Visual maturation of term infants fed long-chain polyunsaturated fatty acid–supplemented or control formula for 12 mo[1–3]

Eileen E Birch, Yolanda S Castañeda, Dianna H Wheaton, David G Birch, Ricardo D Uauy, and Dennis R Hoffman

ABSTRACT

Background: Several studies found a benefit of long-chain polyunsaturated fatty acid (LCP) supplementation for visual or mental development, but others found no benefit. Likely contributors to differences among studies are the amount of LCP supplementation, functional outcomes, and sample size.

Objective: We evaluated LCP supplementation in amounts typical for human milk (based on local and worldwide surveys) in a large cohort of infants by using sweep visual evoked potential (VEP) acuity as the functional outcome.

Design: The study was a double-masked, randomized, controlled clinical trial in 103 term infants. By age 5 d, infants were randomly assigned to receive either formula with no docosahexaenoic acid (DHA) or arachidonic acid (ARA) or formula supplemented with DHA and ARA as 0.36% and 0.72%, respectively, of total fatty acids. Sweep VEP acuity was the primary outcome. Random dot stereoacuity, blood lipid profile, growth, and tolerance were secondary outcomes.

Results: VEP acuity in the LCP-supplemented group was significantly better than that in the control group at ages 6, 17, 26, and 52 wk. Stereoacuity in the LCP-supplemented group was significantly better than that in the control group at age 17 wk but not at ages 39 and 52 wk. By ages 17 and 39 wk, the red blood cell DHA concentration in the LCP-supplemented group was more than double and more than triple, respectively, that in the control group. Growth of infants fed LCP-supplemented and control formulas did not differ significantly, and both diets were well tolerated.

Conclusion: LCP supplementation of term infant formula during the first year of life yields clear differences in visual function and in total red blood cell lipid composition.

KEY WORDS Docosahexaenoic acid, infant, visual evoked potential, acuity, random dot stereoacuity

INTRODUCTION

Whereas infant dietary requirements for lipids have traditionally been considered in terms of energy metabolism, it is now clear that some lipids may play an important role in the brain, especially during development. Between 50% and 60% of the brain's dry weight is lipid (1). Approximately half of these lipids are polyunsaturated fatty acids, primarily long-chain polyunsaturated fatty acids (LCPs). Nearly all are structural lipids that are not available for energy metabolism (1).

The n−3 LCP docosahexaenoic acid (DHA; 22:6n–3) has been the focus of several recent clinical trials of retinal and brain development, which have been reviewed by Lauritzen et al (2) and San Giovanni et al (3, 4). Early randomized clinical trials in preterm infants compared experimental formulas providing preformed DHA with commercial formulas containing very little α-linolenic acid (ALA), lacked DHA, or both. Human milk, which in most Western countries provides 0.2–0.4% of fatty acids as preformed DHA (2), was used as the gold standard in many of the preterm studies. In comparison with human milk, formula that lacked DHA and had very little ALA failed to support optimal retinal and brain development (5–9). Two studies supported a specific benefit of providing preformed DHA (5, 6).

Commercial formula manufacturers in the United States added ALA to their products by 1992, and it is no longer ethically feasible in randomized clinical trials to evaluate formulas lacking sufficient ALA. Because all term infant clinical trials were initiated after this date, they compared experimental formulas providing preformed DHA with formulas providing adequate ALA. Several randomized trials found a specific benefit of dietary DHA for retinal maturation, visual acuity development, or mental development in term infants (10–16). Others have found no benefit (17–22).

Lauritzen et al (2) pointed to 3 aspects of study design as likely contributors to differences among term infant study outcomes. First, studies that provided $<0.25\%$ of total fatty acids as DHA were less likely to show a functional benefit than were studies that provided $>0.35\%$. Second, studies that used the Teller acuity cards (Stereo Optical Co, Chicago, IL) as an outcome measure were less likely to show a functional benefit than were visual evoked potential (VEP) studies. Third, studies with sample sizes of <20/diet group were less likely to show a functional benefit than were studies with sample sizes of >20/diet group. To date, there is only a single large study of LCP-supplemented formula fed for 12 mo that used VEP acuity as an outcome measure. That study provided a relatively low amount of LCP supplementation

[1] From the Retina Foundation of the Southwest, Dallas, TX (EEB, YSC, DHW, DGB, and DRH), the Department of Ophthalmology (EEB and DGB), UT Southwestern Medical Center, Dallas, TX, and INTA, University of Chile, Santiago, Chile (RDU).

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(0.2% of total fatty acids as DHA) and failed to find a functional benefit of LCP supplementation (19–21).

The aim of the current randomized trial was to evaluate functional effects of LCP supplementation in amounts typical for human milk according to local and worldwide surveys in a protocol in which assigned formulas were fed to term infants at a single site throughout the first year of life. Sweep VEP acuity, the primary functional outcome, provided an objective measure of the maturity of the visual cortex. Random dot (RD) stereoacuity (another measure of visual cortical function), blood lipid profile, growth, and tolerance made up the secondary outcome measures.

SUBJECTS AND METHODS

Subjects

One hundred three term infants were enrolled in the study; all were exclusively formula-fed from birth. All were born at 37–40 wk after conception, as ascertained by sonogram, date of the mother's last menstrual period, and physical and neurodevelopmental assessment of the infant at birth. Only singleton births with birth weights appropriate for gestational age were included. Exclusion criteria were family history of milk protein allergy or genetic or familial eye disease (eg, hereditary retinal disease or strabismus); maternal vegetarian or vegan dietary patterns; maternal metabolic disease, anemia, or infection; presence in the infant of a congenital malformation or infection; jaundice, perinatal asphyxia, or meconium aspiration; and any perinatal event that resulted in placement of the infant in the neonatal intensive care unit.

Parents of eligible formula-fed neonates were provided a brief information sheet about the dietary study only after hospital records noted that they had elected to feed formula. If a positive response to the information sheet was obtained, the study coordinator reviewed the protocol with the parents and obtained their written informed consent before enrollment. This research protocol observed the tenets of the Declaration of Helsinki and was approved by the institutional review boards of the University of Texas Southwestern Medical Center (Dallas), Presbyterian Medical Center (Dallas), and Medical City Columbia Hospital (Dallas).

Randomization

Infants were randomly assigned on the day of enrollment (age range: 1–5 d; $\bar{x} \pm$ SD age: 3.6 $\pm$ 1.3 d) to 1 of 2 diets, which are described below. Infants were recruited from 2 hospitals to encourage ethnic and socioeconomic diversity in the cohort. All infants were randomly assigned with the use of a single randomization schedule at a central location. Each diet was masked by 2 color and 2 number codes, for a total of 4 possible diet assignments. The randomization schedule had random-length blocks (block length varied from 6 to 12) and was provided in individual sealed envelopes to the study site.

Diets

Study diets were commercial infant formula (Enfamil with iron; Mead Johnson Nutritional Group, Evansville, IN) alone or the infant formula supplemented with DHA (22:6n–3) at 0.36% of total fatty acids and arachidonic acid (ARA; 20:4n–6) at 0.72% of total fatty acids. The fatty acid compositions of the control (commercial) formula and of the LCP-supplemented formula are summarized in **Table 1**. Both formulas provided ≈15% linoleic acid (LA; 18:2n–6) and 1.5% ALA (18:3n–3). DHA+ARA–supplemented formulas contained single-cell oils (DHASCO and ARASCO; Martek Biosciences, Columbia, MD). Both formulas were supplied in 946-mL ready-to-feed cans and provided 14.7 g protein/L, 37.5 g fat/L, 69.0 g carbohydrate/L, and 2805 kJ/L. All nutrients met existing standards for commercial formula established by the Infant Formula Act. Assigned diets were fed from the time of enrollment through age 52 wk. None of the infants had solid food before age 17 wk.

TABLE 1
Fatty acid profiles of control and long-chain polyunsaturated fatty acid (LCP)–supplemented study diets[1]

	Control diet	LCP diet
	g/L	
Individual fatty acids		
LA (18:2n–6)	8.48 (14.6)[2]	8.37 (14.9)
20:3n–6	0	0.01 (0.05)
ARA (20:4n–6)	0	42 (0.72)
ALA (18:3n–3)	0.86 (1.49)	0.86 (1.53)
EPA (20:5n–3)	0	0
DHA (22:6n–3)	0	0.21 (0.36)
Totals		
C6–12 saturates[3]	11.67 (20.1)	10.97 (19.5)
C14–24 saturates[4]	21.69 (34.4)	20.36 (33.6)
Total monounsaturates[5]	17.11 (29.4)	16.42 (29.2)
Ratios		
Polyunsaturate:saturate	0.30	0.33
n–6:n–3 Polyunsaturate	9.6	8.3

[1] Values are the mean from ≥ 3 determinations. LA, linoleic acid; ARA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
[2] Percentage of total fatty acid in parentheses.
[3] Includes 6:0, 8:0, 10:0, and 12:0.
[4] Includes 14:0, 16:0, 17:0, 18:0, 20:0, 22:0, and 24:0.
[5] Includes 14:1, 16:1, 18:1, 20:1, 22:1, and 24:1.

General protocol

The overall study was composed of 2 substudies, the VEP substudy and the electroretinogram (ERG) substudy. Information about both substudies was provided to parents of eligible neonates, and the parents chose the substudy into which their child was enrolled. In the VEP substudy, 71 infants were evaluated at 4 time points (ages 6, 17, 39, and 52 wk) for growth, VEP acuity, and RD stereoacuity. In the ERG substudy, 32 infants were identically evaluated at the same 4 time points but underwent an ERG rather than VEP acuity testing on the initial visit (at age 6 wk). On subsequent visits, all infants were evaluated for growth, VEP acuity, and RD stereoacuity. This report presents data on VEP acuity and RD stereoacuity outcomes for all of the infants; ERG data will be published separately. Blood samples were obtained from the infants in the VEP substudy at ages 17 and 39 wk and from the infants in the ERG substudy at ages 6 and 39 wk. The schedule of testing is summarized in **Table 2**. All testing was conducted at a single site within $\pm$ 2 wk of target ages, according to age from conception.

Sample size

Sample sizes were estimated by using the method described by Rosner (23) for $\alpha = 0.05$ and $1\text{-}\beta = 0.80$ to detect mean differences

TABLE 2
Summary of enrollment, loss to follow-up, and testing schedule[1]

	1–5 d		6 wk		17 wk		39 wk		52 wk	
	LCP group	Control group	LCP group	Control group	LCP group	Control group	LCP group	Control group	LCP group	Control group
Enrollment (active patients, *n*)	51	52	47	48	46	46	44	46	42	44
Subjects tested (*n*)										
Growth	51	52	47	48	46	46	44	46	42	44
Blood lipids			14	16	32	30	44	46		
ERG			14	16						
VEP acuity			33	32	46	46	44	46	42	44
Stereoacuity					45	45	44	45	41	43
Loss due to gastrointestinal intolerance (*n*)			4	3	0	2	1	0	0	0
Infant illness unrelated to formula (*n*)			0	0	1	0	1	0	0	1
Loss due to inability to contact parents (*n*)			0	1	0	0	0	0	2	1

[1] LCP, long-chain polyunsaturated fatty acid; ERG, electroretinogram; VEP, visual evoked potential.

of ≥1.0 SD in VEP acuity among diet groups. Sample sizes were estimated by using SDs of 0.06 log of the minimal angle for resolution (logMAR) for term infant sweep VEP acuity and of 1.5 log of the minimal detectable binocular disparity (logsec) for term infant RD stereoacuity. On the basis of these SDs, the final sample size required at age 52 wk is 21 infants. Anticipating a 25% loss to follow-up over 52 wk, we planned a recruitment of 30 infants for each of the diet groups in the VEP substudy. Because enrollment for the ERG substudy was concurrent, and these patients joined the VEP substudy at age 17 wk, our final sample size at 52 wk was >40/diet group. Recruitment and the number of infants completing the protocol at each visit are summarized in Table 2. The greatest loss during follow-up occurred in the first 6 wk after enrollment, as a result of pediatricians' recommendation to switch to a soy protein–based formula after the occurrence of symptoms suggestive of lactose or cow milk protein intolerance before the 6-wk visit (7 infants) or because of an inability to contact the parents (1 infant). Of the 95 infants who remained in the protocol to age 6 wk (the age at which vision was first evaluated), 86 (90.5%) completed the protocol through age 12 mo.

Blood lipids

Blood samples (2.0 mL) were collected in EDTA-containing microtainer tubes via heel stick, aided by infant heel-warming packs. Plasma and red blood cells (RBCs) were separated by centrifugation; lipids were extracted and transmethylated with 14% boron trifluoride in methanol, and methylesters were analyzed by using capillary column gas chromatography with flame ionization detection (13). Results were obtained as the percentage of total fatty acids and as mass concentration (μg/mL of packed RBCs) on the basis of the addition of an internal standard (10 μg 23:0 fatty acid). Thirty-one fatty acid peaks were identified by comparison with a standard mixture of fatty acid methyl esters (GLC68 + 11: GLC68A; NuCheck Prep, Elysian, MN, and 11 individual standards; Sigma Chemical Co, St Louis, MO, and Cayman Chemical Co, Ann Arbor, MI), and a custom software program (written by a contract programmer working for the Retina Foundation of the Southwest) was used to semiautomate data processing. Ninety-one percent of the chromatographic peaks were identified for analysis.

Because the focus of this report is visual function outcomes, reported results of fatty acid composition analyses are limited to diet-induced differences in major n−6 and n−3 fatty acids in RBC total lipids as indexes of compliance with diets and of neural membrane composition. If differences were found, our aim was to ascertain whether they were correlated with visual function outcome.

Growth

Weight was measured by using a pediatric strain-gauge scale (Healthometer, Bridgeview, IL) that is accurate to 1 g. Length was measured by using length boards (Ellard Instrumentation Ltd, Seattle, WA) that are accurate to 0.1 cm. Growth data were normalized and expressed as *z* scores for term infants of the appropriate age and sex by using the least-squares mean (LSM) values provided in the data files from the Centers for Disease Control and Prevention growth charts released in 2000 by the Department of Health and Human Services as part of the third National Health and Nutrition Examination Survey (*see* www.cdc.gov/growthcharts/).

Sweep VEP acuity

VEP acuity was assessed according to the swept values protocol developed by Norcia et al (24–26) using vertical gratings with phase reversing at 6.6 Hz. Details of the protocol have been described previously (15, 27, 28). Briefly, 2 bipolar placements of O_z versus O_1 and O_2 were used to record (gain = 10 000–20 000, −3 dB cutoff at 1 and 100 Hz) the electroencephalogram that was adaptively filtered in real time to isolate the VEP (397-Hz sampling rate). Amplitude and phase of the response at the second harmonic of the stimulation frequency were calculated for each channel. Noise was measured by determining the amplitude and phase of the 2 adjacent nonharmonic frequencies. Grating acuity was estimated with the use of an automated algorithm that examined signal-to-noise ratio and phase coherence and that performed a linear regression for the final descending limb of the vector-averaged function (minimum of 3 trials; typically 5 trials) that related VEP second harmonic amplitude (amplitude at the reversal frequency of 13.2 Hz) to spatial frequency.

Sweep VEP acuities were expressed in logMAR (eg, 20/20 corresponds to a MAR of 1-min arc and logMAR of 0.0, whereas 20/200 corresponds to a MAR of 10-min arc and logMAR of 1.0). As noted in Table 2, the sweep VEP test could not be completed for one of the control infants at age 6 wk because of an equipment malfunction.

Stereoacuity

RD stereoacuity was assessed by using forced-choice preferential looking and Infant Random Dot Stereocards (Stereo Optical Co; 29, 30). RD stereoacuity was chosen as an outcome measure because it directly reflects cortical processing; detection of the disparate stimulus depends on a cortical combination of monocular images that lack any form information. The Random Dot Stereocards consist of a series of test cards with disparities ranging from 1735 s to 45 s in steps of approximately an octave each. The cards are presented in a 2 down–1 up staircase protocol. The infant views the test cards while wearing polarizing filters mounted in spectacle frames especially designed for infants, and an observer judges on each trial whether the infant prefers to look at a disparate or a nondisparate stereogram. Stereoacuity is obtained by calculating the geometric mean of the last 6 of 8 reversals or by maximum likelihood estimation. To avoid bias introduced by "basement effects" in low-vision eyes, we have established criteria for switching over to the block method (29, 30). Stereoacuity was expressed in logsec (eg, 40 s disparity corresponds to 1.60 logsec). As noted in Table 2, the stereoacuity test could not be completed in all infants at all visits because conjunctivitis prevented the polarized glasses from being placed on the child (1 LCP-supplemented infant and 1 control infant at age 17 wk) or because the child refused to wear the glasses (1 control infant at age 39 wk; 1 LCP-supplemented infant and 1 control infant at age 52 wk).

Statistical analysis

During the course of the study, all data were handled in a coded manner. Data analyses were conducted with repeated-measures analysis of variance (ANOVA) after verification that the data met normality criteria. To avoid the need for imputation of missing VEP acuity data at 6 wk for the infants in the ERG substudy, 2 separate repeated-measures ANOVAs for VEP acuity were conducted, one for the infants in the VEP substudy (who were tested at ages 6,17, 39, and 52 wk) and a second for data from all infants in the study (combining the data from both substudies for the 3 common visits at ages 17, 39, and 52 wk). Planned comparisons were carried out to compare means of the 2 diet groups at each age point. Because 4 pairwise comparisons were conducted for each of the vision outcome variables, only planned comparisons with $P < 0.01$ were considered significant (Bonferroni adjustment = 0.05/4 = 0.0125). Linear regression was conducted to examine the association between RBC lipid concentrations and visual outcomes. Because linear regression was conducted to examine the relation between 4 major fatty acids (ie, LA, ALA, ARA, and DHA) and visual outcomes, only regression coefficients associated with $P < 0.01$ were considered significant (Bonferroni adjustment = 0.05/4 = 0.0125).

RESULTS

Demographics of the cohort

The ethnicity of the cohort was representative of the greater Dallas area: 82% were white, and 18% were minorities. Sex representation in the cohort was 50% male and 50% female. Maternal variables included a mean age of 31 ± 4 y, mean prepregnancy weight of 64 ± 15 kg, and mean height of 166 ± 7 cm. Paternal variables included a mean age of 34 ± 5 y, mean weight of 89 ± 20 kg, and mean height of 181 ± 7 cm. At least 2 y of college education was completed by 62% of the mothers and 76% of the fathers. Demographic information for the individual diet groups is summarized in **Table 3**. There were no significant differences between the groups in sex representation, ethnicity, or assessed maternal and paternal variables. Also presented in Table 3 are the demographic data for the 86 infants who

TABLE 3
Demographic characteristics of the cohort[1]

	At enrollment		At study completion	
	LCP group (n = 51)	Control group (n = 52)	LCP group (n = 42)	Control group (n = 44)
Sex (M/F)	25/26	26/26	21/21	22/22
White/minority (%)	82/18	81/19	76/24	82/18
Maternal age (y)	30.6 ± 4.2[2]	32.2 ± 4.4	31.0 + 4.1	32.2 + 4.6
Maternal weight (kg)	63.0 ± 11.9	64.4 ± 17.6	61.4 + 10.2	61.7 + 12.9
Maternal height (m)	1.66 ± 0.06	1.66 ± 0.07	1.65 + 0.06	1.65 + 0.07
Paternal age (y)	32.8 ± 4.6	34.3 ± 6.7	33.8 + 4.7	34.1 + 6.8
Paternal weight (kg)	91.1 ± 20.8	86.8 ± 19.3	90.2 + 22.6	87.6 + 20.5
Paternal height (m)	1.81 ± 0.06	1.81 ± 0.08	1.80 + 0.06	1.81 + 0.07
Maternal education (%)				
High school	40	35	40	39
College	46	41	48	36
Postgraduate	14	24	12	25
Paternal education (%)				
High school	25	24	24	27
College	56	41	55	43
Postgraduate	19	35	21	30

[1] LCP, long-chain polyunsaturated fatty acid. There were no significant differences between groups for any of the demographic variables.
[2] $\bar{x}$ ± SD (all such values).

completed the entire 12-mo study. There were no significant differences between the groups in sex representation, ethnicity, or assessed maternal and paternal variables.

Red blood cell lipids

Mean concentrations of major n−3 and n−6 fatty acids in RBC total lipids for both randomized diet groups at each age are summarized in **Table 4**. All 92 infants who remained in the study through at least age 17 wk (46 in the LCP-supplemented group and 46 in the control group) provided a blood sample; 30 participants in the ERG substudy provided a blood sample at age 6 wk, and 62 participants in the VEP substudy provided a blood sample at age 17 wk. All 90 infants who remained in the study through at least age 39 wk provided a blood sample.

n−3 Fatty acids

At age 6 wk, the concentration of DHA was 29% higher in the LCP-supplemented group than in the control group ($P < 0.001$). At age 17 wk, DHA concentration was 142% higher in the LCP-supplemented group than in the control group, and, at age 39 wk, it was 215% higher in the LCP- supplemented group than in the control group (both: $P < 0.00001$). There were no significant differences in ALA concentration at any age. Concentrations of eicosapentaenoic acid (EPA; 20:5n–3) and docosapentaenoic acid (DPA; 22:5n–3) were significantly lower in the LCP-supplemented group than in the control group at ages 17 and 39 wk ($P < 0.001$). Total RBC n−3 was significantly higher in the LCP-supplemented group than in the control group at ages 17 and 39 wk ($P < 0.001$).

n−6 Fatty acids, saturates, and monounsaturates

At ages 6, 17, and 39 wk, the concentration of ARA was 15–18% higher in the LCP-supplemented group than in the control group ($P < 0.001$). The concentration of LA was significantly lower in the LCP-supplemented group than in the control group at ages 6, 17, and 39 wk ($P < 0.001$). Both 20:3n−6 and n−6 DPA (22:5n–6) concentrations were lower in the LCP-supplemented group than in the control group at ages 17 and 39 wk ($P < 0.00001$). Concentrations of 22:4n–6, total RBC n−6, saturates, and monounsaturates did not differ significantly between diet groups at any age.

Ratios

The ratio of DHA to DPA n−6 was significantly higher in the LCP-supplemented group than in the control group at ages 6, 17, and 52 wk ($P < 0.001$). The ratios of n−6 to n−3 and of Mead acid (20:3n–9) to ARA (20:4n−6) were significantly lower in the LCP-supplemented group than in the control group only at ages

TABLE 4
Fatty acid profiles in total red blood cell lipids[1]

	6 wk		17 wk		39 wk	
	Control group ($n = 16$)	LCP group ($n = 14$)	Control group ($n = 30$)	LCP group ($n = 32$)	Control group ($n = 44$)	LCP group ($n = 46$)
	μg/mL		*μg/mL*		*μg/mL*	
n–3 Fatty acids						
ALA	2.62 ± 0.99	2.55 ± 1.10	2.14 ± 0.99	2.04 ± 1.17	2.75 ± 1.27	2.29 ± 1.07
EPA	1.96 ± 0.41	1.57 ± 0.57	2.33 ± 0.62	1.50 ± 0.39[2]	2.73 ± 0.85	1.70 ± 0.44[2]
n–3 DPA	7.66 ± 2.36	6.48 ± 1.64	15.1 ± 2.7	7.54 ± 1.45[2]	16.6 ± 3.6	7.33 ± 1.91[2]
DHA	48.1 ± 9.9	62.1 ± 9.7[3]	31.6 ± 7.4	76.5 ± 12.1[2]	23.8 ± 10.6	74.9 ± 17.6[2]
n–6 Fatty acids						
LA	186 ± 38	151 ± 23[3]	161 ± 24	139 ± 25[3]	197 ± 40	159 ± 24[2]
20:3n–6	25.5 ± 6.9	19.7 ± 3.3	21.7 ± 4.4	11.6 ± 2.3[2]	21.5 ± 5.6	11.3 ± 2.5[2]
ARA	151 ± 17	178 ± 19[3]	164 ± 23	188 ± 25[3]	158 ± 28	183 ± 24[3]
22:4n–6	44.3 ± 9.0	44.6 ± 6.6	46.8 ± 6.6	43.8 ± 7.0	46.1 ± 7.4	41.2 ± 6.7
n–6 DPA	17.9 ± 3.6	15.5 ± 2.9	12.4 ± 2.5	7.1 ± 1.4[2]	11.1 ± 2.5	6.0 ± 1.6[2]
Totals						
Saturates[4]	498 ± 66	482 ± 60	460 ± 68	458 ± 63	461 ± 74	466 ± 60
Monounsaturates[5]	266 ± 46	243 ± 36	226 ± 33	220 ± 36	252 ± 43	242 ± 40
n–3 LCP[6]	57.9 ± 11.2	70.7 ± 10.9	49.3 ± 9.8	85.9 ± 13.0[2]	43.6 ± 12.3	84.3 ± 17.7[2]
n–6 LCP	244 ± 30	263 ± 28	251 ± 34	256 ± 33	243 ± 40	247 ± 32
Fatty acids	1264 ± 171	1210 ± 137	1156 ± 138	1166 ± 146	1224 ± 158	1208 ± 137
Unsaturation index[7]	1967 ± 243	2029 ± 209	1833 ± 222	2031 ± 235[3]	1863 ± 281	2039 ± 261
Ratios						
DHA:n–6 DPA	2.79 ± 0.78	4.09 ± 0.88[3]	2.59 ± 0.62	10.9 ± 1.5[2]	2.26 ± 1.68	12.9 ± 3.5[2]
n–6:n–3 LCP	4.35 ± 0.88	3.77 ± 0.42	5.21 ± 0.74	3.00 ± 0.30[2]	5.80 ± 0.96	3.06 ± 0.85[2]
Mead acid:ARA[8]	0.029 ± 0.012	0.022 ± 0.012	0.007 ± 0.004	0.003 ± 0.002[2]	0.007 ± 0.002	0.003 ± 0.003[2]

[1] All values are $\bar{x}$ ± SD. LCP, long-chain polyunsaturated fatty acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; ARA, arachidonic acid. Percentage of total fatty acids = (individual fatty acid value/total fatty acid value) × 100.

[2,3] Significantly different from control (Bonferroni adjustment of Student's *t* test): [2]$P < 0.00001$, [3]$P < 0.001$.

[4] Includes 14:0, 16:0, 17:0, 18:0, 20:0, 22:0, and 24:0.

[5] Includes 16:1, 18:1, 20:1, 22:1, and 24:1.

[6] LCPs are of >18 carbon chain length.

[7] Unsaturation index = sum of (no. of double bonds × mass of each fatty acid).

[8] 20:3n–9/20:4n–6.

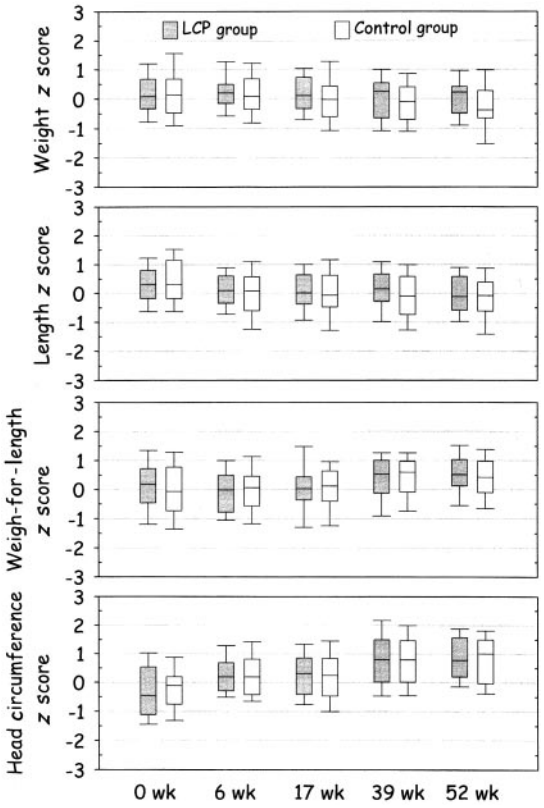

FIGURE 1. Weight, length, weight-for-length, and head circumference z scores for each diet group, based on the standards of the third National Health and Nutrition Examination Survey. The horizontal line in each box corresponds to the mean z score. The extent of the box corresponds to the 25th and 75th percentiles. The whiskers on each box correspond to the 10th and 90th percentiles. The number of infants measured at ages 0, 6, 17, 39, and 52 wk was 51, 47, 46, 44, and 42 for the LCP-supplemented group and 52, 48, 46, 46, and 44 for the control group. No significant main effect of diet was found for weight, length, or head circumference ($P > 0.58$ for all variables; repeated-measures ANOVA). A significant main effect of age was found for each of the growth variables ($P < 0.0006$ for all variables). None of the age × growth variable interactions were significant. LCP, long-chain polyunsaturated fatty acid.

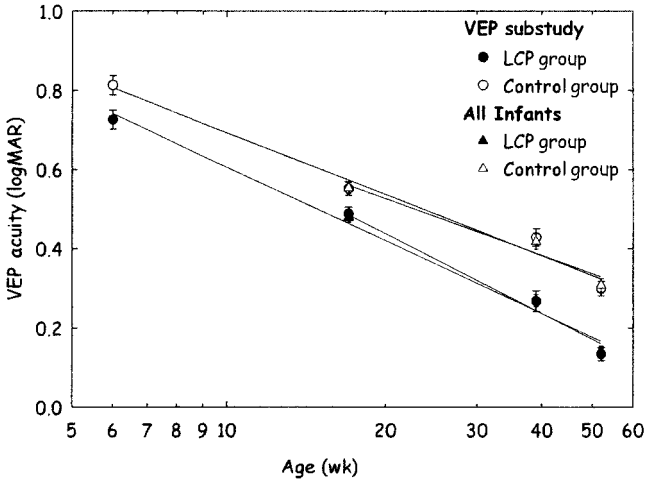

FIGURE 2. Mean (±SE) sweep visual evoked potential (VEP) acuity for both diet groups as a function of age. Data from infants in the VEP substudy are shown as circles (for ages 6, 17, 39, and 52 wk). The number of infants measured at ages 6, 17, 39, and 52 wk was 33, 32, 31, and 30 for the long-chain polyunsaturated fatty acid (LCP)–supplemented group and 32, 30, 30, and 29 for the control group. There were significant main effects of diet ($P < 0.001$) and age ($P < 0.001$) and a significant diet × age interaction ($P < 0.02$) (all: repeated-measures ANOVA). In the planned comparisons, VEP acuity in the control group was significantly poorer than that in the LCP-supplemented group at ages 6 ($P = 0.01$), 17 ($P = 0.01$), 39 ($P < 0.001$), and 52 ($P < 0.001$) wk. Data from the combined VEP and electroretinogram substudies are shown as triangles (for ages 17, 39, and 52 wk). The number of infants measured at ages 17, 39, and 52 wk was 46, 44, and 42 for the LCP-supplemented group and 46, 46, and 44 for the control group. There were significant main effects of diet ($P < 0.001$) and age ($P < 0.001$) and a significant diet × age interaction ($P < 0.01$) (all: repeated-measures ANOVA). In the planned comparisons, VEP acuity in the control group was significantly poorer than that in the LCP-supplemented group at ages 17, 39, and 52 wk ($P < 0.001$). The lines represent the best fit by linear regression of VEP acuity (logMAR) on log age in weeks for each data set. logMAR, log of the minimal angle of resolution.

17 and 39 wk ($P < 0.00001$). The unsaturation index in the RBC lipids was significantly higher among the LCP-supplemented group at age 17 wk than among the control group ($P < 0.001$).

Growth

The z scores for length, weight, and head circumference for both diet groups are shown in box plots in **Figure 1**. All anthropometric outcome data were normally distributed. With the use of repeated-measures ANOVA, no significant main effect of diet was found for weight, length, or head circumference ($P > 0.58$ for all variables). A significant main effect of age was found for each of the growth variables (all: $P < 0.0006$). None of the interactions between age and growth variables were significant.

Sweep VEP acuity

Mean sweep VEP acuity for both diet groups at each age is summarized in **Figure 2**. Data from infants in the VEP substudy are shown as circles (for ages 6, 17, 39, and 52 wk), and data from the combined VEP and ERG substudies are shown as triangles (for ages 17, 39, and 52 wk). All acuity outcome data were normally distributed. For the VEP substudy, there were significant main effects of diet ($P < 0.001$) and age ($P < 0.001$) and a significant diet × age interaction ($P < 0.02$). In the planned comparisons, VEP acuity in the control group was significantly poorer than that in the LCP-supplemented group at ages 6, 17, 39, and 52 wk ($P = 0.01, 0.01, < 0.001$, and < 0.001, respectively). For the combined ERG and VEP substudies, there were significant main effects of diet ($P < 0.001$) and age ($P < 0.001$) and a significant diet × age interaction ($P < 0.01$). In the planned comparisons, VEP acuity in the control group was significantly poorer than that in the LCP-supplemented group at ages 17, 39, and 52 wk ($P < 0.001$). In both analyses, the VEP acuity in the control group was ≈0.12 logMAR poorer than that in the LCP-supplemented group overall; this corresponds to a little more than a one-line difference in reading a standard eye chart.

Random dot stereoacuity

Mean RD stereoacuity for both diet groups at each age is summarized in **Figure 3**. There were significant main effects of diet ($P < 0.001$) and age ($P < 0.001$) and a significant diet × age interaction ($P = 0.001$). In planned comparisons, the LCP-supplemented group had significantly better stereoacuity than did the control group at age 17 wk ($P < 0.001$) but not at ages 39 and 52 wk ($P = 0.37$ and 0.06, respectively).

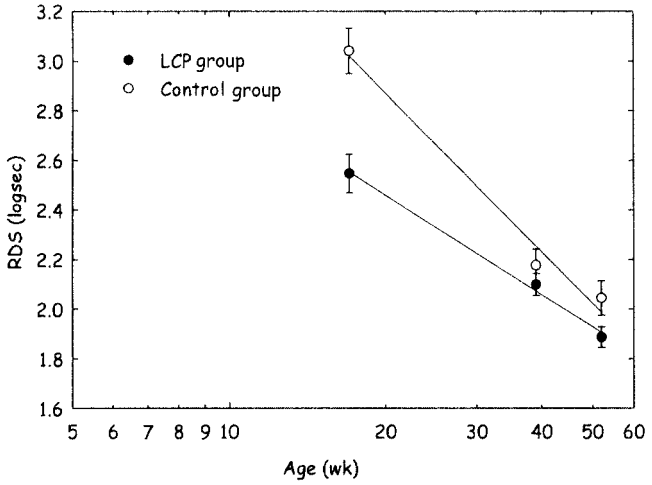

FIGURE 3. Mean (±SE) random dot stereoacuity (RDS) for both diet groups as a function of age. The number of infants measured at ages 17, 39, and 52 wk was 45, 44, and 41 for the long-chain polyunsaturated fatty acid (LCP)–supplemented group and 45, 45, and 43 for the control group. There were significant main effects of diet ($P < 0.001$) and age ($P < 0.001$) and a significant diet × age interaction ($P = 0.001$) (all: repeated-measures ANOVA). In planned comparisons, the LCP-supplemented group had significantly better stereoacuity than did the control group at age 17 wk ($P < 0.001$) but not at ages 39 and 52 wk ($P = 0.37$ and 0.06, respectively). Lines represent the best fit by linear regression of stereoacuity (logsec) on log age in weeks. logsec, log of the minimal detectable binocular disparity.

Linear regression of vision outcomes on red blood cell lipids

The relation between RBC LCP composition at ages 17 and 39 wk and sweep VEP acuity at ages 17, 39, and 52 wk was examined by linear regression. Because sweep VEP acuities were expressed in logMAR, negative regression coefficients indicate that better acuity is associated with a higher concentration of the LCP, whereas positive regression coefficients indicate that poorer acuity is associated with a higher concentration of the LCP. Outcomes from the linear regression analysis are summarized in **Table 5**.

Better sweep VEP acuity at age 17 wk was associated with higher DHA concentration at that age but not with ALA, ARA, or LA concentration. Better sweep VEP acuity at age 39 wk was associated with higher DHA and ARA concentrations at ages 17 wk and 39 wk but not with ALA or LA concentration at either age. Better sweep VEP acuity at age 52 wk was associated with higher DHA concentration at ages 17 wk and 39 wk and with higher ARA concentration at age 39 wk but not with ALA or LA concentration at either age. Better sweep VEP acuity was associated with a lower n−6:n−3 and a higher DHA:n−6 DPA at ages 17, 39, and 52 wk.

Better RD stereoacuity at age 17 wk was associated with higher DHA concentration at that age but not with ALA, ARA, or LA concentration (data not shown). Better RD stereoacuity at ages 39 and 52 wk was associated with lower LA concentration at ages 17 and 39 wk but not with DHA, ARA, or ALA concentration at either age (data not shown). Better RD stereoacuity at age 17 wk was associated with lower n−6:n−3 and higher DHA:n−6 DPA (data not shown), but RD stereoacuity at ages 39 and 52 wk was significantly associated with neither (data not shown).

DISCUSSION

Supplementation of term infant formula with 0.36% DHA and 0.72% ARA during the first year of life yields clear differences in total RBC lipid composition and in visual function. By infant age 17 wk, DHA concentration in RBCs in the LCP-supplemented group was more than twice that in the control group; by infant age 39 wk, it was more than 3 times as high. VEP acuity in the control group was significantly poorer than that in the LCP-supplemented group at ages 6, 17, 39, and 52 wk. Stereoacuity in the control group was significantly poorer than that in the LCP-supplemented group at age 17 wk, but not at ages 39 and 52 wk. The growth of infants fed LCP-supplemented and control formulas did not differ significantly, and both diets were well tolerated.

Some earlier randomized trials also found a specific benefit of dietary DHA for term infant retinal maturation, visual acuity development, or mental development (10–16). Others have found no benefit (17–22). Our trial met each of the criteria that Lauritzen et al (2) proposed for a study design to be sensitive to the functional effects of LCP supplementation: DHA concentration >0.35% of total fatty acids, outcome measure of VEP acuity,

TABLE 5
Correlations between red blood cell (RBC) long-chain polyunsaturated fatty acid composition and sweep visual evoked potential (VEP) acuity at ages 17, 39, and 52 wk[1]

VEP acuity		RBC lipids at 17 wk						RBC lipids at 39 wk					
		LA	ALA	ARA	DHA	n–6:n–3 LCPs	DHA:n–6 DPA	LA	ALA	ARA	DHA	n–6:n–3 LCPs	DHA: n–6 DPA
17 wk	*r*	0.252	−0.002	−0.270	−0.371	0.361	−0.361						
	n	92	92	92	92	92	92						
	P	NS	NS	NS	0.003	0.004	0.004						
39 wk	*r*	0.317	0.044	−0.346	−0.523	0.457	−0.520	0.218	0.073	−0.288	−0.494	0.472	−0.500
	n	90	90	90	90	90	90	90	90	90	90	90	90
	P	NS	NS	0.006	< 0.0001	< 0.0001	< 0.0001	NS	NS	0.006	< 0.0001	0.000	< 0.0001
52 wk	*r*	0.325	0.230	−0.234	−0.556	0.541	−0.632	0.276	−0.061	−0.448	−0.582	0.512	−0.568
	n	86	86	86	86	86	86	86	86	86	86	86	86
	P	NS	NS	NS	< 0.0001	< 0.0001	< 0.0001	NS	NS	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[1] LA, linoleic acid, ALA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid. Sweep VEP acuity was expressed in log for minimal angle of resolution (logMAR), so negative regression coefficients indicate that better acuity is associated with a higher concentration of the LCP, and positive regression coefficients indicate that poorer acuity is associated with a higher concentration of the LCP.

and sample size >20/diet group. In comparison, the only other large study of LCP-supplemented formula fed for 12 mo using VEP acuity as an outcome measure provided a relatively low amount of LCP supplementation (0.2% of total fatty acids as DHA) and failed to find a functional benefit of LCP supplementation (19–21).

The differences between diet groups in sweep VEP acuity and stereoacuity are subtle but statistically significant. For example, the difference in VEP acuity at age 39 wk is equivalent to one line on reading a standard eye chart (eg, 20/37 rather than 20/52 vision). However, the rationale for VEP acuity and RD stereoacuity as outcome measures in infant nutrition studies is not the detection of gross visual impairment requiring treatment but rather the quantification of subtle differences among diet groups that reflect diet-related modifications in the developmental course of structure and function in the brain and retina. Thus, even a subtle or transient difference in visual function may provide an important clue to the nutritional requirements of the central nervous system during critical periods of its development.

Alterations in fatty acid composition can have profound effects on neural membrane function. Membrane phospholipids serve as precursors in the production of intramembrane and intermembrane messengers (31, 32). The degree of unsaturation also contributes to membrane fluidity, although the relation is complex (33, 34); the degree of unsaturation also affects membrane thickness, deformability, and curvature (34–36). Membrane phospholipids may also act as an antioxidant buffer, because they are more easily repaired than is oxidative damage to proteins or DNA (37). Because some proteins retain association with specific phospholipids even when solubilized (38), it has been hypothesized that lipids influence the optimal function of the protein in situ; ie, annular lipids or microdomain lipids create a suitable membrane thickness, curvature, order, and mobility for optimal function (39–41). In addition, phospholipids can act as chaperones in the folding of newly synthesized proteins (42), offer neural cells protection from apoptotic cell death (43), and influence gene expression in the fetal retina (44).

Direct membrane effects of free or esterified DHA on neural receptors, pumps, and channels may be transient, but differences in nerve cell signaling during infancy could lead to permanent changes in the cytoarchitecture of the brain by affecting the processes of synapse formation and the elimination of supernumerary synapses during critical periods of development. If so, long-lasting effects of the dietary supply of DHA during infancy on brain function may be expected.

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EEB, DGB, RDU, and DRH participated in study design, interpretation of the data, and writing the manuscript. EEB and DRH supervised all aspects of the study, were responsible for data analysis, and wrote the first draft of the manuscript. YSC coordinated eligibility screening, recruitment, blood samples, assessments of growth and tolerance, and data management. DHW coordinated blood lipid analyses. None of the authors had a conflict of interest.

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