

The DIAMOND (DHA Intake And Measurement Of Neural Development) Study: a double-masked, randomized controlled clinical trial of the maturation of infant visual acuity as a function of the dietary level of docosahexaenoic acid¹⁻³

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ABSTRACT

Background: The range of human milk docosahexaenoic acid (DHA) concentrations worldwide is much broader than the range explored in randomized clinical trials to date.

Objective: The primary objective was to determine the effect of 4 amounts of DHA supplementation on the visual acuity of formula-fed infants at 12 mo of age. Secondary objectives were to evaluate visual acuity maturation, red blood cell fatty acids, tolerance, anthropometric measures, and adverse events.

Design: This double-masked, randomized trial was conducted at 2 sites (Dallas and Kansas City). Three hundred forty-three healthy, term, formula-fed infants were enrolled at 1–9 d of age and were randomly assigned to be fed 1 of the following 4 infant formulas containing equivalent nutrient amounts, except for long-chain polyunsaturated fatty acids: control (0% DHA), 0.32% DHA, 0.64% DHA, or 0.96% DHA; DHA-supplemented formulas also provided 0.64% arachidonic acid. Visual acuity was measured by visual evoked potentials in 244 infants who completed the 12-mo primary outcome examination.

Results: Infants fed control formula had significantly poorer visual evoked potential visual acuity at 12 mo of age than did infants who received any of the DHA-supplemented formulas ($P < 0.001$). There were no significant differences in visual evoked potential visual acuity between the 3 amounts of DHA supplementation for either site at any age tested.

Conclusions: DHA supplementation of infant formula at 0.32% of total fatty acids improves visual acuity. Higher amounts of DHA supplementation were not associated with additional improvement of visual acuity. This trial was registered at clinicaltrials.gov as NCT00753818. *Am J Clin Nutr* 2010;91:848–59.

INTRODUCTION

Docosahexaenoic acid (DHA; 22:6 ω -3, or 22:6n-3) is a long-chain polyunsaturated fatty acid (LCPUFA) in the omega-3 (n-3) family. DHA is present in high concentrations in the central nervous system (CNS) and accumulates during the third trimester prenatally and the first 2 y postpartum (1–4). After birth, human milk is a source of DHA for infants (5). In 2002, DHA and arachidonic acid (ARA; 20:4 ω -6, or 20:4n-6), another LCPUFA found in high concentrations in the CNS, were added to US infant formulas.

Randomized clinical trials of the effects of DHA supplementation on visual and cognitive maturation in preterm and term infants were the basis for the addition of DHA and ARA to infant formulas. Studies in preterm infants, with rare exception, have shown benefits of supplementation for visual and/or cognitive development, whereas studies in term infants have been mixed (6–16). It has been noted that clinical trials that fed formulas with a higher DHA content, fed the formulas for longer periods of time, or used more sensitive electrophysiologic rather than psychophysical assessments of visual acuity were more likely to show a benefit of DHA supplementation (17–19).

The range of human milk DHA concentrations worldwide is much broader than the range of concentrations that have been explored in randomized clinical trials (20). To date, there has not been a dose-response study of DHA supplementation of infant formula. Moreover, meta-analyses of published data are limited in value as a guide for designing infant formulas with optimal LCPUFA concentrations because the DHA concentration is not the sole difference in formula composition between studies; formulas also differ in the source of DHA as well as in the amounts and sources of ARA and in the essential fatty acids linoleic acid (LA; 18:2 ω -6, or 18:2n-6) and α -linolenic acid (ALA; 18:3 ω -3, or 18:3n-3). In addition, duration of feeding and outcome measures differ between studies. We report here the results of a double-masked, randomized, controlled, parallel-

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² Supported by Mead Johnson Nutrition.

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Received August 20, 2009. Accepted for publication December 22, 2009. First published online February 3, 2010; doi: 10.3945/ajcn.2009.28557.

group prospective trial designed to determine the effect of 4 different levels of DHA supplementation on visual acuity maturation at 12 mo of age. The DIAMOND Study (DHA Intake And Measurement Of Neural Development) was conducted at 2 clinical sites in the United States. Secondary objectives were to evaluate the effect of 4 different amounts of DHA supplementation on visual acuity at 1.5, 4, and 9 mo of age and red blood cell (RBC) fatty acids at 4 and 12 mo of age. Anthropometric measures, formula intake, tolerance, and prevalence of adverse events were monitored. The results of the primary and secondary objectives are reported here. Other site-specific assessments of neural development during the first year of life and at later ages are being collected and will be reported later with reference to this parent trial.

SUBJECTS AND METHODS

Study population

Healthy, term, formula-fed, singleton-birth infants (37–42 wk gestation; 2490–4200 g birth weight) born in 5 hospitals in the Dallas metropolitan area and in 2 hospitals in the Kansas City metropolitan area between 3 September 2003 and 25 September 2005 were eligible for the study. Infants who had received human milk within 24 h of randomization or who had diseases or congenital abnormalities likely to interfere with normal growth and development or with the normal maturation of visual or cognitive function, poor formula intake, or known or suspected intolerance to cow milk infant formula were excluded from the study. Also excluded were infants born to mothers with chronic illness, such as HIV disease, renal or hepatic disease, type 1 or type 2 diabetes, alcoholism, or substance abuse.

Institutional Review Boards at the Dallas hospitals of Arlington Memorial Hospital, Presbyterian Hospital, University of Texas Southwestern Medical Center, and Medical City Hospital and at the University of Kansas Medical Center (Kansas City, KS) and University of Missouri Kansas City (Kansas City, MO) approved the study protocol. The infants' parents or guardians provided written informed consent.

Randomization to infant formulas

Between 1 and 9 d of age, infants were randomly assigned to 1 of 4 cow milk-based term infant formulas that had the same nutrient amounts and ingredients except for LCPUFAs: control with no DHA or ARA (previously marketed Enfamil with Iron; Mead Johnson Nutrition, Evansville, IN), 0.32% DHA with 0.32% fatty acids from DHA (17 mg/100 kcal; marketed Enfamil LIPL; Mead Johnson Nutrition), and the experimental formulas with 0.64% DHA (34 mg/100 kcal) or 0.96% DHA (51 mg/100 kcal). All DHA-supplemented formulas provided 0.64% fatty acids as ARA (34 mg/100 kcal). The sources of DHA and ARA were single-cell algal (*Cryptocodinium cohnii*) and fungal (*Mortierella alpina*) oils, respectively (Martek Biosciences, Columbia, MD). The content of other major fatty acids, including LA (16.9–17.5% fatty acids) and ALA (1.61–1.68% fatty acids), were similar between all 4 formulas. Additional details of formula composition are available as Online Supplemental Material (see "Supplemental data" in the online issue).

Randomization for the study was performed by the study sponsor. A computer program, using a random-number generator function, created 2 randomization lists for each study site, 1 for males and 1 for females. Blocking was used to keep formula group sizes similar. Codes were used to designate the study formulas. Each of the 4 formulas had 2 different codes, for a total of 8 codes. Only the study sponsor knew which codes designated which study formula. On the basis of the randomization lists, sealed envelopes were labeled with consecutive numbers, with each of the envelopes containing the code of the study formula that was to be assigned. On entry into the study, the next sequential numbered envelope for the infant's sex was opened at the study site to provide the code of the study formula to be consumed by the infant. Study formula, packaged by the study sponsor and identifiable only by its code, was then provided for the infant. The infants' study formula group allocation was masked until all infants reached 12 mo of age and data collection had been completed, validated, and locked.

The study formulas were fed for the first 12 mo of life and were to be the sole source of nutrition until \approx 4 mo of age, when additional foods could be introduced as directed by the infants' physicians. The amount of formula provided to the infant was not limited. Families were to avoid using commercial DHA-supplemented or DHA-enriched foods until the infant was older than 12 mo.

Data collection

Infants were evaluated at the study sites at enrollment, 1.5 mo (42 ± 7 d), 4 mo (120 ± 7 d), 6 mo (180 ± 7 d), 9 mo (275 ± 7 d), and 12 mo (365 ± 7 d) of age. Target test ages were based on days since expected date of delivery by sonogram rather than on days since birth because vision matures as a function of post-conceptual age (for review see 21). The evaluations conducted at each visit are listed in **Table 1**.

Visual evoked potential

At both sites, experienced electrophysiologists (2 at each site) assessed VEP visual acuity using Power Diva hardware and software (Smith Kettlewell Institute, San Francisco, CA) according to the swept parameter protocol developed by Norcia et al (9, 22–24) using vertical gratings with phase reversing at 6.6 Hz. Two bipolar placements of Oz versus O1 and O2 were used to record (gain = 10,000–20,000, –3 dB cutoff at 1 and 100 Hz) the electroencephalogram that was adaptively filtered in real time to isolate the VEP (397 Hz sampling rate).

All sweep VEP records were scored by the same trained expert (EB, Dallas, TX), who was unaware of formula group assignment. Amplitude and phase of the response at the second harmonic of the stimulation frequency were calculated for each channel. Noise was measured by determining the amplitude and phase of the 2 adjacent nonharmonic frequencies. Individual trials were reviewed for the presence of recording artifacts missed by the software and manually rejected. Grating acuity was estimated with an automated algorithm that examined signal-to-noise ratio and phase coherence and performed a linear regression for the final descending limb of the vector averaged function (minimum of 3 good quality trials; typically 5 trials) relating VEP second harmonic amplitude (amplitude at the reversal frequency



TABLE 1
Study outcome evaluations¹

Age	Birth	1.5 mo	4 mo	6 mo	9 mo	12 mo
Visual evoked potential acuity		X	X		X	X
Blood fatty acids			X			X
Anthropometric measures	X	X	X	X	X	X
Formula intake and tolerance		X	X	X	X	X

¹ X, time point at which outcome measure was collected.

of 13.2 Hz) to spatial frequency. The minimum peak signal-to-noise ratio for the vector average to be able to determine VEP visual acuity was 3.00. Sweep VEP visual acuities were expressed in logMAR (log of the minimum angle of resolution; eg, 20/20 corresponds to a minimum angle of resolution of 1-min arc and logMAR of 0.0, whereas 20/200 corresponds to a minimum angle of resolution of 10 min arc and logMAR of 1.0). Lower logMAR values represent better or more mature visual acuity.

Fatty acid analysis

All fatty acid analyses were conducted at the Retina Foundation of the Southwest in Dallas, TX, under the direction of experts (DH and DW), who were unaware of formula group assignment. Fatty acid data were merged with the VEP visual acuity and other results after all other data had been entered and validated. Blood samples (2.0 mL) were collected by venipuncture (Kansas City site) or via heel stick aided by infant heel-warming packs (Dallas site) into EDTA-containing evacuated tubes. Blood collection was the only methodologic difference between study sites; all other procedures were harmonized. Plasma and RBCs were separated by centrifugation (3000 × g × 10 min), and the fractions were immediately separated and frozen at −70°C until analyzed. The samples collected in Kansas City were immediately separated, frozen at −70°C, and shipped on dry ice monthly via overnight express to Dallas.

Lipids were extracted and transmethylated with 14% boron trifluoride in methanol, and methyl esters were analyzed by using capillary column gas chromatography (0.25 mm × 30 m Omegawax 250 column; Supelco, Bellefonte, PA) with flame ionization detection. The results were expressed in concentrations (μg/mL packed RBCs) based on the addition of internal standard (10 μg 23:0 fatty acid; Nu-Chek Prep Inc, Elysian, MN). Fatty acid peaks were identified by comparison with GLC68+11 standard and quantified by semiautomated data processing. Fatty acid analysis methods were described in detail by Hoffman et al (25). The results of RBC fatty acid analysis reported here are limited to diet-induced differences in DHA and ARA in RBC total lipids as indexes of DHA and ARA status and of neural membrane composition (26).

Anthropometric measurements

Infants' birth weight, length, and head circumference measurements were obtained from their birth records. Infants were weighed one time at each study visit on a pediatric scale, and weight was recorded in grams. Length was measured to the nearest millimeter or 1/8th inch with length boards after po-

sitioning the head at the top of the board and ensuring that the body and legs were straight. Head circumference was measured with a flexible, nonstretchable cloth or vinyl tape to the nearest millimeter or 1/8th inch. Anthropometric data were normalized by expressing them as z scores based on term infants of the same age and sex by using parameters provided in the data files from the Centers for Disease Control and Prevention (CDC) growth charts released in 2000 (www.cdc.gov/growthcharts/).

Formula consumption, tolerance, and adverse events

Formula consumption and tolerance data were collected by 24-h diet and tolerance recalls that parents completed for the day before each study visit. Parents provided information on the amount of formula consumed (number of ounces consumed in the past 24 h), infants' stool characteristics (number, color, and consistency), occurrence of constipation or diarrhea, and whether the infant was unusually fussy or gassy. Parents were also asked to provide information about which weaning foods were fed to the infant since the time of the last study visit. Adverse events, coded by body system symptoms, were recorded at each study visit and from medical records obtained from the child's primary medical care provider. Adverse events were defined as any unfavorable and unintended sign, symptom, or disease temporally associated with participation in the clinical study whether or not related to study formula. An adverse event was considered serious if it was life-threatening, required hospitalization, prolonged existing hospitalization, resulted in persistent or significant disability, was a congenital anomaly or birth defect, or resulted in death.

Sample size and statistical methods

The primary response variable was VEP visual acuity at 12 mo of age. This age was chosen because it corresponds to the end of the formula-feeding period; ie, the maximum cumulative consumption of the assigned formulas. The sample size was calculated so that the study would have a power of 80% to detect a clinically relevant difference of 0.1 logMAR. Assuming an SD of 0.12 logMAR (9, 11, 23, 24, 27, 28) and at an α level of 0.008 (to account for planned multiple comparisons), 37 participants per formula group at each site were required; however, many more were enrolled at each site to have enough statistical power to independently assess other site-specific developmental outcomes beyond infancy.

VEP visual acuity, RBC fatty acid, anthropometric, and frequency of bowel movements data were analyzed with analysis of variance (ANOVA) that included study site, formula group, and study site by formula group interaction. VEP visual acuity results were also analyzed with repeated-measures ANOVA; similar conclusions were reached with both the per protocol and repeated-measures analyses. Because the repeated measures did not provide further insights, they are not presented.

Study formula intake was analyzed with ANOVA and included terms for study site, sex, formula group, and sex-by-formula group interaction. The consistency and color of bowel movements were analyzed with the chi-square test. Fussiness, gas, diarrhea, constipation, and discontinuation rates were analyzed with Fisher's exact test. Fisher's exact test was also used to



compare the formula groups' proportion of participants who had adverse events.

All of the analyses noted above were preplanned and conducted as intent-to-treat. *P* values were based on 2-tailed tests. All testing was conducted at an α level of 0.05. The Tukey-Kramer adjustment was used for multiple pairwise comparisons to control the overall error rate to 0.05. All possible pairwise comparisons were carried out when pairwise comparisons were performed. Analyses were performed by using SAS version 9 (SAS Institute Inc, Cary, NC). All means presented are least-squares means \pm SEs.

For VEP visual acuity, a significant study site-by-formula group interaction ($P < 0.05$) was observed at 4, 9, and 12 mo of age. Accordingly, in addition to the combined site data, the data also are shown by study site. To better understand the underlying cause of these interactions, a series of post hoc exploratory analyses, using ANOVA, were undertaken. Various explanatory factors were considered in these models, including race, ethnicity, education of parent, and sex. Race, ethnicity, and education of parent could not be thoroughly evaluated because of the lack of statistical power caused by the occurrence of the small subclass numbers within interactions involving study sites. However, sex appeared to contribute to the underlying cause of the study site-by-formula group interaction. Therefore, we also provide the study site by formula group-by-sex interaction means for VEP visual acuity.

RESULTS

Subject accountability and infant characteristics

A total of 343 infants were enrolled in the study (Figure 1). Two hundred forty-four infants completed the study; completion rates were similar between the formula groups: 56/85 (66%) in the control group, 64/83 (77%) in the 0.32% DHA group, 59/84 (70%) in the 0.64% DHA group, and 65/87 (75%) in the 0.96% DHA group. The reasons for discontinuation were also similar between the formula groups. Of the 95 infants who discontinued the study, 43 (45%) discontinued before the 1.5-mo (6-wk) visit. Of 181 infants who were enrolled and consumed formula at Dallas, 141 (78%) completed the study, whereas a significantly lower percentage (103/158, or 65%; $P = 0.011$) completed at Kansas City. Within sites, discontinuation rates were similar between the 4 formula groups. There were no significant differences between formula groups in race, ethnicity, maternal education, or paternal education for those who were dropped. Three infants who did not meet the protocol inclusion criteria were inadvertently enrolled in the study and were included in the statistical analyses: 1 was 10 d old at randomization (0.96% DHA group) and 2 were breastfed within 24 h before randomization (control and 0.96% DHA groups).

Infant characteristics at birth are presented in Table 2 for infants enrolled and for infants who completed the 12-mo primary outcome visit. Because there was a significant study site-

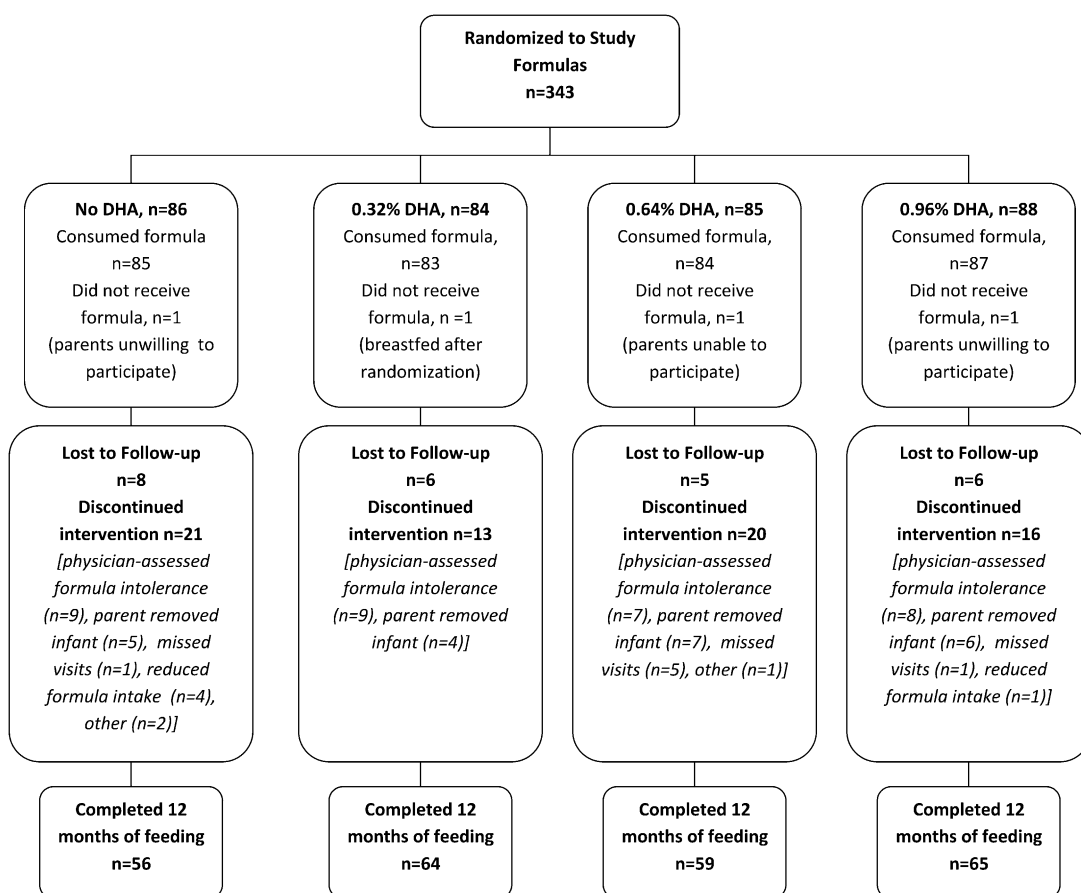


FIGURE 1. Flow chart showing study completion in each formula group. DHA, docosahexaenoic acid.

TABLE 2
 Characteristics at birth of infants enrolled into the randomized controlled trial and of infants who completed the 12-mo primary outcome visit¹

	Study site				Formula group							
	Dallas		Kansas City		Control		0.32% DHA		0.64% DHA		0.96% DHA	
	Enrolled	Completed	Enrolled	Completed	Enrolled	Completed	Enrolled	Completed	Enrolled	Completed	Enrolled	Completed
No. of subjects [n (% male)] ²	181 (57)	56(52)	158 (47)	56(52)	85 (49)	56(52)	83 (53)	64 (52)	84 (54)	59 (56)	87 (54)	65 (51)
Duration of gestation (wk) ^{3,4}	39 ± 0.1 ⁵	39.4 ± 0.1	39.5 ± 0.1	39.4 ± 0.1	39.2 ± 0.1	39.4 ± 0.1	39.3 ± 0.1	39.3 ± 0.1	39.3 ± 0.1	39.1 ± 0.1	39.3 ± 0.1	39.2 ± 0.1
Apgar score, 5 min	9.0 ± 0.03	8.9 ± 0.06	9.0 ± 0.03	8.9 ± 0.06	9.0 ± 0.04	8.9 ± 0.06	9.0 ± 0.04	8.9 ± 0.05	9.0 ± 0.04	9.0 ± 0.06	9.0 ± 0.04	9.0 ± 0.05
Anthropometric measures												
Weight (g)	3411 ± 27	3405 ± 48	3387 ± 29	3405 ± 48	3350 ± 40	3425 ± 40	3425 ± 40	3454 ± 45	3434 ± 40	3379 ± 48	3388 ± 39	3386 ± 45
Length (cm)	50.2 ± 0.2	50.1 ± 0.2	50.2 ± 0.2	50.1 ± 0.2	49.9 ± 0.2	50.2 ± 0.2	50.2 ± 0.2	50.4 ± 0.2	50.3 ± 0.2	50.1 ± 0.2	50.3 ± 0.2	50.2 ± 0.2
Head circumference (cm)	34.4 ± 0.1	34.5 ± 0.2	34.3 ± 0.1	34.5 ± 0.2	34.3 ± 0.2	34.5 ± 0.1	34.5 ± 0.1	34.5 ± 0.2	34.4 ± 0.1	34.3 ± 0.2	34.2 ± 0.1	34.3 ± 0.2
Race [n (%)] ^{3,4,6}												
Black	25 (14)	22 (39)	97 (61)	22 (39)	38 (45)	22 (39)	22 (27)	16 (25)	29 (35)	18 (31)	33 (38)	22 (34)
White	137 (76)	30 (54)	59 (37)	30 (54)	43 (51)	30 (54)	50 (60)	39 (61)	52 (62)	39 (66)	51 (59)	40 (62)
Other ⁷	19 (11)	4 (7)	2 (1)	4 (7)	4 (5)	4 (7)	11 (13)	9 (14)	3 (4)	2 (3)	3 (3)	3 (5)
Ethnicity [n (%)] ⁸												
Hispanic	25 (14)	5 (9)	10 (6)	5 (9)	8 (9)	5 (9)	8 (10)	6 (9)	10 (12)	10 (17)	9 (10)	6 (9)
Non-Hispanic	156 (86)	51 (91)	148 (94)	51 (91)	77 (91)	51 (91)	75 (90)	58 (91)	74 (88)	49 (83)	78 (90)	59 (91)
Maternal education [n (%)] ^{3,4}												
Did not complete high school	3 (2)	6 (11)	35 (29)	6 (11)	8 (11)	6 (11)	11 (14)	11 (17)	9 (12)	7 (12)	10 (12)	6 (9)
Completed high school	67 (37)	32 (57)	83 (68)	32 (57)	41 (56)	32 (57)	32 (42)	25 (39)	34 (47)	27 (47)	43 (54)	38 (58)
Completed college	90 (50)	13 (23)	4 (3)	13 (23)	19 (26)	13 (23)	25 (32)	19 (30)	24 (33)	18 (31)	26 (33)	21 (32)
Postgraduate	21 (12)	5 (9)	0 (0)	5 (9)	5 (7)	5 (9)	9 (12)	8 (13)	6 (8)	6 (10)	1 (1)	0 (0)
Information not collected	0	0	36	0	12	0	6	1	11	1	7	0
Paternal education [n (%)] ^{3,4,6}												
Did not complete high school	1 (1)	4 (8)	32 (28)	4 (8)	6 (9)	4 (8)	8 (10)	6 (10)	11 (15)	9 (16)	8 (11)	5 (8)
Completed high school	61 (34)	29 (55)	76 (67)	29 (55)	36 (51)	29 (55)	42 (55)	35 (56)	23 (32)	18 (32)	36 (49)	30 (49)
Completed college	92 (51)	18 (34)	4 (4)	18 (34)	26 (37)	18 (34)	16 (21)	15 (24)	32 (45)	28 (49)	22 (30)	20 (33)
Postgraduate	25 (14)	2 (4)	1 (1)	2 (4)	2 (3)	2 (4)	11 (14)	7 (11)	5 (7)	2 (4)	8 (11)	6 (10)
Information not collected	2	3	45	3	15	3	6	1	13	2	13	4

¹ Includes infants who were randomly assigned and consumed study formulas. DHA, docosahexaenoic acid.

^{2,4} Significant difference between sites for infants who completed the 12-mo primary outcome visit: ²*P* = 0.020, ⁴*P* < 0.001.

^{3,8} Significant difference between sites for infants enrolled in the study: ³*P* < 0.001, ⁸*P* = 0.031.

⁵ Mean ± SE (all such values).

⁶ Significant difference between formula groups for infants enrolled in the study, *P* < 0.05.

⁷ Includes Asian, Native American/Alaskan Native, and more than one race.

by-formula group interaction ($P < 0.05$) for the primary outcome of VEP visual acuity at 12 mo of age, data for infant characteristics are presented separately by study site as well as by formula group. For all infants enrolled, there were significant differences in duration of gestation, race, ethnicity, and parents' highest level of achieved education between study sites and significant differences between groups for racial distribution and paternal education. Among infants who completed the primary outcome 12-mo visit, significant differences between study sites were present for duration of gestation, race, parents' highest level of achieved education, and sex, but no significant differences were found between formula groups in any of the infant characteristics.

Visual acuity

More than 97% of infants were evaluated within ± 7 d of the study visit, as specified in the study protocol. Ranges for actual times of evaluations were as follows: 1.5-mo (42 d) visit, -8 to $+7$ d; 4-mo (120 d) visit, -12 to $+12$ d; 9-mo (275 d) visit, -9 to $+15$ d; and 12-mo (365 d) visit, -12 to $+48$ d. For the primary outcome (Figure 2), VEP visual acuity at 12 mo of age, infants fed control formula had significantly poorer visual acuity than did all groups who received DHA-supplemented formulas ($P < 0.001$). Visual acuity did not differ significantly between the supplemented groups. Similar visual acuity results were obtained at each of the other assessment ages (1.5, 4, and 9 mo).

A significant study site-by-formula group interaction ($P < 0.05$) was observed at 4, 9, and 12 mo of age (Figure 3). At 3

ages, infants at Dallas who were fed control formula with no DHA had significantly poorer visual acuity than did all groups who received DHA-supplemented formulas ($P < 0.001$); visual acuity did not differ between the supplemented groups. At the Kansas City site, visual acuity did not differ between the formula groups at 4 mo. At 9 mo, infants at Kansas City who were fed control formula had significantly poorer visual acuity than did infants fed formula with 0.64% DHA ($P = 0.036$); visual acuity did not differ significantly between the 3 supplemented groups. At 12 mo, infants at Kansas City who were fed control formula had poorer visual acuity than did infants fed 0.64% and 0.96% DHA formula ($P = 0.017$ and $P = 0.050$, respectively); visual acuity did not differ significantly between the 3 supplemented groups. Infants at Kansas City who were fed control formula had significantly better VEP visual acuity than did infants at Dallas who received control formula ($P = 0.045$), whereas infants at Kansas City who received 0.32% DHA had significantly poorer visual acuity than did infants from Dallas who received the same formula ($P = 0.040$).

Sex was evaluated through post hoc analysis to better understand its contribution to the significant formula group-by-study site interactions at 4, 9, and 12 mo. In Figure 4, the 3 supplemented formula groups are combined, because there were no significant differences in VEP visual acuity between the 3 amounts of DHA supplementation for either site at any age tested; also, because this was a post hoc exploratory analysis, no statistical significance is provided. The figure suggests improvements in visual acuity with the addition of DHA to formula for males and females at the Dallas site, whereas, males, but not females, appear to show improvements in visual acuity with the addition of DHA to formula at the Kansas City site.

RBC fatty acids

RBC DHA concentrations were significantly different ($P < 0.001$) between all formula groups at both 4 and 12 mo of age and increased as the percentage of DHA in the formula increased (Table 3). There were significant study site-by-formula group interactions ($P < 0.001$) for RBC DHA concentrations ($\mu\text{g/mL}$) at 4 and 12 mo of age. At the Dallas site, RBC DHA concentrations were significantly different ($P < 0.001$) between all formula groups at both 4 and 12 mo of age and increased as the percentage of DHA in the formula increased. In infants at 4 mo of age at Kansas City, RBC DHA concentrations increased as the percentage of DHA in the formula increased in the control, 0.32% DHA, and 0.64% DHA groups ($P < 0.01$), but did not differ between the 0.64% DHA and 0.96% DHA formula groups. For infants at Kansas City at 12 mo of age, RBC DHA concentrations increased significantly from control to 0.32% DHA to 0.96% DHA, but infants who received 0.64% DHA formula had RBC DHA values that did not differ significantly from those who received 0.32% DHA or 0.96% DHA. At 4 mo of age, infants at Kansas City who were fed formula containing 0.96% DHA had significantly lower RBC DHA concentrations than did infants at Dallas fed the same formula ($P < 0.001$). At 12 mo of age, infants at Kansas City who were fed formula containing 0.64% or 0.96% DHA had significantly lower RBC DHA concentrations than did infants at Dallas fed the same formulas ($P < 0.001$ and $P < 0.001$, respectively). RBC DHA

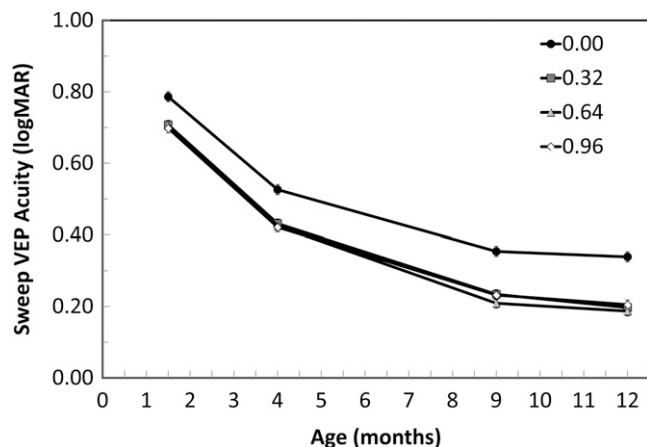


FIGURE 2. Mean (\pm SE) sweep visual evoked potential (VEP) visual acuity (logMAR, log of the minimum angle of resolution) for each diet group as a function of age. Lower visual acuity values indicate better acuity. The numbers of infants at each age were as follows: 1.5 mo [72 in the control group, 71 in the 0.32% docosahexaenoic acid (DHA) group, 73 in the 0.64% DHA group, and 78 in the 0.96% DHA group], 4 mo (64 in the control group, 68 in the 0.32% DHA group, 67 in the 0.64% DHA group, and 69 in the 0.96% DHA group), 9 mo (59 in the control group, 65 in the 0.32% DHA group, 62 in the 0.64% DHA group, and 67 in the 0.96% DHA group), and 12 mo (56 in the control group, 63 in the 0.32% DHA group, 57 in the 0.64% DHA group, and 65 in the 0.96% DHA group). VEP visual acuity data were analyzed by using ANOVA that included study site, formula group, and study site-by-formula group interaction. All possible pairwise comparisons were carried out when pairwise comparisons were performed. The Tukey-Kramer adjustment was used for multiple pairwise comparisons to control the overall error rate to 0.05. At each age, visual acuity in the control group was significantly poorer than in the 0.32% DHA group ($P \leq 0.002$), the 0.64% DHA group ($P \leq 0.001$), and the 0.96% DHA group ($P < 0.001$).

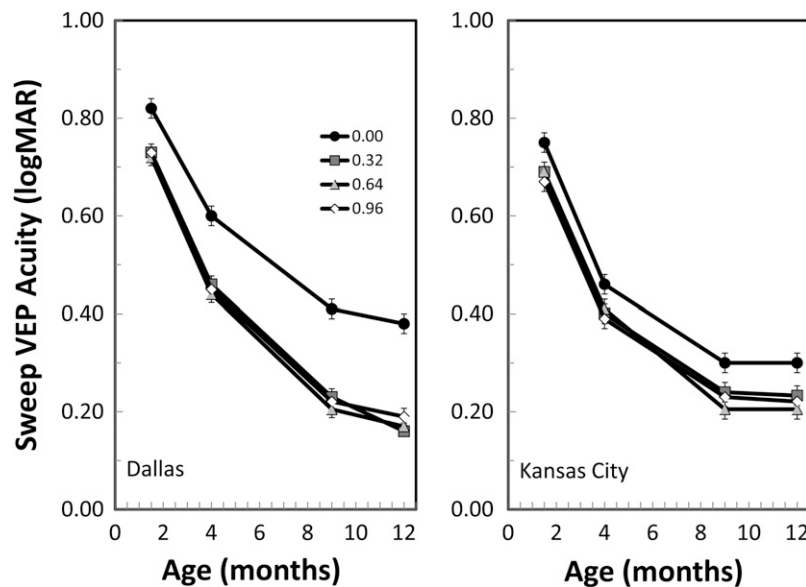


FIGURE 3. Mean (\pm SE) sweep visual evoked potential (VEP) visual acuity (logMAR, log of the minimum angle of resolution) by study site for each diet group as a function of age. The numbers of infants at Dallas at each age were as follows: 1.5 mo [37 in the control group, 39 in the 0.32% docosahexaenoic acid (DHA) group, 42 in the 0.64% DHA group, and 41 in the 0.96% DHA group], 4 mo (35 in the control group, 37 in the 0.32% DHA group, 40 in the 0.64% DHA group, and 38 in the 0.96% DHA group), 9 mo (35 in the control group, 36 in the 0.32% DHA group, 37 in the 0.64% DHA group, and 38 in the 0.96% DHA group), and 12 mo (34 in the control group, 35 in the 0.32% DHA group, 36 in the 0.64% DHA group, and 36 in the 0.96% DHA group). The number of infants at Kansas City at each age were as follows: 1.5 mo (35 in the control group, 32 in the 0.32% DHA group, 31 in the 0.64% DHA group, and 37 in the 0.96% DHA group), 4 mo (29 in the control group, 31 in the 0.32% DHA group, 27 in the 0.64% DHA group, and 31 in the 0.96% DHA group), 9 mo (24 in the control group, 29 in the 0.32% DHA group, 25 in the 0.64% DHA group, and 29 in the 0.96% DHA group), and 12 mo (22 in the control group, 28 in the 0.32% DHA group, 21 in the 0.64% DHA group, and 29 in the 0.96% DHA group). VEP visual acuity data were analyzed by using ANOVA that included study site, formula group, and study site-by-formula group interaction. All possible pairwise comparisons were carried out. The Tukey-Kramer adjustment was used for multiple pairwise comparisons to control the overall error rate to 0.05. Significant differences ($P < 0.05$) are reviewed in the text.

concentrations correlated with visual acuity at 4 mo ($r = -0.508$, $P < 0.001$) and at 12 mo ($r = -0.513$, $P < 0.001$).

All DHA-supplemented formulas also provided 0.64% ARA, and control formula provided 0% ARA. Overall RBC ARA concentrations were significantly different between formula groups at 4 and 12 mo ($P < 0.001$ at both ages), and a significant difference was observed between study sites at 4 mo ($P = 0.021$). A comparison of formula groups within sites showed that infants at both the Dallas and Kansas City sites fed formula supplemented with 0.32% DHA had significantly higher RBC ARA concentrations than did infants fed control formula at 4 mo of age ($P < 0.008$ for both comparisons). In addition, infants at the Dallas site fed formula with 0.96% DHA had significantly lower RBC ARA concentrations than did infants fed control formula ($P = 0.028$). At 12 mo of age, a similar trend was seen, although a statistically significant difference at the Kansas City site was not observed between infants fed formula with 0.32% DHA and those fed control formula ($P = 0.11$). At 4 mo of age, infants at the Kansas City site had a significantly higher RBC ARA concentration than did infants at the Dallas site ($P = 0.021$).

Anthropometric measurements

Means for anthropometric data, presented as z scores, are provided for both study sites and all formula groups (Table 4).

Weight z scores

At 1.5 mo, there were no significant effects of study site or formula group on weight z scores. At all ages except 1.5 mo,

we found significant site-by-formula interactions. The site-by-formula interactions were related to the low weight z scores of the 0.32% DHA formula group at the Dallas site. At 4, 6, and 9 mo, the weight z scores of the 0.32% DHA group at Dallas were significantly lower than those of the 0.96% DHA group at Kansas City (0.00 ± 0.14 compared with 0.67 ± 0.16 , $P = 0.022$; -0.19 ± 0.14 compared with 0.48 ± 0.16 , $P = 0.040$; and -0.41 ± 0.15 compared with 0.29 ± 0.17 , $P = 0.049$; respectively). At 6 mo, the weight z score of the 0.32% DHA group at Dallas was also significantly less than that of the 0.32% DHA group at Kansas City (-0.19 ± 0.14 compared with 0.50 ± 0.16 ; $P = 0.036$). At 12 mo, although there was a statistically significant site-by-formula interaction, none of the pairwise comparisons were significant.

Length z scores

There were no significant effects of formula group on length z scores at 1.5, 6, 9, and 12 mo. However, infants at the Kansas City site had significantly greater length z scores than did those at Dallas at 4, 6, 9, and 12 mo ($P = 0.007$, $P < 0.001$, $P = 0.013$, and $P < 0.001$, respectively). There also were significant site-by-formula group interactions for length z scores at 1.5, 4, and 6 mo of age. No statistically significant pairwise comparisons were identified at 1.5 mo. Pairwise comparisons at 4 mo found that infants fed the 0.32% DHA formula at Kansas City had significantly greater length z scores (0.62 ± 0.14) than did those fed control formula at Kansas City (-0.06 ± 0.15 ; $P = 0.024$), those fed 0.32% DHA formula at Dallas (-0.10 ± 0.13 ; $P = 0.006$), and those fed 0.96% formula at

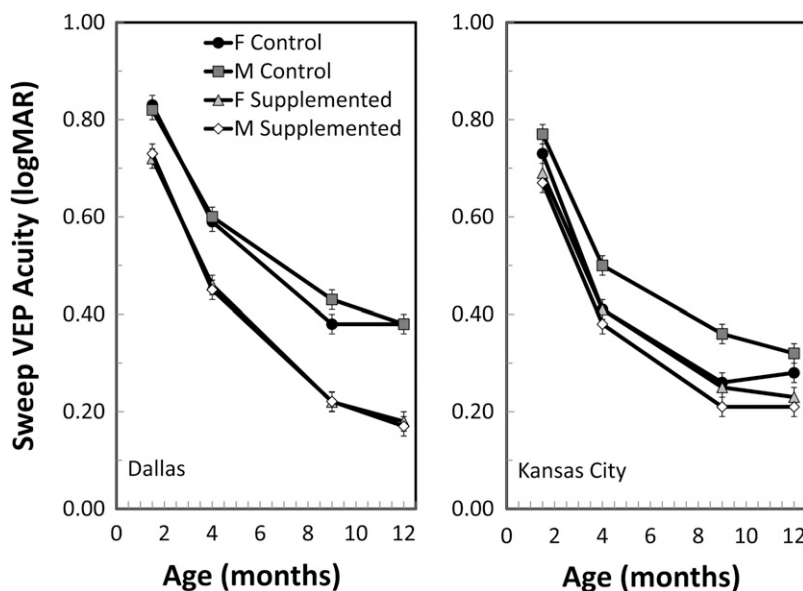


FIGURE 4. Results of post hoc exploratory analysis. Mean (\pm SE) visual evoked potential (VEP) visual acuity (logMAR, log of the minimum angle of resolution) by study site for each age by sex and formula group. The numbers of infants at Dallas measured at each age were as follows: 1.5 mo (21 male controls, 74 males supplemented, 16 female controls, and 48 females supplemented), 4 mo (19 male controls, 70 males supplemented, 16 female controls, and 45 females supplemented), 9 mo (19 male controls, 68 males supplemented, 16 female controls, and 43 females supplemented), 12 mo (18 male controls, 65 males supplemented, 16 female controls, and 42 females supplemented). The numbers of infants at Kansas City measured at each age were as follows: 1.5 mo (16 male controls, 46 males supplemented, 19 female controls, and 54 females supplemented), 4 mo (14 male controls, 37 males supplemented, 15 female controls, and 52 females supplemented), 9 mo (12 male controls, 35 males supplemented, 12 female controls, and 48 females supplemented), and 12 mo (11 male controls, 33 males supplemented, 11 female controls, and 45 females supplemented). VEP visual acuity data were analyzed by using ANOVA that included study site, formula group, sex, and all interactions. All possible pairwise comparisons were carried out. The Tukey-Kramer adjustment was used for multiple pairwise comparisons to control the overall error rate to 0.05.

Dallas (-0.02 ± 0.13 ; $P = 0.024$). Pairwise comparisons at 6 mo found that infants fed 0.32% DHA formula at Kansas City had significantly greater length z scores (0.66 ± 0.16) than did those fed 0.32% DHA formula at Dallas (-0.02 ± 0.14 ; $P = 0.029$) and that infants fed 0.96% DHA formula at Kansas City had significantly greater length z scores (0.59 ± 0.15) than did those fed 0.96% formula at Dallas (-0.07 ± 0.14 ; $P = 0.024$).

Weight-for-length z scores

Weight-for-length z scores also differed by study site. At 1.5 mo, infants from Kansas City had greater weight-for-length z scores ($P = 0.036$), whereas those from Dallas had greater weight-for-length z scores at 6 and 12 mo ($P = 0.029$ and $P = 0.011$, respectively). Also, at 6 mo, there was a significant effect of formula group ($P = 0.039$); no statistically significant pairwise comparisons were identified.

Head circumference z scores

Infants at the Dallas site had significantly greater head circumference values than did those at Kansas City at 1.5, 4, and 12 mo ($P = 0.032$, $P = 0.01$, and $P = 0.01$, respectively). At 9 mo of age, there was a significant effect of formula group ($P = 0.026$); the only significant pairwise difference between formula groups was that the control formula group had greater head circumference z scores than did the 0.64% DHA formula group ($P = 0.031$).

Formula intake and tolerance

The formula groups did not differ in the amount of study formula consumed in the 24 h before study visits at 1.5, 6, 9 or 12 mo of age (data not shown). At 4 mo of age, the control group consumed significantly ($P = 0.007$) more formula (1103 ± 26 mL) than did the 0.64% DHA group (985 ± 26 mL). Male infants consumed significantly more formula than did female infants at ages 1.5 and 9 mo (1.5 mo: 961 ± 17 mL for males and 911 ± 18 mL for females, $P = 0.04$; 9 mo: 958 ± 25 mL for males and 878 ± 26 mL for females, $P = 0.031$). At 6, 9, and 12 mo of age, there were significant differences between study sites in amounts of formula consumed. At 6 mo of age, infants from the Kansas City site consumed significantly more formula than did infants at the Dallas site (1053 ± 25 mL compared with 985 ± 21 mL; $P = 0.041$). At 9 and 12 mo of age, however, infants at the Kansas City site consumed significantly less formula than did infants at the Dallas site (9 mo: 875 ± 28 mL compared with 961 ± 24 mL, $P = 0.018$; 12 mo: 441 ± 39 mL compared with 772 ± 34 mL, $P < 0.001$).

At each visit, the parents were also asked to provide information about which weaning foods were fed to their infants since the time of the last study visit. Fewer than 2.2% of parents reported feeding DHA-supplemented foods to their child at any visit. At the 1.5-mo visit, 9% reported feeding cereal. At the 4-mo visit, 49% reported feeding cereal; 19% fruit and vegetables; 0.7% cheese, eggs, and milk; and 2% fish, poultry, and red meat. At the 6-mo visit, 90% reported feeding cereal; 81% fruit and vegetables; 13% cheese, eggs, and milk; and 16% fish, poultry, and red meat. At the 9-mo visit, 94% reported feeding cereal; 99% fruit and vegetables; 61% cheese, eggs, and milk;

TABLE 3
Red blood cell docosahexaenoic acid (DHA) and arachidonic acid (ARA) concentrations at 4 and 12 mo of age

	Study site		Formula group				P for interaction
	Dallas	Kansas City	Control	0.32% DHA	0.64% DHA	0.96% DHA	
	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	
DHA							
4 mo	115.7 \pm 1.7 (150) ^f	110.3 \pm 2.1 (99)	46.9 \pm 2.8 (58)	113.4 \pm 2.7 (63)	139.4 \pm 2.7 (63)	152.4 \pm 2.6 (65)	<0.001
12 mo	108.2 \pm 1.7 (141)	96.9 \pm 2.4 (80)	40.5 \pm 3.0 (53)	100.7 \pm 2.8 (57)	123.3 \pm 3.0 (54)	144.1 \pm 2.9 (57)	<0.001
ARA							
4 mo	245.0 \pm 2.7 (150)	254.8 \pm 3.3 (99)	233.7 \pm 4.4 (58)	268.6 \pm 4.2 (63)	253.9 \pm 4.3 (63)	243.4 \pm 4.1 (65)	NS
12 mo	235.1 \pm 2.4 (141)	233.6 \pm 3.1 (80)	224.3 \pm 4.0 (53)	248.6 \pm 3.8 (57)	235.4 \pm 4.0 (54)	229.1 \pm 3.8 (57)	<0.001

^f Mean \pm SE; n in parentheses (all such values).

and 76% fish, poultry, and red meat. At the 12-mo visit, 93% reported feeding cereal; 100% fruit and vegetables; 92% cheese, eggs, and milk; and 92% fish, poultry, and red meat. Significant differences between weaning foods provided in Dallas and those provided in Kansas City were found at 6, 9, and 12 mo; Kansas City had a consistently higher rate of feeding cheese, eggs, and milk and fish, poultry, and red meat than did Dallas.

Throughout the study, there were no differences between formula groups in the number of bowel movements occurring in the 24 h before study visits (data not shown). At 1.5 and 4 mo of age, there were significant differences between sites in the number of bowel movements reported (1.5 mo: 2.5 ± 0.11 in Kansas City and 2.2 ± 0.10 in Dallas; $P = 0.044$; 4 mo: 2.4 ± 0.11 in Kansas City and 1.89 ± 0.09 in Dallas; $P < 0.001$). At other ages, there were no differences between sites. We found no statistical differences in consistency or color of bowel movements, frequency of diarrhea or constipation, or frequency of unusual gas or fussiness between formula groups at any time (data not shown).

Adverse events

There were no statistical differences between formula groups in the number of participants reporting at least one adverse event of any type during the study: 75/85 (88%) in the control group, 76/83 (92%) in the 0.32% DHA group, 80/84 (95%) in the 0.64% DHA group, and 80/87 (92%) in the 0.96% DHA group. There were no statistical differences between the formula groups in 86 body-system symptoms recorded as adverse events, except for watery eyes: 0/85 (0%) in the control group, 1/83 (1%) in the 0.32% DHA group, 4/84 (5%) in the 0.64% DHA group, and 0/87 (0%) in the 0.96% DHA group ($P = 0.021$). In addition, there were no differences between formula groups in the number of participants having at least one serious adverse event: 7/85 (8%) in the control group, 6/83 (7%) in the 0.32% DHA group, 6/84 (7%) in the 0.64% DHA group, and 6/87 (7%) in the 0.96% DHA group. The participants' physicians evaluated the infants with serious adverse events and determined that for 24 of the 25 participants, serious adverse events were not related to study formula. The relation to study formula of one report of sepsis in the 0.64% DHA group was not determined.

DISCUSSION

The DIAMOND study is the first double-masked, randomized, controlled, parallel-group, prospective, dose-response study of DHA in term infant formula. We compared the VEP visual acuity of infants who were fed formulas containing 0% (control), 0.32%, 0.64%, or 0.96% DHA. Infants fed control formula had significantly poorer VEP visual acuity at 12 mo of age than did infants fed any of the DHA-supplemented formulas ($P < 0.001$). There were no significant differences in VEP visual acuity between the 3 DHA supplementation groups for either site at any age. At the Dallas site, 0.32% DHA enhanced VEP visual acuity maturation compared with control formula; higher amounts of DHA, up to 0.96% of fatty acids, did not provide additional enhancement of VEP visual acuity maturation. On the other hand, at the Kansas City site, 0.64% DHA was the lowest amount of DHA to show a statistically significant benefit for VEP visual acuity maturation

TABLE 4
Anthropometric z scores¹

z Scores	Study site			Formula group						P for interaction
	Dallas	Kansas City	P	Control	0.32% DHA	0.64% DHA	0.96% DHA	P		
Weight										
1.5 mo	0.11 ± 0.06 (159) ²	0.25 ± 0.06 (135)	NS	0.17 ± 0.08 (72)	0.13 ± 0.08 (72)	0.16 ± 0.08 (72)	0.27 ± 0.08 (78)	NS	NS	
4 mo	0.34 ± 0.07 (150)	0.45 ± 0.08 (119)	NS	0.34 ± 0.11 (64)	0.31 ± 0.11 (68)	0.32 ± 0.11 (67)	0.60 ± 0.10 (70)	NS	0.014	
6 mo	0.22 ± 0.07 (149)	0.33 ± 0.08 (111)	NS	0.27 ± 0.11 (60)	0.16 ± 0.11 (66)	0.23 ± 0.11 (65)	0.44 ± 0.11 (69)	NS	0.013	
9 mo	-0.03 ± 0.08 (146)	0.07 ± 0.09 (107)	NS	0.02 ± 0.12 (59)	-0.13 ± 0.12 (65)	0.04 ± 0.12 (62)	0.16 ± 0.11 (67)	NS	0.022	
12 mo	-0.11 ± 0.08 (141)	-0.05 ± 0.09 (103)	NS	-0.10 ± 0.13 (56)	-0.20 ± 0.12 (64)	-0.08 ± 0.12 (59)	0.05 ± 0.12 (65)	NS	0.016	
Length										
1.5 mo	-0.02 ± 0.06 (159)	-0.05 ± 0.06 (135)	NS	-0.10 ± 0.08 (72)	0.08 ± 0.09 (72)	-0.05 ± 0.09 (72)	-0.07 ± 0.08 (78)	NS	0.040	
4 mo	0.02 ± 0.07 (150)	0.29 ± 0.07 (119)	0.007	0.02 ± 0.10 (64)	0.26 ± 0.10 (68)	0.09 ± 0.10 (67)	0.24 ± 0.10 (70)	NS	0.005	
6 mo	0.03 ± 0.07 (149)	0.43 ± 0.08 (111)	<0.001	0.13 ± 0.11 (60)	0.30 ± 0.10 (66)	0.23 ± 0.10 (65)	0.25 ± 0.10 (69)	NS	0.036	
9 mo	-0.07 ± 0.07 (146)	0.21 ± 0.08 (107)	0.013	0.04 ± 0.11 (59)	0.12 ± 0.11 (65)	0.03 ± 0.11 (62)	0.09 ± 0.10 (67)	NS	NS	
12 mo	-0.13 ± 0.07 (141)	0.31 ± 0.08 (103)	<0.001	0.13 ± 0.11 (56)	0.08 ± 0.10 (64)	0.12 ± 0.11 (59)	0.05 ± 0.10 (65)	NS	NS	
Weight-for-length										
1.5 mo	-0.12 ± 0.07 (159)	0.11 ± 0.08 (135)	0.036	0.06 ± 0.11 (72)	-0.22 ± 0.11 (72)	-0.04 ± 0.11 (72)	0.18 ± 0.10 (78)	NS	NS	
4 mo	0.34 ± 0.08 (150)	0.18 ± 0.09 (119)	NS	0.35 ± 0.12 (64)	0.04 ± 0.12 (68)	0.20 ± 0.12 (67)	0.46 ± 0.12 (70)	NS	NS	
6 mo	0.44 ± 0.08 (149)	0.17 ± 0.09 (111)	0.029	0.41 ± 0.13 (60)	0.04 ± 0.12 (66)	0.25 ± 0.12 (65)	0.52 ± 0.12 (69)	0.039	NS	
9 mo	0.48 ± 0.09 (146)	0.35 ± 0.10 (107)	NS	0.51 ± 0.13 (59)	0.13 ± 0.13 (65)	0.47 ± 0.13 (62)	0.55 ± 0.13 (67)	NS	NS	
12 mo	0.53 ± 0.09 (141)	0.21 ± 0.10 (103)	0.011	0.37 ± 0.14 (56)	0.19 ± 0.13 (64)	0.37 ± 0.14 (59)	0.57 ± 0.13 (65)	NS	NS	
Head circumference										
1.5 mo	-0.16 ± 0.07 (159)	-0.38 ± 0.08 (135)	0.032	-0.31 ± 0.10 (72)	-0.24 ± 0.10 (72)	-0.20 ± 0.10 (72)	-0.31 ± 0.10 (78)	NS	NS	
4 mo	0.10 ± 0.08 (150)	-0.21 ± 0.09 (119)	0.010	-0.05 ± 0.12 (64)	-0.14 ± 0.12 (68)	-0.06 ± 0.12 (67)	0.01 ± 0.12 (70)	NS	NS	
6 mo	0.26 ± 0.08 (148)	0.05 ± 0.09 (111)	NS	0.32 ± 0.12 (59)	0.08 ± 0.12 (66)	0.01 ± 0.12 (65)	0.21 ± 0.11 (69)	NS	NS	
9 mo	0.33 ± 0.08 (146)	0.11 ± 0.10 (106)	NS	0.55 ± 0.13 (59)	0.09 ± 0.12 (65)	0.05 ± 0.13 (61)	0.21 ± 0.12 (67)	0.026	NS	
12 mo	0.54 ± 0.08 (141)	0.21 ± 0.10 (103)	0.010	0.57 ± 0.13 (56)	0.28 ± 0.12 (64)	0.34 ± 0.13 (59)	0.30 ± 0.12 (65)	NS	NS	

¹ DHA, docosahexaenoic acid.

² Mean ± SE; n in parentheses (all such values).

compared with control formula; no additional benefit was derived from 0.96% DHA.

Whereas differences between the control and supplemented-formula groups in sweep VEP visual acuity are subtle (ie, 1–2 lines on an eye chart), they are important because they suggest diet-related modifications in the developmental course of structure and function in the brain and/or retina. Any differences in the cytoarchitecture of the developing brain that result from differences in the dietary supply of DHA during infancy may have long-lasting effects on brain function. Indeed, some but not all studies of long-term outcomes of DHA-supplementation of infant formula have documented differences in visual and cognitive function at 18 mo to 4 y of age (8, 10, 29–31).

We have no explanation for the interaction between formula group and site with regard to VEP visual acuity, RBC DHA response to DHA supplementation, and anthropometric measures. We noted differences between the sites in race, ethnicity, and parents' education, consistent with the lower socioeconomic status of families at the Kansas City site compared with the Dallas site. These population factors or a related factor may in some way influence the outcomes measured in this study. Many factors that potentially moderate the effects of DHA supplementation on functional outcomes are associated with socioeconomic status, such as DHA status at birth, maternal alcohol use, and maternal tobacco use (32). With the data that were collected, we could not conclusively discount or confirm that these population factors or a related factor may in some way influence the outcomes measured in this study. Our study was not designed to evaluate these factors.

Differences in the rate of neurologic maturation between the cohorts at the 2 sites were apparent. Kansas City infants, particularly girls, had more mature visual acuity at 12 mo of age than did those in Dallas when fed control formula. We also noted that the Kansas City control group, particularly girls, had more mature visual acuity at 12 mo of age compared with control data in the literature (7, 9, 23), whereas the 0.32% DHA-supplemented group had visual acuity similar to that of published data for the same diet (23).

The dose-response relation between amount of DHA supplementation and RBC DHA concentration had a smaller dynamic range for the Kansas City cohort than for the Dallas cohort at both 4 and 12 mo of age. There is no evidence to suggest that blood collection by venipuncture in Kansas City, as opposed to blood collection by heel stick in Dallas, contributed to these observed differences in RBC DHA at the 2 sites. The excellent agreement between the 2 sites in the dynamic range of DHA RBC concentrations measured in the control group and in the 0.32% DHA group (Table 3) further suggests that differences in the blood collection method did not contribute to differences in the dynamic range of RBC DHA at the 2 sites. Nor can the smaller dynamic range of RBC DHA response in the Kansas City group be explained by differences in DHA intake between the 2 cohorts. At 9 and 12 mo of age, there were significantly lower intakes of formula at Kansas City than at Dallas; this may have accounted for the overall lower DHA at the Kansas City site than at the Dallas site but not for differences between formula groups. Weaning practices and weaning foods did differ significantly between the 2 sites but, because foods that were potential sources of DHA (dairy products, fish, meat, and poultry) were fed to more children in the Kansas City cohort than in the Dallas cohort,

weaning practices cannot explain the lower RBC DHA concentration found at the Kansas City site.

Our finding of a different dose-response relation at the 2 study sites, despite careful harmonization of protocols, is consistent with the hypothesis that some of the heterogeneity in functional outcomes of DHA supplementation of infant formula among published studies may result from genetic heterogeneity. A single nucleotide polymorphism for FADS2 has been reported in children to interact with human milk intake to produce a significant cognitive benefit (33). Another single nucleotide polymorphism of FADS 1/2 has been linked to higher ARA, but the data suggest that higher DHA too would have been associated if the study sample had been large (34). Whereas these studies did not establish that any genetic variant influences DHA status, such a finding in the future would not be surprising. Indeed, the authors suggested that FADS2 genetic variations may influence the biosynthesis of LCPUFAs from their precursors, condition the feedback regulation of PUFAs, alter gene expression in LCPUFA pathways or, more directly, alter the expression of genes involved in synaptic plasticity (33).

Infants tolerated all formulas well and had normal growth throughout the first 12 mo of life. In a recent consensus statement published under the auspices of the World Association of Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation (30), the recommended LCPUFA content in infant formulas includes the addition of $\geq 0.2\%$ of fatty acids as DHA, with at least the same amount of ARA, but they caution that DHA amounts should not exceed 0.5% of fatty acids because systematic evaluation of higher intakes had not been published as of 2007. Our data speak directly to the safety and tolerance profiles of DHA amounts as high as 0.96% of fatty acids in infant formula. The safety and tolerance of these higher DHA concentrations was expected, because they are within the range of DHA concentrations found in human milk worldwide (20).

We identified no sources of bias or confounding to explain our findings. The follow-up visit rate was high at both sites. Infants, parents, and investigators were masked to the assigned amount of DHA supplementation. VEP visual acuity testing was performed by a standardized protocol by using a VEP testing instrument developed specifically for the study of visual maturation in infants, and harmonization procedures were in place to ensure consistency of testing across both sites. With the sample size of 244 infants having a 12-mo VEP visual acuity score, and using the observed SD of the outcome VEP visual acuity scores, the statistical power to detect a group difference of 0.1 logMAR (ie, one line on a standard eye chart) in VEP visual acuity was 96% at Dallas and 85% at Kansas City. Thus, it seems unlikely that we missed a true VEP visual acuity difference of meaningful magnitude between the different amounts of DHA supplementation.

In summary, supplementation of infant formula with 0.32% to 0.64% DHA appears to be sufficient to promote VEP visual acuity maturation during infancy. Careful harmonization of methods between sites supports the conclusion that the site-specific results for VEP visual acuity and RBC DHA response to DHA intake reflect true differences between the control and supplemented formula groups. Whether differences in long-term outcomes will be observed between control and supplemented formula groups and whether they follow a similar



dose-response function as the primary 12-mo outcome remains to be determined.

We appreciate the considerable contribution of time and effort from the parents, caregivers, and infants who participated in the study. We are grateful to Kim Merkel for study monitoring, to Paul Ferguson for guidance on statistical analyses, to Julia Boettcher for assistance with writing, to Sara Hildebrand and Lindsey Weidemann for assistance with the fatty acid analysis, and to Sarah Morale and Christina Cheng Patel for assistance with VEP testing.

The authors' responsibilities were as follows—EEB, SEC, DRH, and DAD-S: participated in the conception and design of the study; YSC, LM, and DM: participated in the study coordination, participant enrollment, and medical oversight of participants; KMF-G, VLNF, JRD, YSC, LM, DM, and DKHW: participated in data collection; EEB, SEC, DRH, KMF-G, JM, and DAD-S: participated in the data analysis and interpretation; EEB: wrote the article; and SEC, DRH, KMF-G, JM, and DAD-S: provided critical revisions. JM and DAD-S are employees of Mead Johnson Nutrition. SEC and DRH have served on speakers' panels at scientific and educational conferences on behalf of Mead Johnson. None of the other authors had a potential conflict of interest related to this study.

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