subtest was discontinued. Each scale is age-standardized with a normative mean of 10 (SD = 3), with higher scores indicative of better performance. The scaled scores of these 5 subtests were summed to form an overall visual perceptual score.

Statistical analyses

Data were analyzed by using SPSS version 20. Demographic and perinatal characteristics were compared between participating and nonparticipating children by using t tests for continuous data or chi-square for categorical data. Between-group differences (between high-dose DHA group compared with standard-dose DHA group) were first analyzed by t tests, and

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mean differences and 95% CIs were calculated. The analyses were repeated by using linear regression to determine whether there were any interaction effects for sex, birth weight (<1250 compared with ≥1250 g), and parity (multiple birth compared with singleton birth); the follow-up assessment at 18 mo corrected age reported an interaction between sex and dietary treatment in assessments of global development (36).

The prevalences of impairment (dichotomous outcome data) in each of the visual-processing domains in the high-dose DHA and standard-dose DHA groups were contrasted by using the chi-square test, and ORs and 95% CIs were calculated. Clinical impairment (impaired compared with normal) was determined by well-accepted cutoffs for VA, CS, and stereopsis, or the 10th percentile in the case of the visual perceptual variables. With 49 and 55 participants, the study had 81% power ($\alpha = 0.05$) to detect a difference of 0.56 SDs between groups for continuous variables, which is a moderate-sized clinical effect.

RESULTS

A total of 104 children (91% of the 114 eligible children) participated in the 7-y visual function follow-up assessment (49 in the high-DHA group and 55 in the standard-DHA group), although some children had missing data for some items. There were no significant differences between those who were followed up and those who were not followed up ($n = 12$) for perinatal variables (**Table 1**). The perinatal and demographic characteristics of the high-DHA and standard-DHA groups were mostly similar. Although the high-DHA group had more participants who required surgery in the neonatal period ($P = 0.03$) and the standard-DHA group had more participants diagnosed with retinopathy of prematurity stage 3 or greater in either eye ($P = 0.05$), these complications were uncommon in both groups.

Visual-processing outcomes

There was no evidence of differences between the high-DHA and standard-DHA groups, although the high-DHA group tended to perform poorer across most (12 of 13) measures of visual processing ($P > 0.05$) (**Table 2**). There were no interaction effects for sex, birth weight, or parity (data not shown).

Consistent with the continuous data, the rates of impairment in visual-processing outcomes were not different between groups, and the high-DHA group showed higher rates of clinical impairment across most domains than did the standard-DHA group (**Table 3**), except for both spatial relation tasks (judgment of line orientation and spatial relations from the TVPS-3).

DISCUSSION

This study compared visual-processing functions in very preterm 7 y olds who were randomly allocated to receive either breast milk and/or formula supplemented with 1% DHA or who or were fed according to current practice (~0.3% of total dietary fatty acids). We did not find any visual-processing benefit associated with high DHA, which is notable given the purported early benefits of DHA for retinal and visual outcomes. Predetermined subgroup analyses that examined the interaction effect of sex, birth weight (<1250 compared with ≥1250 g), and parity on visual-processing outcomes did not yield any significant relations.

Although the outcomes between the groups were not significantly different, the high-DHA group showed higher rates than the standard group of clinical impairment in most of the visual-processing

TABLE 1
Demographic characteristics of very preterm participants who were followed up and those who were not followed up: high-DHA group compared with control group[1]

	Followed up		Not followed up	
	High-dose DHA ($n = 49$)	Controls ($n = 55$)	High-dose DHA ($n = 12$)	Controls ($n = 8$)
Neonatal characteristics				
Male sex,[2] n (%)	25 (51.0)	29 (52.7)	6 (50)	2 (25)
Gestational age at birth,[3] wk	29.6 ± 2.0[4]	29.4 ± 2.1	29 ± 2.7	29.3 ± 3.0
Birth weight,[3] g	1417 ± 412	1345 ± 359	1356 ± 534	1391 ± 429
SGA,[2] n (%)	2 (4.1)	4 (7.3)	1 (8.3)	0 (0)
Multiple birth,[2] n (%)	15 (30.6)	25 (45.5)	4 (33.3)	2 (25)
Diagnosed with brain injury,[2,5] n (%)	10 (20.4)	11 (20.0)	2 (16.7)	2 (25)
Antenatal corticosteroids,[2] n (%)	43 (87.8)	44 (80.0)	12 (100)	6 (75)
Oxygen therapy at 36 wk PMA,[2] n (%)	6 (12.2)	8 (14.5)	2 (16.7)	0
Surgery,[2] n (%)	4 (8.2)	0 (0)*	2 (16.7)	1 (12.5)
ROP stage ≥3 in either eye,[2] n (%)	0	4 (7.3)	1 (8.3)	0
Sepsis,[2] n (%)	8 (16.3)	10 (18.2)	2 (16.7)	0
Neurosensory disability[2]				
Cerebral palsy, n (%)	3 (6.1)	3 (5.5)	0	0
IQ <85, n (%)	7 (14.3)	4 (7.5)	—	—

[1]*$P < 0.05$ (high-dose DHA compared with controls in the followed-up group). IQ, intelligence quotient; PMA, postmenstrual age; ROP, retinopathy of prematurity; SGA, small for gestational age.
[2]Categorical data were compared by using chi-square tests.
[3]Continuous data were compared by using independent t tests.
[4]Mean ± SD (all such values).
[5]Diagnosed with brain injury = periventricular leukomalacia or grade III or IV intraventricular hemorrhage.

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TABLE 2
Visual-processing outcomes for the high-dose DHA and control groups[1]

Variable	High-dose DHA (n = 49)	Control (n = 55)	Mean difference (95% CI)	P
logMAR VA				
Left ↓	0.11 ± 0.35[2]	0.06 ± 0.24	0.05 (−0.08, 0.17)	0.44
Right ↓	0.11 ± 0.33	0.09 ± 0.37	0.02 (−0.12, 0.17)	0.77
Better eye ↓	0.05 ± 0.33	−0.01 ± 0.17	0.06 (−0.05, 0.17)	0.26
logCS ↑	1.63 ± 0.34	1.69 ± 0.22	−0.06 (−0.18, 0.06)	0.32
Vernier acuity ↑	132 ± 104	133 ± 108	−1 (−45, 43)	0.97
Binocular stereopsis ↓	221 ± 300	149 ± 220	72 (−50, 195)	0.24
Judgment of line orientation ↑	−0.62 ± 1.19	−0.78 ± 1.04	0.16 (−0.31, 0.63)	0.51
Visual discrimination (SS) ↑	8.49 ± 3.05	8.75 ± 3.23	−0.26 (−1.50, 0.97)	0.67
Spatial relations (SS) ↑	11.57 ± 3.81	12.25 ± 4.83	−0.68 (−2.40, 1.05)	0.43
Form constancy (SS) ↑	7.10 ± 3.53	7.90 ± 3.50	−0.80 (−2.19, 0.59)	0.26
Figure ground (SS) ↑	9.02 ± 3.45	9.55 ± 4.06	−0.53 (−2.01, 0.96)	0.48
Visual closure (SS) ↑	7.47 ± 3.74	8.80 ± 3.70	−1.32 (−2.79, 0.14)	0.08
Total score ↑	43.6 ± 13.3	47.49 ± 13.8	−3.8 (−9.2, 1.5)	0.16

[1]Comparisons of the high-DHA and control groups were conducted by using independent t tests. logCS, logarithm of contrast sensitivity score; logMAR, log of the minimum angle of resolution; SS, scaled score; VA, visual acuity; ↓, lower scores are better; ↑, higher scores are better.
[2]Mean ± SD (all such values).

measures assessed, except for both spatial relations tasks. This may be clinically meaningful or may reflect chance. Interestingly, research has shown that DHA differentially affects parts of the visual system, such as rod photoreceptors and M retinal ganglion cells. M ganglion cells that project to the lateral geniculate nucleus are fast-responding and specialized in processing low spatial frequency information, such as the general size and shape of an object. Because they respond transiently to the presentation of visual stimuli, M cells are important for motion perception, spatial relations, and directing actions (56–58). Although it is appealing to suspect that DHA had a specific beneficial effect on these specific tasks it is important to reiterate that the differences were not significant and the effect sizes small. Furthermore, other parts of the visual system that also appear to be affected by concentrations of DHA, and thus also have specific functional roles, did not show any benefit. For example, rods are more adversely affected by inadequate DHA than cone cells and are located mostly in the peripheral part of the retina; they are ∼500 times more sensitive to light than cone cells, enable night vision, and are more sensitive to motion than cone cells. Consequently, visual functions such as CS, perception of motion, and visual fields may be influenced by DHA supplementation, in addition to visual acuity. This premise is supported by animal research; in rats, deficiency in n–3 fatty acids resulted in decreased amplitude of the electroretinogram (59) and impairment in the ability to learn a visual discrimination task (60), whereas in primates, the deficient animals had reduced visual acuity (61) and an abnormal electroretinograph (62). This premise was not supported by the current study findings.

Our findings are also contrary to earlier trials that reported short-term visual-processing benefits associated with DHA supplementation. There are a number of methodologic explanations for this difference: *1*) measurement, *2*) age at assessment, *3*) DHA concentration, *4*) mode of feeding (breast milk compared with formula), and *5*) cohort size and characteristics. Limitations with regard to the measurement of visual processing represent one of the major differences between our study and previous trials. Specifically, previous trials that reported short-term benefits of DHA supplementation for retinal and cortical visual function in both term (63–66) and preterm (29, 35, 62, 67, 68) infants typically used a single measure of visual function, either visual evoked potentials (VEPs) or grating acuity, both of which assess very low level visual function (69). The visual system is complex and a single measure, such as VEPs or grating acuity, is unable to provide an accurate representation of functional vision as a whole and provides little information as to functional vision, such as CS and visual perception. In contrast to these previous trials, the current study evaluated a range of visual functions including those that have been shown to differentially benefit from DHA, such as functions subsumed primarily by rod photoreceptors and M retinal ganglion cells, including CS, spatial relations, and stereopsis (70–77). In addition, although there appear to be several studies that suggested benefit for

TABLE 3
Rates of impairment in visual-processing outcomes contrasted between high-dose DHA and control groups[1]

Visual impairment	High-dose DHA (n = 49)	Control (n = 55)	OR (95% CI)	P
logMAR VA better eye >0.2	8 (17.8)	6 (12.2)	1.55 (0.49, 4.88)	0.45
logCS	11 (25.6)	7 (14.6)	2.01 (0.70, 5.78)	0.19
Binocular stereopsis	15 (44.1)	14 (31.1)	1.75 (0.69, 4.41)	0.23
Judgment of line orientation	9 (21.4)	12 (25.5)	0.80 (0.30, 2.13)	0.65
Visual discrimination	9 (18.4)	6 (11.3)	1.76 (0.58, 5.40)	0.32
Spatial relations	2 (4.1)	5 (9.6)	0.40 (0.07, 2.17)	0.27
Form constancy	17 (34.7)	11 (21.2)	1.98 (0.82, 4.81)	0.13
Figure ground	7 (14.3)	5 (9.4)	1.60 (0.47, 5.42)	0.45
Visual closure	20 (40.8)	13 (24.5)	2.12 (0.91, 4.95)	0.08

[1]Values are n (%) unless otherwise indicated. Comparisons of the higher-DHA and control groups were conducted by using chi-square test. logCS, logarithm of contrast sensitivity score; logMAR, log of the minimum angle of resolution; VA, visual acuity.

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DHA supplementation of preterm infants, most of these compared preterm infants fed DHA-supplemented formulas with those fed unsupplemented formulas (29, 62, 67, 68). This is an important methodologic issue because breast milk is the milk feeding of choice for the clinical management of preterm infants in the neonatal intensive care unit (i.e., decreases the risk of necrotizing enterocolitis and sepsis). There are only 2 trials that reflect enteral feedings that match the in utero accretion rate (~1% of total fatty acids) and are inclusive of breast-milk feeding practices (36, 37, 78). The current trial (DINO) is one of these and previously showed that DHA supplementation enhanced visual acuity at 4 mo corrected age in a subset of children from the DINO trial different from those who participated in the current study (35); the other did not evaluate visual processing (37, 78).

The current findings suggest that there is no long-term benefit for visual processing in either visual sensory or perceptual tasks. There is some precedence for this finding: for example, Birch et al. (79) assessed rod electroretinograms in very-low-birth-weight neonates who received either mother's milk or 1 of 3 infant formulas; and although they reported significant differences in rod ERP function between high- and low-DHA formulas at 36 wk postconception, these differences were no longer present at 57 wk postconception. Similarly, Carlson et al. (80) found only a temporary benefit for DHA supplementation in preterm infants without bronchopulmonary dysplasia who were fed formula (i.e., at 2 mo, but not at 4, 6, 9, or 12 mo). Although some animal research has shown that excess supplementation with DHA or a high DHA:AA ratio can have negative consequences for development and retinal structure/function, the concentration of DHA in the current study was comparable to what the fetus would accrue in the uterus during the last trimester and, as stated above, did not alter the naturally occurring concentration of AA in breast milk (39, 63). Furthermore, previous research investigating the effect of 4 amounts of DHA supplementation on VA clearly showed that infants fed a control formula (0% DHA) had significantly poorer VEP VA at 12 mo of age than did infants fed any of the DHA-supplemented formulas (0.32%, 0.64%, or 0.96% DHA; $P < 0.001$). All DHA-supplemented formulas provided 0.64% of fatty acids as AA (34 mg/100 kcal), which shows that differences in VA were not related to AA:DHA imbalance (63). There appears to be very little evidence to suggest that AA is especially relevant to visual-processing outcomes.

The major strength of this 7-y follow-up study in very-preterm-born children is that it is the only study, to our knowledge, to evaluate the effect of enteral feedings matching the in utero accretion rate of DHA in infancy on an extensive battery of visual functions. Furthermore, the study was large enough to detect a moderate-sized clinical effect if one existed, and the attrition rate was low. Lack of power is unlikely to be an explanation for not finding a benefit of high-dose DHA on visual functioning, because the direction of the effect favored the control group for all but one variable in Table 2. We acknowledge that the intervention did not quite reach the target amount of DHA (1%) (41, 81), and the "full dose" of DHA was not achieved until infants were receiving all enteral feedings; however, it seems unlikely that the results would differ had it done so.

In conclusion, supplementing human milk with DHA at a dose of ~1% of total fatty acids given in the first months of life to very preterm infants does not appear to confer any long-term benefit for visual processing at school age.

The authors' responsibilities were as follows—CSM: contributed to designing the research (project conception, development of overall research plan), contributed to conducting the research (hands-on conduct of the experiments and data collection), analyzed data, performed statistical analysis, contributed to writing the manuscript, and had primary responsibility for final content; SS: contributed to conducting the research (hands-on conduct of the experiments and data collection), analyzed data, and contributed to writing the manuscript; MM: contributed to designing the research (project conception, development of overall research plan, and study oversight) and writing the manuscript; CTC and PJA: contributed to designing the research (project conception, development of overall research plan, and study oversight), analyzed data, and contributed to writing the manuscript; LWD: contributed to designing the research (project conception, development of overall research plan, and study oversight), analyzed data, contributed to writing the manuscript. CTC and MM have received nonfinancial support from Clover Corporation and Nestlé Nutrition for research outside that of the submitted work. MM serves on scientific advisory boards for Nestlé, Fonterra, and Nutricia. Associated honoraria for MM are paid to her institutions to support conference travel and continuing education for postgraduate students and early-career researchers. MM, through the Women's and Children's Health Research Institute, has a patent pending "Methods and compositions for promoting the neurological development of an infant." None of the other authors declared a conflict of interest.

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