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Effect of Docosahexaenoic Acid Supplementation vs Placebo on Developmental Outcomes of Toddlers Born Preterm
A Randomized Clinical Trial

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IMPORTANCE Intake of dietary docosahexaenoic acid (DHA) among toddlers is low. Supplementation may benefit developmental outcomes of toddlers who were born preterm.

OBJECTIVE To determine whether 6 months of daily DHA supplementation improves developmental outcomes of toddlers who were born preterm.

DESIGN, SETTING, AND PARTICIPANTS A randomized, fully masked, placebo-controlled trial was conducted from April 26, 2012, to March 24, 2017, at a large US pediatric academic center with 9 neonatal intensive care units. Children born at less than 35 weeks' gestation who were 10 to 16 months corrected age underwent 6 months of intervention. Of 2363 children assessed, 982 were eligible, 605 declined, and 377 enrolled and were randomized. Analyses were according to intent to treat.

INTERVENTIONS One-to-one allocation to receive daily microencapsulated DHA, 200 mg, and arachidonic acid (AA), 200 mg (DHA+AA), or microencapsulated corn oil (placebo).

MAIN OUTCOMES AND MEASURES The primary outcome specified a priori was Bayley Scales of Infant and Toddler Development, third edition (Bayley-III), cognitive composite score at 16 to 22 months corrected age. Secondary outcomes were Bayley-III language and motor composite scores and Infant Behavior Questionnaire–Revised and Early Childhood Behavior Questionnaire effortful control and activity level scores. Subgroup analyses defined a priori were by income, sex, and birth weight.

RESULTS Among 377 children randomized and included in the analysis (182 girls and 195 boys; median corrected age, 15.7 months), 338 children (89.7%) had complete data on the primary outcome. Bayley-III cognitive scores did not differ between the DHA+AA and placebo groups (difference in change, 0.5 [95% CI, −1.8 to 2.8]; effect size, 0.05; $P = .66$). Assignment to the DHA+AA group had a small to medium negative effect on Bayley-III language scores among children with lower birth weights (eg, a child with a birth weight of 1000 g assigned to receive DHA+AA experienced a 4.1-point relative decrease, while a child assigned to placebo did not; $P = .03$ for interaction). Supplementation had a similar negative effect on effortful control scores among children with annual household incomes greater than $35 000 (difference in change, −0.3 [95% CI, −0.4 to −0.1]; effect size, −0.37; $P = .01$). Bayley-III motor scores and activity level scores were unaffected.

CONCLUSIONS AND RELEVANCE Daily supplementation with 200 mg of DHA and 200 mg of AA for 6 months resulted in no improvement in cognitive development and early measures of executive function vs placebo, and may have resulted in negative effects on language development and effortful control in certain subgroups of children. These findings do not support DHA supplementation in the second year of life for children who are born preterm.

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Supplemental content

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Cognitive deficits are common, burdensome long-term consequences of prematurity.[1] Around age 1 year, many preterm children enter early intervention programs, which can improve long-term outcomes. However, these programs are costly, require substantial family involvement, and do not eliminate the developmental gap.[2-4] Children with mild deficits may receive no intervention but are most of those born preterm and remain at risk for poor outcomes.[5] Disparities in outcomes also exist by socioeconomic status.[6]

Docosahexaenoic acid (DHA), an ω-3 long-chain polyunsaturated fatty acid, is the brain's dominant structural fatty acid.[7] Docosahexaenoic acid is crucial for neurotransmitter function, signal transduction, gene expression, neurogenesis, and anti-inflammation.[8-12] Preterm infants are born DHA deficient.[13] In some trials, dietary DHA supplementation initiated in the neonatal period has improved global developmental outcomes in preterm infants.[14,15] Certain sex, birth weight, or socioeconomic groups may experience differential effects of DHA supplementation, but results have been inconsistent.[16,17]

The second year of life is also a period of DHA deficiency because dietary intake of DHA decreases from approximately 50 mg/d in midinfancy to 20 mg/d after the child weans from breast milk or formula.[18,19] Nevertheless, the brain continues incorporating DHA rapidly to at least age 2 years.[7] Despite no rigorous trial evidence supporting DHA supplementation during the second year of life for children born preterm, numerous DHA-supplemented foods and formula products are marketed for toddlers. This trial tested the efficacy of DHA supplementation in improving developmental outcomes among toddlers born across a spectrum of premature gestational ages.

Key Points

Question What is the effect of dietary supplementation with docosahexaenoic acid during the second year of life on the development of children who were born preterm?

Findings In a randomized clinical trial of 377 US children aged 1 year who were born preterm, 6 months of daily docosahexaenoic acid supplementation resulted in no overall effect on Bayley Scales of Infant and Toddler Development, third edition cognitive composite scores compared with placebo.

Meaning In toddlers born prematurely, daily supplementation with docosahexaenoic acid did not result in improvements in global cognitive development.

Methods

Trial Design and Setting

Omega Tots was a randomized, fully masked, placebo-controlled trial that began with a seed grant–funded phase to enroll 112 children; enrollment expanded with subsequent funding (trial protocol in Supplement 1). The study physician reviewed adverse events. The study was approved by the Nationwide Children's Hospital (NCH) Institutional Review Board. Children's caregivers provided written informed consent.

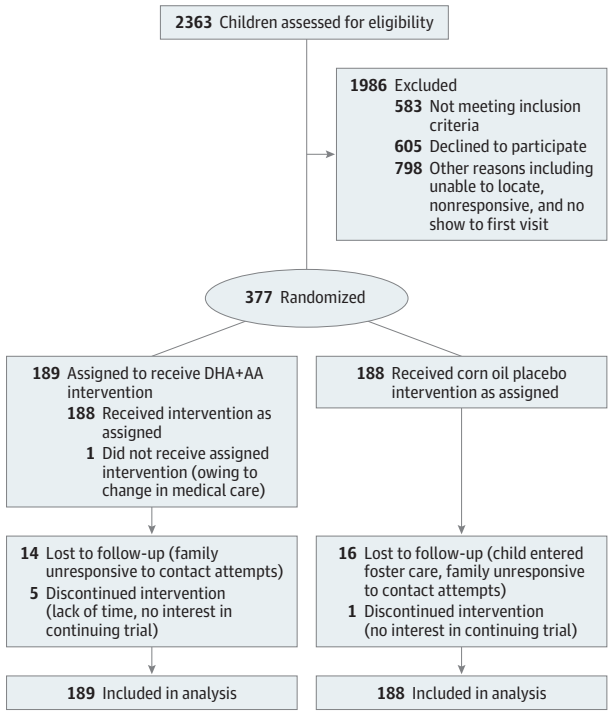
Participants and Trial Procedures

A census identified all children aged 10 to 16 months (adjusted for prematurity) born at less than 35 weeks' gestation who were former patients in 1 of 9 NCH-affiliated neonatal intensive care unit or referred to the neonatology clinic for clinical follow-up. Electronic medical records provided initial eligibility and neonatal data. Eligible children weighed between the 5th and 95th percentiles for corrected age and sex per World Health Organization standards,[20] had discontinued consumption of human milk and formula, and had English as their primary language. Children were excluded for consuming fatty acid supplements, fatty fish, or nutritional support beverages with DHA more than twice weekly; having allergies to fish, corn, or soy; planning to relocate; or having a major malformation or feeding, metabolic, or digestive disorder precluding participation or absorption. Recruitment occurred from April 26, 2012, to September 2, 2016; all children completed the study by March 24, 2017.

Caregivers were contacted by mail and telephone to invite participation. Three study visits occurred: baseline (day 0), interim (day 60 ± 14), and final (day 180 ± 20, at 16-22 months' corrected age). Baseline questionnaires included demographics, infant feeding, health care, and a brief food frequency questionnaire previously validated against blood fatty acid values for intake of DHA and eicosapentaenoic acid (EPA, a precursor to DHA).[21] Caregivers could select 1 or more race and ethnicity category for their child to enable comparison with the US preterm population. The Bayley Scales of Infant and Toddler Development, third edition (Bayley-III) was administered by a trained research assistant.[22] Bayley-III was selected to offer comparability with prior infant trials and because it is used clinically to evaluate preterm children.[15,17,23,24] It provides subscale composites for cognitive, language (receptive and expressive), and motor (fine and gross) skills (standardized mean, 100; SD, 15). Caregivers completed the Infant Behavior Questionnaire-Revised (IBQ-R; very short form) to assess effortful control (12 items) and activity level (3 items).[25] The IBQ-R measures early temperament-based inhibitory control, a key component of executive function as children mature. Scores range from 1 to 7, with higher scores indicating greater frequency of behaviors in the past week. Effortful control and activity level were selected as secondary outcomes because children born preterm often exhibit impairments in executive functions, such as working memory and effortful control, and dysregulated behaviors, such as hyperactivity and inattention, and these impairments are associated with poor academic outcomes.[26,27] Hyperactivity and inattention have been shown to be reduced with ω-3 supplementation.[28]

The final visit repeated the Bayley-III and food frequency questionnaire. Caregivers completed the Early Childhood Behavior Questionnaire very short form, the companion to the IBQ-R very short form but for older children, with demonstrated longitudinal continuity and identical score interpretations.[29,30] Caregivers were asked to guess their child's randomization assignment. Families with difficulty keeping appointments were rescheduled even if outside the target window.

Figure. Participant CONSORT Flow Diagram, Omega Tots Trial, 2012-2017



DHA+AA indicates daily microencapsulated docosahexaenoic acid, 200 mg, and arachidonic acid, 200 mg.

Randomization, Masking, and Intervention

Children were allocated to 1 of 4 masked color codes—2 for the treatment group, which received DHA and arachidonic acid (AA), and 2 for placebo—using a randomization scheme with varying block size of multiples of 4 and 8 and with 1:1 allocation to the DHA+AA group or placebo group (**Figure**). A statistician (A.N.T.) with no contact with participants prepared the scheme; assigned ID numbers; prepared opaque, sealed, tamper-resistant envelopes; and conducted the statistical analysis with another statistician (J.R.). Twins and triplets were randomized as sets to prevent crossover. Each sequentially numbered envelope was prelabeled with the next ID number and was opened by a research assistant to assign children to the DHA+AA group or placebo group upon caregiver's provision of written informed consent. All caregivers, investigators, and staff remained masked throughout. One of the statisticians (J.R.) was unmasked after data collection ended. The principal investigator (S.A.K.) was unmasked after analysis.

The DHA+AA group was assigned to 180 days of daily oral supplementation with dissolvable, microencapsulated DHA, 200 mg (from *Schizochytrium* species algal oil), and AA, 200-mg (from fungal *Mortierella alpina* oil) powder (DSM Nutritional Products). The dose was selected to approximate the peak daily DHA consumed per body weight during infancy (ie, for a typical 4-month-old infant, 20 mg/kg/d). The 180-day period was selected to ensure significant incorporation of DHA into neuronal cell membranes while maximizing enrollment and retention. This product is a dietary supplement under US Food and Drug Administration regulations, and an Investigational New Drug application was filed (No. 112885). The placebo group was assigned to receive 400 mg of daily microencapsulated corn oil powder. Both products came in identical foil packets and were dissoluble in milk. The investigational drug service dispensed the masked products directly to families.

Outcomes

The Bayley-III cognitive composite score was chosen a priori as the primary outcome measure because prior trials of preterm neonates have most consistently reported effects on global cognitive development measured with the Bayley Scales of Infant Development-2 Mental Development Index.[15,17,24,31] The Bayley-III language and motor composite scores and the IBQ-R and Early Childhood Behavior Questionnaire effortful control and activity level scores were specified a priori as secondary outcomes.

Fatty Acid Biomarkers

Blood was collected via venipuncture to examine erythrocyte fatty acid levels at baseline and the final visit. One funder of the study prohibited collection of biospecimens, so children enrolled after May 2015 (1 month after the funding started and procedures were put in place to manage this issue) were randomly allocated before recruitment to undergo blood collection or not. Red blood cells were separated from whole blood by centrifugation at collection and stored at -80°C. Esterified fatty acids were isolated using organic extraction, then hydrolyzed and methylated using standard techniques.[32,33] Resultant fatty acid methyl esters were separated and quantified using gas chromatography with flame ionization detection. The fatty acid contents were quantified using experimentally derived standard curves.

Adherence and Follow-up

Adherence was calculated as the packets consumed out of those dispensed based on diaries. Families were contacted regularly to address adherence and participation barriers.

Statistical Analysis

A sample size of 448 was planned to achieve greater than 80% power to detect a 4.2-point difference (0.27 SD) in Bayley-III cognitive composite scores with 10% loss to follow-up. The product manufacturer discontinued production per preexisting business plans once 377 participants were enrolled. This discontinuation had a negligible effect on power; a sample size of 377 provided 80% power to detect a 0.29-SD difference in Bayley-III scores. This effect size is similar to those reported for several subgroups in the largest DHA trial involving preterm neonates and would be meaningful at the population level.[15]

Except when noted otherwise, analyses were conducted according to intent to treat (all randomized children were included). No interim analyses were conducted. No correction for multiple comparisons was made because only 1 primary outcome was analyzed. The Bayley-III plus IBQ-R and Early Childhood Behavior Questionnaire continuous scores, calculated per the manuals and age-corrected for prematurity when pre-

scribed, served as the outcome measures.[22] To address statistical nonindependence because twins and triplets were included, all analyses included a random effect for family within a mixed model.

Analyses compared the change between baseline and the final visit in outcome scores between groups, controlling for baseline scores, using a mixed-effects regression approach (analogous to analysis of covariance) that leverages maximum likelihood to include children with missing outcome data.[34] Treatment-by-time interaction terms were included as fixed effects and served as estimates of treatment effect. No demographic or clinical characteristics were included as covariates. Group mean differences divided by the SD of the residuals from an analysis of covariance model were calculated as standardized effect sizes (ie, Cohen *d*) for each outcome based on the model already mentioned except that the random within-family effect was excluded to enable estimation of effect size using a conventional approach. A post hoc sensitivity analysis repeated the primary analysis, with outcome data treated as missing for children whose last visit occurred more than 200 days after randomization.

Prespecified subgroup analyses were conducted to explore effects by household income (≤$35 000 vs >$35 000), child sex, and birth weight (<1250 g vs ≥1250 g); the latter 2 variables mirror the trial by Makrides et al.[15] Interactions were tested by 3-way interaction terms including time, treatment, and the moderator. Post hoc analyses explored alternative specifications of categorical moderators (income, ≤$20 000 vs >$20 000 and birth weight as a continuous variable) when the prespecified analyses revealed interactions with $P < .20$.

The Bayley-III analysis was repeated with post hoc dichotomization at a score of 85 (mild or moderate delay) and analyzed using generalized estimating equations and logistic regression, adjusted for baseline score. The number of adverse events per child, the change in erythrocyte fatty acids, and the change in dietary DHA and EPA (aside from the supplement) were examined by treatment group using mixed-effects regression, with a random effect for within-family dependence. Analyses used SAS, version 9.3 (SAS Institute Inc). All *P* values were from 2-sided tests, and results were deemed statistically significant at $P < .05$.

Results

The children screened for eligibility, enrolled, and followed up through the trial are displayed in the Figure. All but 1 of 377 children received their allocated intervention. None of the children were randomized out of sequence. Primary outcome data were available for 338 children (89.7%; same proportion in each group). Characteristics were similar across treatment groups (**Table 1**). Gestational age ranged from 23 to 34 completed weeks, and 64 children (17.0%) were small for gestational age.

Adherence, Adverse Events, and Masking

Children consumed a mean of 80.6% of the packets (of children with adherence data, mean of 80.6% in the DHA+AA group and mean of 80.7% in the placebo group). No complaints about products' sensory characteristics (eg, taste or smell) were reported. A total of 256 children experienced at least 1 adverse event, totaling 683 events (1.7 events per child in the DHA+AA group and 2.0 events per child in the placebo group; group difference, -0.37; 95% CI, -0.07 to 0.80; $P = .10$), mainly minor gastrointestinal illness and respiratory tract infections. No adverse events were judged to be serious and related to the intervention. At the last visit, 69 of 126 caregivers with children in the DHA+AA group (54.8%) and 73 of 122 caregivers with children in the placebo group (59.8%) guessed that their child was assigned to the DHA+AA group ($P = .42$). No participant or staff was unmasked.

Erythrocyte Fatty Acids and Diet

Of 206 children assigned to undergo blood collection, 205 had analyzable samples at the baseline visit and 171 had analyzable samples at the final visit. Baseline DHA levels were low, and the DHA+AA group experienced statistically significantly greater mean (SD) increases in erythrocyte DHA and AA compared with the placebo group (reported as percentage of total fatty acid: DHA, 1.1% [1.0%] vs -0.1% [0.2%]; and AA, 2.0% [2.6%] vs -0.2% [1.1%]) (**Table 2**). Dietary DHA and EPA intake unrelated to the intervention increased similarly in both groups (median DHA+EPA intake at the end of the trial for DHA+AA group, 67.0 mg/d; placebo group, 60.0 mg/d; group difference in change, -0.70 mg; 95% CI, -20.9 to 19.5; $P = .95$).

Primary Outcome

The change in primary outcome did not differ between the DHA+AA group and placebo group (0.5-point difference in change; both groups declined, but DHA+AA experienced less decline; effect size = 0.05; $P = .66$) (**Table 3**). Mean (SD) baseline Bayley-III cognitive composite scores (based on corrected age, 100.5 [12.7]; 36 of 376 [9.6%] with a score <85) were similar to norms. No statistically significant interactions were observed between treatment assignment and income (≤$35 000 vs >$35 000), sex, or birth weight (<1250 g vs ≥1250 g). Supplementation had no effect on the odds of mild or moderate developmental delay (**Table 4**). The sensitivity analysis, in which the primary outcome was set to missing for 34 children whose final visit was late (median, 17 days), yielded results similar to the main analysis (-0.07-point difference in change; 95% CI, -2.50 to 2.36; $P = .95$).

Secondary Outcomes

No difference was detected between the DHA+AA and placebo groups in the change in Bayley-III language or motor composite scores. Mean (SD) language scores (92.9 [12.1]; 82 of 375 [21.9%] with a score <85) and motor scores (96.2 [12.5]; 51 of 375 [13.6%] with a score <85) at baseline were lower than norms. A significant interaction was observed with birth weight (continuous) in post hoc analysis for the language outcome, with a moderate negative effect of DHA+AA supplementation observed among children with lower birth weights (eg, a child with a birth weight of 1000 g assigned to receive DHA+AA experienced a relative 4.1-point decrease in language scores compared with a child assigned to receive placebo based on the model estimate; regression slope of treatment effect for 1-g in-

Table 1. Participant Demographic and Clinical Characteristics at Baseline

Characteristic	Participants, No. (%)		
	DHA+AA (n = 189)	Placebo (n = 188)	All (N = 377)
Maternal educational level[a]			
High school or GED or less	46/187 (24.6)	56/184 (30.4)	102/371 (27.5)
Some college or associate's degree	73/187 (39.0)	59/184 (32.1)	132/371 (35.6)
Bachelor's degree or higher	68/187 (36.4)	69/184 (37.5)	137/371 (36.9)
Spouse or partner educational level			
High school or GED or less	36/126 (28.6)	25/113 (22.1)	61/239 (25.5)
Some college or associate's degree	48/126 (38.1)	45/113 (39.8)	93/239 (38.9)
Bachelor's degree or higher	42/126 (33.3)	43/113 (38.1)	85/239 (35.6)
Maternal age, mean (SD), y	30.6 (6.1)	30.7 (7.1)	30.7 (6.6)
Maternal marital status			
Married or living with partner	126/185 (68.1)	115/175 (65.7)	241/360 (66.9)
Separated or divorced	7/185 (3.8)	8/175 (4.6)	15/360 (4.2)
Single: never married or not living with partner	52/185 (28.1)	52/175 (29.7)	104/360 (28.9)
Household size, median (IQR)	4.0 (3.0-5.0)	4.0 (3.0-5.0)	4.0 (3.0-5.0)
Public or no health insurance	96/188 (51.1)	91/184 (49.5)	187/372 (50.3)
Annual household income, $			
<20 000	43/188 (22.9)	42/184 (22.8)	85/372 (22.8)
20 000 to <35 000	42/188 (22.3)	47/184 (25.5)	89/372 (23.9)
35 000 to <45 000	38/188 (20.2)	37/184 (20.1)	75/372 (20.2)
≥45 000	65/188 (34.6)	58/184 (31.5)	123/372 (33.1)
Have a medical home	58/105 (55.2)	49/100 (49.0)	107/205 (52.2)
Sets of twins or triplets	29	26	55
Female sex	84 (44.4)	98 (52.1)	182 (48.3)
Child's race			
White	114 (60.3)	122 (64.9)	236 (62.6)
Black or African American	52 (27.5)	53 (28.2)	105 (27.9)
Asian or Pacific Islander	2 (1.1)	1 (0.5)	3 (0.8)
Other or multiple	21 (11.1)	12 (6.4)	33 (8.8)
Child's ethnicity, Hispanic	7 (3.7)	10 (5.3)	17 (4.5)
Gestational age at delivery, median (IQR), completed wk	32.0 (30.0-34.0)	32.0 (30.0-34.0)	32.0 (30.0-34.0)
Birth weight, mean (SD), g[b]	1705 (534)	1749 (570)	1727 (552)
Birth weight <1250 g	45/188 (23.9)	36 (19.1)	81/376 (21.5)
Small for gestational age	32/188 (17.0)	32 (17.0)	64/376 (17.0)
Child weight at baseline, mean (SD), g	10 164 (1041)	10 144 (1139)	10 154 (1090)
Attended neonatology follow-up clinic	102 (54.0)	107 (56.9)	209 (55.4)
Received community-based early intervention services	66 (34.9)	79 (42.0)	145 (38.5)
Received breast milk	168 (88.9)	163 (86.7)	331 (87.8)
Breast milk feeding duration, median (IQR), d	117 (44-269)	101 (41-209)	113 (44-239)
Dietary DHA+EPA intake before randomization, median (IQR), mg/d	44.3 (29.8-83.5)	55.3 (26.0-95.0)	48.0 (26.8-90.8)
Child age at baseline (corrected for prematurity), median (IQR), mo	15.7 (13.8-16.5)	15.6 (13.1-16.5)	15.7 (13.6-16.5)
Child age at baseline (unadjusted for prematurity), median (IQR), mo	17.3 (15.7-18.3)	17.4 (15.3-18.4)	17.3 (15.6-18.4)

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GED, General Education Development certificate; IQR, interquartile range.

[a] Children with missing data include 6 for maternal education, 2 for partner's education (of the 241 with a partner), 5 for maternal age, 17 for maternal marital status, 2 for household size, 5 for health insurance, 5 for income, 0 for medical home (only the 205 last-enrolled families were surveyed), 1 for birth weight and small for gestational age, 4 for breast milk feeding duration (among those who were fed breast milk), and 1 for dietary DHA+EPA intake.

[b] Birth weight ranged from 595 to 3545 g among those who received any breast milk (n = 167 in the DHA+AA group and n = 160 in the placebo group).

crease in birth weight, 0.0046; 95% CI, 0.0005-0.0088; $P = .03$ for interaction) (eTable in Supplement 2).

There was no overall treatment effect on the secondary outcomes of effortful control and activity level, but in prespecified subgroup analyses, children from households with income greater than $35 000 exhibited a small to medium decline in effortful control scores when assigned to the DHA+AA group compared with those assigned to the placebo group (difference in change, -0.3; effect size, -0.37; $P = .01$; $P = .17$ for interaction) (eTable in Supplement 2). Post hoc analysis revealed

Table 2. Changes in Erythrocyte Fatty Acid Levels[a]

Erythrocyte Fatty Acid[b]	Median (IQR), mol%				Change, Mean (SD), mol%		Difference in Change (95% CI), mol%
	Baseline		End of Trial				
	DHA+AA (n = 99)	Placebo (n = 106)	DHA+AA (n = 85)	Placebo (n = 88)	DHA+AA	Placebo	
Linoleic acid (18:2n-6)	30.2 (27.6 to 32.6)	29.3 (26.6 to 31.9)	28.5 (24.9 to 31.2)	29.9 (27.2 to 32.6)	−2.1 (5.2)	0.6 (4.4)	−2.2 (−3.4 to −0.9)
Arachidonic acid (20:4n-6)	5.7 (4.9 to 6.6)	5.6 (4.8 to 6.4)	7.7 (6.0 to 9.4)	5.4 (4.5 to 6.2)	2.0 (2.6)	−0.2 (1.1)	2.2 (1.7 to 2.8)
Total n-6	37.3 (34.1 to 39.4)	36.0 (33.7 to 38.9)	37.5 (33.8 to 40.2)	37.1 (33.6 to 39.7)	0.2 (6.0)	0.4 (5.0)	0.4 (−1.0 to 1.7)
α-Linolenic acid (18:3n-3)	0.7 (0.5 to 0.9)	0.7 (0.5 to 0.9)	0.6 (0.5 to 0.8)	0.6 (0.5 to 0.7)	−0.1 (0.4)	0.0 (0.3)	0.0 (−0.1 to 0.1)
Eicosapentaenoic acid (20:5n-3)	0.4 (0.3 to 0.4)	0.4 (0.3 to 0.4)	0.3 (0.3 to 0.4)	0.3 (0.3 to 0.4)	0.0 (0.1)	−0.1 (0.2)	0.0 (0.0 to 0.1)
Docosapentaenoic acid (22:5n-3)	0.4 (0.4 to 0.5)	0.4 (0.4 to 0.5)	0.3 (0.3 to 0.4)	0.4 (0.4 to 0.5)	−0.1 (0.1)	0.0 (0.1)	−0.1 (−0.1 to 0.0)
Docosahexaenoic acid (22:6n-3)	1.0 (0.8 to 1.1)	0.9 (0.8 to 1.1)	2.3 (1.1 to 3.0)	0.8 (0.7 to 1.1)	1.1 (1.0)	−0.1 (0.2)	1.2 (1.0 to 1.5)
Total n-3	2.5 (2.2 to 2.8)	2.5 (2.3 to 2.8)	3.6 (2.6 to 4.3)	2.3 (2.1 to 2.6)	0.9 (1.1)	−0.2 (0.5)	1.2 (0.9 to 1.4)
n-6:n-3 ratio	14.9 (13.0 to 16.4)	14.4 (12.7 to 15.6)	10.6 (8.4 to 12.8)	15.9 (14.2 to 17.8)	−3.2 (3.9)	1.6 (2.8)	−4.6 (−5.5 to −3.7)

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; IQR, interquartile range; mol%, percentage of total quantified fatty acids.

[a] A total of 205 children had analyzable samples at the baseline visit.

[b] The DHA+AA supplement contained only DHA and AA. Because units are determined as a percentage of total fatty acids, other fatty acids may show a decrease because DHA and AA increased.

Table 3. Change From Baseline to Trial Completion in Developmental Outcomes

Outcome	DHA+AA/Placebo, No. at Baseline	Baseline Score, Mean (SD)		DHA+AA/Placebo, No. at End of Trial	Score at End of Trial, Mean (SD)		Change, Mean (SD)		Difference in Change (95% CI)[a]	ANCOVA Effect Size	*P* Value
		DHA+AA	Placebo		DHA+AA	Placebo	DHA+AA	Placebo			
Cognitive composite	188/188[b]	99.7 (13.9)	101.3 (11.4)	169/169	97.7 (11.8)	97.7 (11.5)	−2.6 (13.3)	−3.7 (12.2)	0.5 (−1.8 to 2.8)	0.05	.66
Language composite	187/188	92.5 (12.1)	93.2 (12.1)	169/169	92.5 (12.3)	93.2 (13.2)	−0.5 (12.0)	0.2 (12.6)	−0.7 (−3.1 to 1.6)	−0.07	.55
Motor composite	187/188	96.1 (13.8)	96.3 (11.1)	168/169	96.3 (10.9)	96.0 (12.5)	−0.6 (12.5)	−0.4 (12.7)	0.2 (−2.1 to 2.4)	0.02	.88
Effortful control	188/185	5.3 (0.7)	5.2 (0.8)	160/163	4.6 (0.7)	4.7 (0.8)	−0.7 (0.8)	−0.5 (0.8)	−0.1 (−0.3 to 0.0)	−0.16	.13
Activity level	188/186	4.8 (1.2)	4.9 (1.2)	159/163	5.4 (1.1)	5.4 (1.1)	0.6 (1.5)	0.5 (1.5)	0.0 (−0.2 to 0.3)	0.01	.76

Abbreviations: AA, arachidonic acid; ANCOVA, analysis of covariance; DHA, docosahexaenoic acid.

[a] Difference in Change column is based on a mixed-effects model (analogous to ANCOVA) using maximum likelihood to account for missing data (39 children did not participate in the last trial visit) and thus may differ from the results of the raw summary statistics presented for the within-group mean change and SD. Positive numbers indicate the effect tended to favor DHA even if not statistically significant.

[b] One child's baseline Bayley-III assessment was deemed invalid.

Table 4. Odds of Mild or Moderate Developmental Delay

Outcome	DHA+AA/Placebo, No. at Baseline	Baseline Score <85, No. (%)		DHA+AA/Placebo, No. at End of Trial	Trial End Score <85, No. (%)		Odds Ratio (95% CI)[a]
		DHA+AA	Placebo		DHA+AA	Placebo	
Cognitive composite	188/188	21 (11.1)	15 (8.0)	169/169	14 (8.3)	11 (6.5)	1.26 (0.55-2.91)
Language composite	187/188	43 (23.0)	39 (20.7)	169/169	41 (24.3)	38 (22.5)	1.03 (0.59-1.78)
Motor composite	187/188	25 (13.4)	26 (13.8)	168/169	14 (8.3)	20 (11.8)	0.81 (0.36-1.81)

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid.

[a] All models adjusted for baseline score (<85 vs ≥85).

that the effect applied to all groups with income greater than $20 000, while children from families with income of $20 000 or less experienced no negative effect (difference between treatment and placebo groups in change comparing ≤$20 000 and >$20 000 groups, -0.44; 95% CI, -0.81 to -0.06; P = .02).

Two other interactions with .05 < P < .20 were observed: income and motor composite scores as well as sex and activity level. In post hoc analyses, alternative specification of the income variable did not reveal a statistically significant result for the test for interaction.

Discussion

In this randomized, masked trial of 1-year-old children born preterm, 6 months' daily DHA+AA supplementation did not improve cognitive development compared with placebo. Motor development and activity level ratings were also unaffected. Subgroup analyses by income, sex, and birth weight with prespecified categories (≤$35 000 vs >$35 000 and <1250 g vs ≥1250 g) suggested treatment effects in certain birth weight groups on the secondary outcome of language and treatment effects in certain income groups on the secondary outcome of effortful control. Specifically, DHA supplementation resulted in moderate negative effects on early language ability for the children with the lowest birth weight and moderate declines in effortful control among children from all but the poorest households.

To our knowledge, this is the first trial to test the effect of supplementation on developmental outcomes among children born preterm just after weaning to cow's milk and foods low in long-chain ω-3 fatty acids. Despite the developmental differences between infants and toddlers, comparison of our findings with prior trials of preterm neonates is informative. The findings for our primary outcome are compatible with the largest trial of Australian preterm neonates that reported no overall short-term cognitive benefit from high-dose DHA based on the Bayley Mental Developmental Index at age 18 months.[15] However, girls in that study assigned to receive high-dose vs low-dose DHA exhibited a 4.5-point advantage on the Bayley Mental Developmental Index. Other trials in infants have reported no cognitive benefit at 12 to 18 months or reported benefits specific to boys only.[17,35] We observed no sex-specific differences in our study.

Makrides et al[15] also observed a 43% reduced risk of mild mental delay from high-dose supplementation in children with birth weights less than 1250 g. Our result for poorer language outcomes among children with the lowest birth weight who received DHA contrasts that finding, although the Bayley-II, used in the study by Makrides et al,[15] did not separately assess language. For the smallest children who are at particular risk for language delay, this possible negative effect is concerning.

To our knowledge, this is the first trial involving preterm children to consider socioeconomic status as a potential treatment effect modifier. The moderate negative effect of DHA on effortful control among all but the very lowest income groups may have been a chance finding and should be interpreted with caution but is concerning given the importance of self-regulation for long-term academic and social outcomes.[36,37] The previously mentioned Australian trial[15] and another trial that studied DHA supplementation in pregnant women reported poorer parent-reported executive function and more dysregulated behavior in their intervention groups when followed up to 4 or 7 years of age.[38-40] Although the Omega Tots toddlers in our study were too immature to evaluate fine-grained aspects of executive function, ours is the earliest report of negative effects in this domain to our knowledge. Effortful control was reported by caregivers, but caregivers were poor at guessing their child's group assignment and so were unlikely to give biased reports.

Effects may have been isolated to the children with the lowest birth weight and higher income groups perhaps because these children were not truly DHA deficient, and supplementation upset a previously healthy balance of fatty acids in the brain, resulting in poorer neurotransmitter function and consequent changes in behavior in children in the higher income groups. The ω-6 to ω-3 ratio has previously been shown to be associated with executive function in children.[41] However, baseline DHA status in this sample appeared to be lower than reported for several other samples of young children, although measurement units vary across studies.[42-44]

Strengths and Limitations

Strengths of this study include its large, socioeconomically diverse sample, broad inclusion criteria to improve generalizability to the preterm population, masking, and multiinformant assessment of outcomes. The sample was smaller than planned, but this factor had a negligible effect. The study was powered for the primary outcome, not to detect

subgroup differences. Some children were lost to follow-up, but our statistical methods leveraged their baseline data in estimating effects. As a tradeoff for our broad inclusion criteria, the median gestational age of children in this study was higher than in some infant trials. This increased heterogeneity may have hampered the detection of treatment effects specific to children with the lowest birth weights. Finally, the children were too young for robust evaluation of executive function and cognitive ability, which limited estimation of long-term outcomes, but follow-up would help clarify whether supplementation during toddlerhood has detectable effects later.

Conclusions

Among 1-year-old children born at less than 35 weeks' gestation, DHA+AA supplementation had no developmental benefit vs placebo. Small- to medium-magnitude negative effects were observed for language ability among children of lower birth weight and for effortful control for children from all but the very lowest income strata. Ongoing follow-up is needed, particularly given the ubiquity of DHA-supplemented formulas and foods for children and the cognitive deficits associated with prematurity.

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