

## Maturation of Visual Acuity Is Accelerated in Breast-Fed Term Infants Fed Baby Food Containing DHA-Enriched Egg Yolk<sup>1,2</sup>

Dennis R. Hoffman,\* \*\*<sup>3</sup> Richard C. Theuer,<sup>†4</sup> Yolanda S. Castañeda,\*  
Dianna H. Wheaton,\* Rain G. Bosworth,\* Anna R. O'Connor,\*<sup>5</sup> Sarah E. Morale,\*  
Lindsey E. Wiedemann,\* and Eileen E. Birch\*<sup>†</sup>

\*Anderson Vision Research Center, Retina Foundation of the Southwest, Dallas, TX 75231; Departments of <sup>†</sup>Ophthalmology and \*\*Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX 75390; and <sup>‡</sup>Beech-Nut Nutrition Corporation, St. Louis, MO 63102

**ABSTRACT** Between 6 and 12 mo of age, blood levels of the (n-3) long-chain PUFA, docosahexaenoic acid (DHA), in breast-fed infants typically decrease due to diminished maternal DHA stores and the introduction of DHA-poor solid foods displacing human milk as the primary source of nutrition. Thus, we utilized a randomized, clinical trial format to evaluate the effect of supplemental DHA in solid foods on visual development of breast-fed infants with the primary outcome, sweep visual-evoked potential (VEP) acuity, as an index for maturation of the retina and visual cortex. At 6 mo of age, breast-fed infants were randomly assigned to receive 1 jar (113 g)/d of baby food containing egg yolk enriched with DHA (115 mg DHA/100 g food;  $n = 25$ ) or control baby food (0 mg DHA;  $n = 26$ ). Gravimetric measures were used to estimate the supplemental DHA intake which was 83 mg DHA/d in the supplemented group and 0 mg/d in controls. Although many infants in both groups continued to breast-feed for a mean of 9 mo, RBC DHA levels decreased significantly between 6 and 12 mo (from 3.8 to 3.0 g/100 g total fatty acids) in control infants, whereas RBC DHA levels increased by 34% from 4.1 to 5.5 g/100 g by 12 mo in supplemented infants. VEP acuity at 6 mo was 0.49 logMAR (minimum angle of resolution) and improved to 0.29 logMAR by 12 mo in controls. In DHA-supplemented infants, VEP acuity was 0.48 logMAR at 6 mo and matured to 0.14 logMAR at 12 mo (1.5 lines on the eye chart better than controls). At 12 mo, the difference corresponded to 1.5 lines on the eye chart. RBC DHA levels and VEP acuity at 12 mo were correlated ( $r = -0.50$ ;  $P = 0.0002$ ), supporting the need of an adequate dietary supply of DHA throughout 1 y of life for neural development. *J. Nutr.* 134: 2307–2313, 2004.

**KEY WORDS:** • (n-3) fatty acids • docosahexaenoic acid • neural development

Long-chain PUFA (LCPUFA)<sup>6</sup> have an important role in visual development during infancy. Compared with infants fed commercial formulas lacking LCPUFA, breast-fed infants have more advanced electroretinographic function as early as 6 wk of age (1) and more mature visual acuity by 4 mo of age (2). In longitudinal assessment of the effect of maternal and infant dietary factors in infant visual development, Williams

et al. (3) reported that the variable most associated with stereoacuity at 3.5 y of age was breast-feeding. Children who had nursed for even short periods during infancy had more mature visual stereoacuity than children who had never received human milk. The (n-3) LCPUFA, docosahexaenoic acid [DHA; 22:6(n-3)], which is present in human milk but absent in unsupplemented infant formulas, may be the major factor responsible for this benefit.

Direct evidence for a role of DHA in visual development is that term infants fed formula with adequate amounts of DHA and a balanced amount of the 20-carbon (n-6) LCPUFA, arachidonic acid [ARA; 20:4(n-6)], have improved visual and mental development with no adverse effect on growth (4–6). Furthermore, DHA in infant formula was associated with shorter-look duration to novel stimuli on the Fagan test (7) and with improved visual acuity in a multistudy meta-analysis (8). Critical reviews of this literature were published recently (9–11).

A woman producing milk for her infant derives a major portion of milk LCPUFA from her endogenous stores (12). Human milk can vary considerably in its LCPUFA content depending on the diet of the mother and the amount of

<sup>1</sup> Presented in part at the American College of Nutrition, October 2003, Nashville, TN [Hoffman, D. R., Theuer, R. C., Castañeda, Y. S., Wheaton, D. H., Morale, S. E., Wiedemann, L. E. & Birch, E. E. (2003) Maturation of visual acuity in breast-fed term infants weaned to baby food containing DHA-enriched egg yolk. *J. Am. Coll. Nutr.* 22: 468 (abs.)].

<sup>2</sup> Supported in part by National Institutes of Health grant HD22380 and a grant from Beech-Nut Nutrition.

<sup>3</sup> To whom correspondence should be addressed.  
E-mail: dhoffman@retinafoundation.org.

<sup>4</sup> Present address: Theuer Research & Consulting, Raleigh, NC 27615.

<sup>5</sup> Present address: Department of Orthoptics, University of Liverpool, Liverpool, L69 3GB UK.

<sup>6</sup> Abbreviations used: ARA, arachidonic acid [20:4(n-6)]; DHA, docosahexaenoic acid [22:6(n-3)]; DPA, docosapentaenoic acid [22:5(n-3) and 22:5(n-6)]; EPA, eicosapentaenoic acid [20:5(n-3)]; LCPUFA, long-chain PUFA; LA, linoleic acid [18:2(n-6)];  $\alpha$ -LNA,  $\alpha$ -linolenic acid [18:3(n-3)]; MAR, minimum angle of resolution; VEP, visual-evoked potential.

LCPUFA mobilized from her tissues over the course of the current and any preceding pregnancies and/or lactations (13). The concentration of DHA in human milk varies from as little as 0.1% of total fatty acids in women consuming Western diets to as much as 1.4% in Inuit women in North America and 2.78% in Chinese women from a fishing village, both consuming large amounts of marine animal foods (14–16).

The rationale for this study was that at ~6 mo of age, infants are beginning to be fed semisolid foods and thus are likely to have a reduction in dietary DHA as reflected in decreased blood DHA levels (4,6). This reduction in the infant's DHA intake may be due to a concomitant reduction in consumption of human milk (17) combined with increasing intake of DHA-poor weaning foods (18).

The objective of this randomized clinical trial was to determine whether DHA-enriched baby food provided as a supplemental source of DHA to breast-fed infants in the second 6 mo of life altered blood lipid fatty acid profiles and modified visual development. In addition, to assess whether the long-chain fatty acids affected infant metabolism, we evaluated total antioxidant capacity, blood chemistry, and hematology. Supplementary DHA was provided in the form of ready-to-feed baby foods made with DHA-enriched egg yolk providing DHA and ARA.

## SUBJECTS AND METHODS

**Subjects.** Healthy term infants receiving human milk born at either Presbyterian Hospital of Dallas or Medical City Dallas Hospital were enrolled in the study. Additional infants were recruited through advertisements. Inclusion criteria were a gestational age at birth >37 wk, a birthweight > 2800 g, exclusive breast-feeding in hospital and for the first 4 mo of life with a maternal intention to continue breast-feeding, a good possibility of long-term follow-up, and informed consent to the protocol. Exclusion criteria were any underlying disease or congenital malformation that was judged likely to interfere with the evaluation of the study material, any abnormal maternal dietary patterns, and any evidence of maternal metabolic disease.

Informed consent was obtained from one or both parents before the infant's participation. 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 Hospital of Dallas, and Medical City Dallas Hospital.

Computer-generated randomization codes with variable-length blocks of 8–12 were used to assign 55 infants to 1 of 2 groups at 6 mo of age. Both groups received study baby foods and were directed to feed the baby 1 jar of study food per day. As incentive, all parents received store coupons for purchasing commercial baby foods at their local store. One group received control baby foods, and the other group received baby foods containing DHA-enriched egg yolk.

Fifty-one infants completed the study: 26 in the group receiving the control baby food, with 1 drop-out (due to viral infection), and 25 in the group receiving the baby foods made with DHA-enriched egg yolks with 3 drop-outs (2 due to constipation and 1 refusal to eat solid foods at 6 mo of age), yielding a 93% completion rate.

**Baby foods.** DHA-enriched eggs were obtained from hens receiving a diet essentially free of LCPUFA but containing flaxseed and soybean meal as sources of the DHA precursor,  $\alpha$ -linolenic acid ( $\alpha$ -LNA) (19). The egg yolks were separated, pasteurized, and spray-dried. The dried egg yolks at 120 g/kg food (12%) were used to prepare semisolid, ready-to-feed DHA-enriched baby foods as described by Theuer et al. (20,21); all foods were packaged in hermetically sealed jars containing 113 g food. The control baby foods were devoid of egg yolk but otherwise contained the same ingredients as the DHA-enriched foods. The dried egg yolk and the baby foods were analyzed for fatty acids by Medallion Laboratories (22) (see Table 1). The dried egg yolk contained ~2% of fatty acids as DHA; the DHA-enriched baby foods contained ~115 mg DHA/100 g food (i.e.,

TABLE 1

Distribution of fatty acids in study baby foods<sup>1</sup>

Individual fatty acids	Control	DHA-egg yolk enriched
	mg fatty acid/100 g	
18:2(n-6) (LA)	113	1076
18:3(n-6)	0	10
20:3(n-6)	1	13
20:4(n-6) (ARA)	1	78
22:5(n-6) [DPA(n-6)]	0	0
18:3(n-3) ( $\alpha$ -LNA)	11	366
18:4(n-3)	0	2
20:3(n-3)	0	8
20:5(n-3) (EPA)	0	4
22:5(n-3) [DPA(n-3)]	0	17
22:6(n-3) (DHA)	0	115
Totals	g/100 g	
Total fat	0.81	6.48
Total saturates <sup>2</sup>	0.41	2.21
Total monounsaturates <sup>3</sup>	0.21	2.28
Trans fat	0.02	0.08
Total (n-6) PUFA	0.12	1.18
Total (n-3) PUFA	0.01	0.51
(n-6)/(n-3) PUFA ratio	9.8	2.3
Vitamin E, mg/100 g	0.64	1.66

<sup>1</sup> Values are from analyses of 4 representative flavor varieties.

<sup>2</sup> Includes 14:0, 16:0, 17:0, 18:0, 20:0, 22:0, and 24:0.

<sup>3</sup> Includes 16:1, 18:1, 20:1, 22:1, and 24:1.

~130 mg DHA/113-g baby food jar). The fat content of the different flavors of DHA-enriched baby foods (5.8–8.1 g/100 g) was 5- to 6-fold higher than that of control foods (0.1–2.3 g/100 g), resulting in a higher energy density of the DHA-enriched baby foods (greater by ~230 kJ/100 g).

Five varieties of study foods were shipped directly to each participant's home; a minimum of 216 jars was provided for the 6-mo feeding period with a goal of providing 1 jar of study food/d. Vegetable, cereal with fruit, and fruit custard varieties were included. Food intake was estimated for the first 2 mo of the trial by collecting and weighing the opened jars to determine the unused portion. Potential discrepancies between food intake and food disappearance (amount missing from each jar) were minimized by instructing the parent to spoon out only a small amount of food at a time and then to spoon out additional food as needed. The food disappearance data served to estimate compliance and the overall food and DHA consumption of study infants.

**General protocol.** Informed consent was obtained and randomization occurred at the 6-mo visit. The assigned foods were shipped within 3–7 d. Visual function (sweep VEP acuity and stereoacuity) and growth were assessed at 6, 9, and 12 mo, and blood samples were taken at 6 and 12 mo.

**Sweep VEP acuity.** VEP acuity was the primary outcome measure and was assessed according to the swept parameter protocol developed by Norcia and colleagues (23,24) with the use of vertical-gratings phase reversing at 6 Hz. Details of the protocol were described previously (4). Sweep VEP acuities were expressed in logMAR (minimum angle of resolution; e.g., the Snellen equivalents of 20/20 correspond to a MAR of 1 min arc and logMAR of 0.0 whereas 20/200 corresponds to an MAR of 10 min arc and logMAR of 1.0).

**Stereoacuity.** Random dot stereoacuity was assessed with the use of forced-choice preferential looking and the Infant Randot Stereocards (25) as described previously (26). Random dot stereoacuity was chosen as an outcome measure because it reflects cortical processing; detection of the disparate stimulus depends on the cortical combination of monocular images that lack any form information. Stereo-

acuity was expressed in log arc s (log of the minimum detectable binocular disparity; e.g., a 40 arc s disparity corresponds to 1.60 log arc s).

**Growth.** Weight, length, head circumference, and triceps and subscapular skinfold thickness measures were described previously (4) and were obtained at 6, 9, and 12 mo. Growth data were normalized by expression as Z-scores derived for term infants of appropriate age and sex by comparison with published normative data by the Department of Health and Human Services as part of the National Health and Nutrition Examination Survey III (27).

**Blood lipids.** Blood samples (2.0 mL) were collected at 6 and 12 mo by heel stick aided by infant heel warming packs into tubes containing EDTA. Plasma and RBC were separated by centrifugation at  $3000 \times g$  for 10 min at 4°C, lipids were extracted and transmethylated with boron trifluoride:methanol, and methyl esters were analyzed by capillary column GC with flame ionization detection [see (6) for details]. The fatty acid level was reported as mass concentration for baby foods and both the relative percentage of total fatty acids and mass concentrations [ $\mu\text{mol/L}$  plasma (data not presented) or packed RBC on the basis of the addition of an internal standard (23:0)].

**Total antioxidant capacity.** Total antioxidant capacity of plasma was measured using an enhanced chemiluminescence modification of the total peroxyl radical trapping parameter [TRAP assay (28)]. The antioxidant capacity of an aliquot (20  $\mu\text{L}$ ) of citrate anticoagulated plasma diluted 1:10 with isotonic sodium chloride solution was established by its ability to quench a horseradish peroxidase-catalyzed reaction generated by a chemiluminescence kit (cat. # RPN 190; Ortho-Clinical Diagnostics). Quenching capacity was assayed on a luminometer (Turner Designs) with subsequent quantification by comparison to a standard curve (10–100  $\mu\text{mol/L}$ ) of the synthetic vitamin E derivative, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Aldrich). Antioxidant activity was expressed as  $\mu\text{mol/L}$  equivalents of this vitamin E derivative.

**Blood hematology and chemistry.** At 6 and 12 mo, aliquots of whole EDTA-anticoagulated blood were sent to a central clinical laboratory (LabCorp) for hematological assay. The analysis included platelet count, red and white blood cell counts, hemoglobin and hematocrit determination, mean corpuscular volume, and hemoglobin quantitation (Coulter LH750 Hematology Analyzer; Beckman Coulter). At 12 mo, blood chemistry was analyzed in serum samples sent to the same laboratory. The analysis included the following: determination of glucose, blood urea nitrogen, creatinine, blood urea nitrogen:creatinine ratio, sodium, potassium, chloride, carbon dioxide, calcium, total protein, albumin, globulin, albumin:globulin ratio, total bilirubin, and activities of alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase (Modular Hitachi, Roche).

**Sample size.** Sample sizes were estimated using the method described by Rosner (29) for  $\alpha = 0.05$  and  $1 - \beta = 0.90$ . With the use of standard deviations for sweep VEP [0.1 logMAR corresponds to 1 line on an eye chart (4)] from our present and past studies of term infants, the final sample size per group at 12 mo required to detect a 1-SD difference between groups was 21 infants. This sample size was also sufficient to detect a 1-SD difference between groups in random dot stereoacuity [0.2 log arc s; e.g., 40 arc s compared with 60 arc s; (25)] and a <1% difference in the DHA or ARA fatty acid composition of RBC lipids (5). Measurements of antioxidant capacity, hematological and blood chemistry variables were not obtained in our previous studies of infant nutrition; thus, the experimental data required to estimate potential dietary effects of these secondary analyses were not available. Anticipating a 10% loss to follow-up over 12 mo, we planned to recruit 25 infants for each diet group. In actuality, 55 infants were enrolled and 51 completing the study, for a 93% completion rate.

**Statistical analyses.** All data were handled in a coded manner. The data were analyzed with two-way repeated-measures ANOVA after verifying that they met normality criteria. Planned comparisons were carried out to compare the means of the 2 diet groups at each age point. Because 4 pairwise comparisons were conducted for each of the vision outcome variables (acuity and stereoacuity), only planned comparisons with  $P < 0.01$  were considered significant (Bonferroni adjustment of 0.05/4 or 0.0125). Because linear regression was simi-

larly conducted between visual acuity and the 4 major fatty acids (linoleic acid [LA; 18:2(n-6)],  $\alpha$ -LNA, ARA, and DHA),  $P < 0.01$  was considered significant. The multiple comparisons of RBC fatty acids were considered significant at  $P < 0.002$ . Values in the text are means  $\pm$  SD.

## RESULTS

Despite the expressed intention to continue breast-feeding their infants, mothers in the control and DHA-enriched groups breast-fed for 9.7 mo and 8.8 mo, respectively (see **Table 2**); 65% of the infants in the control group and 80% of infants in the supplemented group were weaned from human milk to formula before 12 mo of age. The study was largely completed before the commercial availability of infant formula fortified with LCPUFA in the United States. Only 1 infant in the control group and 3 in the DHA-enriched group were weaned from human milk to infant formula containing DHA and ARA during the 6-mo trial interval. Removal of these infants from data analysis did not affect the results of the statistical analyses.

Dietary sources of DHA for infants in the study included both human milk and enriched baby foods. The estimated intakes of DHA from human milk during the trial were 37 mg/d in control infants and 28 mg/d in the supplemented group. Consumption of baby food by the control group was  $84 \pm 23$  g food/d ( $\sim 0.75$  jar/d; **Table 2**). Based on gravimetric measures, control infants consumed 0 and 0.3 mg supplemental DHA and ARA/d, respectively, from baby food during the 6-mo study. Infants in the DHA-enriched group consumed  $72 \pm 31$  g baby food/d (about 0.66 jar/d); this was not different from controls ( $P = 0.12$ ). Infants fed DHA-enriched baby foods were estimated to have consumed 83 mg supplementary DHA/d and 56 mg supplementary ARA/d during the 6-mo trial.

The fatty acid content of RBC lipids did not differ between groups at the start of the trial (6 mo; **Table 3**); however, by 12 mo, the groups differed in RBC lipid DHA, docosapentaenoic acid [DPA; 22:5(n-6)], and total (n-3) LCPUFA ( $P < 0.002$ ). RBC DHA levels decreased in the control group from 3.8% at 6 mo to 3.0% at 12 mo ( $P = 0.012$ ). In contrast, RBC DHA levels increased ( $P < 0.002$ ) in the DHA-enriched group from 4.1% at 6 mo to 5.5% at 12 mo. RBC DHA levels expressed

**TABLE 2**

*Intakes of food and DHA (estimated) in infants randomly assigned to DHA-enriched baby food or control baby food at 6 mo of age<sup>1</sup>*

	Control	Supplemented
<i>n</i>	26	25
<b>Food</b>		
Human milk intake, <i>mo</i>	$9.7 \pm 2.2$	$8.8 \pm 2.4$
Study baby foods, <sup>2</sup> <i>g/d</i>	$84 \pm 23$	$72 \pm 31$
<b>Estimated DHA intake (6–12 mo)</b>		
Human milk, <sup>3</sup> (50 mg/d), <i>g/6 mo</i>	$\sim 7$	$\sim 5$
Study baby foods, <sup>2</sup> (83 mg/d), <i>g/6 mo</i>	0	$\sim 15$
<b>Totals</b>		
<i>g DHA/6 mo</i>	$\sim 7$	$\sim 20$
<i>mg DHA/(kg body weight · d)</i>	$\sim 4.4$	$\sim 13.4$

<sup>1</sup> Values are means  $\pm$  SD or estimations.

<sup>2</sup> Based on gravimetric measures of food consumed between 6 and 8 mo.

<sup>3</sup> See text for calculations of estimates.

TABLE 3

Fatty acid profiles in total RBC lipids of breast-fed infants randomly assigned to DHA-enriched baby food or control baby food at 6 mo of age<sup>1</sup>

	6 mo		12 mo	
	Control	Supplemented	Control	Supplemented
<i>n</i>	26	25	26	25
	<i>g/100 g total fatty acids</i>			
(n-3) Fatty acids				
$\alpha$ -LNA	0.16 $\pm$ 0.08	0.19 $\pm$ 0.09	0.22 $\pm$ 0.10	0.29 $\pm$ 0.13
EPA	0.32 $\pm$ 0.25	0.39 $\pm$ 0.33	0.65 $\pm$ 0.34	0.71 $\pm$ 0.28
DPA(n-3)	1.65 $\pm$ 0.27	1.68 $\pm$ 0.31	1.45 $\pm$ 0.44	1.38 $\pm$ 0.33
DHA	3.80 $\pm$ 0.94	4.10 $\pm$ 0.84	3.00 $\pm$ 1.25	5.50 $\pm$ 1.67*
(n-6) Fatty acids				
LA	12.2 $\pm$ 1.8	12.5 $\pm$ 1.7	12.7 $\pm$ 1.6	12.5 $\pm$ 1.4
20:3(n-6)	1.51 $\pm$ 0.39	1.40 $\pm$ 0.28	1.52 $\pm$ 0.43	1.33 $\pm$ 0.49
ARA	15.1 $\pm$ 1.8	15.2 $\pm$ 1.9	14.1 $\pm$ 2.2	14.3 $\pm$ 1.7
22:4(n-6)	3.53 $\pm$ 0.55	3.39 $\pm$ 0.55	3.52 $\pm$ 0.55	3.06 $\pm$ 0.58
DPA(n-6)	1.06 $\pm$ 0.27	0.95 $\pm$ 0.15	0.93 $\pm$ 0.19	0.69 $\pm$ 0.25*
Totals				
Saturates <sup>2</sup>	38.8 $\pm$ 2.4	38.1 $\pm$ 2.8	40.0 $\pm$ 2.8	39.4 $\pm$ 1.9
Monounsaturates <sup>3</sup>	20.9 $\pm$ 2.4	21.1 $\pm$ 2.3	21.0 $\pm$ 3.1	19.9 $\pm$ 1.3
(n-3) LCPUFA	5.89 $\pm$ 1.18	6.32 $\pm$ 1.99	5.13 $\pm$ 1.4	7.63 $\pm$ 1.70*
(n-6) LCPUFA	21.7 $\pm$ 2.3	21.4 $\pm$ 2.4	20.5 $\pm$ 2.5	19.9 $\pm$ 1.9
Ratios <sup>4</sup>				
DHA/DPA(n-6)	3.88 $\pm$ 1.90	4.41 $\pm$ 1.45	3.40 $\pm$ 1.89	9.34 $\pm$ 4.83*
(n-6)/(n-3)				
LCPUFA	3.66 $\pm$ 0.68	3.44 $\pm$ 0.63	4.26 $\pm$ 1.02	2.77 $\pm$ 0.85*
Mead acid/ARA	0.004 $\pm$ 0.002	0.005 $\pm$ 0.006	0.006 $\pm$ 0.002	0.004 $\pm$ 0.002*
Unsaturation index	165 $\pm$ 11	168 $\pm$ 10	158 $\pm$ 12	169 $\pm$ 10*
Mass concentration	$\mu$ mol DHA/L RBC			
DHA	174 $\pm$ 36	195 $\pm$ 49	155 $\pm$ 52	287 $\pm$ 76*

<sup>1</sup> Values are means  $\pm$  SD for the relative percentage of total fatty acids in packed RBC. Different from 12 mo control,  $P < 0.002$ .

<sup>2</sup> Includes 14:0, 16:0, 17:0, 18:0, 20:0, 22:0, and 24:0.

<sup>3</sup> Includes 16:1, 18:1, 20:1, 22:1, and 24:1.

<sup>4</sup> Mead acid/ARA ratio = 20:3(n-9)/20:4(n-6). The unsaturation index is the sum of (# of double bonds  $\times$  the percentage of each fatty acid).

as mass concentration showed similar changes (Table 3,  $P < 0.002$ ).

The sufficiency indices for (n-3) fatty acids [(n-6)/(n-3) LCPUFA ratio], DHA [DHA/DPA(n-6)], and essential fatty acids [Mead acid [20:3(n-9)]/ARA] improved in the DHA-supplemented group. The unsaturation index was significantly elevated in RBC of supplemented infants; this summation of double bonds is reflective of an increase in the fluidity of RBC membranes. Treen et al. (30) reported that an increase in the unsaturation index of 10 units (based on the percentage of total fatty acids) in cultured retinoblastoma cells increased the lateral mobility in the membrane bilayer (i.e., an  $\sim$ 30% increase in pyrene eximer formation) and increased transport of choline across the cell membrane by 12%.

In control infants, VEP acuity at 6 mo was  $0.49 \pm 0.13$  logMAR; it improved to  $0.45 \pm 0.14$  logMAR at 9 mo and to  $0.29 \pm 0.11$  logMAR at 12 mo (Fig. 1). In the DHA-supplemented group, VEP acuity was  $0.48 \pm 0.10$  logMAR at 6 mo and improved to  $0.31 \pm 0.13$  logMAR at 9 mo and to  $0.13 \pm 0.1$  logMAR at 12 mo. Compared with controls, infants in the DHA-supplemented group had improved visual acuity by 0.14 and 0.16 logMAR at 9 and 12 mo, respectively, ( $P < 0.002$ ), equivalent to  $\sim$ 1.5 lines on an eye chart.

VEP acuity at 12 mo was correlated with RBC DHA levels at 12 mo ( $r = -0.50$ ;  $P = 0.0002$ ) (Fig. 2) such that infants

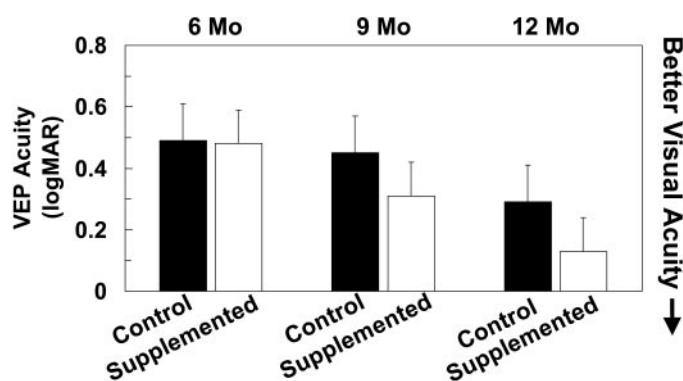
with high DHA levels had lower logMAR values, i.e., more mature acuity. In addition, the estimated dietary intake of DHA from human milk and baby food on an individual basis was correlated with VEP acuity ( $r = -0.49$ ,  $P < 0.0002$ ) as well as with RBC DHA levels ( $r = 0.57$ ,  $P < 0.0002$ ).

Stereoacuity at 6 mo was  $2.54 \pm 0.54$  log arc s and improved to  $2.25 \pm 0.47$  log arc s at 12 mo in the control infants. In the supplemented infants, stereoacuity was  $2.37 \pm 0.34$  log arc s at 6 mo and improved to  $2.22 \pm 0.55$  log arc s at 12 mo. However, stereoacuity at 12 mo did not differ between the 2 diet groups ( $P = 0.8$ ).

Despite differences in energy and fat content of the study baby foods, the groups did not differ in weight, length, head circumference, or skin-fold thicknesses at 6, 9, and 12 mo ( $P > 0.3$  for all measures; data not shown).

Total plasma antioxidant capacity did not differ between the 2 diet groups at the onset of the trial ( $345 \pm 78$   $\mu$ mol/L for controls and  $328 \pm 107$   $\mu$ mol/L Trolox equivalents for the supplemented group;  $P = 0.53$ ) or at 12 mo ( $335 \pm 63$  vs.  $321 \pm 109$   $\mu$ mol/L Trolox equivalents;  $P = 0.58$ , respectively).

Protocol compliance was excellent and the DHA-enriched foods were well tolerated. There were 3 adverse events recorded for controls: 2 were not diet-related (neuroblastoma and occluded tear duct, both requiring surgery), and 1 infant had a 3-fold elevation in aspartate aminotransferase at 12 mo.



**FIGURE 1** Sweep VEP acuity of infant groups at 6, 9, and 12 mo after randomization to DHA-enriched baby food or control baby food at 6 mo of age. Values are means  $\pm$  SD,  $n = 26$  (control) or  $n = 25$  (supplemented). Lower logMAR values reflect more mature visual acuity; differences of 0.1 logMAR are equivalent to 1 line on the eye chart. The groups differed at 9 and 12 mo ( $P < 0.002$ ).

In the supplemented group, there were 3 adverse events: 2 were not diet-related (genetically associated elevation in alkaline phosphatase and eczema since birth) and 1 infant had a 5-fold elevation in alkaline phosphatase at 12 mo. All events were reported to the Institutional Review Boards and to patients' pediatricians. The groups did not differ in hematological measures at either 6 or 12 mo of age ( $P > 0.1$ ). Similarly, their blood chemistries did not differ at 12 mo ( $P > 0.15$ ). Upon termination of the study, neither group had mean hematological results that were outside of the normal range, although both groups had levels of creatinine (for control and supplemented groups, 32.2 and 30.4  $\mu\text{mol/L}$ , respectively) and carbon dioxide (18.2 and 18.5 mEq/L) that were marginally below normal and albumin levels (43.6 and 42.8 g/L) that were slightly higher than normal; none were of clinical importance.

## DISCUSSION

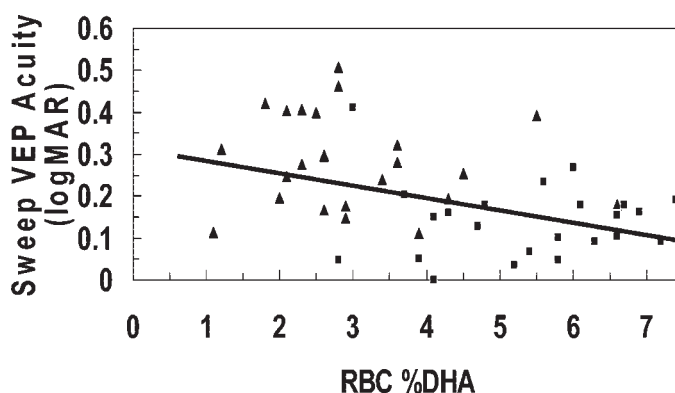
In the current randomized clinical trial, breast-fed infants receiving LCPUFA-enriched baby foods during mo 6–12 of life had an 83% elevation in RBC DHA levels (Table 3) resulting from an approximately 2-fold greater intake of DHA compared with unsupplemented infants (Table 2). In addition, DHA-supplemented infants had more mature VEP acuity than control infants at 9 and 12 mo of age (by 0.14 and 0.16 logMAR, i.e.,  $\sim 1.5$  lines on an eye chart; Fig. 1). Furthermore, the blood lipid level of DHA was significantly correlated with VEP acuity such that infants with higher levels of RBC DHA had better visual acuity (Fig. 2). Metabolic measures were equivalent in both groups with no major diet-related adverse events.

No benefit to stereoacuity attributable to DHA-enriched baby food was evident in the current trial; this is consistent with a previous trial using LCPUFA-enriched infant formula (31). In both of these studies, infants received human milk for the first 4–6 mo of life, which may have provided sufficient nutrition for optimal development of stereoacuity. However, in a separate trial in which infants were randomized to receive control formula or LCPUFA supplemented formula beginning at 6 wk of life, this environmental influence was evident at a 4-mo time point but not later (26). Thus, a "critical period of sensitivity" appears to occur up to 6 mo of age in the maturational susceptibility of stereoacuity to environmental influences (e.g., dietary factors).

The biochemical and functional results from this study are consistent with an earlier randomized clinical trial of breast-fed infants weaned between 4 and 6 mo of age to receive either DHA + ARA-enriched or nonenriched infant formula (31). At 12 mo, infants fed a nonenriched diet had a 50% reduction in RBC-DHA concentration compared with weaning levels. In contrast, infants fed the LCPUFA-enriched formula had a 24% higher RBC DHA content compared with weaning levels and at 12 mo had a 1.5-fold higher DHA level than that in the nonenriched infant group. In this formula trial, we estimated that the supply of DHA was  $\sim 0.2$ – $0.4$  g DHA/6 mo in the control group (primarily due to endogenous DHA synthesis from  $\alpha$ -LNA) compared with a dietary intake of  $\sim 22$  g DHA/6 mo in LCPUFA-supplemented infants. The 1-y-old supplemented infants had improved VEP acuity by 0.103 log MAR (1 line on the eye chart) compared with the nonsupplemented group.

The average amount of human milk consumed each day between 6 and 9 mo of age decreases from  $\sim 750$  mL to about 625 mL (17). Because the average fat content of human milk is  $\sim 37$  g/L (17), the daily intake of human milk fat over this period would be  $\sim 25$  g. With an average DHA content of human milk fat in the United States of  $\sim 0.2$  g/100 g total milk fatty acids (27,32), the DHA intake of exclusively breast-fed older infants in the United States would be  $\sim 50$  mg/d. Between 6 and 9 mo, the average dietary DHA intake of infants fed the baby foods made with DHA-enriched egg yolks was estimated to be 133 mg/d from both human milk (50 mg/d) and solid food sources (83 mg/d), whereas between 9 and 12 mo, the majority of infants were weaned and solid foods alone contributed DHA at an average of 83 mg/d. Thus, for the entire 6-mo trial period, the supplemented infants received an average of 108 mg DHA/d compared with 38 mg DHA/d in control infants who received only human milk until 9.7 mo of age. This corresponded to an approximate 2-fold increase in DHA intake by the supplemented group (7 vs. 20 g/6 mo; Table 2).

Body weight over the 6- to 12-mo period averaged 8.4 kg; thus, the mean intake of DHA for the DHA-supplemented infants was 13 mg/(kg  $\cdot$  d). However, intake for these infants from 6 to 9 mo while still breast-feeding was 17 mg/(kg  $\cdot$  d) but dropped to  $\sim 9$  mg DHA/(kg  $\cdot$  d) from 9 to 12 mo when the only source of DHA was from enriched baby foods. These amounts are  $\sim 15$  and 55% lower than the 20 mg DHA/(kg  $\cdot$  d)



**FIGURE 2** Association between RBC DHA (g/100 g total fatty acids) and sweep VEP acuity (given as logMAR) in infants randomly assigned to DHA-enriched baby food or control baby food at 6 mo of age. The line represents a Pearson linear regression ( $r = -0.50$ ;  $P = 0.0002$ ;  $n = 51$ ). Lower logMAR values reflect more mature visual acuity.

recommended by the FAO/WHO Joint Expert Consultation (33). By comparison, the mean DHA intake for the control group during the 6-mo study was only 4.5 mg/(kg · d).

Human milk and supplemented infant formula are among the few foods available to infants in the United States that contain a nutritionally relevant amount of DHA + ARA. Infant formula is the logical choice as a vehicle for providing DHA and ARA to younger infants who are not breast-fed. During weaning to solid foods, the North American infant receives very little DHA from the diversified mixture of ordinary foods customarily included in the weaning diet. This assumption was validated in Australia (18) and in Finland (34).

Only 3 foods common in the U.S. diet contain significant amounts of DHA, i.e., egg yolks, chicken, and oily fish. Both regular egg yolks and those from chickens fed special diets to increase the (n-3) fatty acid content contain measurable quantities of DHA. A large egg yolk contains between 25 and 140 mg DHA, depending on the diet of the hen. Egg yolks have long been recognized as a safe food for babies and were used in various ancient cultures as a first solid food (35). Egg yolk was recommended >40 y ago to be started between 4 and 6 mo of age unless there was allergy in the family (36). More recent suggested guidelines for infants during the first 6 mo of life include the introduction of egg yolk at 5–6 mo (37). Most recently, Gibson et al. (38) and Makrides et al. (39) reported the effects of feeding normal and DHA-enriched egg yolks to formula-fed and breast-fed infants in the second 6 mo of life. Consuming 4 DHA-enriched egg yolks weekly significantly increased RBC DHA levels at 12 mo in breast-fed infants. Blood cholesterol levels were no higher than those of breast-fed infants. Gibson et al. (38) also found that infants fed egg yolk had improved iron status, as measured by higher serum iron levels and higher transferrin saturation. Based on estimates of food intake (Table 1) and the content of yolk in baby foods (12%), the consumption of yolk (~67 g/wk), and thus, cholesterol and iron, in the current study was nearly equivalent to that in the Gibson study (38,39). Egg yolk is also a rich source of choline-rich lecithin; choline is a vital constituent of membrane phospholipids and was shown to be an essential nutrient for brain development (40).

Chicken meat contains only a small amount of DHA. Pureed chicken with broth intended for infant use contains ~7 mg of DHA (and 43 mg of ARA) in the 55-g Recommended Amount Customarily Consumed Per Eating Occasion (41). Although chicken is an important source of DHA for adults (42), the low concentration of DHA in chicken makes this a poor source of DHA for infants.

In the United States, no commercial foods intended for infants contain fish. Fish is perceived to be highly allergenic by U.S. pediatricians and parents, even though oily fish such as salmon and tuna have reduced allergenicity if canned commercially (43). A more difficult issue nutritionally is that oily fish contain substantial amounts of DHA and eicosapentaenoic acid (EPA) but very little ARA. Pureed baby foods available in Europe made with trout and nasello (hake; whiting) supply, per 100 g, 100 to 200 mg of DHA and 25 to 70 mg of EPA but only 3 to 6 mg of ARA. Human milk contains some EPA if the maternal diet contains an EPA source (e.g., fish); thus, normal infant growth and development can occur in the presence of small amounts of EPA. However, providing supplemental EPA to infants without sufficient ARA is problematic. EPA inhibits the elongation of LA to ARA (44). A DHA-enriched (0.31% DHA) infant formula made with a low-EPA fish oil and containing relatively little EPA (0.08% of total fatty acids) but even less ARA (0.03%) significantly

depressed RBC phospholipid ARA levels at 4 mo of age (45). Thus, the level of EPA in the infant diet should be limited (44). Infant formulas containing fish oils with a substantial EPA content were shown not to support (46) and to support (47) normal growth in preterm infants.

This trial demonstrates that the visual maturation of healthy infants is improved by continued supplies of DHA from both human milk and DHA-enriched baby foods well into 1 y of life. Modifications later in childhood to visual function and other neural processes by this DHA supplementation in baby foods are currently under investigation.

## ACKNOWLEDGMENTS

The authors are indebted to Kathy James and Myla Tuazon (Retina Foundation) for assistance with biochemical analysis and data processing, to Robert Harvey (Beech-Nut Nutrition) for providing support information on food composition, to Gerald Shaul, Mary Cool, Virginia SanFanandre, and Terry Rocklin (Beech-Nut Nutrition) for preparing the experimental foods, and to Jodi Diemert (Beech-Nut Nutrition) for coordinating the shipments of experimental products. We also acknowledge the contributions of time and effort by parents of participating infants.

## LITERATURE CITED

- Birch, D. G., Birch, E. E., Hoffman, D. R. & Uauy, R. D. (1992) Retinal development in very-low-birth-weight infants fed diets differing in omega-3 fatty acids. *Investig. Ophthalmol. Vis. Sci.* 33: 2365–2376.
- Birch, E., Birch, D., Hoffman, D., Hale, L., Everrett, M. & Uauy, R. (1993) Breast-feeding and optimal visual development. *J. Pediatr. Ophthalmol. Strabismus* 30: 33–38.
- Williams, C., Birch, E. E., Emmett, P. M. & Northstone, K. (2001) Stereoacuity at age 3.5 y in children born full-term is associated with prenatal and postnatal dietary factors: a report from a population-based cohort study. *Am. J. Clin. Nutr.* 73: 316–322.
- Birch, E. E., Hoffman, D. R., Uauy, R., Birch, D. G. & Prestidge, C. (1998) Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. *Pediatr. Res.* 44: 201–209.
- Birch, E. E., Garfield, S., Hoffman, D. R., Uauy, R. & Birch, D. G. (2000) A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. *Dev. Med. Child Neurol.* 42: 174–181.
- Hoffman, D. R., Birch, E. E., Birch, D. G., Uauy, R., Castañeda, Y. S., Lapus, M. G. & Wheaton, D. H. (2000) Impact of early dietary intake and blood lipid composition of long-chain polyunsaturated fatty acids on later visual development. *J. Pediatr. Gastroenterol. Nutr.* 31: 540–543.
- Carlson, S. E., Werkman, S. H. & Tolley, E. A. (1996) Effect of long-chain n-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia. *Am. J. Clin. Nutr.* 63: 687–697.
- SanGiovanni, J. P., Berkey, C. S., Dwyer, J. T. & Colditz, G. A. (2000) Dietary essential fatty acids, long-chain polyunsaturated fatty acids, and visual resolution acuity in healthy full term infants: a systematic review. *Early Hum. Dev.* 57: 165–188.
- Carlson, S. E. & Neuringer, M. (1999) Polyunsaturated fatty acid status and neurodevelopment: a summary and critical analysis of the literature. *Lipids* 34: 171–178.
- Gibson, R. A. & Makrides, M. (1999) Polyunsaturated fatty acids and infant visual development: a critical appraisal of randomized clinical trials. *Lipids* 34: 179–184.
- Neuringer, M. (2000) Infant vision and retinal function in studies of dietary long-chain polyunsaturated fatty acids: methods, results, implications. *Am. J. Clin. Nutr.* 71: 256S–267S.
- Koletzko, B., Rodriguez-Palmero, M., Demmelmair, H., Fidler, N., Jensen, R. & Sauerwald, T. (2001) Physiological aspects of human milk lipids. *Early Hum. Dev.* 65 (suppl.): S3–S18.
- Al, M. D., van Houwelingen, A. C. & Hornstra, G. (1997) Relation between birth order and the maternal and neonatal docosahexaenoic acid status. *Eur. J. Clin. Nutr.* 51: 548–553.
- Innis, S. M. (1992) Human milk and formula fatty acids. *J. Pediatr.* 120: S56–S61.
- Koletzko, B., Thiel, I. & Abiodun, P. O. (1992) The fatty acid composition of human milk in Europe and Africa. *J. Pediatr.* 120: S62–S70.
- Chulei, R., Xiaofang, L., Hongsheng, M., Yiulan, M., Guizheng, L., Gianhoug, D., DeFrancesco, C. A. & Connor, W. E. (1995) Milk composition in women from five different regions of China: the great diversity of milk fatty acids. *J. Nutr.* 125: 2993–2998.
- Heinig, M. J., Nommsen, L. A., Pearson, J. M., Lonnerdal, B. & Dewey, K. G. (1993) Energy and protein intakes of breast-fed and formula-fed infants

during the first year of life and their association with growth velocity: The DARING study. *Am. J. Clin. Nutr.* 58: 152–161.

18. Jackson, K. A. & Gibson, R. A. (1989) Weaning foods cannot replace breast milk as sources of long-chain polyunsaturated fatty acids. *Am. J. Clin. Nutr.* 50: 980–982.

19. Scheideleer, S. E. (1999) Feed to produce omega-3 fatty acid enriched eggs and method for producing such eggs. U.S. Patent 5,897,890.

20. Theuer, R. C., Shaul, G. E., Rocklin, T. L., Cool, M. B. & San Fanandrusso, V. A. (2000) Egg yolk-containing baby food compositions and methods therefore. U.S. Patent 6,149,964. Beech-Nut Nutrition Corporation, St. Louis, MO.

21. Theuer, R. C., Shaul, G. E., Rocklin, T. L., Cool, M. B. & San Fanandrusso, V. A. (2003) Baby-food compositions containing egg yolk and methods therefore. U.S. Patent 6,579,551. Beech-Nut Nutrition Corporation, St. Louis, MO.

22. AOAC (2002) AOAC Official Method 996.06, Fat (Total, Saturated, and Unsaturated) in Foods, Hydrolytic Extraction Gas Chromatographic Method, 17th ed. AOAC International, Gaithersburg, MD.

23. Norcia, A. M. & Tyler, C. W. (1985) Spatial frequency sweep VEP: visual acuity during the first year of life. *Vision Res.* 25: 1399–1408.

24. Norcia, A. M. (1993) Improving Infant Evoked Response Measurement. Oxford University Press, New York, NY.

25. Birch, E. E. & Salomao, S. (1998) Infant random dot stereoacuity cards. *J. Pediatr. Ophthalmol. Strabismus* 35: 86–90.

26. Birch, E. E., Hoffman, D. R., Castañeda, Y. S., Fawcett, S. L., Birch, D. G. & Uauy, R. (2002) A randomized controlled trial of long-chain polyunsaturated fatty acid supplementation of formula in term infants after weaning at 6 wk of age. *Am. J. Clin. Nutr.* 75: 570–580.

27. Centers for Disease Control and Prevention (2000) CDC Growth Charts: United States. CDC, Atlanta, GA.

28. Whitehead, T. P., Thorpe, G.H.G. & Maxwell, S.R.J. (1992) Enhanced chemiluminescent assay for antioxidant capacity in biological fluids. *Anal. Chim. Acta* 266: 265–277.

29. Rosner, B. (1990) *Fundamentals of Biostatistics*, 3rd ed. Duxbury Press, Boston, MA.

30. Treen, M., Uauy, R. D., Jameson, D. M., Thomas, V. L. & Hoffman, D. R. (1992) Effect of docosahexaenoic acid on membrane fluidity and function in intact cultured Y-79 retinoblastoma cells. *Arch. Biochem. Biophys.* 294: 564–570.

31. Hoffman, D. R., Birch, E. E., Castañeda, Y. S., Fawcett, S. L., Wheaton, D. H., Birch, D. G. & Uauy, R. (2003) Visual function in breast-fed term infants weaned to formula with or without long-chain polyunsaturates at 4 to 6 months: a randomized clinical trial. *J. Pediatr.* 142: 669–677.

32. Jensen, C. L., Maude, M., Anderson, R. E. & Heird, W. C. (2000) Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and maternal and infant plasma phospholipids. *Am. J. Clin. Nutr.* 71: 292S–299S.

33. FAO/WHO (1994) *Fats and Oils in Human Nutrition: Report of a Joint Expert Consultation*, pp. 1–55. Food and Agriculture Organization of the United Nations and the World Health Organization. FAO, Rome, Italy.

34. Luukkainen, P., Salo, M. K., Visakorpi, J. K., Raiha, N. C. & Nikkari, T. (1996) Impact of solid food on plasma arachidonic and docosahexaenoic acid status of term infants at 8 months of age. *J. Pediatr. Gastroenterol. Nutr.* 23: 229–234.

35. Simopoulos, A. P. & Salem, N. J. (1992) Egg yolk as a source of long-chain polyunsaturated fatty acids in infant feeding. *Am. J. Clin. Nutr.* 55: 411–414.

36. Spock, B. (1957) *Baby and Child Care*. Pocket Books, Inc., New York, NY.

37. CDA/SSDA (1988) The Chicago Dietetic Association and the South Suburban Dietetic Association. In: *Manual of Clinical Dietetics*. American Dietetic Association, Chicago, IL.

38. Gibson, R. A., Makrides, M. & Hawkes, J. S. (1998) Eggs as a source of essential docosahexaenoic acid (DHA) in the diets of weaning infants. *Rural Industries Research and Development Corporation*, Barton, ACT, Australia.

39. Makrides, M., Hawkes, J. S., Neumann, M. A. & Gibson, R. A. (2002) Nutritional effect of including egg yolk in the weaning diet of breast-fed and formula-fed infants: a randomized controlled trial. *Am. J. Clin. Nutr.* 75: 1084–1092.

40. Zeisel, S. H. (2000) Choline: needed for normal development of memory. *J. Am. Coll. Nutr.* 19: 528S–531S.

41. Food Safety and Inspection Service (2003) Reference Amounts Customarily Consumed Per Eating Occasion. U.S. Department of Agriculture. Title 9, Section 381.412, Code of Federal Regulation. U.S. Government Printing Office, Washington, DC.

42. Raper, N. R., Cronin, F. J. & Exler, J. (1992) Omega-3 fatty acid content of the US food supply. *J. Am. Coll. Nutr.* 11: 304–308.

43. Bernhisel-Broadbent, J., Strause, D. & Sampson, H. A. (1992) Fish hypersensitivity. II: Clinical relevance of altered fish allergenicity caused by various preparation methods. *J. Allergy Clin. Immunol.* 90: 622–629.

44. Barham, J. B., Edens, M. B., Fonteh, A. N., Johnson, M. M., Easter, L. & Chilton, F. H. (2000) Addition of eicosapentaenoic acid to  $\gamma$ -linolenic acid-supplemented diets prevents serum arachidonic accumulation in humans. *J. Nutr.* 130: 1925–1931.

45. Lapillonne, A., Picaud, J. C., Chirouze, V., Goudable, J., Reygrobelle, B., Claris, O. & Salle, B. L. (2000) The use of low-EPA fish oil for long-chain polyunsaturated fatty acid supplementation of preterm infants. *Pediatr. Res.* 48: 835–841.

46. Carlson, S. E. & Werkman, S. H. (1996) A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until two months. *Lipids* 31: 85–90.

47. Uauy, R., Hoffman, D. R., Birch, E. E., Birch, D. G., Jameson, D. M. & Tyson, J. (1994) Safety and efficacy of omega-3 fatty acids in the nutrition of very low birth weight infants: soy oil and marine oil supplementation of formula. *J. Pediatr.* 124: 612–620.