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Growth and Development in Preterm Infants Fed Long-Chain Polyunsaturated Fatty Acids: A Prospective, Randomized Controlled Trial

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ABSTRACT. *Objectives.* **A randomized, masked, controlled trial was conducted to assess effects of supplementing premature infant formulas with oils containing the long-chain polyunsaturated fatty acids, arachidonic acid (AA; 20:4n6), and docosahexaenoic acid (DHA; 22:6n3) on growth, visual acuity, and multiple indices of development.**

Methods. **Infants ($N = 470$) with birth weights 750 to 1800 g were assigned within 72 hours of the first enteral feeding to 1 of 3 formula groups with or without long-chain polyunsaturated fatty acids: 1) control ($N = 144$), 2) AA+DHA from fish/fungal oil ($N = 140$), and 3) AA+DHA from egg-derived triglyceride (egg-TG)/fish oil ($N = 143$). Infants were fed human milk and/or Similac Special Care with or without 0.42% AA and 0.26% DHA to term corrected age (CA), then fed human milk or NeoSure with or without 0.42% AA and 0.16% DHA to 12 months' CA. Infants fed exclusively human milk to term CA (EHM-T; $N = 43$) served as a reference.**

Results. **Visual acuity measured by acuity cards at 2, 4, and 6 months' CA was not different among groups. Visual acuity measured by swept-parameter visual-evoked potentials in a subgroup from 3 sites (45 control, 50 AA+DHA [fish/fungal]; 39 AA+DHA [egg-TG/fish]; and 23 EHM-T) was better in both the AA+DHA (fish/fungal; least square [LS] means [cycle/degree] ± standard error [SE; octaves] 11.4 ± 0.1) and AA+DHA (egg-TG/fish; 12.5 ± 0.1) than control (8.4 ± 0.1) and closer to that of the EHM-T group (16.0 ± 0.2) at 6 months' CA. Visual acuity improved from 4 to 6 months' CA in all but the control group. Scores on the Fagan test of novelty preference were greater in AA+DHA (egg-TG/fish; LS means ± SE, 59.4 ± 7.7) than AA+DHA (fish/fungal; 57.0 ± 7.5) and control (57.5 ± 7.4) at 6 months' CA, but not at 9 months' CA. There were no differences in the Bayley Mental Development Index at 12 months' CA. However, the Bayley motor development index was higher for AA+DHA (fish/fungal; LS means ± SE, 90.6 ± 4.4) than control (81.8 ± 4.3) for infants ≤1250 g. When Spanish-speaking infants and twins were excluded from the analyses, the MacArthur Communicative Development Inventory revealed that control infants (LS means ± SE, 94.1 ± 2.9) had lower vocabulary comprehension at 14 months' CA than AA+DHA (fish/fungal) infants (100.6 ± 2.9) or AA+DHA (egg-TG/fish) infants (102.2 ± 2.8). There were no consistent differences in weight, length, head circumference, or anthropometric gains.**

Conclusion. **These results showed a benefit of supplementing formulas for premature infants with AA and DHA from either a fish/fungal or an egg-TG/fish source from the time of first enteral feeding to 12 months' CA.** *Pediatrics* **2001;108:359–371;** ***premature infants, docosahexaenoic acid, arachidonic acid, long-chain polyunsaturated fatty acids, infant formulas.***

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ABBREVIATIONS. AA, arachidonic acid; DHA, docosahexaenoic acid; SD, standard deviation; CA, corrected age; EPA, eicosapentaenoic acid; egg-TG, egg-derived triglyceride; SSC, Similac Special Care; SDAY 1, study day 1; EHM-T, exclusively human milk-fed to term CA; PC, phosphatidylcholine; PE, phosphatidylethanolamine; RBC, red blood cells; NEC, necrotizing enterocolitis; SAE, serious and/or unexpected adverse event; VEP, visual evoked potential; EEG, electroencephalogram; S:N, signal to noise ratio; ANCOVA, analysis of covariance; LS means, least square means; SE, standard error.

Whether or not formulas designed for the premature infant should be supplemented with long-chain polyunsaturated fatty acids, including arachidonic acid (AA; 20:4n-6) and docosahexaenoic acid (DHA; 22:6n-3) has become one of the most controversial issues in infant nutrition today. Several lines of logic suggest that prema-

ture infants fed formulas without AA and DHA may be at increased risk of slower development related to suboptimal blood and tissue levels of these fatty acids compared with the term infant. First, DHA accumulates in the brain and retina most rapidly during the last intrauterine trimester and during the early months after birth,[1,2] implying that the physiologic requirement for DHA is highest during the perinatal period. Clandinin et al[1] reported that ~80% of intrauterine AA+DHA accumulation occurs during the last trimester of pregnancy. Second, the physiologic supply of preformed AA+DHA to the premature infant is limited by early termination of maternal-to-fetal transfer of these fatty acids. Third, supply may also be limited by immature de novo synthesis of AA+DHA from their dietary essential precursor fatty acids, linoleic (18:2n-6) and α-linolenic (18:3n-3) acids, respectively. It has been shown that premature infants are capable of de novo synthesis of AA+DHA,[3,5] but it is not clear whether synthesis can meet the physiologic requirements for tissue accretion of these long-chain polyunsaturated fatty acids.[6] Furthermore, the impact of standard treatment modalities in the neonatal intensive care unit (eg, drugs, oxygen therapy) and negative energy balance on these biosynthetic pathways is unknown.

Results of randomized, controlled trials with premature infants fed formulas containing DHA in the absence of AA have been interpreted to suggest more rapid maturation of retinal function,[7] visual function,[8–10] and neurodevelopment.[11–12] However, there are also reports of slower growth in preterm infants[10,13,14] and lower scores on a test of infant language development in term infants[15] fed formulas containing DHA without AA. As early nutrition and growth can be a significant predictor of later development,[16,17] it is difficult to judge whether early improvements in visual and neurodevelopment are sufficient to warrant the feeding of DHA at the expense of slower growth.

Carlson et al[18] hypothesized that combined addition of AA and DHA to formulas would offset the observed negative impact of DHA on growth. To our knowledge, only the study by Vanderhoof et al[19] is of sufficient power (able to detect ≥0.5 standard deviation [SD] difference between groups) to test this hypothesis, although, arguably it may not have been of sufficient duration. It is particularly important that such studies feed both AA and DHA and examine growth until at least 4 months' corrected age (CA) and ideally after, because it is in this period that growth decelerated in previous studies[10,14] where a low eicosapentaenoic acid (EPA) source of DHA was fed. Furthermore, no studies to date, including that of Vanderhoof et al,[19] have examined the impact of feeding AA- and DHA-containing formula to premature infants until 12 months' CA, the recommended duration of formula feeding for term infants not fed human milk.[20] In addition, no studies have examined the impact of feeding AA+DHA beyond 2 months' CA as part of a nutrient-enriched feeding regimen specifically designed for premature infants. Lucas et al[21] demonstrated that the use of a nutrient-enriched formula to 9 months' CA resulted in greater linear growth and weight gain among premature infants than a formula designed for the term infant. Furthermore, none of the studies to date attempted to control for the potentially confounding effects of home environment and maternal intelligence on early infant development.[22]

Therefore, we conducted a comprehensive, randomized, controlled trial with adequate power and duration to assess the suitability and possible benefits of supplementing nutrient-enriched formulas designed for premature infants with oils containing AA+DHA to 12 months' CA.

MATERIALS AND METHODS

Study Sample Selection

Four hundred seventy preterm infants (<33 weeks' gestational age) with birth weights of 750 to 1805 g were enrolled between October 1996 and January 1998 from neonatal intensive care units in Cleveland, Ohio ($N = 48$); Kansas City, Missouri ($N = 84$); Little Rock, Arkansas ($N = 24$); Nottingham and Leeds, United Kingdom ($N = 85$); Louisville, Kentucky ($N = 74$); Portland, Oregon ($N = 88$); New York, New York ($N = 16$); and Santiago, Chile ($N = 51$). To assess the impact of study feeding on early feeding tolerance, infants were to be enrolled within 72 hours of the first enteral feeding (including trophic feeds or water). To broaden recruitment beyond the healthiest of infants in the nursery, infants could be enrolled as long as enteral feeding was initiated by the 28th day of life. Singleton and twin births and small-for-gestational age infants were allowed to participate. Infants with serious congenital abnormalities that could affect growth and development or who had undergone major surgery before randomization were not eligible to participate. Other exclusion criteria included periventricular/intraventricular hemorrhage greater than Grade II, maternal incapacity (including maternal cocaine or alcohol abuse during pregnancy or at time of enrollment), liquid ventilation, asphyxia resulting in severe and permanent neurologic damage, or uncontrolled systemic infection at the time of enrollment.

Experimental Design

After informed written consent from at least 1 parent or guardian, infants were randomized to 1 of 3 study formula groups with or without added long-chain polyunsaturated fatty acids; 1) control, 2) AA+DHA (fish/fungal oil), and 3) AA+DHA (egg-derived triglyceride [egg-TG]/fish oil). The centrally computer-generated randomization schedule was stratified for site, gender, and birth weight stratum (750–1250 g and 1251–1800 g) using a random permuted blocks algorithm. After randomization, participants were fed human milk and/or the assigned inhospital preterm formula (modified version of Similac Special Care ready-to-feed [24 kcal/fl oz]; SSC) with or without AA- and DHA-enriched oils until term CA. At term CA, infants were transitioned to an assigned postdischarge nutrient-enriched formula (modified version of NeoSure powder [22 kcal/fl oz]) with and without the same sources of AA+DHA and/or human milk to 12 months CA. The modified versions of SSC and NeoSure used for both control and AA+DHA-containing formulas in the present study differed from the commercial versions of these products in that they contained nucleotides (mean = 84.6 and 80.5 mg of free nucleotides/L for SSC and NeoSure, respectively), had a modified whey to casein ratio (approximately 50:50), and contained β-carotene (0.60 and 0.50 mg/L for SSC and NeoSure, respectively) and natural vitamin E (RRR α-tocopherol; 40.2 and 33.0 IU/L for SSC and NeoSure, respectively). The NeoSure product also contained a higher proportion of lactose (75% vs 50% carbohydrate).

The fatty acid composition of study formulas is found in Table 1. Formulas provided the dietary essential fatty acids, linoleic and α-linolenic acids (16%–20% and 2.5% of total fatty acids, respectively). The fat blend in SSC consisted of 30% soy, 20% coconut, and 50% medium-chain triglyceride oils. The fat blend in NeoSure powder consisted of 28% soy, 20% coconut, 25% medium-chain triglyceride, and 27% high-oleic safflower oils. The levels of coconut oil were reduced in the AA+DHA-supplemented formulas to keep total fat constant. In the AA+DHA-supplemented groups, AA and DHA provided 0.42% and 0.26% of fatty acids for the SSC

TABLE 1. Fatty Acid Composition of the Study Formulas

Fatty Acid	In-Hospital Formula			Postdischarge Preterm Formula		
	Control	AA+DHA (Fish/Fungal)*	AA+DHA (Egg-TG/Fish)	Control	AA+DHA (Fish/Fungal)	AA+DHA (Egg-TG/Fish)
Number of batches	3	3	3	5	6	5
Saturated						
6:0 (caproic acid)	0.38 ± 0.2†	0.34 ± 0.2	0.32 ± 0.2	0.24 ± 0.1	0.27 ± 0.1	0.22 ± 0.1
8:0 (caprylic acid)	30.4 ± 0.3	29.8 ± 0.4	29.6 ± 0.6	16.1 ± 0.9	17.0 ± 1.0	15.8 ± 0.6
10:0 (capric acid)	21.3 ± 0.6	21.2 ± 0.8	20.9 ± 0.6	11.2 ± 0.7	10.6 ± 1.3	10.6 ± 0.6
12:0 (lauric acid)	9.4 ± 0.2	8.1 ± 0.1	5.8 ± 0.3	9.5 ± 0.3	8.5 ± 0.4	6.1 ± 0.4
14:0 (myristic acid)	3.6 ± 0.1	3.2 ± 0.1	2.3 ± 0.1	3.7 ± 0.1	3.4 ± 0.2	2.4 ± 0.2
16:0 (palmitic acid)	5.3 ± 0.1	5.5 ± 0.1	6.4 ± 0.1	6.3 ± 0.2	6.4 ± 0.1	7.4 ± 0.1
18:0 (stearic acid)	2.6 ± 0.1	2.7 ± 0.1	3.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.0	3.2 ± 0.9
Monounsaturated						
18:1 (oleic acid)	8.2 ± 0.5	8.4 ± 0.7	9.8 ± 0.7	28.3 ± 0.6	27.9 ± 0.6	29.8 ± 0.4
Polyunsaturated						
18:2n-6 (linoleic acid)	16.0 ± 0.9	16.8 ± 1.0	17.5 ± 0.9	19.1 ± 1.1	19.5 ± 0.7	20.3 ± 0.4
18:3n-3 (α-linolenic acid)	2.4 ± 0.1	2.6 ± 0.3	2.5 ± 0.3	2.4 ± 0.2	2.4 ± 0.2	2.4 ± 0.2
20:4n-6 (AA)	ND	0.43 ± 0.02	0.41 ± 0.0	ND	0.43 ± 0.01	0.41 ± 0.02
20:5n-3 (EPA)	ND	0.08 ± 0.01	ND	ND	ND	ND
22:6n-3 (DHA)	ND	0.27 ± 0.04	0.24 ± 0.01	ND	0.16 ± 0.01	0.15 ± 0.02

ND indicates none detected.
* The purveyors of the fish, fungal, and egg-TG oils were Mochida International (Tokyo, Japan), Suntory Ltd (Osaka, Japan), and Eastman Chemicals Co (Kingsport, TN), respectively.
† Values are mean ± SD expressed as g/100 g total fatty acids. Fatty acid levels were determined by Analytical Research and Services, Ross Products Division, Abbott Laboratories, Columbus, Ohio.

formula and 0.44% and 0.16% for the NeoSure formula. In one of the AA+DHA-supplemented groups, fungal oil and low-EPA fish oil (DHA to EPA ratio ~3.5:1) were added to both SSC and NeoSure to provide AA and DHA, respectively. The EPA content was 0.08% of fatty acids in the SSC study formula, but was undetectable in the NeoSure study formula. In the other AA+DHA-supplemented group, egg-TG/fish provided both AA and DHA and low-EPA fish oil provided additional DHA in the SSC formula only. The EPA content was undetectable in these formulas.

During the planning phase, it was apparent that most infants in the participating neonatal intensive care units were neither exclusively formula nor human milk-fed, but rather fed a combination of formula and human milk. Hence, the study was designed to accommodate human milk feeding. At the time of first enteral feeding (study day 1 [SDAY 1]), infants could be 1) exclusively human milk-fed, 2) exclusively formula-fed, or 3) fed a combination of human milk and formula. There were no protocol restrictions that limited the amount or duration of human milk feeding. Whenever study infants were fed formula (eg, were being weaned from human milk), the protocol required the infant be fed the assigned study formula unless there was a medical indication to do otherwise. Infants who discontinued the assigned study feeding before 12 months' CA did not have subsequent blood samples drawn and were not administered the Fagan Test of Infant Intelligence. As planned a priori, a reference group consisting of infants exclusively human milk-fed until term CA (EHM-T) was identified from the pool of infants randomized to the 3 study formula groups. Exclusive human milk feeding was defined as <100 mL/kg birth weight formula for the total duration of their initial hospital stay and >80% of all feedings as human milk (fortified or unfortified) at term CA.

Demographic Data

Neonatal, perinatal, and family characteristics of enrolled infants were obtained from medical records or parental report. The HOME Inventory[23] was administered as an in-office questionnaire to the biological mother (if she was living in the home) to assess the quality and quantity of cognitive, social, and emotional support available to each infant in the home environment. The verbal scale of the Wechsler Adult Intelligence Scale-Revised (WAIS-R),[24] serving as a proxy of maternal intelligence, was individually administered to the biological mother (if living in the home) or primary caregiver at the 9-month CA visit.

Blood Fatty Acid Analyses

If blood was drawn at SDAY 1 and at hospital discharge as part of routine clinical practice, then additional blood was drawn for determination of the fatty acid composition of plasma and the phosphatidylcholine (PC) and phosphatidylethanolamine (PE) membrane fractions of red blood cells (RBCs). Furthermore, an attempt was made to obtain blood from all study infants who remained on human milk and/or study formulas at 4 and 12 months' CA for blood fatty acid analyses. Blood samples were processed and frozen at −70°C, and shipped on dry ice to a central laboratory (Analytical Research and Services, Ross Products Division, Columbus, OH) for analysis.[25]

Growth

Weight, length, and head circumference of infants were measured according to standardized procedures[26] at SDAY 1 (± 7 days) and at term (± 7 days), 2 (± 7 days), 4 (± 7 days), 6 (± 7 days), 9 (± 7 days) and 12 (± 10 days) months' CA. At each assessment, infants were weighed at least once in-hospital and twice after hospital discharge using an electronic or double-beam balance accurate to either ± 10 g (in-hospital) or ± 20 g (postdischarge). Recumbent length was measured to the nearest 0.1 cm using a length board with a fixed headboard and a movable footboard (Ellard Length Board, Seattle, WA). Head circumference was measured with a nonstretchable tape measure (InserTape, Ross Products Division, Columbus, OH).

In-Hospital Feeding Tolerance and Clinical Problems

The percentage of infants who had enteral feedings withheld for at least 1 day, the percentage of infants who had enteral feedings withheld because of gastric residuals, and the number of days to reach full enteral feeding (100 kcal/kg/d) were determined by reviewing the medical records for each infant for each day of initial hospitalization. Likewise, the incidence of suspected necrotizing enterocolitis (NEC), confirmed NEC (roentgenographic, surgical or postmortem evidence of pneumatosis, intra-abdominal free air or gas in the portal tract, or perforation), suspected systemic infection, confirmed systemic infection (positive blood culture), and chronic lung disease (supplemental oxygen beyond 1-month postnatal or 36 weeks' CA) were extracted from medical records.

Serious and/or Unexpected Adverse Events (SAEs)

A SAE was defined as any event that occurred during the clinical trial that resulted in death or was life-threatening, disabling, required hospital admission, or required intervention to prevent permanent impairment. This definition excluded nonlife-threatening emergency department visits. During the initial hospitalization period, the site research teams were instructed not to

include SAEs (other than infant death) which were expected in the natural history of the preterm infant but to include SAEs that, in the opinion of the investigator, could be, or were associated with the use of the study product.

Each SAE was reviewed and assigned an α-numeric organ system and severity score by a neonatologist (P Pollack, MD, Ross Products Division, Columbus, OH) masked to study feeding groups. Main categories included: 1) death; 2) pulmonary central, autonomic (eg, apnea, sudden cyanosis); 3) pulmonary parenchymal (eg, pneumonia, respiratory syncytial virus, asthma, wheezing); 4) other serious nonpulmonary disease (eg, diarrhea, dehydration, emesis, fever, sepsis); and 5) definitely unrelated to study feeding (eg, laser therapy for retinopathy, hernia repairs).

Visual Acuity

Behavioral Acuity

Behavioral visual acuity was assessed using the Teller Acuity Card Procedure (Vistech Inc, Dayton, OH)[27] at 2, 4, and 6 months' CA (± 7 days) after formal training and certification of each tester (primary tester and back-up). One of every 4 study infants, plus a small cohort of nonstudy infants ($N = 184$), was tested by 2 trained testers at each site to determine reliability. Tester agreement was within 0.59 and 0.50 octaves (1 line on a Snellen eye chart) for 95% and 78% of tests, respectively.

Visual Evoked Potential (VEP) Acuity

Visual acuity at 4 and 6 months' CA (± 7 days) was estimated using a VEP procedure[28,29] at the Kansas City, New York, and Portland sites only. The electroencephalogram (EEG) was recorded using 3 gold-cup EEG electrodes placed along the midline of the head with the active site at O_z, referenced to the vertex (C_z) and grounded midway between these 2 locations (P_z). An ENFANT recording system (Neuroscientific Corp, Morrisville, PA) was used to generate the visual stimuli, record the electrophysiological signals, and store the data (gain = 20K, bandpass = 0.5–100 Hz). Black and white (100% contrast) horizontal square-wave gratings (ie, black and white stripes) using a swept-parameter technique were presented on the stimulus display (noninterlaced frame rate = 59.9809 Hz, mean space-average luminance 100 cd/m^2; Nokia RGB monitor, Raleigh, NC) and contrast-reversed at 7.4976 Hz.[28,29] Infants were seated on a parent's lap in a darkened room at a distance of 114 cm from the stimulus display. At this distance, the screen subtended a visual angle of 10 × 10 degrees.

A discrete Fourier transform was performed on each 1-second epoch of the EEG. The sine and cosine components of the second harmonic response for each corresponding epoch (either 5 or 10 sweeps) were vector-averaged to yield a mean response. Amplitude and phase values were derived from these means. The Tcirc-squared statistic was used to estimate a 95% confidence circle about the mean vector and obtain a signal-to-noise ratio (S:N).[29,30] Acuity was estimated by linear interpolation between 2 adjacent points to a S:N = 1 (1 point with S:N >1 and the other point with S:N <1).

General Developmental Level

The Bayley Scales of Infant Development[31] (Psychological Corp, San Antonio, TX) was administered by a single, certified tester at each site (except where there was a turnover of study staff) at 12 months' CA (± 10 days) to assess cognitive and motor development (Mental Developmental Index; Psychomotor Developmental Index; respectively). After a centralized training session, testers videotaped sessions in which they administered the Bayley to 12 months' CA nonstudy infants. A tester was considered certified when s/he had administered 3 sessions in which there was 80% agreement with the central tester (Dr R Arendt, Cleveland, OH) on items for the Mental Developmental Index and Psychomotor Development Index. One out of approximately every 10 study infants ($N = 41$) was videotaped during the administration of the Bayley and these videotapes were scored centrally, independent of the site tester. The average percent agreement on scoring between site testers and the central testers was 91% (range, 71%–100%) and 93% (range 73%–100%) for the mental and motor development indices, respectively.

Information Processing

The Fagan Test of Infant Intelligence[32] (Infantest Corporation, Cleveland, OH) was administered at 6 and 9 months' CA (± 7 days) to infants who remained on study feeding at the time of the clinic visit by trained and certified testers. Novelty preference, a measure of visual recognition memory, was computed by determining the percent of total looking time spent looking at a novel versus familiar face stimuli during the test phase. In addition, the mean duration of individual looks, construed as a measure of efficiency of information processing, was computed for the familiarization period, an abbreviated time during the familiarization period and during the paired comparison procedure test period.[11,12,33]

Language

The vocabulary checklist from the infant version of the MacArthur Communicative Development Inventories,[34] a standardized parent-report instrument, was completed at 9 months' CA (± 7 days) and 14 months' (± 10 days) CA. This checklist of words was used to provide information about each child's vocabulary comprehension (words the child understands) at 9 and 14 months' CA, and vocabulary production (words the child says) at 14 months' CA. Percentile scores were computed from age- and gender-specific norms and transformed to standard scores.

Statistical Methods

The primary analysis for this intent-to-treat study included all enrolled infants as randomized. Based on anticipated protocol deviations in this high-risk patient population over the ~16-month study period, a planned subgroup analysis included data through the last collection time at which infants strictly adhered to the feeding protocol, defined as remaining on the feeding protocol at term CA and after term CA consuming >80% of milk feedings (study formula, human milk, nonstudy formula, cow's milk) as study formula and/or human milk at each visit.

A sample size of 140 per group was estimated for detection of a 0.5 SD difference with 80% power and $\alpha = 0.05$ for the Bayley at 12 months' CA among the 3 study formula groups. This sample size estimate took into account anticipated infant attrition (20%), a possible blunting effect of human milk intake on outcome variables (25%), and the formation of an EHM-T intake reference.

Categorical variables were analyzed using χ^2 or Cochran-Mantel-Haenszel tests and continuous variables using analysis of variance (analysis of variance) and/or analysis of covariance (ANCOVA). Data obtained at >1 time point were analyzed using repeated-measures analyses that accommodate missing observations (SAS PROC MIXED, SAS Institute, Inc, Cary, NC).[35] As defined a priori, statistical comparisons among the 3 study formula groups included site as a covariate. Because of small numbers of infants, the Little Rock and New York sites were treated as a single site in the statistical analyses, except for the VEP analyses, for which data from the New York and Kansas City sites were pooled. In addition, analyses of continuous outcome variables included enrollment strata as covariates (gender and birth weight [750–1250 g or 1251–1800 g]), interactions between study group and enrollment strata covariates, and a covariate for human milk intake.

Human milk intake was defined as an ordered categorical variable based on the classification of infants at the term CA visit: exclusively formula-fed, <50% in-hospital enteral energy intake from formula, and ≥50% in-hospital enteral energy intake from formula. Additional preplanned covariates included size for gestation (for growth outcomes); size for gestation, gestational age, HOME Inventory and the vocabulary component of the WAIS-R (for developmental outcomes); and gestational age, HOME Inventory, prenatal smoking, in-home smoking at hospital discharge, and the vocabulary component of the WAIS-R (vision outcomes). All statistical tests of hypotheses were 2-tailed with $\alpha = 0.05$ for main effects and $\alpha = 0.10$ for interaction effects. When multiple comparisons were made for feeding regimens, gender, visit, and/or birth weight stratum, Bonferroni-adjusted α-levels were used.

RESULTS

Study Sample

Three hundred seventy-six (80%) of the 470 infants enrolled completed the study to 12 months' CA. Forty-three infants were classified as EHM-T feeders based on human milk intake until term CA. Of the 144 infants in the formula control group, 126 (88%) and 91 (63%) remained on study feeding at term and 12 months' CA, respectively. Similarly, of the 140 infants enrolled in the AA+DHA (fish/fungal) group, 120 (86%) and 89 (64%) remained on study feeding at term and 12 months' CA, and of the 143 infants enrolled in the AA+DHA (egg-TG/fish) group, 126 (88%) and 91 (64%) remained on study feeding at term and 12 months' CA. At term CA, 35%, 28%, and 33% of infants in the control, AA+DHA (fish/fungal), and AA+DHA (egg-TG/fish) groups, respectively, consumed human milk at least once per day. By 4 months' CA, only 14%, 12%, and 12% of infants in the control, AA+DHA (fish/fungal), and AA+DHA (egg-TG/fish) groups, respectively, consumed human milk. Nineteen (13%), 20 (14%), 11 (8%), and 1 (2%) of infants in the control, AA+DHA (fish/fungal), AA+DHA (egg-TG/fish), and EHM-T groups, respectively, discontinued study feeding because of symptoms typically associated with feeding intolerance (primary reason for discontinuation provided by site investigator). During the course of the study, 6, 3, 6, and 0 infants randomized to the control, AA+DHA (fish/fungal), AA+DHA (egg-TG/fish), and EHM-T groups, respectively, died. No infant deaths were related to study feedings as judged by the investigator at each site. No statistically significant differences existed among formula groups with respect to the aforementioned exit outcomes.

Infant and Family Demographics

Infant and family baseline demographics did not differ among study formula groups, with the exception of scores on the HOME Inventory (Tables 2 and 3). HOME Inventory scores were higher (better) in infants ≤ 1250 g randomized to the control group (LS mean ± SE, 36.0 ± 0.7) than those randomized to the AA+DHA (fish/fungal) group (33.7 ± 0.7, $P = .029$). HOME Inventory scores were lower in infants in the > 1250 g birth weight stratum randomized to the AA+DHA (egg-TG/fish) group (LS mean ± SE, 33.6 ± 0.7) than for the control (36.2 ± 0.6, $P = .006$) and the AA+DHA (fish/fungal) groups (36.2 ± 0.6, $P = .004$). A marginally statistically significant difference in multiple birth status (twin vs singleton birth) across the 3 study formula groups was also observed ($P = .054$). Approximately 17%, 20%, and 28% of participants were twins in the control, AA+DHA (fish/fungal), and AA+DHA (egg-TG/fish) group, respectively. In view of this somewhat disproportionate distribution, the primary developmental outcomes were analyzed with and without twins (intent-to-treat). Except for language development, results for developmental outcomes did not change when twins were excluded.

Blood Fatty Acid Analyses

At SDAY 1, the study formula groups did not differ significantly with respect to the levels (g/100 g) of AA and DHA in the plasma or in the PE or PC fractions of RBCs (data not shown). By hospital discharge, infants consuming AA+DHA-supplemented formulas had generally higher blood levels of AA and DHA than infants in the control group. For example, infants in the control, AA+DHA (fish/fungal), AA+DHA (egg-TG/fish), and EHM-T groups, had LS mean ± SE levels of plasma phospholipid AA (wt%) of 10.3 ± 0.5, 12.7 ± 0.5, 12.8 ± 0.5, and 13.9 ± 0.6, respectively, and the least square (LS) mean (± standard error [SE]) levels of DHA were 2.7 ± 0.2, 3.5 ± 0.2, 3.3 ± 0.2, and 3.5 ± 0.2, respectively, at hospital discharge (control < AA+DHA fish/fungal, and AA+DHA egg-TG/fish; $P < .001$). The mean number of days between SDAY 1 and hospital discharge was ~41.

With the exception of AA levels in RBC PE at 4 and 12 months' CA, infants fed the AA+DHA-supplemented formulas had higher levels of AA and DHA in plasma and RBC phospholipids than those fed the control formulas ($P < .0001$; Table 4). Infants fed AA+DHA (fish/fungal) but not AA+DHA (egg-TG/fish), had higher levels of AA in RBC PE than infants fed control formulas ($P < .02$).

Growth

In the intent-to-treat population, few and inconsistent differences were found in weight, length, or head circumference gains from SDAY 1 to term, SDAY 1 to 4 months' CA, or SDAY 1 to 12 months' CA or in repeated measures analyses of absolute weight, length, and head circumference measurements through 12 months' CA (Table 5 & Fig 1). These differences were not seen when analysis of the intent-to-treat population excluded infants consuming >50% of initial in-hospital energy from human milk.

Similarly, among strict feeding protocol followers (infants who consumed >80% of their feeding as study formula and/or human milk), few and inconsistent differences were found in anthropometric gains or in repeated measures analyses of anthropometric measurements across study visits. Mean length gain from SDAY 1 to 4 months' CA was greater among control (LS Mean ± SE, 8.7 ± 0.1 mm/wk) than among AA+DHA (egg-TG/fish)-fed infants (8.3 ± 0.1 mm/wk, $P = .04$). There was a statistically significant interaction between feeding and gender for head circumference gain from SDAY 1 to term CA. Mean head circumference gain from SDAY 1 to term CA was greater among female control than among female AA+DHA (egg-TG/fish)-fed female infants (9.2 ± 0.2 mm/wk vs 8.4 ± 0.2 mm/wk, $P = .003$).

In-Hospital Feeding Tolerance and Clinical Problems

In both the intent-to-treat and strict protocol follower subgroup analyses, no differences were found among study formula groups with respect to the percentage of infants who had feedings withheld for

TABLE 2. Neonatal and Perinatal Characteristics of the Total Study Population of Preterm Infants: Intent-to-Treat Analysis

Characteristics	Control	AA+DHA (Fish/Fungal)	AA+DHA (Egg-TG/Fish)	EHM-T*
Birth weight,† g				
Mean ± SD	1287 ± 272 (142)	1305 ± 293 (138)	1309 ± 286 (140)	1275 ± 312 (43)
Median	1286	1333	1290	1260
First, third quartile	1090, 1495	1065, 1540	1091, 1805	1020, 1580
Gestational age at birth,† wk				
Mean ± SD	29.6 ± 1.9 (143)	29.8 ± 2.1 (138)	29.7 ± 2.0 (141)	29.7 ± 2.1 (43)
Median	29.9	30.0	30.0	30.0
First, third quartile	28.3, 31.0	29.0, 31.0	28.9, 31.3	28.0, 31.6
Size at birth‡, *n*				
Small for gestational age	9	14	14	7
Appropriate for gestational age	133	124	126	36
Unknown	1	0	1	0
Gender,‡ *n*				
Male	77	77	77	20
Female	66	61	64	23
Multiple birth status,‡ *n*				
Singleton	119	110	101	39
Twin	24	28	40	4
Apgar score‡				
Mean ± SD	8.0 ± 1.4 (143)	8.0 ± 1.4 (136)	8.0 ± 1.4 (141)	8.3 ± 1.2 (43)
Median	8.0	8.0	8.0	8.0
First, third quartile	7.0, 9.0	8.0, 9.0	8.0, 9.0	8.0, 9.0
Ethnicity, *n*				
White	81	80	85	32
Black	22	20	28	2
Hispanic/Latino	20	21	19	0
Other	20	17	9	0
Study Day 1				
Weight†, g				
Mean ± SD	1207 ± 276 (142)	1208 ± 274 (138)	1219 ± 279 (140)	1198 ± 326 (43)
Median	1204	1240	1200	1200
First, third quartile	995, 1400	1005, 1450	1014, 1448	950, 1410
Length,† cm				
Mean ± SD	38.6 ± 3.1 (131)	38.9 ± 3.1 (130)	39.1 ± 2.8 (132)	38.6 ± 3.1 (41)
Median	38.9	39.0	39.3	38.7
First, third quartile	36.5, 41.0	36.5, 41.4	37.2, 41.00	36.0, 40.0
Head circumference,† cm				
Mean ± SD	27.2 ± 2.0 (133)	27.3 ± 2.0 (129)	27.4 ± 1.9 (135)	27.0 ± 2.2 (40)
Median	27.2	27.5	27.5	27.2
First, third quartile	26.0, 28.6	25.8, 29.0	26.0, 29.0	25.7, 28.5
Postnatal age,† d				
Mean ± SD	5.5 ± 3.9 (142)	5.0 ± 2.9 (138)	4.6 ± 2.8 (140)	5.5 ± 2.5 (43)
Median	4.0	4.0	4.0	5.0
First, third quartile	3.0, 7.0	3.0, 6.0	3.0, 5.0	4.0, 7.0

No statistically significant differences were found ($P < .05$); multiple birth status ($P = .054$).
* Exclusively human milk-fed during the early neonatal period. Reference group only, these data were not included in the statistical analyses.
† Differences across formula groups were assessed by ANCOVA controlling for site, gender, birth weight stratum, feeding* gender, and feeding* birth weight stratum.
‡ Differences across formula groups were assessed using χ^2 analyses controlling for site.

at least 1 day, the percentage of infants who had enteral feedings withheld because of gastric residuals, and number of days to reach full enteral feeding (Table 6). Likewise, there were no differences among study formula groups in the incidence of chronic lung disease or in suspected or confirmed cases of systemic infection or NEC.

SAEs

The percentage of infants who had at least 1 SAE did not differ among study formula groups with 44%, 46%, and 47% of infants randomized to the control, and AA+DHA (fish/fungal), AA+DHA (egg-TG/fish) groups, respectively having at least 1 SAE. Thirty-eight percent, 39%, and 43% of infants randomized to the control, AA+DHA (fish/fungal) and AA+DHA (egg-TG/fish) groups, respectively, had at least 1 hospital readmission. The number of SAEs and hospital readmissions did not differ when comparisons among feeding groups were made within each birth weight stratum (750–1250 g or 1251–1800 g). Finally, no statistically significant feeding differences were found within each SAE numerical and alphabetical system and severity rating.

Visual Acuity

Regardless of whether statistical analysis was performed on the intent-to-treat population or strict feeding protocol followers, no significant effect of study feeding on behavioral acuity was found using preplanned statistical comparisons (Fig 2). Likewise, no statistically significant effect of study feeding on VEP acuity was found at 4 months' CA. In contrast, at 6 months' CA, the mean VEP acuity of infants

TABLE 3. Family Characteristics: Intent-to-Treat Analysis

Characteristics	Control	AA+DHA (Fish/Fungal)	AA+DHA (Egg-TG/Fish)	EHM-T*
Maternal age,† y	27.2 ± 6.3 (143)	27.0 ± 6.3 (138)	27.0 ± 7.0 (141)	29.7 ± 5.1 (43)
Maternal education†				
US, y	12.9 ± 2.4 (99)	13.1 ± 2.4 (96)	12.8 ± 2.3 (99)	15.1 ± 2.0 (34)
Chile, y	10.5 ± 1.6 (16)	10.1 ± 2.2 (19)	8.8 ± 3.1 (16)	Not applicable
UK, highest qualification obtained				
None	9	4	6	1
< 3 O-level‡	5	6	2	0
> 3 O-level§	6	9	12	2
A levels	1	0	1	2
Degree +	2	3	4	4
Maternal smoking during pregnancy,‖ *n* (%)				
Yes	40 (28.0)	35 (25.4)	41 (29.3)	2 (4.7)
No	103 (72.0)	103 (74.6)	99 (70.7)	41 (95.3)
Postnatal smoking in the home,‖ *n* (%)				
Yes	37 (27.4)	39 (29.1)	44 (32.1)	4 (9.5)
No	98 (72.6)	95 (70.9)	93 (67.9)	38 (90.5)
Prenatal care,‖ *n* (%)				
First trimester	119 (83.8)	115 (83.9)	114 (80.9)	42 (97.7)
Second trimester	19 (13.4)	17 (12.4)	20 (14.2)	0 (0.0)
Third trimester or none	4 (2.8)	5 (3.6)	7 (5.0)	1 (2.3)
HOME Inventory Score†¶	36.3 ± 5.3 (123)	35.3 ± 5.5 (127)	34.8 ± 6.5 (109)	39.4 ± 3.4 (40)
Maternal WAIS-R Raw Vocabulary Score†	39.3 ± 12.4 (119)	37.5 ± 15.0 (126)	37.0 ± 15.2 (108)	53.2 ± 8.9 (40)

Values are mean ± SD (number of participants) unless otherwise noted.
* Exclusively human milk-fed during the early neonatal period. Reference group only, these data were not included in the statistical analyses.
† Differences across study formula groups were assessed by ANCOVA controlling for site, gender, birth weight stratum, feeding* gender, and feeding* birth weight stratum.
‡ UK education equivalents: <3 Certificate of Education or General CSE below C grade.
§ UK education equivalents: >3 Certificates of Education or any O levels or General Certificates of Education grade A–C.
‖ These data were analyzed using χ^2 analyses controlling for site.
¶ A statistically significant feeding* birth weight interaction was found ($P = .0043$) for the HOME Inventory Score. Infants ≤1250 g birth weight stratum randomized to the control group (LS mean ± SE, 36.0 ± 0.7) had higher HOME Inventory scores than those randomized to AA+DHA (fish/fungal) group (LS Mean ± SE, 33.7 ± 0.7; $P = .0285$). Infants >1250 g birth weight stratum randomized to the control group (LS mean ± SE 36.2 ± 0.6) had higher HOME Inventory Scores than those randomized to AA+DHA (egg-TG/fish) (33.6 ± 0.7, $P = .0059$). Infants >1250 g birth weight stratum randomized to AA+DHA (fish/fungal; 36.3 ± 0.6) had higher HOME Inventory Scores than those randomized to AA+DHA (egg-TG/fish; 33.6 ± 0.7, $P = .0039$).

randomized to either AA+DHA (fish/fungal; LS mean [cy/d] ± SE [octaves], 11.4 ± 0.1; $P = .0098$) or AA+DHA (egg-TG/fish; 12.5 ± 0.1, $P = .0018$) was greater than for infants in the control formula group (8.4 ± 0.1, Fig 3). Furthermore, the mean VEP acuity of infants randomized to the AA+DHA-supplemented formulas increased between 4 and 6 months' CA, but the mean VEP acuity of those in the control group did not. Among infants who consumed >80% of their feeding as study formula and/or human milk, the mean VEP acuity of infants fed AA+DHA (egg-TG/fish; 12.9 ± 0.1) was greater than for control-fed (8.5 ± 0.1) infants at 6 months' CA ($P = .002$). There was a marginally statistically significant difference showing higher visual acuity among AA+DHA (fish/fungal)-fed infants (10.6 ± 0.1) than control infants aged 6 months' CA ($P = .08$). VEP acuity of infants fed AA+DHA (egg-TG/fish) did not differ significantly from that of infants fed AA+DHA (fish/fungal) at either 4 or 6 months' CA.

General Development Level

Regardless of whether the statistical analysis of the data included all infants randomized into the study or included only those infants who strictly adhered to the feeding protocol, no differences were found among study formula groups in the Bayley mental index (Table 7). However, a statistically significant feeding by birth weight stratum interaction was observed for Bayley motor development index ($P = .005$) among infants who consumed >80% of their feeding as study formula and/or human milk. The mean Bayley motor index score of infants in the ≤1250 g birth weight subgroup who strictly followed the feeding protocol was greater in infants fed AA+DHA (fish/fungal; LS mean ± SE, 90.6 ± 4.4) than control infants (81.8 ± 4.3; $P = .007$), even after adjusting for a number of covariates including the HOME inventory, maternal WAIS-R, and human milk intake. The Bayley motor index of AA+DHA (egg-TG/fish)-fed infants (84.7 ± 4.3) did not differ statistically from either the control or AA+DHA (fish/fungal) groups.

The percentage of participants in the intent-to-treat or subgroup populations who had significantly delayed mental or motor performance did not differ statistically by study formula group. As expected in a premature population, approximately 4% and 12% of all infants tested had Bayley mental and motor scores, respectively, <70, a level indicative of significantly delayed performance (intent-to-treat population).

Information Processing

A statistically significant feeding by visit interaction was observed for novelty preference ($P = .10$), and average look duration for an abbreviated time

TABLE 4. Fatty Acid Levels (wt%) in Plasma and RBC Phospholipids at 4 and 12 Months' CA: Strict Feeding Protocol Followers

	Control	AA+DHA (Fish/Fungal)	AA+DHA (Egg-TG/Fish)	EHM-T*
Plasma				
20:4n-6 (AA)†				
4 mo CA	8.3 ± 1.8 (63)	12.1 ± 2.0 (64)	11.8 ± 2.1 (53)	13.0 ± 1.7 (10)
12 mo CA	8.6 ± 2.5 (61)	11.3 ± 2.2 (58)	11.1 ± 2.3 (51)	10.6 ± 2.5 (22)
22:6n-3 (DHA)†‡				
4 mo CA	2.2 ± 1.0 (63)	3.9 ± 0.9 (64)	3.5 ± 0.9 (53)	4.1 ± 0.8 (10)
12 mo CA	1.8 ± 0.8 (61)	3.4 ± 0.8 (58)	3.1 ± 0.8 (51)	2.6 ± 0.9 (22)
RBC Phosphatidylethanolamine				
20:4n-6 (AA)§				
4 mo CA	17.5 ± 7.1 (67)	18.7 ± 7.7 (67)	17.9 ± 7.0 (55)	18.0 ± 7.0 (15)
12 mo CA	17.3 ± 6.1 (61)	18.7 ± 5.9 (58)	18.5 ± 6.5 (56)	19.9 ± 5.5 (26)
22:6n-3 (DHA)†				
4 mo CA	3.2 ± 1.7 (66)	4.7 ± 2.6 (67)	4.3 ± 2.3 (55)	4.1 ± 2.6 (15)
12 mo CA	2.4 ± 1.1 (61)	4.2 ± 2.0 (58)	4.0 ± 1.9 (56)	4.1 ± 2.2 (26)
RBC Phosphatidylcholine				
20:4n-6 (AA)†				
4 mo CA	4.4 ± 1.6 (65)	5.8 ± 2.5 (65)	5.6 ± 2.2 (56)	6.5 ± 2.4 (15)
12 mo CA	4.2 ± 1.8 (61)	5.4 ± 2.0 (58)	5.5 ± 2.0 (56)	6.0 ± 1.9 (26)
22:6n-3 (DHA)†				
4 mo CA	0.9 ± 0.4 (60)	1.4 ± 0.7 (63)	1.4 ± 0.7 (54)	1.5 ± 0.9 (15)
12 mo CA	0.7 ± 0.4 (61)	1.3 ± 0.6 (58)	1.1 ± 0.5 (56)	1.2 ± 0.7 (26)

Values are mean ± SD (number of participants). Differences across formula groups were determined using repeated measures analysis of variance controlling for: site, gender, birth weight stratum, feeding* gender, feeding* birth weight stratum, and feeding* visit.
* Exclusively human milk-fed during the early neonatal period. Reference group only, these data were not included in the statistical analyses.
† Control < AA+DHA (fish/fungal), AA+DHA (egg-TG/fish), $P < .0001$.
‡ AA+DHA (egg-TG/fish) < AA+DHA (fish/fungal), $P = .0169$.
§ Control < AA+DHA (fish/fungal), $P = .0173$.

TABLE 5. Weight, Length, and Head Circumference Gains: Intent-to-Treat Analysis

	Control	AA+DHA (Fish/Fungal)	AA+DHA (Egg-TG/Fish)	EHM-T*
Weight gain, g/kg/d				
SDay 1 to term CA	13.4 ± 1.8 (135)	13.7 ± 1.9 (134)	13.3 ± 1.8 (135)	12.0 ± 1.8 (42)
SDay 1 to 4 mo CA	7.3 ± 0.5 (127)	7.3 ± 0.5 (126)	7.2 ± 0.5 (121)	7.1 ± 0.7 (41)
SDay 1 to 12 mo CA	3.6 ± 0.2 (119)	3.6 ± 0.2 (123)	3.5 ± 0.2 (105)	3.5 ± 0.2 (41)
Length gain, mm/wk				
SDay 1 to term CA	9.8 ± 2.1 (124)	9.8 ± 2.1 (124)	9.6 ± 1.9 (125)	9.1 ± 2.1 (40)
SDay 1 to 4 mo CA	8.4 ± 1.0 (120)	8.3 ± 0.9 (123)	8.1 ± 0.9 (113)	7.9 ± 1.1 (39)
SDay 1 to 12 mo CA†	5.7 ± 0.5 (111)	5.6 ± 0.5 (118)	5.6 ± 0.4 (100)	5.7 ± 0.5 (39)
Head circumference gain, mm/wk				
SDay 1 to term CA‡§	8.7 ± 1.4 (126)	8.7 ± 1.5 (122)	8.4 ± 1.4 (131)	8.0 ± 1.1 (39)
SDay 1 to 4 mo CA	5.5 ± 0.7 (121)	5.5 ± 0.6 (121)	5.5 ± 0.6 (116)	5.5 ± 0.5 (38)
SDay 1 to 12 mo CA	3.1 ± 0.3 (112)	3.1 ± 0.3 (118)	3.1 ± 0.3 (103)	3.1 ± 0.3 (38)

Values are mean ± SD (number of participants). Differences across study formula groups were assessed by ANCOVA controlling for site, gender, birth weight stratum, size for gestation, human milk intake, feeding* gender, and feeding* birth weight stratum.
* Exclusively human milk-fed during the early neonatal period. Reference group only, these data were not included in the statistical analyses.
† There was a statistically significant feeding* birth weight stratum interaction ($P = .0853$). Length gains were greater among ≤1250 g infants in the control versus AA+DHA (fish/fungal) group (LS means 5.74 vs 5.67 mm/week, $P = .0078$).
‡ There was a statistically significant feeding* gender interaction ($P = .0132$). Head circumference gains were greater among female infants in the control vs AA+DHA (egg-TG/fish) groups (LS means 9.1 vs 8.4 mm/week, $P = .0039$).
§ There was a statistically significant feeding* birth weight stratum interaction ($P = .0238$). Head circumference gains were greater among >1250 g infants in the AA+DHA (fish/fungal) versus AA+DHA (egg-TG/fish) group (LS means 9.0 vs 8.4 mm/week, $P = .0029$).

during familiarization ($P = .07$), although pairwise comparisons of study feeding groups at each time point yielded significant differences for novelty preference only (Table 8). The mean novelty preference of AA+DHA (egg-TG/fish)-fed infants (LS means ± SE, 60.0 ± 0.8) was significantly greater than control (57.5 ± 0.8; $P = .02$) and AA+DHA (fish/fungal)-fed (56.6 ± 0.8; $P = .003$) infants at 6 months' CA. The difference between AA+DHA (fish/fungal) and AA+DHA (egg-TG/fish) remained statistically significant using a Bonferroni adjusted α-level of 0.0083.

Language

Vocabulary comprehension did not differ among the 3 study formula groups at either 9 or 14 months' CA in either the intent-to-treat (Table 7) or subgroup analysis. Likewise, there were no study feeding differences in vocabulary production at 14 months' CA. In view of the somewhat disproportionate distribution of twins among the 3 study formula groups, language outcomes were also analyzed with and without twins. The validity of using percentile and gender-specific norms and standard score conver-

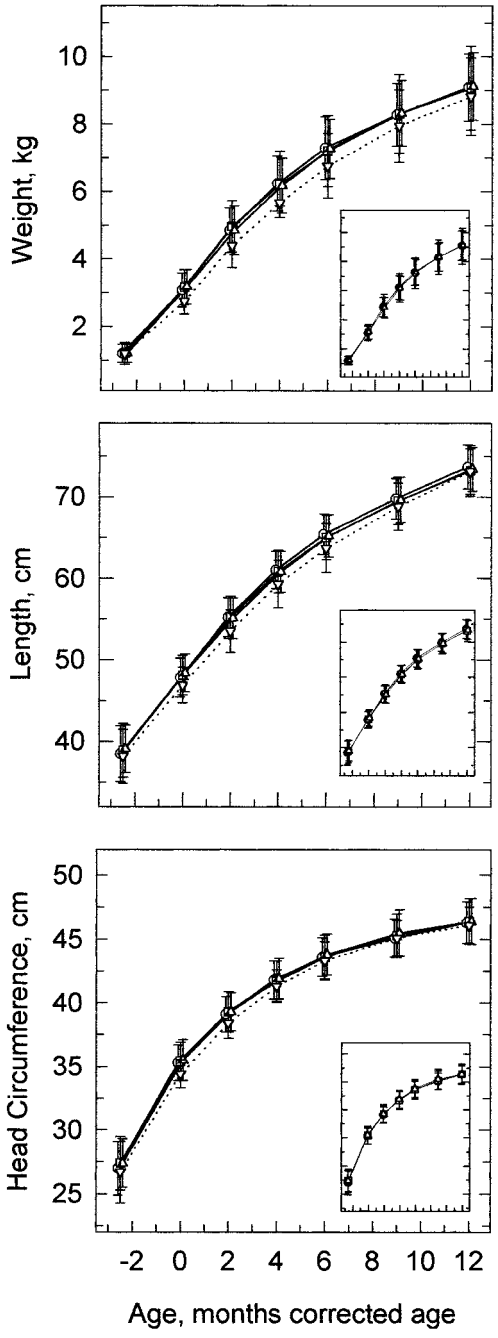

Fig 1. Weight, length, and head circumference of preterm infants fed human milk and/or nutrient-enriched formula with or without AA and DHA from SDAY 1 (median, 5 days of age) to 12 months CA. Values are presented as mean ± SD for the intent-to-treat population (large graph) and strict study feeding protocol followers (small graph). Hatched lines with triangle symbols denote data for infants who were exclusively human milk fed until term CA. Formula groups: 1) control [Circles], 2) AA+DHA (fish/fungal) [Squares], and 3) AA+DHA (egg-TG/fish) [Triangles with solid lines]. The sample size for the intent-to-treat population at each measurement time ranged from 118–135, 123–134, 105–135 and 40–42 for the control, AA+DHA (fish/fungal), AA+DHA (egg-TG/fish) and human milk until term CA groups, respectively. The sample size for the strict study feeding protocol followers at each measurement time ranged from 80–124, 77–115, 76–121 and 35–42 for the control, AA+DHA (fish/fungal), AA+DHA (egg-TG/fish) and human milk fed until term CA, respectively. Repeated measures analysis demonstrated that the weight and lengths of AA+DHA (egg-TG/fish) infants were greater than control infants at term CA (2906 ± 48 g vs 2757 ± 50 g, $P = .03$; 47.1 ± 0.2 cm vs 46.5 ± 0.02 cm, $P = .01$; respectively).

sions established using English-speaking infants is not clearly established for Spanish speakers. When infants from Spanish-speaking families and twins were removed from the intent-to-treat analysis, infants randomized to the control group (LS mean ± SE, 94.1 ± 2.9) had lower vocabulary comprehension than infants randomized to the AA+DHA (egg-TG/fish) (102.2 ± 2.8, $P = .0145$) or AA+DHA (fish/fungal) groups (100.6 ± 2.9, $P = .0422$). Likewise, when infants from Spanish-speaking families and twins were removed from the strict feeding protocol follower analysis, control-fed infants (LS mean ± SE, 95.3 ± 3.3) had lower vocabulary comprehension than AA+DHA (egg-TG/fish)-fed infants (105.4 ± 3.4, $P = .0118$).

DISCUSSION

This is the largest randomized, prospective, longitudinal, and multivariate study to compare AA+DHA-supplemented formulas with unsupplemented control formulas fed to premature infants from the time of first enteral feeding to 12 months' CA. Results from this trial suggest that AA+DHA-supplementation results in improved visual development of preterm infants at 6 months' CA as assessed by VEP acuity. At 6 months' CA, the mean VEP acuity of infants randomized to either AA+DHA (fish/fungal) or AA+DHA (egg-TG/fish) was approximately 0.34 and 0.42 octaves, respectively, higher than that for infants randomized to the control formula. Although there are distinctions between VEP and recognition acuity,[36] the magnitude of this difference corresponds to approximately 1 line on a Snellen eye chart (eg, 20/70 vs 20/50). Unlike AA+DHA-supplemented infants whose VEP acuity improved between 4 and 6 months' CA, the VEP acuity of infants randomized to the control formula did not improve, suggesting a slower rate of development of the visual system in this latter group of infants.

These results are consistent with the higher VEP acuity (1 and 4 months' CA) and the more mature VEP wave latency morphology (3 months' CA) among preterm infants supplemented with DHA alone as reported by Birch et al[37] and Faldella et al[38] Similarly, Carlson et al[9,10] demonstrated improved Behavioral acuity among DHA-supplemented preterm infants at 2 and 4 months' CA and at 2 months' CA among preterm infants fed DHA alone from a low-EPA fish oil source. In the present study, no statistically significant differences in Behavioral acuity were noted among the study groups using preplanned repeated measures comparisons. However, posthoc analysis of Behavioral acuity results at each measurement time (2, 4, and 6 months' CA) revealed that at 4 months' CA infants randomized to AA+DHA (egg-TG/fish; LS mean [cy/d] ± SE [octaves], 1.8 ± 0.1) had significantly higher mean Behavioral acuity scores than those infants randomized to the control formula (1.7 ± 0.1, $P = .0323$); the absolute difference, however, is negligible.

In addition to the benefits to visual development implied by the consistency of the aforementioned study results, there is a growing body of literature

TABLE 6. In-Hospital Feeding Tolerance and Clinical Problems During the Study Period: Intent-to-Treat Analysis*

	Control	AA+DHA (Fish/Fungal)	AA+DHA (Egg-TG/Fish)	EHM-T†
Number of participants	142	138	140	43
Feedings withheld for at least 1 d, % of infants	29	31	31	26
Feedings withheld because of gastric residuals, % of infants	20	17	16	14
Days to reach full enteral feeds (100 kcal/kg/d)	12.8 ± 14.6	12.8 ± 12.1	12.5 ± 14.1	15.8 ± 18.9
Suspected case of NEC, % of infants	24	23	26	21
Confirmed cases of NEC, % of infants	4	4	3	0
Suspected systemic infection, % of infants	33	35	39	33
Confirmed cases of systemic infection, % of infants	14	15	15	7
Chronic lung disease, % of infants	25	25	22	28

* Differences across study formula groups were evaluated by Cochran-Mantel-Haenszel statistics or survival analysis (days to full enteral feeding only) controlling for site. No statistically significant differences were found among study feeding groups for the aforementioned variables either during the study or, not shown in the Table, between birth and SDAY 1.
† Exclusively human milk-fed during the early neonatal period. Reference group only, these data were not included in the statistical analyses.

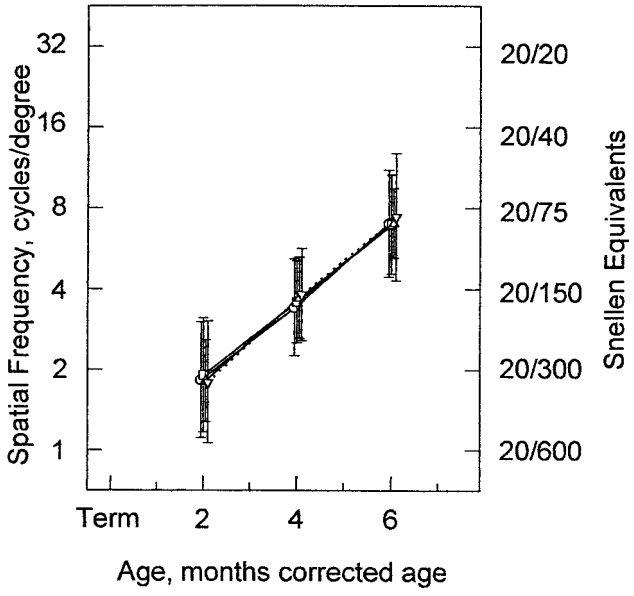

Fig 2. Behavioral visual acuity at 2, 4 and 6 months CA (± 7 days) of preterm infants fed human milk and/or nutrient-enriched formula with or without AA and DHA until 12-mos CA. Data for the intent-to-treat population are shown on the left axis as mean spatial frequency (cycles/degree, cy/deg) ± SD (octaves). Hatched lines with triangle symbols denote data for infants who were exclusively human milk fed until term CA. Formula groups: 1) control [Circles], 2) AA+DHA (fish/fungal) [Squares], and 3) AA+DHA (egg-TG/fish) [Triangles with solid lines]. The sample size at each measurement time ranged from 116–124, 122–129, 111–120 and 40–41 for the control, AA+DHA (fish/fungal), AA+DHA (egg-TG/fish) groups, and human milk until term CA, respectively. No differences among study formula groups for overall behavioral acuity by repeated-measures analysis was found.

suggesting a relationship between the results of early abnormal visual assessments and later motor and cognitive impairment.[39–43] These relationships suggest that the early benefit of AA+DHA-supplementation to the visual system could have long-term implications among preterm infants; although this hypothesis remains untested.

The 4 previously published peer-reviewed clinical trials demonstrating improved visual development secondary to supplementation with DHA alone also report slower growth or were not sufficiently powered to detect subtle differences in growth outcomes.[9,10,37,38,44] Carlson et al[18] hypothesized that despite adequate intakes of the essential fatty acid, linoleic acid, preterm infants may need a dietary source of AA for optimal growth. In contrast, Woltil et al[45] reported that blood levels of AA in premature infants were related to anthropometric measures at 10 days but not at 42 days of age, leading this group to conclude that AA status was related to intrauterine but not postnatal growth. Results from the present study suggest that prolonged feeding of nutrient enriched formulas in combination with AA+DHA-supplementation to at least 6 months' CA

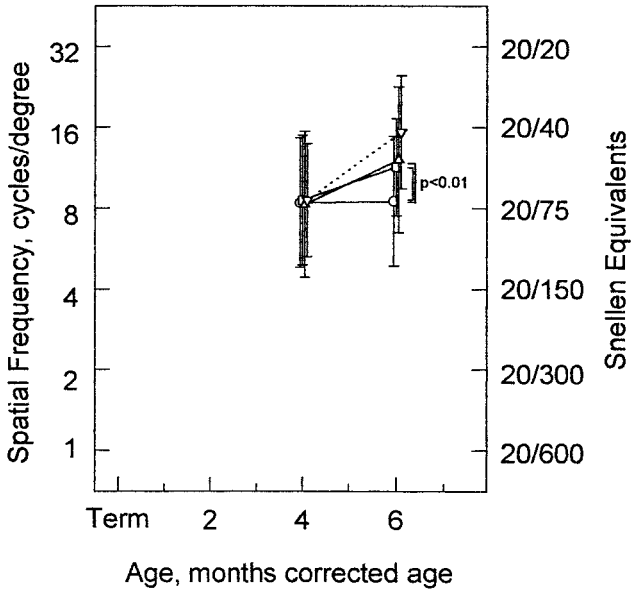

Fig 3. Visual Evoked Potential (VEP) visual acuity at 4- and 6-mos CA (± 7 days) of preterm infants at the Kansas City, New York, and Portland research sites fed human milk and/or nutrient-enriched formula with or without AA and DHA until 12-mos CA. Data for the intent-to-treat population are shown on the left axis as mean (cycles/degree, cy/deg) ± SD (octaves). Hatched lines with triangle symbols denote data for infants who were exclusively human milk fed until term CA. Formula groups: 1) control [Circles], 2) AA+DHA (fish/fungal) [Squares], 3) AA+DHA (egg-TG/fish) [Triangles with solid lines]. The sample size at each measurement time ranged from 39, 46–50, 33–37 and 22 for the control, AA+DHA (fish/fungal), AA+DHA (egg-TG/fish) and human milk until term groups, respectively. At 6-mos CA, the mean VEP acuity of infants randomized to either AA+DHA (fish/fungal) (LS mean ± SE, 11.4 ± 0.1) or AA+DHA (egg-TG/fish) (12.5 ± 0.1) was greater than that of infants randomized to the control (8.4 ± 0.1)

TABLE 7. Cognitive, Psychomotor, and Language Development: Intent-to-Treat Analysis*

	Control	AA+DHA (Fish/Fungal)	AA+DHA (Egg-TG/Fish)	EHM-T†
Bayley's Scales of Infant Development at 12 mo CA				
Mental Development Index	92.2 ± 12.2 (119)	92.8 ± 11.2 (123)	93.4 ± 13.0 (105)	93.1 ± 14.5 (41)
Motor Development Index	86.3 ± 16.2 (118)	87.2 ± 14.2 (123)	85.9 ± 14.4 (105)	86.8 ± 15.2 (41)
MacArthur Communicative Development Inventories				
Vocabulary Comprehension Scores				
9 mo	103.7 ± 21.9 (122)	104.2 ± 19.1 (122)	101.7 ± 19.4 (102)	96.2 ± 17.6 (39)
14 mo	99.9 ± 17.1 (98)	101.6 ± 16.4 (101)	101.2 ± 18.8 (93)	97.0 ± 14.5 (38)
Vocabulary Production Scores				
14 mo	97.8 ± 18.4 (98)	96.6 ± 17.2 (102)	98.3 ± 18.1 (93)	96.6 ± 18.9 (38)

Values are mean ± SD (number of participants).
* Differences across formula groups were determined using ANCOVA controlling for: site, gender, birth weight stratum, feeding* gender, feeding* birth weight stratum, HOME, maternal WAIS-R raw vocabulary score, gestational age, size for gestation, human milk intake, birth order, and the first language of the biological mother. Bayley mental and motor development index scores <50 were excluded from the statistical analyses but are included in the data presented in this Table. No statistically significant effects of study formula feeding, feeding* gender, or feeding* birth weight stratum were found.
† Exclusively human milk-fed during the early neonatal period. Reference group only, these data were not included in the statistical analyses.

TABLE 8. Fagan Test of Infant Intelligence: Strict Feeding Protocol Followers

	Control	AA+DHA (Fish/Fungal)	AA+DHA (Egg-TG/Fish)	EHM-T*
% Novelty preference				
6 mo CA	57.5 ± 7.4[a,b] (96)	57.0 ± 7.5[a] (91)	59.4 ± 7.7[b] (92)	57.9 ± 7.0 (38)
9 mo CA	58.4 ± 7.2 (92)	59.0 ± 7.4 (82)	58.9 ± 7.2 (81)	58.9 ± 7.7 (36)
Average look duration during the familiarization phase, sec				
6 mo CA	2.2 ± 0.8 (96)	2.2 ± 1.0 (91)	2.1 ± 0.8 (92)	2.2 ± 0.8 (38)
9 mo CA	1.4 ± 0.4 (92)	1.4 ± 0.4 (83)	1.5 ± 0.5 (82)	1.6 ± 0.4 (36)
Average look duration for an abbreviated time period of familiarization, sec†				
6 mo CA	1.7 ± 0.7 (95)	1.8 ± 1.4 (93)	1.6 ± 0.8 (92)	1.8 ± 0.8 (38)
9 mo CA	1.3 ± 0.4 (92)	1.4 ± 0.6 (83)	1.5 ± 0.7 (83)	1.4 ± 0.4 (36)
Average look duration during the test phase, sec				
6 mo CA	1.9 ± 0.6 (96)	1.8 ± 0.7 (91)	1.9 ± 0.6 (92)	1.9 ± 0.6 (38)
9 mo CA	1.3 ± 0.3 (92)	1.3 ± 0.4 (83)	1.3 ± 0.4 (82)	1.3 ± 0.3 (36)
Average look duration to novel stimuli during the test phase, sec				
6 mo CA	2.0 ± 0.6 (96)	1.8 ± 0.7 (91)	2.0 ± 0.7 (92)	2.0 ± 0.7 (38)
9 mo CA	1.3 ± 0.3 (92)	1.4 ± 0.4 (83)	1.3 ± 0.4 (82)	1.3 ± 0.3 (36)
Average look duration to the familiar stimuli during the test phase, sec				
6 mo CA	1.9 ± 0.7 (96)	1.8 ± 0.7 (91)	1.8 ± 0.6 (92)	1.9 ± 0.6 (38)
9 mo CA	1.3 ± 0.3 (92)	1.3 ± 0.4 (82)	1.3 ± 0.4 (81)	1.2 ± 0.3 (36)

Values are mean ± SD (number of participants). Differences across formula groups were determined using ANCOVA controlling for site, gender, visit, birth weight stratum, feeding* visit, feeding* gender, feeding* birth weight stratum, HOME, maternal WAIS-R raw vocabulary score, gestational age, size for gestation, parity, maternal age, and human milk intake. A statistically significant feeding* visit interaction was observed for % novelty preference ($P = .10$) and average look duration for an abbreviated time during the familiarization period ($P = .07$). Mean novelty preference of AA+DHA (egg-TG/fish; LS mean ± SE, 60.0 ± 0.8) was greater than control (57.5 ± 0.8; $P = .02$) and AA+DHA (fish/fungal)-fed (56.6 ± 0.8; $P = .003$) infants at 6 months' CA. The difference between AA+DHA (fish/fungal) and AA+DHA (egg-TG/fish) remained significant after Bonferroni adjustment ($\alpha = 0.0083$).
* Exclusively human milk-fed during the early neonatal period. Reference group only, these data were not included in the statistical analyses.
† During the first 10 (6 months' CA) and 6 (9 months' CA) seconds of the first 3 familiarization periods.

provides a mechanism whereby visual development can be supported without slowing growth. In the present study, few and inconsistent differences were found among the greater than 200 statistical comparisons related to weight, length, and head circumference gains (Fig 1, Table 5). Others have also recently reported no growth differences in preterm infants fed formulas containing both AA and DHA (egg phospholipid or microbial oils)[19,46,47]; however, the length of time that AA+DHA-containing formulas were fed was shorter than in studies where growth differences were observed.[10,13,14] Vanderhoof et al[19] recently reported on infants fed nutrient-enriched premature formulas to term CA and AA+DHA to 2 months' CA only, although growth was followed until each infant's 12 month CA birthdate. As reported herein, no differences in growth were found between AA+DHA supplemented and unsupplemented infants. It should be noted, however, that infants in the Vanderhoof et al[19] study probably represent a healthier subset of the premature infant population than those in the present study in that they were larger at birth, and were withdrawn from study if they did not meet prescribed enteral feeding

targets or exceeded the protocol limits for oxygen and corticosteroid use.

In addition to improved visual development in preterm infants, there was evidence of improved motor development among infants ≤1250 g birth weight randomized to the AA+DHA (fish/fungal) group who strictly adhered to the feeding protocol through 12 months' CA. The Bayley motor index measures gross motor abilities such as sitting, walking, standing, stair climbing, and hand and finger fine motor skills. Infants in the AA+DHA (fish/fungal) group with birth weights ≤1250 g had Bayley motor index scores that were 8 points higher than for infants in the same birth weight stratum fed the control formula and similar to those of EHM-T-fed infants in this birth weight stratum (LS mean ± SE, 89.6 ± 2.3). Bayley motor index scores of AA+DHA (egg-TG/fish)-fed infants were intermediate to the control and AA+DHA (fish/fungal) groups. As far as we are aware, this is the first prospective randomized trial demonstrating an improvement in motor scores among premature infants with AA+DHA-supplementation.

There did not seem to be any consistent effect of AA+DHA-supplementation on measures of novelty preference and on average look duration during the familiarization period. Carlson and Werkman[11] and Werkman and Carlson[12] demonstrated lower novelty preference in preterm infants supplemented with DHA only but more and shorter looks to novelty stimuli. These authors' hypothesize that collectively these data suggest that DHA supplementation can increase the information-processing speed of premature infants. No differences in language comprehension (9 or 14 months' CA) or language production (14 months' CA) were found using preplanned statistical comparisons. In these analyses, Spanish-speaking infants and twins were included by computing percentile and gender-specific norms and standard score conversions validated using English-speaking infants.[34] Jackson-Maldonado[48] reported that the trajectories of language acquisition are similar for Spanish- and English-speaking children, justifying this approach. Nonetheless, when Spanish-speaking infants and twins were removed from the intent-to-treat analysis, infants randomized to the control group had lower vocabulary comprehension at 14 months' CA than infants randomized to the AA+DHA (fish/fungal) or AA+DHA (egg-TG/fish) groups. No difference among study formula groups was found with respect to indicators of feeding tolerance, incidence of chronic lung disease, systemic infection, or NEC. Likewise, the percentage of infants who had at least 1 SAE and the type and severity of SAEs did not differ among study formula groups.

CONCLUSION

Results from this comprehensive, randomized, clinical trial suggest a benefit to feeding formula-fed preterm infants AA and DHA from either a fish/fungal oil or egg-TG/fish oil source from the time of first enteral feeding to 12 months' CA. Furthermore, no contraindications or concerns emerged related to the addition of AA+DHA to nutrient-enriched formulas from either a fish/fungal or egg-TG/fish source at the studied levels. On average, fish/fungal oils provided AA, DHA, and EPA at levels of 0.43, 0.27, and 0.08% fatty acids, respectively, to term CA and 0.43, 0.16 and 0% of fatty acids, respectively, from term to 12 months' CA. Egg-TG/fish oils, on average, provided AA, DHA, and EPA acid at levels of 0.41, 0.24, and 0% of fatty acids, respectively, to term CA and 0.41, 0.15, and 0% fatty acids from term to 12 months' CA.

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LIKE RUSSIAN DOLLS

The nature and limits of scientific method are hotly debated among academic philosophers. But this much, at least, is suggested . . . First, that the search for causes involves a search for laws; secondly, that laws are statements of probability; thirdly, that laws are themselves explained through wider and more general laws. . .

Scruton R. *An Intelligent Person's Guide to Philosophy*. New York, NY: Penguin Press; 1996

Submitted by Student

Growth and Development in Preterm Infants Fed Long-Chain Polyunsaturated Fatty Acids: A Prospective, Randomized Controlled Trial

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