Visual, Cognitive, and Language Assessments at 39 Months: A Follow-up Study of Children Fed Formulas Containing Long-Chain Polyunsaturated Fatty Acids to 1 Year of Age

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ABSTRACT. ***Objective.*** **Docosahexaenoic acid (DHA) and arachidonic acid (ARA) are long-chain polyunsaturated fatty acids found in breast milk and recently added to infant formulas. Their importance in infant nutrition was recognized by the rapid accretion of these fatty acids in the brain during the first postnatal year, reports of enhanced intellectual development in breastfed children, and recognition of the physiologic importance of DHA in visual and neural systems from studies in animal models. These considerations led to clinical trials to evaluate whether infant formulas that are supplemented with DHA or both DHA and ARA would enhance visual and cognitive development or whether conversion of linoleic acid and α-linolenic acid, the essential fatty acid precursors of ARA and DHA, respectively, at the levels found in infant formulas is sufficient to support adequately visual and cognitive development. Visual and cognitive development were not different with supplementation in some studies, whereas other studies reported benefits of adding DHA or both DHA and ARA to formula. One of the first trials with term infants that were fed formula supplemented with DHA or both DHA and ARA evaluated growth, visual acuity (Visual Evoked Potential; Acuity Card Procedure), mental and motor development (Bayley Scales of Infant Development), and early language development (MacArthur Communicative Developmental Inventories). Growth, visual acuity, and mental and motor development were not different among the 3 formula groups or between the breastfed and formula-fed infants in the first year of life. At 14 months of age, infants who were fed the formula with DHA but no ARA had lower vocabulary production and comprehension scores than infants who were fed the unsupplemented control formula or who were breastfed, respectively. The present follow-up study evaluated IQ, receptive and expressive vocabulary, visual-motor function, and visual acuity of children from the original trial when they reached 39 months of age.**

Methods. **Infants were randomized within 1 week after birth and fed a control formula (n = 65), one containing DHA (n = 65), or one containing both ARA and DHA (n = 66) to 1 year of age. A comparison group (n = 80) was exclusively breastfed for at least 3 months after which the infants continued to be exclusively breastfed or were supplemented with and/or weaned to infant formula. At 39 months, standard tests of IQ (Stanford Binet IQ), receptive vocabulary (Peabody Picture Vocabulary Test-Revised), expressive vocabulary (mean length of utterance), visual-motor function (Beery Visual-Motor Index), and visual acuity (Acuity Card Procedure) were administered. Growth, red blood cell fatty acid levels, and morbidity also were evaluated.**

Results. **Results were analyzed using analysis of variance or linear regression models. The regression model for IQ, receptive and expressive language, and the visual-motor index controlled for site, birth weight, sex, maternal education, maternal age, and the child's age at testing. The regression model for visual acuity controlled for site only. A variable selection model also identified which of 22 potentially prognostic variables among different categories (feeding groups, the child and family demographics, indicators of illness since birth, and environment) were most influential for IQ and expressive vocabulary. A total of 157 (80%) of the 197 infants studied at 12 months participated in this follow-up study. Characteristics of the families were representative of US families with children up to 5 years of age, and there were no differences in the demographic or family characteristics among the randomized formula groups. As expected, the formula and breastfed groups differed in ethnicity, marital status, parental education, and the prevalence of smoking. Sex, ethnicity, gestational age at birth, and birth weight for those who participated at 39 months did not differ from those who did not. The 12-month Bayley mental and motor scores and 14-month vocabulary scores of the children who participated also were not different from those who did not. At 39 months, IQ, receptive and expressive language, visual-motor function, and visual acuity were not different among the 3 randomized formula groups or between the breastfed and formula groups. The adjusted means for the control, ARA+DHA, DHA, and breastfed groups were as follows: IQ scores, 104, 101, 100, 106; Peabody Picture Vocabulary Test, 99.2, 97.2, 95.1, 97.4; mean length of utterance, 3.64, 3.75, 3.93, 4.08; the visual-motor index, 2.26, 2.24, 2.05, 2.40; and visual acuity (cycles/degree), 30.4, 27.9, 27.5, 28.6, respectively. IQ was positively associated with female sex and**

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maternal education and negatively associated with the number of siblings and exposure to cigarette smoking in utero and/or postnatally. Expressive language also was positively associated with maternal education and negatively associated with the average hours in child care per week and hospitalizations since birth but only when the breastfed group was included in the analysis. The associations between maternal education and child IQ scores are consistent with previous reports as are the associations between prenatal exposure to cigarette smoke and IQ and early language development. Approximately one third of the variance for IQ was explained by sex, maternal education, the number of siblings, and exposure to cigarette smoke. Growth achievement, red blood cell fatty acid levels, and morbidity did not differ among groups.

Conclusions. **We reported previously that infants who were fed an unsupplemented formula or one with DHA or with both DHA and ARA through 12 months or were breastfed showed no differences in mental and motor development, but those who were fed DHA without ARA had lower vocabulary scores on a standardized, parent-report instrument at 14 months of age when compared with infants who were fed the unsupplemented formula or who were breastfed. When the infants were reassessed at 39 months using age-appropriate tests of receptive and expressive language as well as IQ, visual-motor function and visual acuity, no differences among the formula groups or between the formula and breastfed groups were found. The 14-month observation thus may have been a transient effect of DHA (without ARA) supplementation on early vocabulary development or may have occurred by chance. The absence of differences in growth achievement adds to the evidence that DHA with or without ARA supports normal growth in full-term infants. In conclusion, adding both DHA and ARA when supplementing infant formulas with long-chain polyunsaturated fatty acids supports visual and cognitive development through 39 months.** ***Pediatrics*** **2003;112:e177–e183. URL: http://www.pediatrics.org/cgi/content/full/112/3/e177;** ***infant formula, breastfeeding, long-chain polyunsaturated fatty acids, docosahexaenoic acid, child development.***

ABBREVIATIONS. DHA, docosahexaenoic acid; ARA, arachidonic acid; PPVT-R, Peabody Picture Vocabulary Test-Revised; MLU, mean length of utterance.

Docosahexaenoic acid (DHA) and arachidonic acid (ARA), 2 long-chain polyunsaturated fatty acids in breast milk,[1] have been added to some infant formulas in the United States and other countries. Their importance in infant nutrition was suggested by the rapid accretion of these fatty acids in the brain during the first postnatal year,[2] epidemiologic reports suggesting enhanced intellectual development in breastfed children compared with those who were fed infant formula,[3–7] and the recognition of the importance of DHA in visual and neural systems in animal models.[8] These considerations led to clinical trials to evaluate whether infant formulas supplemented with DHA or with both DHA and ARA would enhance visual and cognitive development. The fundamental question has been whether conversion of linoleic acid and α-linolenic acid—the essential fatty acid precursors of ARA and DHA, respectively—at the levels found in infant formulas is sufficient to support adequately visual and cognitive development.

The early studies with preterm infants, who are more likely to benefit from DHA supplementation than full-term infants, evaluated adding DHA to preterm formulas; ARA was not added in the early studies. Improved visual development was reported for preterm infants who were fed the DHA-supplemented formulas[9–11]; however, some studies also found evidence of slower growth.[11–13] In subsequent studies with preterm infants who were fed formulas containing both ARA and DHA, enhanced visual and cognitive development[14] was found with no adverse effects on growth,[14–17] except for 1 study that reported shorter length at 18 months but not at 9 months.[18]

Similar studies with full-term infants who were fed formulas with DHA or both DHA and ARA, however, found no evidence of slower growth. In these studies, results on tests of visual and cognitive development are mixed. Visual and cognitive development were not improved with supplementation in some studies,[19–23] whereas other studies reported benefits of supplementing formulas with DHA or both DHA and ARA.[24–29] Mixed results were also seen in studies that included comparisons between formula-fed and breastfed infants studied concurrently.[19–22,24–26,30]

In 1 study, infants who were fed a formula supplemented with DHA but not one with both DHA and ARA had lower scores on a test of early language development than infants who were fed an unsupplemented control formula or were breastfed.[19] This study added to the evidence from preterm studies that formulas that are supplemented with DHA should also include ARA. Language depends on higher cortical function and can be followed longitudinally.[31] Therefore, it may be an especially suitable domain in which to evaluate nutritional effects on infant development.[32] The present study is a follow-up evaluation of these study children at 39 months of age. Neurodevelopmental outcomes included IQ, receptive and expressive vocabulary, visual-motor function, and visual acuity. Growth achievement was also determined.

METHODS

This double-masked follow-up study was conducted at the University of Washington (Seattle, WA), Oregon Health and Science University (Portland, OR), and Children's Mercy Hospital (Kansas City, MO). The protocol was approved by the institutional review board(s) at each center and was conducted in accordance with the ethical principles derived from the Declaration of Helsinki. Eligible families were first contacted by telephone, and if they expressed willingness to participate, then a clinic visit was scheduled. Parents gave written informed consent before the follow-up assessments were made.

In the original infant feeding study, the infants were randomized at a median of 2 days after birth to 1 of 3 infant formulas with or without long-chain polyunsaturated fatty acids.[19,20] The study formulas were fed to 12 months of age. A concurrent comparison group of infants were breastfed exclusively to 3 months of age. The control formula contained no ARA or DHA, the ARA+DHA-supplemented formula contained (% total fatty acids) 0.43% ARA and 0.12% DHA from egg-phospholipid, and the DHA-supplemented formula contained 0.23% DHA from fish oil (Table 1). All formulas were liquid ready-to-feed milk-based formulas with (per

TABLE 1. Fatty Acid Composition (g/100 g Total Fatty Acids) of the Infant Formulas Fed in the First Year*

Fatty Acid†	Control	ARA+DHA	DHA
Saturated	28.6	28.6	30.9
Monounsaturated	39.5	42.1	40.2
Omega-6			
18:2n-6	21.9	21.7	20.7
20:4n-6	ND	0.43	-
Omega-3			
18:3n-3	2.2	1.9	1.9
20:5n-3	-	-	-
22:6n-3	-	0.12	0.23

ND indicates not detected.
* From Vanderhoof et al.[16]
† Saturated fatty acids: 12:0, lauric acid; 14:0, myristic acid; 16:0, palmitic acid; 18:0, stearic acid. Monounsaturated fatty acids: 18:1, oleic acid. Omega-6 fatty acids: 18:2n-6, linoleic acid; 20:4n-6, ARA. Omega-3 fatty acids: 18:3n-3, α-linolenic acid; 20:5n-3, eicosapentaenoic acid; 22:6n-3, DHA.

liter) 14.3 to 15.0 g of protein, 72.4 to 74.8 g of carbohydrate, 35.9 to 37.2 g of fat, and 670 to 694 kcal. The fat blends consisted of high-oleic safflower oil, soy oil, and coconut oil with or without the sources of long-chain polyunsaturated fatty acids. The levels of ARA and DHA in breast milk samples from a subset of women (Portland) whose infants were in the breastfed group were 0.48 ± 0.10 and 0.15 ± 0.09%, respectively, generally consistent with the levels of these fatty acids reported in many[23,28,33–35] but not all[26] US studies published in recent years. There was no feeding intervention beyond the first year.

Infants were eligible for the original feeding study when they were ≥37 weeks of gestation at birth, had a birth weight appropriate for gestational age, and were <1 week of age at enrollment. Infants with 5-minute Apgar scores <7 or physical or metabolic defects or those who received intravenous lipid infusions or blood transfusions were not eligible.

Eligibility for the 39-month follow-up study required previous participation in the original feeding trial, including completion of the 12- and/or 14-month assessments,[19,20] informed consent from parents/guardians, and availability for study at 39 months ± 3 weeks. The primary outcome assessments at 39 months were IQ, language development, visual acuity, and growth anthropometrics. Additional assessments included an index of visual-motor function, red blood cell fatty acid levels, and the frequency of illnesses and hospitalizations since birth. Birth demographics were obtained from the original study records. Information about family demographics, child care attendance, and the medical history from birth (frequency of antibiotic use for ear infections, pressure equalization tubes for chronic otitis media, and hospitalizations) was obtained by parent interview at the follow-up clinic visit.

A workshop was held to standardize the procedures to administer the cognitive and language tests. The Stanford-Binet Intelligence Scale Form L-M[36], the Peabody Picture Vocabulary Test-Revised (PPVT-R)[37], and the Beery Visual-Motor Index test[38] were administered according to the guidelines in their manuals. Mean length of utterance (MLU) was derived from a sample of the child's spoken language.[39–41] Briefly, the mother was instructed to engage in conversational play with her child, and the child's spoken language was recorded on an audiocassette recorder for 15 to 30 minutes and/or until 50 utterances were obtained. A written transcript was prepared during the audiocassette recording session. All transcript and audiocassette tapes were analyzed at the Speech and Hearing Clinic, Children's Mercy Hospital, Kansas City. The tapes were transcribed and coded by 2 listeners and analyzed using the 3.0 version of the Basic Systematic Analysis of Language Transcripts Program. In a standardization assessment performed before the data analysis, >90% agreement was found between the 2 individuals who transcribed and coded a random subset of MLU samples. Visual acuity was assessed using the Teller acuity card procedure (Vistech, Inc, Dayton, OH).

The sample sizes varied among the various tests. Some children were uncooperative or lost interest during the testing, 20 children had inaudible audiocassette tapes or did not provide a complete language sample (MLU), and the Beery Visual-Motor Index test was administered incorrectly to 6 children. In addition, the IQ and vocabulary results for 2 bilingual children with minimal exposure to English and for 2 children with vision abnormalities (bilateral amblyopia, exotropia of the right eye) were excluded before the study was unmasked. The number (percentage) of children with assessable results was 147 (94%) for the Stanford-Binet IQ test, 144 (92%) for the visual-motor index, 147 (94%) for receptive vocabulary (PPVT-R), 132 (84%) for expressive vocabulary (MLU), and 147 (94%) for visual acuity (see Table 2 for n/group). The percentage of children without assessable results or children who were tested outside the study window were represented across all feeding groups. The mean age at testing was 39 weeks for all groups, and all testing was completed within a 6-week window (37–43 months of age).

Weight, height, and head circumference were measured at the clinic visits using standard equipment. Blood samples (5 mL) were obtained by venipuncture from a subset of children whose parents agreed to blood draws in Portland and Kansas City. The red blood cells were isolated and analyzed for fatty acid composition as described previously.[20]

Statistical Analyses

Comparisons were made among the 3 randomized formula groups and between the formula and breastfed groups. Analyses were 2-sided at a .05 significance level. Comparability of the subjects who participated in this follow-up assessment with those who did not and among the groups at entry to the study was assessed using analysis of variance and χ^2 tests of association. Subject feeding assignments were included in this analysis to ensure that there were no differences in participation by group. Weight, height, and head circumference were analyzed by 2-way analyses of variance controlling for sex. The Bonferroni procedure was used for pairwise comparisons. A multiple linear regression model was used to analyze the developmental test results. Diagnostic tests for multicollinearity and influential points were conducted, and assumptions of normality were checked using SAS Proc Reg. The regression model for IQ, receptive and expressive language, and the visual-motor index controlled for site, birth weight, sex, maternal education, maternal age, and the child's age at testing. The regression model for visual acuity controlled for site only. As an exploratory analysis, a variable selection model also was used to identify which of 22 potentially prognostic variables among several different categories (feeding groups, the child and family demographics, indicators of illness since birth, and environment) were most influential for IQ and expressive vocabulary.

RESULTS

A total of 157 (80%) of the 197 infants studied at 12 months[19,20] participated in this follow-up study. Twenty-four families could not be located or had moved out of the area, 14 declined participation, and 1 child was ineligible (>42 months of age at study initiation); the results for 1 child, who was mistakenly enrolled and tested at 48 months, were excluded before the study was unmasked. Sex, ethnicity, gestational age at birth, and birth weight for those who participated at 39 months did not differ from those who did not (data not shown). In addition, the 12-month Bayley mental and motor scores and the 14-month vocabulary scores of the children who participated in this follow-up assessment were not different from those who did not (data not shown).

Although there were no differences in the demographic and family characteristics among the randomized formula groups, expected differences between the formula-fed and breastfed groups were found (Table 2). Children in the breastfed group were predominantly white (96%; $P = .03$) and from 2-parent families ($P < .005$). By comparison, up to 20% of those in the formula-fed groups were from other ethnic backgrounds, and only 59% to 79% were

TABLE 2. Demographic and Family Characteristics

	Formula Groups			Breastfed Group (n = 50)
	Control (n = 37)	ARA+DHA (n = 35)	DHA (n = 35)	
Birth weight (kg)	3.65 ± 0.47	3.48 ± 0.44	3.60 ± 0.44	3.69 ± 0.45
Gestational age (wk)	39.8 ± 1.0	39.3 ± 1.3	39.7 ± 1.3	40.0 ± 1.2
Male (n [%])	22 (59)	20 (57)	19 (54)	25 (50)
Ethnicity (n [%])*				
White	30 (81)	28 (80)	32 (91)	48 (96)
Black	4 (11)	1 (3)	2 (6)	0 (0)
Hispanic	2 (5)	1 (3)	0 (0)	1 (2)
Other	1 (3)	5 (14)	1 (3)	1 (2)
Birth order (n [%])				
1	17 (46)	11 (31)	15 (43)	25 (50)
2	10 (27)	13 (37)	7 (20)	16 (32)
≥3	10 (27)	11 (31)	12 (34)	7 (14)
Missing	0	0	1 (3)	2 (4)
Smoking (n [%], yes)				
During pregnancy*	4 (11)	9 (26)	6 (17)	4 (8)
In household (at birth)*	11 (31)	14 (40)	8 (23)	4 (8)
Demographics at 39-month follow-up				
Marital status (n [%])*				
Married	22 (59)	22 (63)	27 (79)	45 (92)
Divorced	5 (14)	2 (6)	2 (6)	2 (4)
Separated	1 (3)	2 (6)	1 (3)	1 (2)
Cohabitating	5 (14)	2 (6)	3 (9)	0 (0)
Single	4 (11)	7 (20)	1 (3)	1 (2)
Maternal age (y)	30.5 ± 4.4	31.7 ± 5.6	31.7 ± 6.0	33.3 ± 4.9
Parental education (y)				
Mother	13.7 ± 2.2‡	14.7 ± 2.4	14.0 ± 2.3	15.3 ± 2.0
Father	13.7 ± 2.6‡	13.6 ± 2.1†	14.8 ± 2.3	15.1 ± 2.7
Attending child care (n [%])	31 (84)	24 (69)	26 (76)	39 (80)
Hours/wk (median; interquartile range)	24 (3–40)	16 (0–35)	25 (2–43)	9 (3–21)
Age (mo; range)	39 ± 1 (38–42)	39 ± 1 (38–42)	39 ± 1 (38–41)	39 ± 1 (38–43)

* Significant differences among the 4 feeding groups by χ^2 ($P < .05$).
† Significantly different from the breastfed group ($P < .05$) by analysis of variance. There were no significant differences among the 3 randomized formula groups.
Values with ± are means ± SD.

from 2-parent families. The prevalence of smoking in the household was lower at birth for the breastfed group (8%) than for the formula-fed groups (23%–40%; $P = .005$). In addition, the level of educational achievement was greater for 1 or both parents in the breastfed group than in the control or the ARA+DHA groups ($P < .005$).

The groups did not differ in weight, length, or head circumference at 39 months of age (Table 3). There were also no differences found among the 3 randomized formula groups or between the breastfed and formula groups for IQ, receptive and expressive language, visual-motor function, or visual acuity (Table 4). Female sex was positively associated with IQ ($P < .01$), receptive vocabulary ($P < .02$), and visual-motor ability ($P < .04$). The number of years of maternal education was positively associated with IQ ($P < .04$) and receptive vocabulary ($P < .04$) when either all 4 feeding groups or only the formula groups were included in the regression model.

The variable selection model identified which of 22 potentially influential variables contributed significantly to the variance for IQ and expressive language. Approximately one third of the variance for IQ ($P < .05$) was explained by 4 factors: sex, years of maternal education, number of siblings, and exposure to cigarette smoke. Positive associations were found for female sex and maternal education, and

TABLE 3. Anthropometric Measures at 39 Months for Children Who Were Fed Formulas With or Without ARA and DHA or DHA Without ARA or Were Breastfed

	Formula Groups			Breastfed Group
	Control	ARA+DHA	DHA	
Weight (kg)				
Boys	15.8 ± 2.4 (22)*	15.9 ± 1.8 (19)	16.4 ± 1.8 (19)	15.6 ± 2.0 (24)
Girls	14.8 ± 1.5 (15)	15.4 ± 2.2 (15)	14.8 ± 1.6 (16)	14.6 ± 1.6 (25)
Height (cm)				
Boys	97.6 ± 5.1 (22)	98.7 ± 3.2 (18)	99.3 ± 4.3 (19)	98.3 ± 3.8 (24)
Girls	96.6 ± 3.8 (15)	97.2 ± 3.3 (14)	96.8 ± 4.0 (16)	96.5 ± 4.0 (25)
Head circumference (cm)				
Boys	50.9 ± 1.6 (21)	50.4 ± 1.1 (20)	51.5 ± 1.3 (21)	51.0 ± 1.5 (24)
Girls	49.6 ± 1.5 (15)	50.2 ± 1.3 (15)	49.8 ± 1.5 (16)	49.7 ± 1.1 (25)

* Values are mean ± SD (n). There was a significant feeding by sex interaction for all measures ($P < .05$) but no significant differences among feeding groups ($P > .05$).

TABLE 4. Developmental Measures at 39 Months for Children Who Were Fed Formulas With or Without ARA and DHA or DHA Without ARA or Were Breastfed*

	Formula Groups			Breast-fed Group	ANCOVA *P* Value†
	Control	ARA+DHA	DHA		
IQ (Stanford-Binet)	103 ± 15 (35)‡	101 ± 13 (34)	99 ± 12 (33)	108 ± 16 (45)	.14
Receptive vocabulary (PPVT-R)	97.3 ± 14.2 (35)	96.6 ± 15.8 (34)	95.1 ± 13.2 (34)	98.9 ± 16.9 (46)	.70
Expressive vocabulary (MLU; morphemes)	3.59 ± 0.87 (34)	3.70 ± 0.88 (31)	3.92 ± 0.76 (29)	4.16 ± 0.81 (38)	.14
Visual-Motor Index Score§	2.18 ± 1.29 (33)	2.23 ± 1.38 (31)	1.91 ± 1.18 (33)	2.42 ± 1.32 (48)	.73
Visual Acuity (Teller Acuity Cards; cy/deg)‖	30.3 ± 0.7 (36)	28.2 ± 0.6 (32)	27.5 ± 0.6 (32)	28.6 ± 0.6 (46)	.74

ANCOVA indicates analysis of covariance; SD, standard deviation.
* Unless otherwise indicated, values are reported as standard scores. (), number of infants tested.
† There were no significant differences among the 3 formula groups or when both the formula and the breastfed groups were included in the analyses. Regression analyses for IQ, receptive and expressive vocabulary, and the visual-motor index controlled for site, birth weight, sex, maternal education, maternal age, and the child's age at testing; regression analyses for visual acuity controlled for site.
‡ Unadjusted mean ± SD (*n*). Adjusted means for the control, ARA+DHA, DHA, and breastfed groups were as follows: IQ, 104, 101, 100, 106; PPVT-R, 99.2, 97.2, 95.1, 97.4; MLU, 3.64, 3.75, 3.93, 4.08; VMI, 2.26, 2.24, 2.05, 2.40; visual acuity, 30.4, 27.9, 27.5, 28.6, respectively.
§ Values shown as raw scores
‖ Mean shown as cy/deg and SD as octaves.

negative associations were found for number of siblings and exposure to cigarette smoking in utero and/or postnatally. Expressive language was positively associated ($P < .05$) with maternal education but negatively associated ($P < .05$) with average hours in child care per week and hospitalizations since birth but only when the breastfed group was included in the analyses.

At 14 months, there was a significant association between vocabulary production and comprehension ($r^2 = 0.24$; $P = .0001$). At 39 months, there was a significant association between receptive language (PPVT-R) and IQ ($r^2 = 0.53$; $P = .0001$) and a small but statistically significant association between receptive (PPVT-R) and expressive (MLU) language ($r^2 = 0.04$; $P < .05$) and between expressive language (MLU) and IQ ($r^2 = 0.04$; $P < .05$). However, no significant associations ($P > .05$) between vocabulary production at 14 months and expressive language (MLU) at 39 months were found.

There was no evidence of differences in health status during the first 39 months after birth. The groups did not differ in the percentage of children who had had 3 or more prescriptions for antibiotics since birth (breastfed, 66%; formula control, 62%; ARA+DHA, 46%; DHA, 57%), had pressure equalization tubes for chronic otitis media (breastfed, 4%; formula control, 8%; ARA+DHA, 11%; DHA, 6%), or were hospitalized at least once since birth (breastfed, 14%; formula control, 19%; ARA+DHA, 29%; DHA, 12%). The percentage of children who were taking prescription medications at the time of the follow-up visit also did not differ significantly among the groups (16%, 25%, 6%, and 12% for the breastfed, formula control, ARA+DHA, and DHA groups, respectively). The levels of DHA and ARA in red blood cell fatty acid phospholipids (g/100 g total fatty acids) at 39 months also were not different among groups (Table 5).

DISCUSSION

Whether infant formulas should be supplemented with long-chain polyunsaturated fatty acids has been hotly debated during the past decade. Some expert committees have published recommendations to add DHA and ARA to infant formulas (eg, FAO/WHO/UNU),[42] whereas others (eg, Raiten et al[43]) recommended reevaluation after completion of clinical trials ongoing at that time. Some studies have reported improved visual acuity[25,26,28,44] and/or cognitive development[24,27,29] with supplementation, whereas others found no benefits[19–23,30] There are several possible reasons for the inconsistent findings in these studies: 1) differences in the amounts of α-linolenic acid (1.1%–2.6% of fatty acids), DHA (0.12%–0.36% of fatty acids), and/or ARA (0.30%–0.72%) in the formulas studied; 2) other differences among the formulas (eg, sources of fats); 3) differences in the test instruments used, testing procedures, or outcomes studied; 4) sample size limitations; or 5) whether the infants were term or preterm.

Among the studies that have followed development of children past the period during which they were fed formulas with DHA or with both DHA and ARA, 39 months is the longest follow-up period reported to date. The present study is the only randomized, prospective supplementation study that evaluated visual and cognitive development through 14 months[19,20] and again at 39 months of age. Other studies have evaluated visual acuity in full-term in-

TABLE 5. AA and DHA in Red Blood Cell Phospholipids (g/100 g Total Fatty Acids) at 39 Months for Children Who Were Fed Formulas With or Without ARA and DHA or DHA Without ARA or Were Breastfed

	Formula Groups			Breast-fed Group
	Control	ARA+DHA	DHA	
20:4n-6 (ARA)	2.2 ± 0.5 (35)*	2.2 ± 0.5 (34)	2.4 ± 0.4 (33)	2.4 ± 0.7 (45)
22:6n-3 (DHA)	14.6 ± 1.7 (35)	14.9 ± 1.4 (34)	14.7 ± 1.0 (34)	14.8 ± 1.5 (46)

* Values are reported as mean ± SD; ( ), number of samples. There were no significant differences among groups.

fants up to 1 year of age[21,23,25,26,28,30,44] and neurodevelopment at different ages between 4 months and 2 years using standardized[21–24,27,45] or investigational[29] test instruments.

Characteristics of the families in the present study were representative of US families with children up to 5 years of age.[46] The primary differences between the formula-fed and breastfed groups, as in other studies, were ethnicity, marital status, parental education, and the prevalence of smoking. The associations between maternal education and child IQ scores in the present study also are consistent with previous reports,[3–7] as are the associations between prenatal exposure to cigarette smoke and IQ and early language development.[47,48] The absence of differences in growth achievement among the children in this follow-up study adds to the evidence that DHA (with or without ARA) supports normal growth in full-term infants,[22,23,25,26,28,49] unlike the apparent slower growth seen in preterm infants who were fed formulas with DHA alone.[11–13] The number of infants in the present 39-month follow-up was larger than in many studies of visual or cognitive development[21,23,25,26,30,49] and included 80% of the children with 12- and/or 14-month assessments.

Because there was no feeding intervention past the first year, the similar amounts of ARA and DHA in red blood cells among groups were not surprising. The red blood cell levels of ARA and DHA in 2- to 13-year-old children published previously[50,51] are similar to those in the present study.

Early language development has been studied in relation to DHA levels in breast milk[52] as well as in another formula study of DHA or both DHA and ARA supplementation.[19,23] Innis et al[52] reported positive correlations between DHA levels in red blood cells and a laboratory-based assessment of speech perception at 9 months. However, given that this was an observational study, selection bias cannot be ruled out and causality cannot be presumed. In a different formula supplementation study,[27] infants were fed formulas with DHA (0.35% of fatty acids) or both DHA and ARA (0.36% and 0.72% of fatty acids, respectively) for the first 4 postnatal months, and vocabulary development at 18 months was assessed using the language subscale of the Bayley Scales of Infant Development (17–20 infants/group). No differences in vocabulary development at 18 months were found between the DHA (no ARA) group and the control group,[27] but instead higher scores for both supplemented groups on the cognitive and motor subscales and the overall mental index were reported. Study design differences between supplementation studies[27] that may explain the different early language results for the DHA (no ARA) groups include differences in feeding duration, sources and amounts of DHA supplementation, sample size, age when tested, and test instruments for early language development.

CONCLUSIONS

We reported previously that children who were fed a formula with DHA through 12 months showed no improvement in visual acuity through 12 months or on the Bayley Scales of Infant Development at 12 months but had lower vocabulary scores on a standardized, parent-report instrument (MacArthur Communicative Developmental Inventories) at 14 months of age when compared with those who were fed a control formula or who were breastfed.[19,20] When infants were reassessed at 39 months using age-appropriate tests of receptive and expressive language as well as visual-motor function and IQ, no differences among groups were found. The present 39-month follow-up results suggest that, within the constraints of sample size limitations (n = 35–50 children/group), the 14-month observation represented a transient effect of DHA (without ARA) supplementation on early vocabulary development or was a chance finding.

Continuity between early vocabulary production and expressive language development has been suggested by an association between 13-month vocabulary scores using a similar parent-report instrument to that in the present study and 20-month expressive language (MLU) results.[32] This contrasts to the results of the present study showing no significant associations between vocabulary production at 14 months and expressive language (MLU) at 39 months. Differences in the sociodemographic characteristics of the populations studied in relation to differential parental reporting biases and "floor" effects for early vocabulary and the nonlinear nature of early language development[53] may explain the inconsistent findings between these studies.

The present follow-up evaluation of growth, visual development, and neurodevelopmental outcomes at 39 months found no adverse effects or benefits of infant formula supplemented with DHA or with both DHA and ARA. It seems that the lower vocabulary scores at 14 months reported previously may have been transient or occurred by chance. In conclusion, providing both DHA and ARA when supplementing infant formulas with long-chain polyunsaturated fatty acids supports visual and cognitive development through 39 months.

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