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Feeding preterm infants milk with a higher dose of docosahexaenoic acid than that used in current practice does not influence language or behavior in early childhood: a follow-up study of a randomized controlled trial[1–3]

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ABSTRACT

Background: The visual and mental development of preterm infants improved after feeding them milk enriched with docosahexaenoic acid (DHA) in amounts matching the fetal accretion rate.

Objective: The objective was to evaluate whether feeding preterm infants milk with a higher DHA content than that used in current practice influences language or behavior in early childhood.

Design: This was a follow-up study in a subgroup of infants enrolled in the DINO (Docosahexaenoic acid for the Improvement in Neurodevelopmental Outcome) trial. In a double-blind randomized controlled trial, infants born at <33 wk of gestation were fed milk containing 1% of total fatty acids as DHA (higher-DHA group) or ≈0.3% DHA (control group) until reaching full-term equivalent age. The longer-term effects of the intervention on language, behavior, and temperament were measured by using the MacArthur Communicative Development Inventory (MCDI) at 26-mo corrected age, the Strengths and Difficulties Questionnaire (SDQ), and the Short Temperament Scale for Children (STSC) between 3- and 5-y corrected age.

Results: Mean (±SD) MCDI scores did not differ significantly (adjusted $P = 0.8$) between the higher-DHA group (308 ± 179, $n = 60$) and the control group (316 ± 192, $n = 67$) per the Vocabulary Production subscale. Composite scores on the SDQ and STSC did not differ between the higher-DHA group and the control group [SDQ Total Difficulties: higher-DHA group (10.3 ± 6.0, $n = 61$), control group (9.5 ± 5.5, $n = 64$), adjusted $P = 0.5$; STSC score: higher-DHA (3.1 ± 0.7, $n = 61$), control group (3.0 ± 0.7, $n = 64$), adjusted $P = 0.3$].

Conclusions: Feeding preterm infants milk containing 3 times the standard amount of DHA did not result in any clinically meaningful change to language development or behavior when assessed in early childhood. Whether longer-term effects of dietary DHA supplementation can be detected remains to be assessed. This trial was registered with the Australia and New Zealand Clinical Trial Registry at www.anzctr.org.au as 12606000327583. *Am J Clin Nutr* doi: 10.3945/ajcn.2009.28603.

INTRODUCTION

Infants born preterm have a life-long increased risk of poor developmental outcomes across a range of cognitive domains compared with infants born full term. In particular, preterm infants are reported to have deficits in language development and behavior (1, 2). Differences in language skills between preterm and full-term infants emerge as early as 1 y, with those born at the earliest gestational ages being at the greatest risk and having the most severe language problems (3). In addition, compared with full-term children, those born preterm are more likely to have a difficult temperament (4), to have a higher prevalence of behavioral problems, and to have nearly twice the rate of abnormal internalizing and externalizing behaviors (1).

Improving the cognitive outcomes of preterm infants has been the subject of many dietary supplementation trials of docosahexaenoic acid (DHA) (5). DHA is a long-chain polyunsaturated fatty acid (LCPUFA) that normally accumulates in neural tissues during fetal and early postnatal development (6). Infants born preterm are denied the usual gestational transfer of DHA. Our systematic review of randomized controlled trials (RCTs) showed that feeding preterm infants formula fortified with 0.2–0.4% DHA (% total fat) improved mental development, as assessed by using the Bayley II, compared with infants fed an unsupple-

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mented formula (5). However, no RCT has studied behavioral outcomes of preterm infants fed DHA-enriched diets, and only limited data are available on communicative skills in preterm infants fed LCPUFA-supplemented formula (7, 8).

In the largest trial of its kind (the DINO trial, Docosahexaenoic acid for the Improvement in Neurodevelopmental Outcome; n = 657), we evaluated the global development of toddlers at 18-mo corrected age (CA) who were born preterm and fed breast milk and formula supplemented with 1% DHA (a level of DHA designed to match the intrauterine tissue accumulation) compared with those fed according to current practice (≈0.3% DHA) (9). Here we report the language and behavioral outcomes of the subset of children who were also assessed for visual acuity development (10). Assessments were conducted at ages when problems in language and behavior begin to emerge and when reliable clinically valid tools are available for assessment.

SUBJECTS AND METHODS

Subjects

Recruitment for the DINO trial began with a pilot phase at the Women's & Children's Hospital, Adelaide, in April 2001 until September 2003 and was expanded to a multicenter trial from October 2003. The subset of 143 infants enrolled during the pilot phase had additional assessments, including visual acuity (10) and erythrocyte membrane and plasma phospholipid fatty acids (11). Participation in the current follow-up involving assessments of language and behavior was open only to the 143 infants enrolled in the pilot phase of the DINO trial. All DINO trial participants were born at <33 wk of gestation. Infants born with major congenital or chromosomal abnormalities were excluded, as were lactating women for whom tuna oil was contraindicated (women with bleeding disorders or taking anticoagulants). Written informed consent was obtained from the caregivers of all participants at enrollment to the trial and at follow-up assessments at ≈2- and 4-y CA. All procedures were conducted in accordance with the trial protocol, which was approved by the Research Ethics Committee of the Children, Youth and Women's Health Service.

Allocation and treatment

The procedure for allocating participants to treatment was previously described (10). Briefly, infants were randomly assigned, by using consecutively numbered sealed opaque envelopes, to milk containing a higher concentration of DHA (1% of total fatty acids, higher-DHA group) or a standard amount of DHA (0.2–0.3% total fatty acids as DHA, control group). The randomization schedule was stratified by sex and birth weight <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 per day. Women in the higher-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 (11). Women in the control group were given soy oil capsules, which contains no DHA and does not alter the DHA content of breast milk (11). Tuna and soy oil capsules were identical in appearance. A formula matching the higher-DHA or standard-DHA content was provided if formula was required. The treatment group formula contained DHA as triglyceride from a mixture of algal and fish oils, whereas the formula for the control group contained DHA as triglyceride from algal oil. The DHA composition of milk and the bioavailability of DHA in the milk were previously shown (11).

Language development

All children who were not withdrawn at 18-mo CA were invited to participate in the language outcome. Language abilities were assessed by parent report by using the MacArthur Communicative Development Inventory (MCDI) at 26-mo CA. The MCDI is a validated assessment of receptive and expressive language use by toddlers and is standardized for age and sex (12). Although developed in the United States, the MCDI is sensitive to differences in language abilities between 2-y-old term and preterm children from English-speaking populations in other countries (13). The MCDI comprises 3 subscales: the Vocabulary Production subscale, which contains a comprehensive checklist to assess vocabulary size; the Irregular Words subscale, which measures the use of words that do not follow common rules of English (eg, mice instead of mouses, children instead of childs); and the Sentence Complexity subscale, which evaluates the syntactic complexity of sentences used by the child (eg, cat table or cat on the table). As part of the MCDI, parents document whether their child is combining words and the Mean Length of Utterance (MLU; parent recall of the longest sentence spontaneously produced by the child in the previous 2 wk). Scores on the Vocabulary Production subscale were the primary language outcome. Parents were sent the MCDI before a home visit by a trial staff member who collected forms and administered a structured questionnaire on language spoken in the family home and the child's exposure to languages other than English. The quality of the home environment was assessed by parent report by using the Home Screening Questionnaire for 3- to 6-y-olds (14).

Behavioral problems and temperament

All children who were not withdrawn at the time of the 26-mo CA language assessment were mailed invitations to participate in the behavior and temperament outcome in 2007, when participants were aged between 3- and 5-y CA. In addition to the posted invitation, nonrespondents and difficult-to-contact participants were telephoned and visited at their last known residence on 3 separate occasions to reestablish contact and optimize trial retention. Parents reported child behavior by using the Strengths and Difficulties Questionnaire (SDQ), which has been validated for use in the Australian population (15). The SDQ consists of 5 subscales: emotional symptoms, hyperactivity, conduct problems, peer problems, and prosocial behavior. The primary outcome at 3–5-y CA follow-up was the Total Difficulties score, which is generated by summing the scores from all subscales except the prosocial scale. Scores indicating abnormal behavior are based on validated cutoff scores, where 10% of children were defined as having behavioral difficulties (16). (Children <4 y of age were assessed with the 3–4-y-old version and those aged >4 y were assessed with the 4–10-y-old version of the SDQ.)

A measure of temperament was included to complement the behavioral assessment. Temperament was assessed by using the Short Temperament Scale for Children (STSC) (17, 18), which consists of 4 subscales, including approach (manner of approaching new people and situations), inflexibility (adjusting to challenges), persistence (in performing difficult tasks), and rhythmicity (the regularity of the child's usual activity patterns). An overall temperament score was determined by summing scores on the approach, inflexibility, and persistence subscales, and scores >1 SD above the mean represent difficult temperament.

In addition to the SDQ and STSC questionnaires, parents were asked to complete questionnaires on other risk factors for problem behavior, which included the quality of the home environment, the effect of major life events, and overall family functioning. The home environment was measured by using the Home Screening Questionnaire (HSQ) (14), family functioning by using the McMaster Family Assessment Device (FAD) (19), and the effect of recent events on family life was evaluated by using the Recent Life Events Questionnaire (20).

Statistical analyses

Statistical analyses were performed by using SPSS for WINDOWS (versions 14.0 and 15.0; SPSS Inc, Chicago, IL) and STATA 8.0 (Stata Corp, College Station, TX). A probability <0.05 was considered significant, and no statistical adjustment was made for multiple comparisons. Adjusted analyses were considered the primary analyses, although unadjusted analyses have been reported for completeness. The primary comparison was the MCDI Vocabulary Production subscale at 26-mo CA and the SDQ Total Difficulties score at 3–5-y CA between the higher-DHA and control groups. At 26-mo CA, secondary outcomes comprised scores on other MCDI subscales and whether children were combining words. At 3–5-y CA, secondary outcomes were subscale scores on the SDQ as well as the total and subscale scores on the STSC. Given that higher-DHA milk had previously been associated with a reduced incidence of developmental delay at 18-mo CA (9), the prevalences of poorer performance on the MCDI subscales (<50th percentile) and abnormal scores on the SDQ between the higher-DHA and control groups were also compared.

Generalized linear models were used to compare normally distributed outcome variables between the higher-DHA and control groups. Although many studies treat twins as independent units, genetic and environmental exposure of twins are shared and language of twins is known to differ from that of singletons (21). The lack of independence of twins was addressed in statistical analyses by using Robust Variance of Estimates (RVE) analysis. RVE is a type of cluster correlation in which the differences in variance observed within a cluster (ie, of twins) is adjusted by using the Sandwich Estimator of Variance method (22). This procedure maximizes sample size and results in a more representative assessment of variance in the overall analysis. However, RVE is not suitable for use in nonparametric analyses; therefore, all variables with a nonparametric distribution were compared by using a Mann-Whitney *U* test, randomly excluding a single twin from the analyses. Categorical variables were compared by using multiple logistic regression and included RVE to address nonindependence due to twinning. The normally distributed and categorical variables were adjusted for preplanned covariates, sex and birth weight, which are known to affect language development and behavior in early childhood. Additional preplanned covariates for the language outcomes included English as first language and number of siblings. In unadjusted analyses, the higher-DHA and control groups were compared by using all available data in independent *t* tests for continuous variables, Mann-Whitney *U* test for nonparametric continuous variables, and chi-square tests for categorical variables.

Sample size and power

With a sample size of 64 per group, we could detect a difference of half an SD between the groups in MCDI Vocabulary Production at 26-mo CA (≈80 words) and SDQ Total Difficulties score (≈2.6 points) at 3–5-y CA, with 95% confidence and 80% power. This moderate effect size is both relevant and appropriate because term and preterm children have large differences in MCDI scores at 2 y of age (21), and more than double the risk of behavioral problems are reported at 3–5 y (23, 24).

RESULTS

Of the 143 preterm infants enrolled in the trial, 128 (90%) participated in the language follow-up at 26-mo CA, and 125 (87%) participated in the behavior follow-up at 3–5-y CA (**Figure 1**). Overall trial retention rates did not differ between the higher-DHA and control groups (chi-square = 0.5, $P = 0.5$), and the baseline characteristics of participants at enrollment and at both phases of follow-up were comparable across the higher-DHA and control groups (**Table 1**). Dietary data collected at the 3–5-y CA follow-up showed the number of children taking DHA-rich supplements [higher-DHA group: $n = 11/61$ (18%); control group: $n = 11/64$ (17%)] or consuming ≥2 meals of fish per week did not differ between the groups [higher-DHA group: $n = 15/61$ (25%); control group: $n = 14/64$ (22%)], which suggests that there was no substantial difference in DHA intake in childhood.

Language

The primary outcome of MCDI Vocabulary Production scores did not differ between the higher-DHA and control groups, after nonindependence of twins and covariate adjustment for sex, birth weight, English as a first language, and the number of siblings were accounted for (**Table 2**). In addition, the higher-DHA and control groups did not differ in any of the other MCDI subscales analyses, the proportion of children combining words, or the proportion of children with MCDI scores below the 50th percentile. Despite observing interactions between sex and dietary treatment in assessments of global development at 18-mo CA (9), no interaction between treatment group and sex or treatment group and birth weight strata (<1250 and ≥1250 g) was found for the language outcomes. Irrespective of treatment group, exploratory analyses showed the mean (±SD) vocabulary production of girls did not differ from boys (girls: 329 ± 192, $n = 70$; boys: 292 ± 177, $n = 57$; mean difference: 37; 95% CI: −28, 102; $P = 0.2$).

Background data on language use collected at the 26-mo CA follow-up showed that a similar proportion of children in each group were exposed to English as a first language [higher-DHA

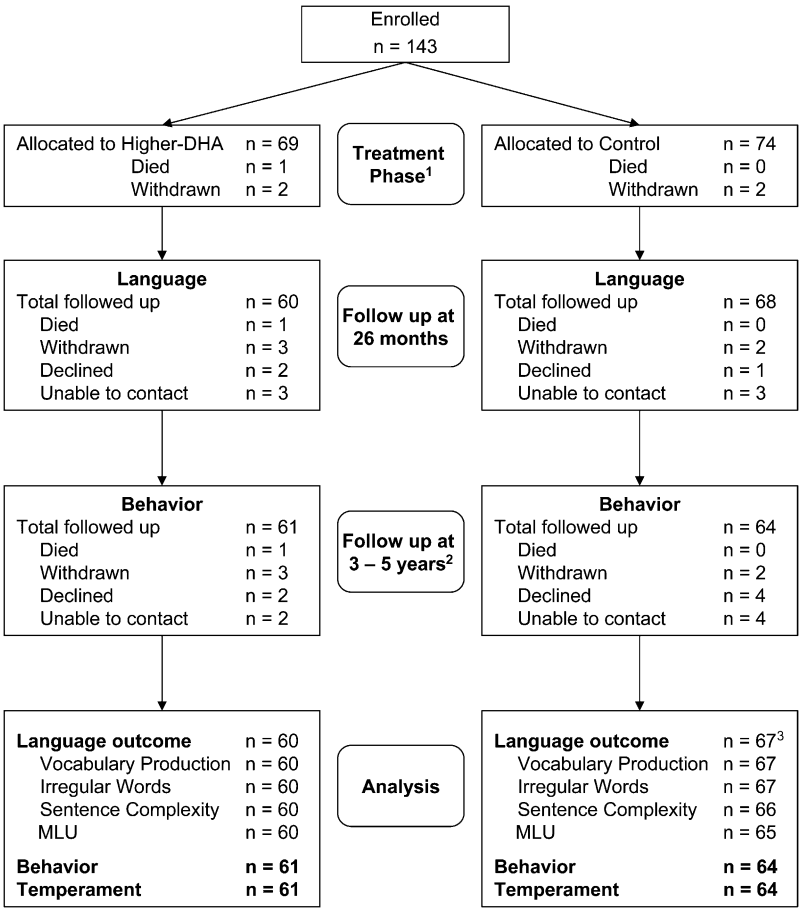

FIGURE 1. Flow of participants throughout the trial. [1]Two infants from both groups were withdrawn at their parents' request, and one infant from the higher-docosahexaenoic acid (DHA) group died before completion of the treatment phase. [2]Participants whose parents declined to participate at 26-mo corrected age were invited to attend the 3–5-y corrected age follow-up. Multiple attempts were made to reestablish communication with difficult-to-contact participants by telephone or visiting their last known address. [3]Language data were not available for one profoundly hearing-impaired child (control group). Two parents (control group) returned incomplete language questionnaires with missing Sentence Complexity (n = 1) and Mean Length of Utterance (MLU) (n = 2) data.

group: n = 60/60 (100%); control group: n = 65/68 (96%)] and to languages other than English [higher-DHA group: n = 17/60 (28%); control group: n = 14/68 (21%)]. Furthermore, no differences [median (interquartile range; IQR)] between the higher-DHA and control groups were found in the quality of the home environment (higher-DHA group: 33 (4.5), n = 53; control group: 35 (6), n = 55; U = 1390; z = –0.42, P = 0.7).

Behavioral problems and temperament

At 3–5-y CA, the primary outcome of the Total Difficulties scores on the SDQ did not differ between the higher-DHA and control groups (**Table 3**). Similarly, secondary analyses involving the SDQ subscales or the proportion of children with abnormal behavioral scores did not differ between the groups. Interestingly, the parents of 13 (21%) children from the higher-DHA group and 9 (14%) from the control group reported consulting a health care professional regarding their child's behavior [odds ratio (OR): 0.7; 95% CI: 0.3, 1.7, P = 0.4], although no children were taking prescription medications for problem behavior.

The STSC summary score of "Easy/Difficult" temperament did not differ between the higher-DHA and control groups, nor did the Approach, Inflexibility, and Rhythmicity subscales (Table 3). Although scores on the Persistence subscale increased (indicating a poorer persistence) in the higher-DHA group compared with in the control group, the size of the difference between the groups was small. Overall, no differences in the number of children with difficult temperament were observed between the groups. The higher-DHA and control groups did not differ on the HSQ [median (IQR)] according to the quality of the home environment [higher-DHA group: 44 (4), n = 53; control group: 45 (5), n = 54; P = 0.6] or recent major life events on the RLEQ [higher-DHA group: 2 (2), n = 53; control group: 1 (2), n = 54; P = 0.2]. However, a comparison of the FAD scores indicated that family functioning was poorer in the higher-DHA group than in the control group, but the difference [median (IQR)] was not within the range of clinical concern [higher-DHA group: 1.5 (0.6), n = 49; control group: 1.3 (0.7), n = 50; P = 0.03].

DISCUSSION

Our study indicates that language development and behavior in early childhood do not appear to be influenced by feeding preterm infants milk with a DHA concentration ≈3-fold higher than concentrations currently used in clinical practice. Language and behavior assessments were conducted by using validated questionnaires with reliable properties that have specificity for

TABLE 1
Demographic characteristics of participating mothers and their children[1]

	At enrollment		At language follow-up		At behavior follow-up	
	Higher-DHA group	Control group	Higher-DHA group	Control group	Higher-DHA group	Control group
Maternal characteristics						
No. of subjects	60	61	53	57	53	54
Maternal age at trial entry (y)[2]	29 ± 6[3]	31 ± 6	29 ± 6	31 ± 6	29 ± 6	31 ± 6
Mother completed secondary school [*n* (%)][4,5]	42 (61)	41 (55)	31 (58)	32 (56)	35/53 (66)	32/54 (62)
Child characteristics						
No. of subjects	69	74	60	68	61	64
Male [*n* (%)][4]	34 (49)	36 (49)	27 (45)	31 (46)	29 (48)	29 (45)
Singletons [*n* (%)][4]	51 (74)	46 (62)	45 (75)	43 (63)	44 (72)	42 (66)
Birth weight (g)[2]	1312 ± 439	1358 ± 433	1333 ± 447	1356 ± 444	1292 ± 447	1335 ± 450
Gestational age at birth (wk)[2]	29 ± 3	30 ± 2	29 ± 3	29 ± 2	29 ± 3	29 ± 2
Participants with older siblings [*n* (%)][4]	27 (39)	32 (43)	25 (36)	31 (46)	23 (38)	26 (41)
Age at follow-up (y)[2]	NA	NA	2.2 ± 0.1	2.2 ± 0.1	4.5 ± 0.7	4.5 ± 0.8

[1] DHA, docosahexaenoic acid; NA, not applicable.
[2] Independent *t* tests were used to compare normally distributed variables between the higher-DHA and control groups at follow-up. No statistically significant differences were observed.
[3] Mean ± SD (all such values).
[4] Chi-square tests were used to compare categorical variables between the higher-DHA and control groups at follow-up. No statistically significant differences were observed.
[5] Calculated as the percentage of mothers participating in the follow-up study.

diagnoses of language and behavior problems (12, 15). Although both questionnaires are likely to be sensitive to clinically relevant effects, they may not be as robust as a full clinical evaluation. Because of the absence of differences in early language development or behavioral outcomes in response to a neonatal diet rich in DHA, it might be argued that the benefits to mental development observed at 18 mo may not confer advantages to other developmental domains known to be problematic for preterm children. In the present study, the assessments were timed to the emergence of reliable communicative skills and behavioral characteristics—a period when any change in the rate of development is more likely to be detected.

Only 2 other RCTs have reported assessments of communicative development in preterm infants fed DHA-enriched milks (7, 8). Both trials assessed participants between 6- and 14-mo CA, before the rapid increase in vocabulary size and use of complex language skills in early childhood. O'Connor et al (7) reported no differences in intention-to-treat comparisons of vocabulary

TABLE 2
Language development at 26-mo corrected age[1]

	Participants assessed[2]			
	Higher-DHA group (*n* = 60)	Control group (*n* = 67)	Unadjusted *P*[3]	Adjusted *P*[3]
Vocabulary production[4]	308 ± 179[5]	316 ± 192	0.8	0.8
Sentence complexity[6]	7 (13)	7 (13)	0.8	0.9
Irregular words[6]	4.5 (8)	4 (6)	0.6	0.9
MLU[4]	3.6 ± 1.5	3.7 ± 2.0	0.6	0.3
Combining words [*n* (%)][7]	43 (62)	43 (58)	0.5	0.5
Vocabulary Production score >50th percentile [*n* (%)][7]	17 (25)	23 (31)	0.5	0.7

[1] DHA, docosahexaenoic acid; MLU, Mean Length of Utterance.
[2] The language assessment was not possible in one profoundly hearing-impaired child, and incomplete reporting by parents contributed to missing responses for sentence complexity (*n* = 1) and MLU (*n* = 2) outcomes, all from the control group. All available data were included in the analyses.
[3] $P < 0.05$ was considered significant.
[4] For normally distributed outcomes, unadjusted comparisons of the higher-DHA and control groups were conducted by using independent *t* tests. In adjusted analyses, the higher-DHA and control groups were compared by using generalized linear models and including adjustment for sex, birth weight, English as first language, and number of children in the family home with Robust Variance of Estimates applied to account for the lack of independence of twins. At 26-mo corrected age, the primary outcome was the adjusted analysis of Vocabulary Production scores between the higher-DHA and control groups.
[5] Mean ± SD (all such values).
[6] Values are medians; interquartile ranges in parentheses. Mann-Whitney *U* tests were used to make unadjusted comparisons of nonparametric outcomes between the higher-DHA and control groups, whereas unadjusted analyses involved randomly excluding one twin from the analysis.
[7] For unadjusted analyses of categorical outcomes, the higher-DHA and control groups were compared by using chi-square tests, whereas adjusted analyses were conducted by using logistic regression with Robust Variance of Estimates and adjustment for sex, birth weight, English as a first language, and the number of children in the family home.

TABLE 3
Behavior and temperament at 4.5-y corrected age[1]

	Subjects assessed[2]			
	Higher-DHA group (n = 61)	Control group (n = 64)	Unadjusted P[3]	Adjusted P[3]
SDQ				
Total Difficulties[4,5]	10.3 ± 6.0[6]	9.5 ± 5.5	0.4	0.5
Emotional symptoms[7]	2 (2)	1 (2)	0.9	0.7
Conduct problems[7]	2 (2)	2 (2)	0.9	0.7
Hyperactivity[7]	4 (3)	4 (3)	0.3	0.5
Peer problems[7]	1 (2)	1 (2)	0.9	0.8
Prosocial behavior[7]	8 (3)	8 (2)	0.7	0.9
Abnormal behavior [n (%)][8,9]	9 (15)	7 (11)	0.6	0.5
STSC				
Overall temperament score[5]	3.1 ± 0.7	3.0 ± 0.7	0.2	0.3
Approach[5]	2.7 ± 1.0	2.9 ± 1.1	0.4	0.5
Persistence[5]	3.5 ± 0.9	3.1 ± 0.9	0.02	0.02
Inflexibility[5]	3.1 ± 1.0	2.9 ± 1.0	0.3	0.3
Rhythmicity[5]	2.9 ± 0.7	2.6 ± 0.8	0.1	0.09
Difficult temperament [n (%)][9,10]	13 (21)	10 (16)	0.5	0.5

[1] DHA, docosahexaenoic acid; SDQ, Strengths and Difficulties Questionnaire; STSC, Short Temperament Scale for Children.
[2] Follow-up was completed in 61 children from 53 families in the higher-DHA group and in 64 children from 54 families in the control group.
[3] $P < 0.05$ was considered significant.
[4] Total Difficulties score was calculated from the sum of emotional symptoms, conduct problems, hyperactivity, and peer problems subscales.
[5] For normally distributed outcomes, unadjusted comparisons of the higher-DHA and control groups were conducted by using independent t tests. In adjusted analyses, the higher-DHA and control groups were compared by using generalized linear models and including adjustment for sex and birth weight with Robust Variance of Estimates applied to account for the lack of independence of twins. At 4.5-y corrected age, the primary outcome was the adjusted analysis of SDQ Total Difficulties scores between the higher-DHA and control groups.
[6] Mean ± SD (all such values).
[7] Values are medians; interquartile ranges in parentheses. Mann-Whitney U tests were used to make unadjusted comparisons of nonparametric outcomes between the higher-DHA and control groups, whereas adjusted analyses involved randomly excluding one twin from the analysis.
[8] Abnormal behavior was determined from normative values and set at scores ≥17.
[9] For unadjusted analyses of categorical outcomes, the higher-DHA and control groups were compared by using chi-square tests, whereas adjusted analyses were conducted by using logistic regression with Robust Variance of Estimates and adjustment for sex and birth weight.
[10] Easy/difficult temperament was calculated from approach, persistence, and inflexibility subscales, and difficult temperament was based on scores >3.7.

comprehension at 9- and 14-mo CA and vocabulary production at 14-mo CA between preterm infants fed a formula containing 0.3% DHA and 0.4% arachidonic acid and those fed formula devoid of LCPUFAs. Exploratory subgroup analyses limited to singleton children from English-speaking families showed that children fed the LCPUFA-enriched formula had a higher vocabulary comprehension than did the placebo group (7). However, the reduced sample size and a disproportionately higher loss of twins in the LCPUFA-supplemented group raise the possibility of bias and random error. Henriksen et al (8) compared preterm infants fed human milk containing a higher dose of DHA (≈1.4% total fatty acids) with those fed a lower dose of DHA (0.7% total fatty acids) and found no difference in communication skills at 6 mo of age. Despite the differences in the ages of assessment, our data are consistent with those of Henriksen et al (8). In addition, the higher prevalence of suboptimal language scores found in the present study is consistent with other research on the language development of preterm infants and suggests that the study sample is probably representative of the wider population of preterm children (3, 13).

Likewise, the increased prevalence of behavior problems in our study sample is similar to that reported in other cohorts of preterm children (1). Although the prevalence of behavioral problems of preterm children has been evident for some time, only 2 clinical trials have assessed the effect of dietary LCPUFAs in infancy on later behavior (25, 26). Unfortunately, both trials involved infants born at term and both reported no differences in behavioral problems between infants fed LCPUFA-enriched formula and those fed an unsupplemented control formula (25, 26). Using the SDQ for assessing behavior, Hibbeln et al (27) showed that maternal intake of fish, a rich source of DHA, during pregnancy is associated with a reduced prevalence of behavioral problems in 7-y-old children. Although the study by Hibbeln et al was observational and involved full-term infants, the maternal intake of DHA occurred during the intrauterine development period, which is comparable with the neonatal period of preterm infants. Whereas the observational research suggests benefits of dietary DHA on behavioral outcomes, our RCT showed that dietary DHA did not influence the behavior of preterm children at 3–5-y CA; however, follow-up studies in later childhood, when more mature behavioral patterns are manifested, may be worthy of further research.

Our study had good internal validity, as indicated by low attrition rates with equal participation across groups, and the integrity of blinding was maintained from the original trial. Consequently, the risk of attrition or participant bias was low. Post hoc calculations based on a moderate effect size (d = 0.5) also indicate that the trial had adequate power (>80%) to detect a difference between the groups in language or behavioral outcomes that is similar in magnitude to differences in vocabulary size and prevalence of behavioral problems between children born preterm compared with those born full term (1, 21).

We speculate that it may be more difficult to detect differences in development when the control group receives a substantial amount of DHA, compared with previous formula trials that have tested infants fed LCPUFA-enriched formula with those fed an unsupplemented formula without DHA. The lack of an effect of dietary DHA in this study may be explained by the fact that the dose of DHA in the control group was adequate or that an effect of dietary DHA may have been obscured by other larger influences on child development, such as family functioning, the quality of the home environment, and genetic potential. This latter point is supported by the data of Casiro et al (28), who found that perinatal characteristics were related to language skills of preterm infants at 1 y but not by 3 y. This suggests that discriminating the longer-term effects of dietary DHA interventions in the neonatal period may become more difficult as children mature (29).

In conclusion, the present study is the first to report the effects of dietary DHA supplementation of preterm infants on developmental outcomes beyond 2 y of age and includes the relatively unexplored areas of language and behavior. Despite observing earlier benefits of a higher-DHA diet in the newborn period to visual acuity and global scores of mental development, the present follow-up does not provide evidence to support enhanced early language or reduced behavioral problems. Because of the wide variance and comparatively slow emergence of behavioral problems, further long-term follow-up studies involving larger sample sizes may be necessary to assess more comprehensively the effect of a DHA-rich milk on behavioral outcomes.

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