Neurodevelopmental Outcomes of Preterm Infants Fed High-Dose Docosahexaenoic Acid

A Randomized Controlled Trial

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Context Uncertainty exists about the benefit of dietary docosahexaenoic acid (DHA) on the neurodevelopment of preterm infants.

Objective To determine the effect of meeting the estimated DHA requirement of preterm infants on neurodevelopment at 18 months' corrected age.

Design, Setting, and Participants Randomized, double-blind controlled trial enrolling infants born at less than 33 weeks' gestation from April 2001 to October 2005 at 5 Australian tertiary hospitals, with follow-up to 18 months.

Intervention High-DHA (approximately 1% total fatty acids) enteral feeds compared with standard DHA (approximately 0.3% total fatty acids) from day 2 to 4 of life until term corrected age.

Main Outcome Measures Bayley Mental Development Index (MDI) at 18 months' corrected age. A priori subgroup analyses were conducted based on randomization strata (sex and birth weight <1250 g vs ≥1250 g).

Results Of the 657 infants enrolled, 93.5% completed the 18-month follow-up. Bayley MDI scores did not differ between the high- and standard-DHA groups (mean difference, 1.9; 95% confidence interval [CI], −1.0 to 4.7). The MDI among girls fed the high-DHA diet was higher than girls fed standard DHA in unadjusted and adjusted analyses (unadjusted mean difference, 4.7; 95% CI, 0.5-8.8; adjusted mean difference, 4.5; 95% CI, 0.5-8.5). The MDI among boys did not differ between groups. For infants born weighing less than 1250 g, the MDI in the high-DHA group was higher than with standard DHA in the unadjusted comparison (mean difference, 4.7; 95% CI, 0.2-9.2) but did not reach statistical significance following adjustment for gestational age, sex, maternal education, and birth order (mean difference, 3.8; 95% CI, −0.5 to 8.0). The MDI among infants born weighing at least 1250 g did not differ between groups.

Conclusion A DHA dose of approximately 1% total fatty acids in early life did not increase MDI scores of preterm infants overall born earlier than 33 weeks but did improve the MDI scores of girls.

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INFANTS BORN BEFORE 33 WEEKS' GESTATION are at high risk of developmental disorders and learning disabilities. Long-term outcome studies of preterm infants show an overall reduction in developmental quotient and a poorer performance on tests of visual-motor integration, spatial relations, quantitative concepts, and classroom behavior compared with reference norms.[1-7] An inadequate nutrient supply in the neonatal period is hypothesized to contribute to this poor developmental outcome. The n-3 long-chain polyunsaturated fatty acid (LCPUFA) docosahexaenoic acid (DHA) is of particular interest in this regard because it is a major lipid in the brain with specific structural and functional roles.

Docosahexaenoic acid accretion into the brain and nervous system is greatest during the last trimester of pregnancy,[8,9] and postmortem studies indicate that average whole-body accretion of DHA during this time is in excess of 50 mg/kg/d,[8] which is equivalent to a dietary DHA content of approximately 1% of total fatty acids. However, preterm infants must rely on the relatively low levels of DHA supplied by human milk or supplemented infant formulas (0.2%-0.35% total fatty acids) and may have an increased requirement for DHA compared with their term counterparts.

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Previous LCPUFA trials have used infant formula interventions comparing DHA supplementation ranging from 0.2% to 0.5% of total fatty acids with formulas containing no dietary DHA and have generally restricted enrollment to preterm infants free of major morbidities.[10-17] On the basis of improvements in visual acuity development reported in some of these trials, 0.3% DHA is added to formulas for preterm infants.[15,16,18] However, formula feeding trials evaluating important neurodevelopmental outcomes have reported inconsistent results.[10-13,19,20] Only 1 study with short-term outcomes has addressed the issue of DHA supplementation in preterm infants fed breast milk.[21]

We conducted a randomized controlled trial to study the long-term efficacy of high-dose dietary DHA in preterm infants. The intervention was designed to reflect the feeding practices of most neonatal units, where expressed breast milk is the nutrition of choice. Lactating women received tuna oil supplements to increase the DHA concentration of their milk,[22] and preterm infant formula with a matching DHA composition was used if there was insufficient breast milk. We hypothesized that high-dose DHA supplementation would improve developmental quotient at 18 months' corrected age.

METHODS

Study Design

We conducted a multicenter, randomized controlled trial in 5 Australian perinatal centers; ethics approval was granted by the local institutional review boards (human research ethics committees) of each center. The trial began with a pilot phase at the Women's and Children's Hospital, Adelaide, and became multicenter when funding was obtained from the National Health and Medical Research Council of Australia. A central trial coordinator monitored data collection, entry, and checking. An independent serious adverse event committee reviewed all deaths.

Infants born before 33 weeks' gestation were eligible, and families were approached by the research nurses within 5 days of their infant receiving any enteral feeds. Infants were excluded if they had major congenital or chromosomal abnormalities, were from a multiple birth in which not all live-born infants were eligible, or were in other trials of fatty acid supplementation. Lactating mothers in whom tuna oil was contraindicated (for example, because of bleeding disorders or therapy with anticoagulants) were also excluded.

Randomization and Trial Entry

After written informed consent was obtained, mother-infant pairs were randomly assigned a unique study number through a computer-driven telephone randomization service according to an independently generated randomization schedule. Stratification was by center, birth weight ($<$1250 g vs $\geq$1250 g), and infant sex. Multiple births were considered a single randomization unit and randomization of twins or triplets was according to the sex and birth weight of the first-born infant. Baseline characteristics, including maternal age, infant race as identified by parents, parental education, birth order, parity, gestational age at birth, birth measurements, and pregnancy and birth complications, were recorded. Race was assessed only to characterize the population.

Dietary Treatments

Lactating mothers allocated to the high-DHA group were asked to consume six 500-mg DHA-rich tuna oil capsules per day to achieve a breast milk DHA concentration that was approximately 1% of total fatty acids without altering the naturally occurring concentration of arachidonic acid (AA) in breast milk.[22] If supplementary formula was required, infants were given a high-DHA preterm formula (approximately 1.0% DHA and 0.6% AA). Mothers with infants allocated to the standard-DHA group were asked to consume six 500-mg placebo soy oil capsules that did not change the fat content or fatty acid composition of their milk.[22] In the event that mothers chose not to breastfeed or could not produce enough breast milk, infants were fed standard preterm infant formula (approximately 0.35% DHA and 0.6% AA).

To facilitate blinding, each treatment group was separately color-coded into 2 groups. All capsules were similar in size, shape, and color and were donated by Clover Corporation, Sydney, Australia. If formula was required in the pilot phase, 2 drops of oil from capsules in matching color-coded containers were added to each 90-mL jar of formula. For the remainder of the trial, Mead Johnson Nutritionals, Evansville, Indiana, specifically manufactured ready-to-feed preterm formula to trial specifications and packaged the formula according to the color codes.

The intervention continued until infants reached their expected date of delivery. During hospitalization, the feeding regimen was under the direction of the infant's clinician and did not interfere with the use of human milk fortifier or supplementary vitamins or minerals. Postterm, breastfeeding mothers were encouraged to continue breastfeeding and those who had weaned to formula were encouraged to use a term formula supplemented with DHA and AA. Parents were reimbursed the difference in cost between unsupplemented term formula and DHA-supplemented term formula.

Treatment Phase Monitoring

During the intervention, the proportion of parenteral and enteral nutrition, human milk and infant formula intakes, and the frequency of interrupted feeds were documented weekly. Confirmed cases of necrotizing enterocolitis, sepsis, intraventricular hemorrhage, retinopathy of prematurity, and oxygen treatment at 36 weeks were also documented. Weight, length, and head circumference were assessed at the expected date of delivery and women who were breastfeeding donated a 5-mL sample of milk to assess the fatty acid composition.[22] At the expected date of delivery, women were also asked to guess their group allocation.

Outcome Assessments

The Mental Development Index (MDI) of the Bayley Scales of Infant Development, Second Edition (BSID-II)[23] evaluates memory, habituation, problem solving, early number concepts, and language. The MDI at 18 months' corrected age was chosen as the primary outcome because it represents a robust assessment of mental delays in children, is reasonably correlated with IQ in preterm children,[24] and allows comparison with other relevant studies. The Psychomotor Development Index (PDI), which evaluates control of the gross muscle groups including movements associated with standing, walking, running, and jumping, as well as fine motor manipulations involved in prehension, adaptive use of writing implements, and imitation of hand movements, was a secondary outcome.

The MDI and PDI scores were standardized to a mean of 100 with a standard deviation of 15 (range, 50-150). If a child performed below the threshold of the tests for either the MDI or the PDI, they were assigned a score of 45. If they were completely untestable because of severe delay, they were assigned a score of 40. At the time of the BSID-II assessment, weight, length, and head circumference were measured and the Home Screening Questionnaire[25] was administered to assess the quality and quantity of cognitive, social, and emotional support available to each infant in the home environment. Parents, clinicians, and all research personnel were blinded to participant study group.

Sample Size and Statistical Analysis

We designed the trial to evaluate the effect of high dietary DHA in the preterm period on infants born before 33 weeks' gestation as well as important subgroups in this heterogeneous population. Sample sizes of 288 children per group would allow us to detect a 4-point difference in MDI scores between the 2 treatment groups at 18 months' corrected age with more than 85% power ($\alpha = .05$). Previous studies indicate that the greatest benefit of high-dose DHA may be in infants born weighing less than 1250 g and have reported differences of 7 to 8 developmental quotient points between infants fed no DHA and those fed low-dose DHA.[11,13] Because 40% of our total sample was expected to be born weighing less than 1250 g, 114 infants per group would allow us to detect in this subgroup a minimum difference in MDI scores between high and standard DHA of 6 points (SD, 0.4) with 85% power ($\alpha = .05$). We also planned a priori to conduct a subgroup analysis based on infant sex because developmental quotient in early childhood often varies according to sex, and differences on the order of 5 to 8 points have been reported.[26-28] With half the study sample expected to be a single sex, 144 infants per group allowed us to detect a 5-point difference in MDI scores with 80% power ($\alpha = .05$) between treatment groups. Overall, our recruitment target was 320 infants per group to allow for 10% loss to follow-up, including deaths.

All analyses were conducted according to the intention-to-treat principle. The a priori level of significance was $P < .05$. Most of the outcomes were analyzed using generalized estimating equations (GEE)[29] to account for the clustering of infants within mother using SAS, version 9.1 (SAS Institute Inc, Cary, North Carolina). Normally distributed outcomes were analyzed using a linear GEE, with the difference in means (95% confidence interval [CI]) as the treatment effect. The subgroup analyses were performed via factorial models to allow testing for an interaction between treatment and subgroup.

Outcomes that were counts were analyzed using Poisson or negative binomial GEE as appropriate, with the ratio of means (95% CI) as the treatment effect. Binary or categorical data were analyzed using log-binomial GEE, with the relative risk (ratio of proportions) (95% CI) as the treatment effect. In secondary analyses, the BSID-II outcomes were also adjusted for the potential confounders of maternal education, infant sex, gestational age at delivery, and birth order. An additional adjustment was made for phase of the study, which made little difference to the results. All other outcomes were adjusted for the potential confounders of infant sex and gestational age at delivery. In post hoc analyses, we also investigated whether groups differed in the proportion of children with mild (score <85) and significant (score <70) mental delay.

Missing data were multiply imputed using regression models (normal, Poisson, or binary) with 50 imputations. Sensitivity analyses were performed using different seeds, increasing the number of imputations or adding further terms to the regression models. The results of these sensitivity analyses were similar to those presented herein.

RESULTS

The number of infants who were screened for the trial, randomly assigned to receive high DHA or standard DHA, and assessed at 18 months' corrected age are shown in the FIGURE. Enrollment for the trial began on April 4, 2001, and ended October 28, 2005. Follow-up commenced on January 17, 2003, and ended September 21, 2007. Adequate data for the analysis of the primary outcome were available for 614 infants, 93.5% of the infants who were originally enrolled in the trial (92.5% in the high-DHA group and 94.3% in the standard-DHA group). Missing data for the other 43 particpants were imputed as described above. The participants were similar to the population of infants in the Australian and New Zealand Neonatal Network.[30] The demographic and clinical characteristics of the infants and their families at randomization were comparable between the 2 groups (TABLE 1).

Median duration of treatment was comparable between the high-DHA and the standard-DHA groups (9.4 weeks [interquartile range, 7.9-11.4 weeks] vs 9.4 weeks [interquartile range, 8.0-11.6 weeks], respectively). Maternal adherence based on capsule returns was 81.1% in the high-DHA group and 81.7% in the standard-DHA group ($P = .88$). Mean DHA concentration in the milk of women in the high-DHA

group was greater than with standard treatment (0.85% [SD, 0.39%] vs 0.25% [SD, 0.13%] total fatty acids; $P<.001$), as was the mean DHA concentration in the 3 batches of preterm formula used for the trial (1.11% [SD, 0.29%] vs 0.42% [SD, 0.05%] total fatty acids; $P<.001$). The mean AA concentration did not differ between groups for human milk (0.41% [SD, 0.09%] vs 0.40% [SD, 0.09%] total fatty acids) or preterm infant formula (0.69% [SD, 0.29%] vs 0.69% [SD, 0.22%] total fatty acids). At the end of dietary treatment, 72% of women in the high-DHA group correctly guessed their group allocation, as indicated by more frequent reports of fishy eructations from the high-DHA group compared with standard treatment (140/322 vs 24/335; unadjusted relative risk, 6.20; 95% CI, 3.79-10.20; $P<.001$). There were no differences between the groups in maternal reports of diarrhea, constipation, nausea, or vomiting.

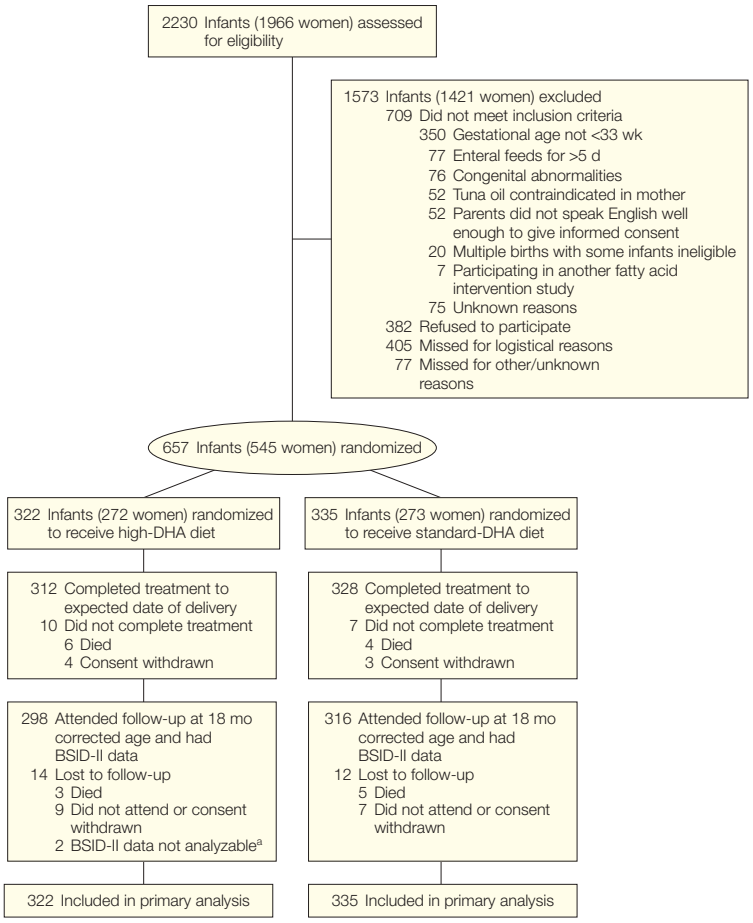
The primary outcome of mean MDI score did not differ between the high-DHA and standard-DHA groups (unadjusted mean difference, 1.9; 95% CI, –1.0 to 4.7; adjusted mean difference, 1.6; 95% CI, –1.2 to 4.3) (TABLE 2). A priori subgroup analyses based on the randomization strata showed interactions between dietary treatment and sex and between dietary treatment and birth weight. The MDI score among girls fed the high-DHA diet was significantly higher than among girls fed the standard-DHA diet in both unadjusted and adjusted analyses (unadjusted mean difference, 4.7; 95% CI, 0.5-8.8; adjusted mean difference, 4.5; 95% CI, 0.5-8.5) (Table 2) while the MDI score among boys did not differ between groups. The MDI score among infants born weighing less than 1250 g and fed a high-DHA diet until the expected date of delivery was higher than that of infants fed standard-DHA diets in the unadjusted comparison (mean difference, 4.7; 95% CI, 0.2-9.2) (Table 2) but did not reach statistical significance following adjustment for gestational age, sex, maternal education, and birth order (mean difference, 3.8; 95% CI, –0.5 to 8.0). The MDI score of infants born weighing at least 1250 g did not differ between groups.

For the PDI score, there was no significant difference between groups (mean difference, 0.9; 95% CI, –1.8 to 3.6; Table 2). There were no interactions between diet and sex or between diet and birth weight strata and, consequently, no differences in PDI between the groups in either of the birth weight strata or for boys vs girls. At 18 months' corrected age, the degree of social and cognitive stimulation available in the home environment did not differ between groups (Home Screening Questionnaire score, 34 [SD, 4] [n=322] in the high-DHA group vs 34 [SD, 3] [n=335] in the standard-DHA group).

Post hoc analyses indicated that, overall, fewer infants had significantly delayed mental development with high-DHA diets compared with standard DHA (TABLE 3). In the prespecified subgroups, there were fewer girls with mild and significant mental delay in the high-DHA group relative to the standard-DHA group but no differences in the boys (Table 3). There were fewer infants born weighing less than 1250 g in the high-DHA group with mildly delayed mental development and fewer infants born weighing at least 1250 g in the high-DHA group with significant mental delay compared with the standard-DHA diet (Table 3).

The secondary clinical outcomes of the infants are shown in TABLE 4. Blindness and hearing impairment requiring

Figure. Participant Flow



BSID-II indicates Bayley Scales of Infant Development, Second Edition.
[a]Two children did not have analyzable BSID-II data. One would not cooperate and the other was tested with the BSID-III (Third Edition) in a remote location.

aids were rare. There were no differences in anthropometric measures between the groups except that at 18 months' corrected age, infants who were allocated the high-DHA diet were longer than infants allocated to the standard diet (Table 4). The extent of breastfeeding did not differ between groups at the expected date of delivery (Table 4) or at 4, 12, or 18 months' corrected age. Other secondary outcomes also did not differ between groups, but fewer infants fed high DHA in the preterm period required oxygen treatment at 36 weeks compared with standard DHA treatment after correction for gestational age at birth and sex (Table 4). There were 2 maternal deaths after the end of the intervention phase (1 suicide and 1 Wolff-Parkinson-White syndrome with secondary substance abuse) in the standard-DHA group.

COMMENT

This trial was designed to resolve uncertainties about the putative role of dietary DHA in improving the developmental outcomes of children born preterm. Previous trials were limited by insufficient dose, lack of power, and targeted formula-fed preterm infants without major morbidities.[10,12,13,19] The current trial had broad inclusion criteria, intervened with a dose of DHA that we estimated to be required to match in utero accretion (3 times the levels used in most previous trials), and enrolled sufficient numbers to allow for evaluation of effects of both birth size and sex. In addition, the DHA intervention was measured against standard practice in which the level of DHA is

Table 1. Baseline Demographic and Clinical Characteristics[a]

Characteristics	High-DHA Diet (n = 322)	Standard-DHA Diet (n = 335)
Recruitment hospital		
Flinders Medical Center	31 (9.6)	32 (9.6)
King Edward Memorial Hospital	65 (20.2)	57 (17.0)
Royal Brisbane and Women's Hospital	46 (14.3)	50 (14.9)
Royal Women's Hospital	61 (18.9)	63 (18.8)
Women's and Children's Hospital	119 (37.0)	133 (39.7)
Mother's age at trial entry, mean (SD), y	29.9 (5.8)	30.2 (5.4)
Mother completed secondary education	205 (63.7)	201 (60.1)
Father completed secondary education	172 (53.5)	188 (56.0)
Mother smoked during pregnancy	82 (25.6)	84 (25.1)
Previous preterm births	51 (15.8)	58 (17.4)
Birth by cesarean delivery	220 (68.3)	235 (70.0)
Antenatal steroids administered	279 (86.6)	302 (90.1)
Multiple pregnancy	98 (30.4)	123 (36.7)
Gestational age at birth, median (IQR), wk	30 (27-31)	30 (27-31)
White race	283 (87.9)	311 (92.8)
Male sex	173 (53.7)	182 (54.3)
Birth weight, mean (SD), g	1308 (423)	1307 (415)
Small for gestational age	61 (18.9)	62 (18.6)
Birth weight <1250 g	147 (45.7)	149 (44.5)
Recumbent length at birth, mean (SD), cm	38.2 (4.0)	38.1 (4.0)
Head circumference at birth, mean (SD), cm	27.2 (2.8)	27.3 (2.7)
Days of partial enteral feeds prerandomization, median (IQR)	2 (1-4)	2 (0-3)
Infant age at randomization, median (IQR), d	4 (3-6)	4 (2-5)
Infants receiving breast milk at trial entry	297 (92.2)	306 (91.3)

Abbreviations: DHA, docosahexaenoic acid; IQR, interquartile range.
[a]Data are expressed as No. (%) unless otherwise indicated.

Table 2. Outcomes on Bayley Scales of Infant Development, Second Edition

Outcomes	High-/Standard-DHA Diet, No.	Mean Scores (SD)		Unadjusted Mean Difference in Scores (95% CI)	Unadjusted *P* Value	Adjusted Mean Difference in Scores (95% CI)[a]	Adjusted *P* Value
		High-DHA Diet	Standard-DHA Diet				
Mental Development Index (MDI)							
Standardized score	322/335	94.9 (14.5)	93.0 (17.3)	1.9 (–1.0 to 4.7)	.20	1.6 (–1.2 to 4.3)	.26
Birth weight <1250 g[b]	147/149	94.8 (15.6)	90.0 (18.4)	4.7 (0.2 to 9.2)	.04	3.8 (–0.5 to 8.0)	.08
Birth weight ≥1250 g[b]	175/186	95.1 (13.4)	95.5 (16.1)	–0.4 (–3.7 to 2.9)	.81	–0.40 (–3.7 to 3.0)	.83
Girls[c]	149/153	99.1 (13.9)	94.4 (17.5)	4.7 (0.5 to 8.8)	.03	4.5 (0.5 to 8.5)	.03
Boys[c]	173/182	91.3 (14.0)	91.9 (17.2)	–0.6 (–4.3 to 3.1)	.76	–1.0 (–4.5 to 2.6)	.60
Psychomotor Development Index (PDI)							
Standardized score	322/335	93.1 (16.1)	92.1 (16.3)	0.9 (–1.8 to 3.6)	.50	0.9 (–1.8 to 3.6)	.51
Birth weight <1250 g[d]	147/149	91.2 (16.8)	89.6 (17.8)	1.6 (–2.7 to 5.9)	.47	0.9 (–3.3 to 5.1)	.67
Birth weight ≥1250 g[d]	175/186	94.7 (15.2)	94.2 (14.8)	0.5 (–2.9 to 3.8)	.78	0.5 (–2.9 to 3.8)	.78
Girls[e]	149/153	94.5 (16.3)	93.9 (16.0)	0.6 (–3.4 to 4.5)	.78	0.5 (–3.4 to 4.4)	.80
Boys[e]	173/182	91.8 (15.8)	90.6 (16.5)	1.2 (–2.6 to 5.0)	.53	1.2 (–2.4 to 4.9)	.51

Abbreviations: CI, confidence interval; DHA, docosahexaenoic acid.
[a]Adjusted for gestational age at delivery, sex, maternal education, and birth order. Further adjustment for pilot phase vs multicenter phase did not alter results.
[b]For MDI × birth weight interaction, *P*=.05 unadjusted, *P*=.07 adjusted.
[c]For MDI × sex interaction, *P*=.06 unadjusted, *P*=.04 adjusted.
[d]For PDI × birth weight interaction, *P*=.68 unadjusted, *P*=.87 adjusted.
[e]For PDI × sex interaction, *P*=.82 unadjusted, *P*=.78 adjusted.

about 0.3% total fats and inclusive of human milk feeding. The consistent and comparable extent of human milk feeding between the 2 groups implies that potential factors in human milk that have the capacity to stimulate neurological development were controlled. The trial showed no overall benefit of the high-DHA diet on mean MDI or PDI scores at 18 months' corrected age. We did, however, detect a benefit of the high-DHA diet on the mean MDI score in girls and not in boys.

Although a number of longitudinal studies examining the outcomes of premature infants have suggested that being male is a risk factor for adverse cognitive outcome,[26-28] few trials were adequately powered to investigate whether there is a differential response to dietary or environmental interventions according to sex. The lack of responsiveness of boys to the intervention is puzzling, and the reasons are unclear but may relate to the higher rate of endogenous synthesis of DHA from the precursor fatty acid α-linolenic acid in girls compared with boys.[31] At the time we commenced the trial, we took a conservative view and designed the dietary intervention to meet estimated in utero accretion, although a proportion of dietary DHA would have been oxidized as an energy source. It may be that the higher synthetic capacity of girls coupled with the extra dietary DHA was sufficient to meet their dietary requirements, while boys may require a higher DHA dose.

Not surprisingly, infants born weighing at least 1250 g had greater MDI scores than infants born weighing less than 1250 g, highlighting the vulnerability of the smallest infants born at the shortest gestations. Our data showed no effect of the high-DHA diet on the MDI scores of infants born weighing at least 1250 g but were suggestive of benefit to infants born weighing less than 1250 g (mean difference, 4.7; 95% CI, 0.2-9.2; $P=.04$) that did not remain significant after adjustment for gestational age, sex, maternal education, and birth order (mean difference, 3.8; 95% CI, –0.5 to 8.0; $P=.08$). Our post hoc analyses demonstrated that the frequency of mild mental delay in smaller infants (<1250 g) was reduced by approximately 45% in the high-DHA group compared with standard DHA treatment. Although these findings should be interpreted with caution, they are consistent with our hypothesis and the concept of dietary deficiency, wherein benefit is expected in individuals with the lowest status or highest requirements. Planned follow-up at 7 years' corrected age of these smallest infants will be important.

Almost all infants were initially fed their mother's breast milk, and by the expected date of delivery about 40% were still exclusively fed human milk. Our intervention reflected clinical practice, in which infant formula is used as complementary nutrition when human milk is insufficient, and we used breast milk as the primary delivery vehicle for DHA to the infants. This was reliant on lactating mothers consuming 6 capsules per day (3 g of oil with about 900 mg of DHA) to deliver about 20% of the consumed DHA to their infants.[22,32] To translate such an intervention to a clinical setting, the adherence of the mothers must be a consideration. Although most women in our trial adhered to the dietary DHA intervention, a more efficient DHA delivery system may be to directly give DHA as a nutritional supplement. The additional fat load to the infant is negligible and could be delivered directly to the infant or added to human milk or formula for delivery via nasogastric tube. This later option may require an emulsification process to ensure that the fat is evenly dispersed through the human milk and less likely to adhere to the plastic nasogastric tubing.[21]

Table 3. Mild and Significant Developmental Delay Derived From BSID-II MDI Outcomes

Outcomes	High-/Standard-DHA Diet, No.	No. (%) of Infants		Unadjusted Relative Risk (95% CI)	Unadjusted *P* Value	Adjusted Relative Risk (95% CI)[a]	Adjusted *P* Value
		High-DHA Diet	Standard-DHA Diet				
All infants							
Mild mental delay (MDI <85)	322/335	64 (19.8)	90 (27.0)	0.73 (0.53-1.01)	.06	0.75 (0.55-1.04)	.08
Significant mental delay (MDI <70)	322/335	17 (5.2)	35 (10.5)	0.49 (0.26-0.97)	.03	0.50 (0.26-0.93)	.03
Birth weight <1250 g							
Mild mental delay (MDI <85)[b]	147/149	27 (18.1)	49 (33.0)	0.55 (0.34-0.87)	.01	0.57 (0.36-0.91)	.02
Significant mental delay (MDI <70)[c]	147/149	11 (7.2)	19 (12.9)	0.56 (0.24-1.28)	.17	0.58 (0.26-1.38)	.17
Birth weight ≥1250 g							
Mild mental delay (MDI <85)[b]	175/186	37 (21.3)	41 (22.1)	0.96 (0.62-1.49)	.86	0.96 (0.62-1.49)	.87
Significant mental delay (MDI <70)[c]	175/186	6 (3.4)	16 (8.6)	0.39 (0.15-1.03)	.06	0.36 (0.14-0.95)	.04
Girls							
Mild mental delay (MDI <85)[d]	149/153	16 (11.0)	40 (26.0)	0.42 (0.22-0.80)	.01	0.43 (0.23-0.80)	.01
Significant mental delay (MDI <70)[e]	149/153	3 (1.9)	16 (10.2)	0.18 (0.04-0.74)	.02	0.17 (0.04-0.72)	.02
Boys							
Mild mental delay (MDI <85)[d]	173/182	47 (27.4)	51 (27.8)	0.98 (0.68-1.44)	.94	1.01 (0.70-1.47)	.94
Significant mental delay (MDI <70)[e]	173/182	14 (8.0)	20 (10.7)	0.74 (0.35-1.56)	.43	0.76 (0.37-1.60)	.47

Abbreviations: BSID-II, Bayley Scales of Infant Development, Second Edition; CI, confidence interval; DHA, docosahexaenoic acid; MDI, Mental Development Index.
[a] Adjusted for gestational age at delivery, sex, maternal education, and birth order. Further adjustment for pilot phase vs multicenter phase did not alter results.
[b] For mild mental delay × birth weight interaction, $P=.04$ unadjusted, $P=.07$ adjusted.
[c] For significant mental delay × birth weight interaction, $P=.59$ unadjusted, $P=.46$ adjusted.
[d] For mild mental delay × sex interaction, $P=.02$ unadjusted, $P=.02$ adjusted.
[e] For significant mental delay × sex interaction, $P=.09$ unadjusted, $P=.08$ adjusted.

Infants included in this study had morbidities typical of infants born earlier than 33 weeks' gestation,[30] and we found no significant differences in the risk of adverse clinical outcome between the 2 treatment groups. In fact, we noted that infants in the high-DHA group may have a lower requirement for oxygen therapy at 36 weeks than infants fed according to standard practice, which is consistent with animal studies that demonstrate that exposure to high DHA increases the production of dipalmatylphosphtidylcholine, the major surfactant lipid, in the fetal and neonatal lung.[33,34] Our study also showed that infants in the high-DHA group were slightly longer than infants in the standard-DHA group at 18 months' corrected age. The effect on growth of adding LCPUFA to infant formula for preterm infants has been a matter of debate, with trials demonstrating positive, neutral, and negative effects of supplementation on infant weight and length.[20] Growth is a dynamic process, and to adequately assess the impact of an intervention, regular anthropometric measures are required. The effect of high DHA on length was small, was noted 18 months after the intervention ended, and should be interpreted with caution.

A limitation of our study was that the majority of women in the high-DHA group correctly guessed their group allocation at the end of dietary treatment. Women in the high-DHA group may have subsequently provided a different environment for their children, and this may have influenced developmental outcome. However, there was no difference between the groups in the quality of the home environment assessed at the time of developmental testing, indicating that the risk of bias was unlikely.

Given the heterogeneous nature of preterm infants, it is perhaps not surprising that we did not detect a mean difference in BSID-II MDI scores following a high-DHA diet. Infants ranged in gestational age from 23 to 33 weeks and, thus, had a range of nutritional stressors, organ immaturity, and morbidities. Despite this, the intervention was sufficiently robust to consistently elicit an improvement in the MDI scores of girls and may point the way for higher-dose interventions in future studies. Given the lack of an alternative therapy for cogni-

Table 4. Secondary Clinical Outcomes

Outcomes	High-DHA Diet (n = 322)[a]	Standard-DHA Diet (n = 335)[a]	Unadjusted Effect (95% CI)	Unadjusted *P* Value	Adjusted Effect (95% CI)[b]	Adjusted *P* Value
Death	9 (2.8)	9 (2.7)	1.04 (0.42 to 2.59)	.93	1.09 (0.44 to 2.66)	.86
Predischarge death	9 (2.8)	6 (1.8)	1.56 (0.56 to 4.34)	.39	1.66 (0.63 to 4.41)	.31
Days in neonatal intensive care unit	22 (3-31)	21 (4-33)	1.02 (0.82 to 1.27)	.87	1.03 (0.88 to 1.20)	.75
Days in hospital care	64 (40-80)	64 (41-80)	1.01 (0.92 to 1.10)	.87	1.00 (0.95 to 1.06)	.92
Days on parenteral nutrition	12 (5-15)	12 (5-14)	1.06 (0.90 to 1.24)	.52	1.03 (0.92 to 1.16)	.59
Days receiving intravenous lipids	8 (0-12)	8 (0-10)	1.06 (0.85 to 1.32)	.59	1.06 (0.87 to 1.30)	.54
Days until full enteral feeds	12 (6-14)	12 (6-14)	–0.2 (–2.0 to 1.6)	.82	–0.2 (–1.0 to 0.6)	.55
Exclusively human milk fed at EDD	142 (44.1)	135 (40.2)	1.10 (0.87 to 1.39)	.42	1.11 (0.88 to 1.40)	.39
Feeding interrupted	106 (32.9)	106 (31.6)	1.04 (0.82 to 1.32)	.74	1.07 (0.87 to 1.31)	.55
Any necrotizing enterocolitis	14 (4.3)	7 (2.1)	2.06 (0.83 to 5.13)	.12	2.14 (0.87 to 5.22)	.10
Bowel surgery	12 (3.7)	9 (2.7)	1.39 (0.58 to 3.33)	.46	1.45 (0.63 to 3.35)	.39
Oxygen treatment at 36 wk	60 (18.6)	84 (25.1)	0.74 (0.54 to 1.02)	.07	0.76 (0.58 to 1.00)	.05
Any intraventricular hemorrhage	45 (14.0)	44 (13.2)	1.06 (0.71 to 1.59)	.77	1.07 (0.72 to 1.58)	.73
Severe intraventricular hemorrhage[c]	9 (2.8)	6 (1.8)	1.56 (0.56 to 4.33)	.39	1.63 (0.61 to 4.33)	.33
Any retinopathy of prematurity	74 (23.0)	73 (21.8)	1.05 (0.77 to 1.45)	.74	1.09 (0.85 to 1.40)	.49
Severe retinopathy of prematurity[d]	14 (4.3)	17 (5.1)	0.86 (0.42 to 1.75)	.67	0.91 (0.46 to 1.80)	.79
Any sepsis	53 (16.6)	48 (14.3)	1.16 (0.79 to 1.69)	.46	1.18 (0.85 to 1.65)	.32
Postnatal steroids	30 (9.3)	34 (10.2)	0.92 (0.56 to 1.51)	.73	0.96 (0.61 to 1.50)	.85
Small for gestational age at EDD	109 (33.8)	105 (31.4)	1.08 (0.85 to 1.37)	.53	1.09 (0.86 to 1.37)	.49
Weight at EDD, mean (SD), g	3175 (553)	3129 (535)	42 (–118 to 203)	.60	42 (–116 to 199)	.60
Weight at 18 mo, mean (SD), g	11 625 (1811)	11 277 (1588)	201 (–237 to 639)	.37	187 (–250 to 623)	.40
Length at EDD, mean (SD), cm	48.7 (3.3)	48.4 (3.3)	0.2 (–0.3 to 0.8)	.42	0.2 (–0.3 to 0.8)	.45
Length at 18 mo, mean (SD), cm	82.8 (5.2)	81.7 (4.7)	0.9 (0.2 to 1.7)	.01	0.9 (0.2 to 1.6)	.01
Head circumference at EDD, mean (SD), cm	35.4 (1.8)	35.4 (1.9)	0.10 (–0.6 to 0.7)	.85	0.10 (–0.6 to 0.7)	.85
Head circumference at 18 mo, mean (SD), cm	47.6 (2.5)	47.6 (2.2)	–0.06 (–0.8 to 0.7)	.86	–0.05 (–0.8 to 0.7)	.88
Seizures at 18 mo	7 (2.0)	17 (5.2)	0.39 (0.15 to 1.04)	.06	0.39 (0.15 to 1.04)	.06
Unilateral or bilateral blindness at 18 mo	0	1 (0.3)				
Severe hearing loss requiring aid at 18 mo	0	1 (0.3)				
Cerebral palsy at 18 mo	13 (3.9)	10 (3.0)	1.31 (0.56 to 3.06)	.53	1.31 (0.56 to 3.06)	.53

Abbreviations: CI, confidence interval; DHA, docosahexaenoic acid; EDD, expected date of delivery.
[a] Data are No. (%) (with relative risk reported as the effect) or mean (interquartile range) (with ratio of means reported as the effect) unless otherwise stated.
[b] Adjusted for gestational age at delivery and sex. Further adjustment for pilot phase vs multicenter phase did not alter the results.
[c] Grade 3 or 4.
[d] Grade 3 or higher.

tive delay in this group of infants and the apparent safety of the current dose of DHA, further studies are warranted.

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