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# Cognitive function in 18-month-old term infants of the DIAMOND study: A randomized, controlled clinical trial with multiple dietary levels of docosahexaenoic acid ☆

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ABSTRACT

*Background:* Studies investigating cognitive outcomes following docosahexaenoic acid (DHA) supplementation of infant formula yield conflicting results, perhaps due to inadequate dietary concentrations.
*Aim:* To determine the optimal DHA concentration in term formula to support cognitive maturation.
*Design:* This was a double-masked, randomized, controlled, prospective trial. A total of 181 infants were enrolled at 1–9 days of age and assigned randomly to receive one of four term infant formulas with one of four levels of docosahexaenoic acid: Control (0% DHA), 0.32% DHA, 0.64% DHA, or 0.96% DHA. All DHA-supplemented formulas contained 0.64% arachidonic acid (ARA). Infants were fed the assigned formulas until 12 months of age. One hundred forty-one children completed the 12-month feeding trial and were eligible for this study. Cognitive function was assessed in 131 children at 18 months of age using the Bayley Scales of Infant Development II (BSID II).
*Results:* There were no diet group differences on the Mental Development Index (MDI), the Psychomotor Development Index (PDI), or the Behavior Rating Scale (BRS) of the BSID II. However, when the scores of children who received any of the three DHA-supplemented formulas were combined and compared to control children, a significant difference emerged: the MDI scores of DHA-supplemented children were higher (104.1 v. 98.4; $p = 0.02$).
*Conclusions:* These results suggest that dietary supplementation of DHA during the first year of life leads to enhanced cognitive development at 18 months of age. DHA concentration of 0.32% is adequate to improve cognitive function; higher concentrations did not confer additional benefit.


Docosahexaenoic acid (DHA) is a conditionally essential long-chain polyunsaturated fatty acid (LCPUFA) found in high concentrations in the phospholipid membranes of the central nervous system. Beginning in the last trimester and throughout the first 18 months of life, the brain undergoes a rapid growth spurt, during which large amounts of DHA are accrued. Prenatally, DHA is obtained from maternal stores, whereas postnatally, it is obtained from breast milk or infant formula. Given the close correspondence between brain growth and the accretion of DHA, it is widely speculated that this fatty acid is critical to cognitive development. Indeed, several studies indicate that breastfeeding, a natural source of DHA, is associated with improved cognitive function [1–6]. In addition, maternal supplementation with DHA during pregnancy and lactation via consumption of high-DHA eggs, fish oil, or capsules results in enhanced cognitive development in the infant [7–9]. Recently, studies investigating the effects of DHA levels in umbilical cord at parturition, a more direct measure of prenatal accretion, report that DHA levels are associated with cognitive development [10]. In contrast, low DHA concentrations in umbilical cord at parturition are associated with greater risk for neurologic impairment [11].

Although these findings suggest that supplementation of infant formulas with DHA might provide cognitive benefits, results are mixed as some studies report beneficial effects [12–15] whereas others do not [16–19]. While the lack of beneficial effects in some studies might be due to insensitive testing procedures, limited statistical power, source of DHA, duration of supplementation, etc., it is also possible that these studies used concentrations of DHA that were too low to confer cognitive benefits. To date, each term formula study has compared only a single DHA concentration to a control formula lacking DHA. Collectively, the range of concentrations implemented in these studies are at the low end of the concentrations

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that have been reported in breastmilk worldwide (0.06–1.4% by weight [20,21]). Therefore, it is plausible that the optimal DHA concentration for the maturation of cognitive function is at a higher concentration than has been studied previously.

In an effort to determine the optimal DHA concentration in infant formula to support cognitive development, we conducted a double-masked, randomized, controlled, prospective trial known as the DIAMOND Study (DHA Intake And Measurement Of Neural Development [22]). This study is of particular importance because it is the first to evaluate cognitive outcomes in infants randomly assigned to multiple DHA concentrations that are representative of the full range found in breastmilk worldwide. Thus, we are able to compare the cognitive effects of several different DHA concentrations. Infants were fed formulas containing one of four different concentrations of DHA supplementation ranging from 0% to 0.96% of total fatty acids for the first 12 months of life. Each infant's cognitive function was assessed at 18 months of age.

## 1. Methods

### 1.1. Participants

Eligible participants were all children who had enrolled in the initial phase of the DIAMOND study [22] at the Dallas site, and had completed the 12-month feeding protocol and the 12-month primary outcome visit (141 children). The DIAMOND study is a prospective, randomized, controlled trial assessing the developmental effects of 3 concentrations of DHA-supplemented formulas as compared to a control formula with no DHA. Phase I of the DIAMOND study was a two-site, randomized, controlled trial conducted in Dallas and Kansas City with visual evoked potential (VEP) acuity at 1 year of age as the primary outcome. Using the sweep VEP paradigm, visual acuity was assessed by measuring the amplitude and phase of evoked potentials for a range of patterned stimuli to determine the finest pattern that evoked a reliable cortical response. These data, which showed a benefit of DHA supplementation of $\geq$0.32% of total fatty acids for VEP acuity at 12 months, and significant differences among all groups in red blood cell (RBC) DHA concentration, were published recently [22]. After completing the primary outcome, each site independently proposed follow-up studies (Phase II) that were reviewed separately by the sponsor (Mead Johnson & Co., Evansville, IN). Although there may be an overlap in the research proposals, the two study sites did not attempt to harmonize protocols, data collection, or data analyses. Thus, the data presented here are from Phase II of the DIAMOND study and include children tested at the Dallas site only.

Participants were born at one of four participating hospitals in the Dallas area between September 2003 and September 2005. Only healthy, term (37 to 42 weeks gestation), formula-fed singleton births with birthweight appropriate-for-gestational-age (2490 to 4200 g) were included in the trial. Infants who had diseases or congenital abnormalities known to affect growth, development, visual or cognitive maturation, or who had poor formula intake did not participate in the study. Infants were also excluded if they had received human milk within 24 h of randomization, or if they were born to mothers with chronic illness such as HIV disease, renal or hepatic disease, type 1 or type 2 diabetes, alcoholism, or substance abuse.

Parents of eligible neonates were provided with a brief information sheet describing the study only after hospital records indicated that they had elected to formula feed exclusively. The sheet included the American Academy of Pediatrics' (AAP) recommendation for breast-feeding through 12 months of age. The letter asked the parents, if they were interested, to call the Study Coordinator before the child was 5 days old to schedule an appointment to obtain written informed assent, randomize, and dispense formula. Infants were recruited from four hospitals to ensure ethnic and economic diversity. The study observed the tenets of the Declaration of Helsinki, and was approved by institutional review boards at Presbyterian Hospital, Medical City Hospital, Arlington Memorial Hospital, and the University of Texas Southwestern Medical Center.

### 1.2. Protocol

Infants were randomly assigned between one and nine days of age to receive one of four cow's milk-based term infant formulas described below. The study sponsor used a computer-based random number generator to create a randomization list. Each formula had two codes for a total of 8 codes, and only the study sponsor knew which code designated which study formula. After obtaining signed assent from a parent, the study coordinator opened the next sequentially-numbered opaque sealed envelope to determine the code of the study formula to be assigned to that infant. All recruiting personnel, parents or guardians, study monitors, researchers, and pediatricians were masked to the infant's assigned formula.

Infants were fed the assigned formula until 12 months of age. This duration was chosen because it reflects the typical duration over which infant formula is provided to infants. Importantly, studies that implement long durations of DHA-supplemented formula feeding are more likely to report enhanced cognitive development than those with shorter durations of supplementation ([23–25], see Discussion below).

Formula was the lone source of nutrition until the introduction of additional foods, as directed by the infants' physician, at 4–6 months of age. No limits were placed on the amount of formula provided to the infant, however, families were instructed not to provide commercial DHA-enriched foods until cessation at 12 months of age. Blood lipid data were collected at 4 and 12 months of age. Cognitive function was assessed at 18 months $\pm$ 2 weeks.

### 1.3. Assessment of cognitive function

Cognitive development was assessed by one of two study authors (SG and SEM) using the Bayley Scales of Infant Development, 2nd edition (BSID II) [26], arguably, the gold standard test of cognitive and motor development from birth to 42 months of age [10,27]. The BSID II consists of three scales or indices. The Mental Development Index (MDI) evaluates memory, problem-solving, discrimination, classification, and language skills. The Psychomotor Development Index (PDI) evaluates the control of both gross motor and fine motor muscle groups and includes standing, jumping, walking, running, prehension, use of writing implements, and imitation of hand movements. The third scale, the Behavior Rating Scale (BRS) evaluates relevant aspects of behavior during test-taking which include emotional regulation, quality of movement, and orientation/engagement. The MDI and PDI scores were converted to standardized scores with a mean of 100 and a standard deviation of 15. Developmental ages for three facets (cognitive, language, and motor) of mental and motor development were analyzed from BSID II results to determine strengths and weaknesses. All BRS items are scored appropriate to the child's age and reported as centile scores; average performance is at the 50th percentile while performance below the 10th percentile is considered non-optimal.

Importantly, the choice of test and age of assessment were based on a previous report from our laboratory that MDI scores at 18 months of age were predictive of Performance IQ, Verbal IQ, and Full IQ of the Wechsler preschool and primary scale of intelligence at 4 years of age [13]. Thus, if a beneficial cognitive effect was found in the present study, it might translate to improved intelligence at 4 years.

### 1.4. Fatty acid analysis

To determine levels of DHA, arachidonic acid (ARA), linoleic acid (LA), and α-linolenic acid (ALA) in RBCs at 4 and 12 months of age, 2.0 mL blood samples were collected by heel stick aided by infant heel

warming packs into EDTA-containing tubes. Fatty acid analyses were conducted under the direction of two of the authors (DH and DW) following methods described previously [28]. Fatty acids were expressed as mass concentration (μg/mL of packed RBCs) based on the addition of internal standard (23:0 fatty acid).

### 1.5. Diet groups

Participants were assigned randomly to one of four cow's milk-based term infant formulas that had the same nutrient levels and ingredients except for DHA and ARA: control with no DHA or ARA (Enfamil® with iron as marketed at the time of the study; Mead Johnson & Co, Evansville, IN); 0.32% DHA with 0.32% fatty acids from DHA (17 mg/100 kcal; marketed as Enfamil LIPIL®); 0.64% DHA, (34 mg/100 kcal); and 0.96% DHA (54 mg/100 kcal). All DHA-supplemented formulas also provided 0.64% ARA (34 mg/100 kcal). Both DHA and ARA were obtained from single cell oils (Martek Biosciences, Columbia, MD).

### 1.6. Sample size and statistical analyses

Sample size was based on the primary outcome for the DIAMOND clinical trial, VEP visual acuity at 12 months of age [22]; i.e., 37 participants per formula group. We anticipated that 75% of the children ($n \geq 28$ per group) enrolled would complete BSID II testing at the 18-month visit. Nutrition studies conducted by our laboratory involve relatively homogenous samples, with all infants tested at the same location, and all assessments conducted by one of two testers who were trained together and harmonized their testing protocol. As a result, we have reported smaller SDs (e.g., 8–11 points on the MDI; [29]) than the population-based studies on which the BSID was standardized ($SD = 15$). With our SDs and an expected sample size of 28 per group at 18 months, we had 75% power to detect a 7 point difference on the MDI (the difference between control and 0.36% DHA supplementation reported by Birch et al. in an earlier study of BSID II outcomes at 18 months of age [29]).

Statistical comparisons for MDI and BRS were conducted using one-way analyses of variance (ANOVA). Because scores on the PDI did not meet normality criteria (Kolmogorov–Smirnov, $p < .05$), these data were analyzed using a Kruskal–Wallis ANOVA. Cognitive, language, and motor developmental facet age scores were also analyzed. These data are ordinal and were analyzed using Kruskal–Wallis ANOVAs. All relevant aspects of behavior evaluated as part of the BRS (emotional regulation, quality of movement, and orientation/engagement) were also analyzed to determine whether there were significant diet group differences. Emotional regulation was distributed normally and analyzed using ANOVA. Quality of movement and orientation/engagement were not distributed normally and were analyzed using Kruskal–Wallis ANOVAs. For all BSID II scales/facets/aspects of behavior, scores of the three DHA-supplemented groups (i.e., 0.32% DHA, 0.64% DHA, and 0.96% DHA) were combined and compared to the control group. If the data from the outcome measure being compared were distributed normally, this comparison was conducted using an ANOVA. If the data from the outcome measure was ordinal and/or not distributed normally, the comparison was conducted using a Mann–Whitney *U* test. Analyses were also conducted to determine the correlations between each of the BSID II scales/facets/aspects of behavior and fatty acid levels in RBCs or sweep VEP visual acuity (logMAR) at 12 months of age [22]. Note that neither RBC-DHA at 4 months of age, nor RBC-LA at 12 months of age was distributed normally (Kolmogorov–Smirnov, $p < .05$). VEP scores and all other fatty acid concentrations in RBCs were distributed normally (Kolmogorov–Smirnov, $p > .05$). If both variables in the correlational analyses were distributed normally, Pearson product correlation coefficients ($r$) were calculated. If one or both variables failed normality tests, Spearman correlation coefficients ($r_s$) were calculated. All analyses were conducted using the Statistica 7.1 software package (StatSoft Inc, Tulsa, OK).

## 2. Results

Of the 181 children enrolled in the DIAMOND study at the Dallas site, 141 (34–36 per diet group) completed the 12-month feeding protocol and were eligible for the 18-month cognitive assessment. Of the 141 eligible children, 131 (93%) completed the 18-month visit (Fig. 1). We were unable to locate and/or schedule 8 children, 1 child was deceased, and 1 parent declined participation. Across all four diet groups, a total 14 children were excluded from the BSID II analysis because the tester (who was blind to the diet assignment) reported that they were tested outside the two-week window ($n = 3$), they had questionable (≤25th percentile) behavioral ratings ($n = 3$), they came from bilingual homes and had speech delay associated with that home environment ($n = 4$), or they were too ill to cooperate fully at the time of testing ($n = 4$). Thus, data from 117 children (28–32 per diet group) were used for the analyses.

### 2.1. Demographic data

Complete demographic data for each diet group are provided in Table 1. To test whether diet groups differed on demographic variables, ANOVAs (age at testing, maternal age and height, and paternal age), Kruskal–Wallis ANOVAs (maternal weight, paternal weight and height), Chi-square analyses (sex and race), or Fisher exact tests (maternal/paternal education) were conducted. There were no significant differences between diet groups (see table for exact p values).

### 2.2. Mental Development Index

The results of three components of the BSID II are presented by diet group in Table 2. The table indicates that on the MDI scale, the mean score of the control group was slightly lower than the normative score of 100, while each of the supplemented groups had a mean score slightly higher than the normative score. Nevertheless, there were no significant diet group differences on the MDI subscale ($F_{3,113} = 2.06$; $p = .110$). Note, however, when the scores of all children who received any of the three DHA-supplemented formulas were combined and compared to children fed control formula, a significant difference emerged ($F_{1,115} = 5.45$, $p = .021$); i.e., overall, the mean score of all DHA-supplemented children was significantly higher than that of control children. Correlational analyses indicated that scores on the MDI did not correlate significantly with RBC concentrations of DHA, ARA, ALA, or LA at 4 months or 12 months of age (all $p > .05$). MDI scores were significantly correlated with VEP visual acuity at 12 months of age ($r = -0.30$, $p = .001$) indicating that better VEP visual acuity at 12 months (.i.e., lower logMAR) was associated with a better MDI score at 18 months.

### 2.3. Psychomotor Development Index

Median PDI scores by diet group are provided in Table 2. Kruskal–Wallis ANOVA indicated that there was no significant effect of diet group on the PDI ($H = 4.49$, $p = .213$). In addition, there were no significant diet group differences when PDI scores of all three DHA-supplemented groups were combined and compared to the control groups (*Mann–Whitney* $U = 983.5$, $p = .089$). Correlational analyses revealed that PDI correlated negatively with RBC-LA concentrations at 4 months of age ($r_s = -0.18$, $p = .049$) indicating that children with higher RBC-LA had lower PDI scores. PDI scores were not correlated with any other RBC fatty acid concentrations, nor were they correlated with VEP scores at 12 months of age (all $p > .05$).

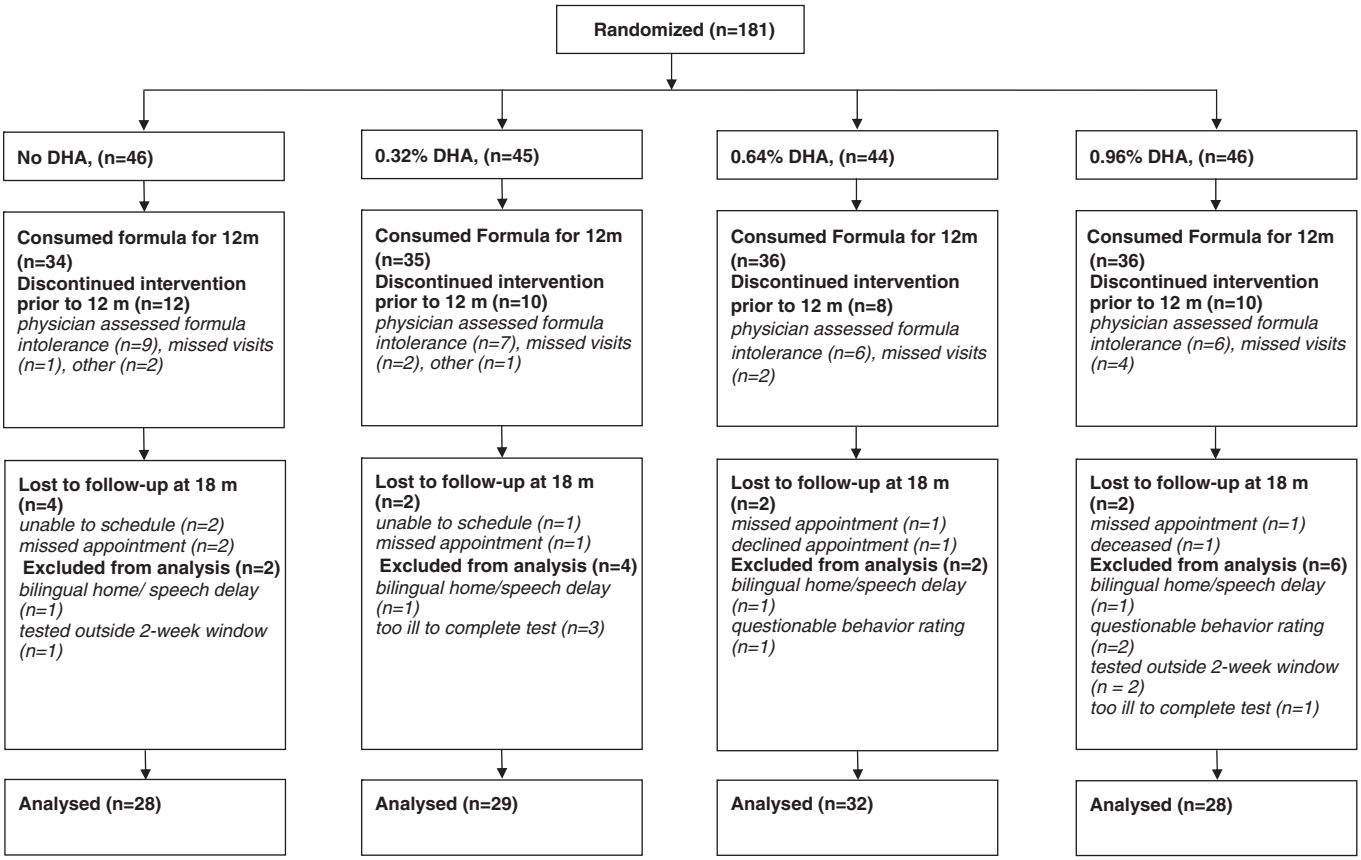


**Fig. 1.** Flow chart showing study completion in each formula group.

### 2.4. Behavior rating scales

Although each supplemented group scored higher than the control group on the BRS (see Table 2), there were no significant diet group differences ($F_{3,113} = 0.627$; $p = .599$). As with the MDI and PDI analyses, the BRS scores of all three-DHA-supplemented groups were combined and compared to the control group. Yet again, there was no significant difference (BRS: $F_{1,115} = 0.828$, $p = .365$). BRS scores did not correlate significantly with fatty acid levels at 4 or 12 months of age or with VEP scores (all $p > .05$). The aspects of behavior that comprise the BRS (emotional regulation, quality of movement, and orientation/engagement) were also analyzed. There were no significant diet group differences on any of these aspects (all $p > .05$). When the three DHA-supplemented groups were combined and compared to the control group, a significant difference emerged only on emotional regulation ($F_{1,115} = 4.53$, $p = .035$). Emotional regulation scores were higher in the DHA-supplemented groups than in the control group (means: 70.0 v. 61.5, respectively). Correlational analyses revealed that both emotional regulation and quality of movement were negatively correlated with VEP scores at 12 months of age (emotional regulation: $r = -0.27$, $p = .003$; quality of movement: $r_s = -0.21$, $p = .024$) such that children with better visual acuity had better scores on these two BRS behaviors. Emotional regulation was also negatively correlated with RBC-LA at 12 months of age ($r_s = -0.19$, $p = .040$) indicating that higher RBC-LA was associated with poorer scores on emotional regulation. There were no other significant correlations between BRS aspects and fatty acid concentrations in RBCs (all $p > .05$).

### 2.5. Cognitive, language, and motor facets

Mean developmental ages for cognitive, language, and motor facets are provided by diet group in Table 2. Although the control group scored the lowest on all three facets, there were no significant diet group differences. When DHA-supplemented groups were combined and compared to the control group, a significant difference emerged on the language facet (*Mann–Whitney* $U = 847.50$, $p = .010$) as the DHA-supplemented group scored higher than the control group. The groups did not differ significantly on the cognitive or motor facets.

Both cognitive and language facets were correlated with VEP scores at 12 months of age (Cognitive: $r_s = -0.23$, $p = .014$; Language: $r_s = -0.24$, $p = .009$), indicating that better performance on these facets was associated with better visual acuity. Motor facet and VEP scores were not correlated significantly ($r_s = -0.16$, $p = .079$). Both the cognitive and language facets were negatively correlated with RBC-LA at 4 months of age (Cognitive: $r_s = -0.19$, $p = .039$; Language: $r_s = -0.21$, $p = .022$) such that children with higher RBC-LA had poorer cognitive and language facet scores. In addition, the language facet and RBC-LA at 12 months of age were negatively correlated ($r_s = -0.19$, $p = .041$). There were no other significant correlations between these facets and fatty acid concentrations in RBCs.

## 3. Discussion

This study is the first double-masked, randomized, controlled, prospective trial to evaluate the cognitive effects of a wide range of DHA concentrations provided in infant formulas. While there were no significant diet group differences, when scores of all DHA-supplemented groups were combined, these groups had a significantly higher mean score on the MDI component of the BSID II compared to children who received control formula. Two facets of the BSID II, language developmental age and cognitive developmental age were also higher in the combined DHA-supplemented groups than in the control group. Yet, it should be noted that developmental age equivalents on these

**Table 1**
Demographic characteristics of diet groups.

| | Formula group | | | | | |
|---|---|---|---|---|---|---|
| | Control (n = 28) | 0.32% DHA (n = 29) | 0.64% (n = 32) | 0.96% (n = 28) | P values for comparisons across diet groups | Combined DHA groups (nc89) |
| Gender (M/F) | 14/14 | 16/13 | 20/12 | 15/13 | 0.80 | 51/38 |
| White/minority (%) | 68/32 | 79/21 | 56/44 | 75/25 | 0.22 | 70/30 |
| Age at testing (m) | 18.1 ± 0.2 | 18.1 ± 0.2 | 18.1 ± 0.2 | 18.1 ± 0.2 | 0.47 | 18.1 ± 0.2 |
| Maternal age (y) | 31.2 ± 4.6 | 30.9 ± 4.2 | 31.6 ± 4.5 | 30.1 ± 3.7 | 0.58 | 30.9 ± 4.2 |
| Maternal weight (kg) | 73.7 ± 20.9 | 68.7 ± 19.8 | 72.8 ± 18.6 | 68.5 ± 15.7 | 0.61 | 70.1 ± 18.1 |
| Maternal height (m) | 1.64 ± 0.08 | 1.65 ± 0.07 | 1.64 ± 0.08 | 1.62 ± 0.07 | 0.41 | 1.64 ± 0.07 |
| Paternal age (y) | 33.0 ± 5.6 | 33.2 ± 3.9 | 34.7 ± 5.6 | 33.1 ± 4.1 | 0.47 | 33.7 ± 4.7 |
| Paternal weight (kg) | 94.2 ± 20.6 | 93.4 ± 17.4 | 92.7 ± 15.7 | 92.3 ± 17.0 | 0.94 | 92.8 ± 16.5 |
| Paternal height (m) | 1.80 ± 0.07 | 1.82 ± 0.06 | 1.80 ± 0.07 | 1.80 ± 0.05 | 0.49 | 1.81 ± 0.06 |
| Maternal Education *(n) | | | | | 0.13 | |
| Did not complete high school | 0 | 0 | 0 | 0 | | 0 |
| Completed high school | 13 | 8 | 10 | 11 | | 29 |
| Completed college | 10 | 14 | 16 | 17 | | 47 |
| Postgraduate | 5 | 7 | 6 | 0 | | 13 |
| Paternal education *(n) | | | | | 0.144 | |
| Did not complete high school | 0 | 0 | 1 | 0 | | 1 |
| Completed high school | 9 | 11 | 6 | 9 | | 26 |
| Completed college | 17 | 11 | 23 | 15 | | 49 |
| Postgraduate | 2 | 7 | 2 | 4 | | 13 |

* Analysis was conducted based on distribution of highest level of education received across diet groups.

facets are not as psychometrically sound as scores on the MDI and PDI. Specifically, the determination of item difficulty for these facets is less precise, often leading to discrepancies when compared to the MDI and PDI. Thus, these developmental age results must be interpreted with caution.

The results presented here suggest that a DHA concentration of 0.32% is adequate to improve cognitive function and that higher concentrations did not confer additional benefits on MDI scores at 18 months of age. This pattern of results is similar to that of the primary outcome study in which all DHA-supplemented groups had

**Table 2**
Means, medians, and upper and lower quartiles for BSID II.

| Index | Control | 0.32% DHA | 0.64% DHA | 0.96% DHA | P values for group comparisons | Combined DHA groups | P values for control v. combined DHA groups |
|---|---|---|---|---|---|---|---|
| *MDI* | | | | | | | |
| Mean (SD) | 98.4 (13.1) | 105.2 (10.7) | 104.2 (9.8) | 102.6 (11.9) | 0.11 | 104.1 (10.7) | 0.02* |
| Median | 95.5 | 105 | 105 | 106 | | 105 | |
| 25th and 75th percentile | 91 to 109 | 99 to 111 | 97 to 113 | 97 to 111 | | 97 to 112 | |
| *PDI* | | | | | | | |
| Mean (SD) | 102.0 (6.3) | 105.8 (9.5) | 106.8 (8.2) | 104.1 (11.3) | 0.21 | 105.6 (9.6) | 0.09 |
| Median | 103 | 103 | 107 | 103 | | 103 | |
| 25th and 75th percentile | 100 to 104 | 99 to 107 | 103 to 109 | 97 to 111 | | 99 to 110 | |
| *BRS* | | | | | | | |
| Mean (SD) | 73.5 (17.5) | 79.1 (17.7) | 77.9 (19.5) | 74.3 (19.0) | 0.60 | 77.2 (18.7) | 0.37 |
| Median | 68 | 80 | 80 | 73 | | 80 | |
| 25th and 75th percentile | 64 to 86 | 68 to 91 | 68 to 94 | 64 to 91 | | 68 to 91 | |
| Facet | Control mean (SD) | 0.32% DHA | 0.64% DHA | 0.96% DHA | P values for group comparisons | Combined DHA groups | P values for control v. combined DHA groups |
| *Cognitive* | | | | | | | |
| Mean (SD) | 17.1 (1.7) | 17.9 (1.9) | 18.0 (1.2) | 17.6 (1.7) | 0.15 | 17.9 (1.6) | 0.08 |
| Median | 17 | 18 | 18 | 17 | | 18 | |
| 25th and 75th percentile | 16 to 18 | 17 to 18 | 17 to 18 | 16.5 to 18 | | 17 to 18 | |
| *Language* | | | | | | | |
| Mean (SD) | 15.6 (2.7) | 17.4 (3.3) | 16.6 (2.0) | 16.8 (2.3) | 0.07 | 16.9 (2.6) | 0.01* |
| Median | 15.5 | 17 | 16.5 | 17 | | 17 | |
| 25th and 75th percentile | 14 to 17 | 16 to 18 | 15 to 18 | 15.5 to 18 | | 16 to 18 | |
| *Motor* | | | | | | | |
| Mean (SD) | 17.7 (1.2) | 18.1 (2.4) | 18.4 (1.8) | 17.9 (2.1) | 0.38 | 18.2 (2.1) | 0.71 |
| Median | 18 | 18 | 18 | 17.5 | | 18 | |
| 25th and 75th percentile | 16.5 to 18.5 | 17 to 18 | 18 to 20 | 16 to 19 | | 17 to 19 | |

* Denotes a significant difference ($p < .05$).

better visual acuity than children who had received control formula, but planned comparisons yielded no significant differences among the three DHA-supplemented groups (i.e., 0.32% v. 0.64% v. 0.96% DHA) [22]. Although our results indicate that the combined DHA-supplemented groups scored higher than the control group on the MDI, RBC-DHA and MDI scores were not correlated. This appears to contradict results from our previous study comparing MDI scores of children who were fed control formula or formula supplemented with 0.36% DHA, in which our laboratory reported a significant, moderate correlation between MDI scores at 18 months of age and RBC-DHA at 4 months of age ($r = 0.29$) [29]. However, when the correlational analysis of the present data set was limited to control and 0.32% diet groups , we did find a significant, moderate correlation between MDI scores and RBC-DHA levels at 4 months ($r_s = 0.31$, $p = 0.017$).

An unexpected finding of the present study was the series of negative correlations between RBC-LA and several measures on the BSID II (PDI, emotional regulation, cognitive developmental age, and language developmental age). Note however, our laboratory has previously reported a negative correlation between MDI scores and RBC-LA at 4 months of age [29]. The negative correlations may be an indirect result of competition between DHA and LA for incorporation into RBC membranes; DHA supplementation reduces RBC-LA [28,30]. This hypothesis is supported by our finding that RBC-DHA is negatively correlated with RBC-LA at both 4 months of age ($r_s = -0.34$, $p = .0002$) and 12 months of age ($r_s = -0.33$, $p = .0002$).

While our results suggest that a concentration of 0.32% DHA provides substantial cognitive benefits, there are alternative explanations for our findings. First, because control formulas contained no ARA while all DHA-supplemented formulas contained 0.64% ARA, it is possible that the benefits demonstrated by children receiving the supplemented formulas were due solely to ARA. Although this explanation is plausible, no study to date has investigated the effects of ARA supplementation alone on cognitive development. Furthermore, a study of preterm infants demonstrated that a DHA-supplemented formula without ARA led to higher MDI scores and improved visual attention compared to a control group fed traditional formula without LCPUFAs [31].

Second, it is possible that the superior visual acuity of the DHA-supplemented groups relative to the control group reported in the primary outcome study [22], enabled superior performance on the MDI in the present study. In fact, our analyses indicate that MDI, emotional regulation, quality of movement, cognitive developmental age, and language developmental age were all correlated with VEP visual acuity. Yet, this explanation is unlikely because the visual acuity of even the control group (0.38 logMAR; Snellen notation = 20/48) was easily sufficient for these children to clearly see and manipulate the test stimuli in the BSID II. It is perhaps more likely that the superior visual acuity of the DHA-supplemented groups is indicative of changes to the cytoarchitecture of the developing brain that also extend into the areas responsible for cognitive function. Furthermore, even if the effects reported in the present study were due to the better visual acuity of the DHA-supplemented groups, it demonstrates that DHA has, at the very least, an indirect beneficial effect on cognitive function.

Although our results suggest that the supplementation of infant formulas with DHA improves cognitive function, the data from similar studies that assess cognitive function with global tests of development (the Brunet–Lezine test, the first edition of the BSID, and the BSID II) are not consistent. While some studies report a cognitive benefit [12,23,29,31,32], others do not [16–19,24,33–35]. There are several potential explanations for the discrepancy between the results reported here and those from the studies cited above that find no effect. First, while cognitive function was assessed at 18 months of age in the present study, some of the other studies assessed cognitive outcome at younger (3, 6, or 12 months [17,19,35]) or older ages (24 months [16,35]). It is possible that cognitive benefits had not yet emerged, or could not be detected at the time of testing. Second, whereas the present study assessed development using the BSID II, several studies used the first edition of the BSID or Brunet–Lezine test [16,19,35]. Some researchers argue that these tests are indices of perceptual and motor skills, and thus, are inadequate to assess cognitive functions [36]. Three studies [33,34,37] included in the recent Beyerlein et al. meta-analysis [38] did assess cognitive benefit using the BSID II at 18 months of age and found no benefit but did not include blood sample collection as part of their protocol and thus, there was no objective measure of compliance with formula feeding protocol or measurement of blood concentrations of DHA.

Third, the present study used DHA obtained from single cell microalgae, while most other studies have used DHA obtained from fish oil [18,19,24] or eggs [16,19,33,37]. This is of particular importance because only DHA from single cell microalgae has been shown to enhance cognitive function in both term infants [29] and preterm infants [23]. The notion that source of DHA can affect developmental outcome is also supported by growth data. Clandinin et al. [23] reported that in a study of 361 preterm infants at 118 weeks of age, both weight and length were greater in those randomized to a formula enriched with DHA from algal oil compared to a tuna fish oil DHA source. In addition, there were no differences in body weight (at 118 weeks) or length (at 79 or 92 weeks) between the preterm infants receiving the algal source of DHA and breast-fed term infants. Yet, the impact of DHA source is difficult to elucidate as the LCPUFA bioavailability results for the different sources are mixed and might be tissue- and/or species-specific. For example, Wijendran et al. [39] reported that the incorporation of ARA from phospholipids (e.g., eggs) into baboon brain, liver, lung, plasma, and RBCs is preferential while Matthews et al. [40] demonstrated that DHA in triglycerides (e.g., algal oils) is preferentially incorporated into piglet plasma. Sala-Vila et al. [41] found equivalent uptakes of DHA from egg phospholipids and algal triglycerides into plasma phospholipids of term infants receiving nutrient-balanced diets.

Fourth, the discrepancy between the results might be due, in part, to differences in duration of formula feeding. Specifically, ten studies cited above implemented protocols in which the duration of formula feeding was 6 months or less [12,16,17,19,29,31,33–35,37]. Only three of those studies reported beneficial effects on cognitive function [12,29,31]. Meanwhile, out of five studies that implemented feeding durations of 9 months or more (the present study, [18,23,24,32]), four report some evidence of enhanced cognitive development (the present study, [23,24,32]).

Fifth, some of the studies that have failed to find a cognitive benefit have enrolled “high-risk” children, including preterm children [35,37] and children from families with low socioeconomic status [33,34]. Preterm children possess a number of health problems which may have led to low BSID scores and precluded cognitive benefits; e.g., two of the studies analyzed by Beyerlein et al. [38] had group means on the MDI that were borderline “sub-optimal”, ranging from 84.3 to 86.9 [24,37]. Low socioeconomic status is linked to a number of factors that can affect DHA status at birth, including maternal alcohol and tobacco use [42] and questionable home environment [33,34], which may in turn lead to low MDI scores. In fact, MDI group means of term infants at 18 months of age in the Lucas et al. [33] study were below the normative BSID II score of 100 (94.5 to 96.0).

Finally, it is possible that differences in dietary DHA supply contributed to the discrepant results. In several of the studies cited above, DHA concentrations in the supplemented formula (0.12–0.23% of total fatty acids, see Refs. [17,19,33,37]) were at the low end reported in breast milk worldwide (0.06–1.4% of total fatty acids [20,21]), and may have been insufficient to confer cognitive benefits. A recent consensus statement published under the auspices of the World Association of Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation [43] recommended that LCPUFA-containing infant formulas include at least 0.20% DHA. We posit that this minimum level should be even higher since only one study

conducted to date has reported significant effects on the BSID I or BSID II when using DHA levels of <0.32% of total fatty acids [31]. Our results suggest a level of 0.32% of total fatty acids is sufficient to confer a cognitive benefit of almost 7 points on the MDI. This result agrees with a finding reported earlier by our laboratory that infants supplemented with 0.36% DHA + 0.72% ARA showed a mean increase of 7 points on the MDI compared to children who received control formula [29].

In light of the conflicting results of previous studies, some researchers note that the examination of the potential cognitive effects of DHA supplementation is hampered by the use of global developmental tests. They point out that the first edition of the BSID and the Brunet–Lezine test do not truly reflect intelligence, nor do they predict later intelligence, but instead indicate the ages at which motor, mental, and behavioral milestones are achieved [42,44]. Nevertheless, we should mention once again that scores on the BSID II are associated with IQ scores at 4 years of age, and have been demonstrated to show a significant effect of DHA supplementation in published studies [13]. Studies that use outcome measures that assess more specific aspects of cognitive function such as means-end problem-solving [14,36] and information processing/stimulus disengagement [45,46] have reported enhanced performance in children fed DHA-supplemented formulas. Based on these results and those presented here, it would be of particular interest to examine the effects of different levels of DHA supplementation on specific aspects of cognitive development. Such a study might indicate more clearly, the optimal level of DHA supplementation. Our laboratory is currently investigating this possibility.

The mechanisms underlying the beneficial effects of DHA supplementation are not currently understood, but a number of possibilities exist. Researchers posit that DHA supplementation can affect a number of specific processes and structures within the central nervous system. DHA influences gene transcription [47] and can perhaps cause post-translational modifications [27]. DHA can also modify the fluidity and thickness of neuronal membranes, thereby affecting receptor function [48,49]. In addition, DHA may facilitate memory formation and information processing by increasing myelination and improving the function of N-methyl-D-aspartate (NMDA) channels [50]. Two important loci of these effects are the hippocampus and the prefrontal cortex, regions that are essential for memory, attentional control, and higher level cognitive processes [51]. In fact, studies of rats, pigs, and nonhuman primates demonstrate that the neuronal membranes within the prefrontal cortex are particularly sensitive to both DHA deficiency [52,53] and DHA supplementation [54,55].

Although much remains to be learned about the anatomical/physiological effects of DHA on the developing brain, the results presented here suggest that LCPUFA supplementation during the first year of life leads to enhanced cognitive development. Specifically, our findings indicate that 0.32% DHA is sufficient to provide cognitive benefits over control formula at 18 months of age. In addition, as reported in the primary outcome manuscript for the cohort studied here, the formulas were well tolerated [22]. There were no significant differences in the incidence of adverse events with this level of supplementation (stool characteristics, diarrhea, constipation, gas or fussiness) or serious adverse events (i.e., life-threatening events requiring hospitalization). In fact, adverse effects were not expected for any of the three levels of DHA supplementation used (0.32%, 0.64%, and 0.96%), because all are well within the range found in breast milk worldwide (0.06 to 1.4% [20,21]). Currently, we are investigating whether beneficial effects are evident in toddlers and preschoolers who received these diets using tests that assess specific domains of cognitive development.

## Conflict of interest statement

Dennis R. Hoffman has served on speakers' panels at scientific and educational conferences on behalf of Mead Johnson; he does not have a financial interest in the company. None of the other authors has a potential conflict of interest related to this study.

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