Essential Fatty Acids in Visual and Brain Development

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ABSTRACT: Essential fatty acids are structural components of all tissues and are indispensable for cell membrane synthesis; the brain, retina and other neural tissues are particularly rich in long-chain polyunsaturated fatty acids (LC-PUFA). These fatty acids serve as specific precursors for eicosanoids, which regulate numerous cell and organ functions. Recent human studies support the essential nature of n-3 fatty acids in addition to the well-established role of n-6 essential fatty acids in humans, particularly in early life. The main findings are that light sensitivity of retinal rod photoreceptors is significantly reduced in newborns with n-3 fatty acid deficiency, and that docosahexaenoic acid (DHA) significantly enhances visual acuity maturation and cognitive functions. DHA is a conditionally essential nutrient for adequate neurodevelopment in humans. Comprehensive clinical studies have shown that dietary supplementation with marine oil or single-cell oil sources of LC-PUFA results in increased blood levels of DHA and arachidonic acid, as well as an associated improvement in visual function in formula-fed infants matching that of human breast-fed infants. The effect is mediated not only by the known effects on membrane biophysical properties, neurotransmitter content, and the corresponding electrophysiological correlates but also by a modulating gene expression of the developing retina and brain. Intracellular fatty acids or their metabolites regulate transcriptional activation of gene expression during adipocyte differentiation and retinal and nervous system development. Regulation of gene expression by LC-PUFA occurs at the transcriptional level and may be mediated by nuclear transcription factors activated by fatty acids. These nuclear receptors are part of the family of steroid hormone receptors. DHA also has significant effects on photoreceptor membranes and neurotransmitters involved in the signal transduction process; rhodopsin activation, rod and cone development, neuronal dendritic connectivity, and functional maturation of the central nervous system.

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Studies over the past five decades have evaluated the effects of nutrition on central nervous system (CNS) development in experimental animals and humans. The results reveal that a reduction in the supply of energy and/or several essential nutrients during the first stages of life has profound effects on the structural and functional development of the nervous system. Malnutrition impairs brain growth and development by decreasing the number of cell replication cycles, reducing total brain DNA, and restricting dendritic arborization, thus reducing neuronal connectivity. Intrauterine and early postnatal malnutrition affect cell number and hyperplastic growth as measured by DNA content (1). Development of the cerebellum is most affected by nutritional deprivation around the time of birth in humans. Synaptic connectivity is particularly affected if malnutrition occurs after birth but before year 3 of life. Alterations in dietary precursors may affect tissue levels of neurotransmitters (serotonin, norepinephrine, dopamine, and acetylcholine) in specific brain regions (2). The supply of essential fatty acids (EFA) affects the structural composition of the brain and myelin sheaths in particular. The functional correlates of these biochemical changes induced by malnutrition include alterations in the waking electroencephalographic activity, visual- and auditory-evoked responses, motor and cognitive development, and social abilities. Sleep-wake cycle organization as well as autonomic nervous system functioning during sleep are perturbed by early human malnutrition (3,4). Most of these effects are potentiated by other environmental factors that interact with poor diet in defining the adverse consequences.

The traditional point of view, that protein and energy deficits *per se* directly affect brain structural development and cognitive performance, has been challenged because protein energy malnutrition coexists with multiple micronutrient deficiencies and with psychosocial deprivation that interact to disrupt brain development. This makes it particularly difficult to tease out the role of specific nutrients such as EFA from multiple deprivations that act in tandem, contributing to the final outcome in terms of growth and mental developmental (5). The major effects on growth and brain development associated with EFA deficiency are explained by the role of fatty acids (FA) as basic components of biological membranes, precursors of eicosanoids, and regulators of gene expression. The effect on growth is most likely linked to the role of EFA in energy balance and eicosanoid-mediated growth factors. The effects on CNS development are likely mediated by the role of EFA on gene expression, membrane structures, and electrophysiologic responses. The purpose of this review is to examine the effects of dietary EFA on the development of the brain and vision and the potential mechanisms by which modulation occurs.

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Abbreviations: AA, arachidonic acid (20:4n-6); ABER, auditory brainstem-evoked response; ALA, α-linolenic acid (18:3n-3); CNS, central nervous system; DHA, docosahexaenoic acid (22:6n-3); EFA, essential fatty acid; ERG, electroretinogram; FA, fatty acids; FPL, forced-choice preferential looking; GLA, γ-linolenic acid (18:3n-6); LA, linoleic acid (18:2n-6); LC-PUFA, long-chain polyunsaturated fatty acids; M I, metarhodopsin I; M II, metarhodopsin II; MDI, Mental Development Index; PC, phosphatidylcholine; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; RBC, red blood cell; RxR, retinoic acid receptor; TR, thyroxine receptor; VEP, visual-evoked potential.

TABLE 1
Controlled Studies of Long-Chain Polyunsaturated Fatty Acid (LC-PUFA) Supplementation and Visual/Neurodevelopment of Preterm Infants[a]

Reference	Study groups (*n*)	LC-PUFA level in formulas (% total lipids)	Evaluation, outcome, age	Main results
6,7	F1 (12) F2 (10) F3 (10) HM (10)	ALA 1.4, DHA 0.35 EPA 0.65 LA 20.8, ALA 2.7 LA 24.2, ALA 0.5	ERG at 36, 57 wk PCA	F3, lower rod amplitude and higher rod threshold at 36 wk. n-3 LC-PUFA related to rod threshold and inversely to amplitude. No difference at 57 wk.
8	F1 (13) F2 (16) F3 (12) HM (9)	ALA 1.4, DHA 0.35 EPA 0.65 LA 20.8, ALA 2.7 LA 24.2, ALA 0.5	VEP at 36, 57 wk FPL at 36, 57 wk PCA	F3, poorer visual acuity by VEP and FPL. F1 and HM similar results
9	F1 (33) F2 (34)	ALA 3.1, DHA 0.2–0.3 ALA 3–4.8	Teller acuity cards, 38, 48, 57, 68, 79, and 92 wk PCA	F1, better acuity at 48 wk. DHA level related to visual acuity at 48 wk.
10	F1 (27) F2 (27)	ALA 3.1, DHA 0.2–0.3 ALA 3–4.8	Bayley Mental and Psychomotor Developmental Index, 92 wk PCA	F1, lower PDI
11	F1 (33) F2 (34)	ALA 3, DHA 0.2, EPA 0.3 ALA 3–4.8	Fagan's NP, 68, 79, 92 wk PCA	F1, less % novel look time
12	F1 (26) F2 (33)	ALA 3.1, DHA 0.2 ALA 4.8	Teller acuity cards, 39, 48, 57, 68,79, 92 wk PCA	F1, better acuity at 48 wk in nonbronchopulmonary dysplasia infants
13	F1 (15) F2 (12)	ALA 3.1, DHA 0.2 ALA 4.8	Fagan's NP, 92 wk PCA	F1, shorter look duration F2, more % novel time
14	F1 (21) F2 (25) HM (12)	DHA 0.3, AA 0.44 ALA 0.9, LA 19.4 DHA 0.16, AA 0.4	Flash VEP ERG ABER at 52 wk PCA	F1 and HM, better acuity Not different Not different
15	F1 (140) F2 (143) F3 (144) HM	DHA 0.25–0.15[b], AA 0.4 DHA 0.25–0.15[b], AA 0.4 ALA 2.4 F1 and F2 supplemented with two AA/DHA LCP sources Egg/marine Fungal/marine DHA 0.25 until 40 wk then 0.15 DHA for 12 mon	Teller acuity cards, 2, 4, 6 mon PCA Fagan's NP, 6, 9 mon MacArthur, 9, 14 mon Bayley II Mental and Psychomotor Development Index, 18 mon Sweep VEP, 2, 4, 6 mon corrected age	Not different Not different Not different F1, F2, 8 point higher PDI for infants with birth weight <1250 g F1, F2, and HM, better visual acuity at 2, 4, 6 mon

[a]Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ERG, electroretinogram; PCA, postconceptional age; LA, linoleic acid; HM, human milk; VEP, visual evoked potential; FPL, forced-choice preferential looking; PDI, Psychomotor Development Index; NP, novelty preference; AA, arachidonic acid; ABER, auditory brainstem evoked response. F1, F2, and F3 correspond to different formulas.
[b]First value refers to content during the period up to the gestational age (40 wk); second value is the content after 40 wk.

EFFECT OF EFA ON BRAIN DEVELOPMENT

Preterm infants (randomized controlled trials). Very-low-birth-weight infants born with a birth weight of <1500 g are considered particularly vulnerable to EFA deficiency given the virtual absence of adipose tissue at birth, the possible insufficiency of FA elongation/desaturation enzymatic pathways, and the inadequate intake of long-chain polyunsaturated fatty acids (LC-PUFA) provided by formula. Randomized controlled clinical trials that have included formula feeding with or without LC-PUFA and functional assessment of visual/neurodevelopment in preterm infants are summarized in Table 1.

Over the past decade, we and others have conducted studies to evaluate the effect of n-3 FA in preterm infant development. These studies examined the effects of α-linolenic acid (ALA), ALA plus docosahexaenoic acid (DHA), or ALA plus DHA and arachidonic acid (AA) supplementation on plasma and tissue lipid composition, retinal electrophysiologic function, maturation of the visual cortex, and on other measures of infant growth and development (6–14). In our initial work, we showed that retinal function, as determined by the electroretinogram (ERG), is affected by n-3 supply in preterm human infants. Infants fed an n-3–deficient corn oil diet (standard formula at the initiation of the study in 1987) had significantly higher rod ERG thresholds (i.e., more light was necessary to elicit a threshold rod ERG response, resulting in a less mature ERG response) than infants receiving either human milk or n-3–supplemented formula. An analysis of the amplitude–intensity behavior of the responses (Naka-Rushton functions) from the infants in the study revealed that the threshold elevation in the n-3–deficient infants was due primarily to a shift in log *k* (i.e., the sensitivity of the rod system was lower). In addition there was a modest but significant de-

crease in the amplitude in the maximal amplitude (V_{max}) that could be elicited from infants in the deficient group. An analysis of the leading edge of the a-wave showed that the decreased sensitivity in the infants fed n-3–deficient formula originated in the rod photoreceptors (7). The results of dietary FA modification on the function of the visual cortex, as measured by pattern reversal visual-evoked potential (VEP) acuity and behaviorally by the forced-choice preferential looking (FPL) visual acuity response, demonstrated that infants in the human milk and marine oil groups (both receiving DHA) had improved maturation of VEP relative to infants fed formulas devoid of DHA (corn and soy oil groups) at the 4-mon adjusted-age followup (8). No differences were found between soybean- and marine oil-containing formula if acuity was assessed by FPL. Visual acuity tests measure the integrity of the neural pathway from the retina to the occipital cortex and provide a surrogate measure of CNS function. The long-term significance of the improved retinal and visual function on later neurodevelopment is presently being explored.

Carlson's first randomized clinical study in preterm infants supplemented with LC-PUFA demonstrated better visual acuity in infants up to 4 mon of age using FPL measurements. After this time, control infants "caught up" in visual function measures. These investigators report evidence of more rapid visual processing as measured by shorter look duration in the Fagan test of visual recognition at 6 and 12 mon of age in LC-PUFA–supplemented infants (9–11). The reduction in AA in blood lipids, when marine oil was provided as a source of n-3 fatty acids, was associated with reduced weight and linear growth (10–13). In a second preterm infant study using low eicosapentaenoic acid (20:5n-3) marine oil for up to 2 mon corrected age, Carlson demonstrated improved visual development at the 2-mon followup; at 12 mon of age, there was a 10-point Bayley Mental Development Index (MDI) difference favoring the DHA-supplemented group (11,12). Furthermore, the DHA-supplemented group had shorter look times in the novelty preference test at 9 mon, suggesting better visual processing (12).

In our recent studies, conducted in Chile, no LC-PUFA effect on auditory brain stem-evoked responses (ABER) was demonstrated; this coincides with the results of a preterm infant study by Faldella (14). One of our centers (INTA) recently participated in a collaborative multicenter study of a large group of preterm infants. This study included 470 preterm infants fed LC-PUFA formula supplemented with different AA and DHA sources, i.e., marine oil and egg phospholipids or fungal oil. The level of DHA was 0.25% of total fat in preterm formulas and 0.15% in follow-up formula; both formulas contained 0.4% AA (15). Significant differences were found in sweep VEP at 6 mon favoring the LC-PUFA–supplemented formula group compared with the control formula group. There were no diet-induced differences in behavioral tests of visual acuity (determined using Teller acuity cards). Mean novelty preference was significantly better at 6 mon in the LC-PUFA–supplemented group. No differences in MacArthur vocabulary tests were found, but an improved 14-mon score was noted in the supplemented group when non-English-speaking subjects were excluded from the analysis. For infants <1250 g at birth, an advantage of 8.8 points in the LC-PUFA–supplemented group in the Bayley II Psychomotor Development Index was observed at 12 mon (15).

Significance of LC-PUFA for term infants (randomized controlled trials). The question of whether healthy full-term infants require LC-PUFA in their formula has received considerable attention over the past decade. The finding of lower plasma DHA and red blood cell (RBC) concentrations in infants fed formula compared with breast-fed infants suggests that formulas provide insufficient ALA or that chain elongation–desaturation enzymes are not sufficiently active during early life to support optimal tissue accretion of DHA. Full-term infants also may be dependent on dietary DHA for optimal functional maturation of the retina and visual cortex (16–38). Controlled trials that have included formula feeding with or without LC-PUFA and functional assessment of visual and neural development are summarized in Table 2.

Makrides *et al.* (25) conducted the first controlled randomized study in term infants using a formula supplemented with 0.36% DHA and 0.27% γ-linolenic acid (GLA) compared with a formula providing ample ALA (1.6%) but no DHA as well as a breast-fed reference group. They demonstrated delayed visual acuity at 4 and 6 mon of age in the formula group lacking DHA. Infants who received breast milk for >16 wk had better VEP acuity than those breast-fed for shorter times or given formula without LC-PUFA. A separate study showed better visual acuity at 2 mon but no benefits after 6 mon of life in breast-fed or 0.1% DHA formula-fed infants relative to control formula (26). Biochemical indices of DHA status and visual acuity maturation of infants receiving only ALA were delayed relative to those receiving formula supplemented with ALA + DHA; these latter infants had maturational indices similar to those of the breast-fed group (27).

Auestad *et al.* (28) compared infants fed standard formula, formula supplemented with 0.2% DHA (from marine oil), or formula with 0.12% DHA and 0.43% AA. No significant differences could be detected among the three diet groups using ERG at 4 mon, visual acuity, or the Bayley Scales of Infant Development test at 12 mon. Furthermore, negative correlations were found between RBC and DHA at 4 mon and language development assessed by the MacArthur Communicative Development Inventory given at 14 mon ($r = -0.20$ to -0.37 depending on the specific item, $P < 0.05$) (29). A beneficial effect of DHA, AA, and GLA supplementation on psychomotor development assessed by the Brunet-Lezine method was reported at 4 and 12 mon but not at 24 mon of age (30,31). This study reported a strong association between the erythrocyte phosphatidylcholine (PC) AA/linoleic acid (LA) ratio and the developmental quotient at 24 mon, but there was no relation to the dietary intervention in the first 4 mon of life (31). In an attempt to control for confounding variables that could affect visual and neural development, Gibson *et al.* (32) supplemented mothers with LC-PUFA to produce DHA-enriched breast milk with concentrations ranging from 0.1 to 1.7% of total FA. The plasma and erythrocyte-phospholipid DHA level

TABLE 2
Controlled Studies of Long-Chain Polyunsaturated Fatty Acid (LC-PUFA) Supplementation and Visual/Neurodevelopment of Term Infants[a]

Reference	Study groups (*n*)	LC-PUFA level in formulas (% total lipids)	Evaluation, outcome, age	Main results
25	F1 (13)	DHA 0.36, EPA 0.58 GLA 0.27	VEP at 16, 30 wk	F1 and HM, better visual acuity
	F2 (19)	LA 16.8, ALA 1.6		
	HM (23)			
26	F1 (19)	DHA 0.35, AA 0.43	Teller acuity cards, 2, 4, 6, 9, 12 mon	F1, better visual acuity only at 2 mon
	F2 (20)	LA 21.9, ALA 2.2		
	HM (19)			
30	F1 (27)	DHA 0.3, AA 0.44	Brunet-Lezine's developmental quotient at 4 mon	F1 and HM, better developmental quotient than F2
	F2 (29)	LA 11.1, ALA 0.7		
	HM (30)			
31	F1 (26)	DHA 0.3, AA 0.44	Brunet-Lezine's developmental quotient at 24 mon	Not different, developmental quotient related to 24-mon plasma DHA level, but not to 4-mon plasma DHA
	F2 (30)	LA 11.1, ALA 0.7		
	HM (25)			
35	F1 (24)	DHA 0.2, AA 0.35	Infant habituation at 4 mon	F1, shorter fixation times
	F2 (24)	LA 11.4, ALA 0.7		Mean scores on habituation not different
	HM (27)			
36	F1 (21)	DHA 0.2, AA 0.35	Problem solving at 10 mon	F1, better
	F2 (22)	LA 11.4, ALA 0.7		
28	F1 (26)	DHA 0.12, AA 0.43	Sweep VEP acuity 2, 4, 6, 9, 12 mon	Not different
	F2 (28)	DHA 0.23		
	F3 (28)	LA 21.9, ALA 2.2	FPL acuity 2, 4, 6, 9, 12 mon	Not different
	HM (38)			
29	F1 (38)	DHA 0.12, AA 0.43	MacArthur's developmental index at 14 mon	F3, more vocabulary comprehension/ production than DHA group
	F2 (33)	DHA 0.23		
	F3 (42)	LA 21.9, ALA 2.2		
	HM (60)		Bayley I Mental and Psychomotor Developmental Index at 12 mon	Not different
27	F1 (14)	DHA 0.3, GLA 0.5	Sweep VEP at 4 mon	HM better than formulas F1 and F2
	F2 (12)	DHA 0.3		
	F3 (11)	LA 12, ALA 1.2		Nonsignificantly better visual acuity than F3
	HM (25)	DHA 0.38, AA 0.4		
33	F1 (23)	DHA 0.36, AA 0.72	FPL and Sweep VEP at 6, 17, 26, 52 wk	Not different
	F2 (22)	DHA 0.35		F1, F2, HM better visual acuity at 6, 17, and 52 wk, but not at 26 wk
	F3 (23)	LA 14.6, ALA 1.49		
	HM (21)	DHA 0.29, AA 0.56		
34	F1 (19)	DHA 0.36, AA 0.72	Bayley II Mental and Psychomotor Developmental Index at 18 mon	F3, worse in MDI
	F2 (17)	DHA 0.35		MDI related to plasma and RBC DHA at 4 mon
	F3 (20)	LA 14.6, ALA 1.49		
38	F1(154)	DHA 0.32, AA 0.3	Bayley I Mental and Psychomotor Developmental Index at 18 mon	Not different
	F2 (155)	LA 12.4, ALA 1.1		
	HM (138)			
40	F1 (24)	DHA 0.34, AA 0.34	Flash VEP, 16, 34 wk	Not different
	F2 (23)	DHA 0.35		
	F3 (21)	LA 16.8, ALA 0.13	Bayley Mental and Psychomotor Developmental Index at 12, 18 mon	Not different and not related to plasma DHA levels
	HM (46)	DHA 0.2, AA 0.4		

[a]Abbreviations: GLA, γ-linolenic acid; MDI, Mental Development Index; RBC, red blood cell; for other abbreviations see Table 1.

of these infants was related to breast milk DHA in a saturable manner; no significant increases were noted in blood DHA levels when the breast milk content of DHA was >0.6%. Infant VEP acuity had no relationship to DHA content of breast milk; the developmental quotient at 12 mon was significantly but weakly correlated with breast milk DHA. At 24 mon, this effect was no longer evident.

Recently, Birch *et al.* (33) showed a persistent benefit in visual acuity development for year 1 of life in DHA-supplemented formula-fed infants compared with infants fed formula with ample ALA but devoid of LC-PUFA. The formula given for the first 17 wk of life contained 0.35% DHA, with or without 0.72% of AA; both LC-PUFA were derived from single-cell oils in the supplemented groups. The dietary effects on vi-

sual acuity development were evident using sweep VEP acuities but absent if the FPL behavioral measure of acuity was used. Supplemented groups receiving DHA or DHA + AA and the breast milk group had better acuity. The differences were significant during the periods of rapid change in development of VEP acuity, i.e., in the first 20 wk and near 12 mon of life. The developmental outcome of these infants was reported recently (34). Scores on the Bayley MDI II at 18 mon of age for the DHA + AA group were significantly better than those observed in the non–LC-PUFA formula-fed infants. A 7-point normalized MDI score difference was highly significant despite the relatively small sample size (n = 20/group). The small variability in developmental score obtained was likely due to the highly homogeneous population studied and the fact that one observer evaluated mental development in all subjects. The DHA-only group had marginally higher MDI scores than controls. This is the first randomized controlled study that reports an LC-PUFA effect on mental development at 18 mon of age. Moreover, positive significant correlations between blood DHA levels at 4 mon with measures of visual acuity at 1 yr and mental development at 18 mon were noted (34). The existence of a relationship between early biochemical and later functional data suggests that visual function and neurodevelopmental phenomena may be related but do not constitute proof of a causal relationship.

A behavioral study of 44 term infants fed a combined DHA- and AA-supplemented formula or a control formula during the first 4 mon demonstrated that visual habituation performance scores of infants at 4 mon of age were better in the LC-PUFA–supplemented formula group (35). Infant cognitive behavior was assessed at 10 mon of age by a means-end problem-solving test (36). The LC-PUFA–supplemented group had significantly more intentional solutions and scored higher than infants fed the non–LC-PUFA–containing control formula. Higher problem-solving scores in infancy have been shown to relate to higher childhood intelligence quotient scores (37). These studies provide a solid indication of efficacy but are limited in their extrinsic validity because of small sample size and rather homogeneous infant populations.

Lucas *et al.* (38), in the largest controlled term-infant study to date, did not find a beneficial effect of LC-PUFA supplementation in a group of 309 infants randomized to formula diets with or without LC-PUFA. A reference group of 138 infants included for comparative purposes were breast-fed. No biochemical data on FA composition of plasma or tissues were obtained in this study, limiting the assessment of compliance to the test formula. Follow-up studies at 18 mon revealed no benefit in cognitive or motor development. No adverse effects of LC-PUFA supplementation were noted in terms of infection, atopy, or formula tolerance. However, the interpretation of these data is limited by the fact that study formulas differed not only by the presence or absence of LC-PUFA but also by several other fatty acids. The expected higher MDI score in breast-fed infants compared with standard formula-fed infants was not apparent in this study, and because there was no plasma or tissue biochemical evaluation of infant EFA status, a relationship between LC-PUFA status and neurodevelopment could not be established (38). In addition, the maternal population studied had a low educational level, i.e., ~20% had no formal education at all and ~70% had not completed a high school education. Mean MDI values for all groups of infants were 4–6 points below the norm, suggesting that these groups may not be representative of the normal population in the United Kingdom but rather a group subjected to an environmental factor that restricted their neurodevelopment. The possible effect of maternal education or ethnicity is raised from the analysis of confounding variables (39). Using the same LC-PUFA–enriched and control formulas, Makrides *et al.* (40) also did not find diet-induced differences in VEP acuity or Bayley MDI scores during year 1 of life. In this case, breast-fed infants had higher MDI scores than formula-fed infants at 2 yr, even after adjusting for environmental variables. No correlations between plasma LC-PUFA levels and neurodevelopment indices were found by these investigators.

Other studies have attempted to optimize ALA to DHA conversion by providing sufficient ALA (>0.7% of total energy) as well as lowering the LA to ALA ratio in the formula (<10:1). This alternative strategy for the formulation of infant diets would be simpler and less expensive to produce than the addition of n-3 LC-PUFA (21,24). Unfortunately, results from infants fed formula with a ratio of 4.8:1 demonstrated poorer growth, possibly due to the lower AA levels found with this dietary regimen (24).

Recent systematic reviews of LC-PUFA supplementation. The results of two recent meta-analyses conducted on the effect of LC-PUFA on visual acuity maturation in preterm and term infants are summarized in Figures 1 and 2. As can be observed in the figures, most studies demonstrated a positive effect for assessments conducted during the first 2–4 mon of life; the results at 6 mon were equivocal and again, at 12 mon, some studies were positive. The conclusions by San Giovanni *et al.* (41,42), in their two reports, is that there is a significant overall advantage for the LC-PUFA–supplemented infants in terms of visual acuity. The main differences in experimental design, subject selection, dietary supplementation, other nutrition-related factors, and the primary outcomes of these studies are summarized in Table 3. Most studies have chosen the prospective, randomized, controlled, double-blind design, but some studies included in the systematic reviews compared human milk-fed with formula-fed infants. This comparison has limited validity because infants of breast-feeding mothers and formula-fed infants usually vary in terms of maternal education, birth weight, home environment, socioeconomic level, and other confounders. To evaluate efficacy, most studies have used "healthy" subjects controlling for birth weight, gestational age, socioeconomic status, and maternal characteristics. These well-controlled studies have strong intrinsic validity. However, these studies cannot be generalized for all infants because the study groups in the controlled studies may not be representative of the population at large. The diets studied have provided 0.1–0.35% DHA in both preterm and term infant studies. These values are in the middle-to-lower

range of mean DHA content found in breast milk as derived from combined data of omnivorous women around the world. No formal controlled DHA dose-response studies evaluating visual maturation and/or mental development at varying levels of supplementation have been conducted in preterm (8–15) or term infants (25–31,33–40).

The duration and reversibility of diet-induced effects is another important consideration in assessing outcome. In evaluating diet-induced changes in visual/neural development, the selection of outcomes is crucial. The sensitivity and variability of the measurements are vital to detect an effect given the presence of multiple known and unknown confounders. The timing of the measurement is also of great importance; most LC-PUFA effects are evident only over the period of rapid development and do not persist once functional maturation has been achieved. The visual acuity results suggest that transient effects reflect the acceleration or the slowing of a maturational process with a fully normal final outcome. Several studies have demonstrated significant effects of dietary LC-PUFA on visual maturation in the first 4 mon of life, but in most studies, visual acuity normalized by 6 mon or at most by 1 yr of age (41,42). However, we need to consider whether this phenomenon can truly be dismissed as transitory and of limited significance and instead consider that we may have failed to detect a significant change later in life. This may be explained by the limitations in our tools, which may not be sufficiently sensitive; moreover, other related functions may indeed have been affected. As an example of this problem, in term infants, we failed to detect differences in visual acuity of breast-fed compared with formula-fed infants at 6 mon but stereo-acuity responses were different at 3 yr of age (16). In this study, most breast-fed infants (92%) had mature operant preferential looking stereo acuity, whereas only 35% of the infants in the formula-fed group met the maturity criteria. Visual recognition in the breast-fed group was also better; only 61% of the formula-fed infants had a perfect score, whereas 93% of the breast-fed group had a perfect score (16). This illustrates the need to select sensitive outcome measures and provide for sufficient follow-up of cohorts in these randomized clinical trials. It is impossible to fully discard the possible long-term consequences of early developmental delays in function unless followup is conducted over a sufficient period of time.

MECHANISMS FOR EFA EFFECTS ON GROWTH AND BRAIN DEVELOPMENT

Molecular regulation of gene expression by nutrients. Regulation of gene expression by nutrients can occur at multiple levels (43,44). For example, FA can bind to specific or nonspecific ligands that interact with response elements in specific DNA motifs usually present in the promoter region of the gene, affecting gene transcription. DHA and other PUFA interact with nuclear factors modulating the activation of specific nuclear proteins such as peroxisome proliferator-activated receptors (PPAR), which in turn act as transcription regulators (45). In this case, the nutrient effect is indirect because it is the activated transcription factor that binds *cis*-regulatory elements of DNA found in target genes. Another form of nutrient ligand interaction includes changes in phosphorylation mediated by the nutrient. At the posttranscriptional level, once mRNA are formed, nutrients may act by modifying native RNA processing, mRNA transport and stability, and breakdown rates. Nutrients may also modify the rate of mRNA translation by the activation of protein synthesis in the polyribosomal complex. Nutrients such as vitamin K can affect gene expression at the posttranslational level beyond the synthesis of protein by modifying the gene products formed. Vitamin K-dependent amino acid carboxylation, which is necessary for active prothrombin synthesis, represents an example of nutrient-induced posttranslational modification of gene products. Finally, nutrients can modify the turnover rates of proteins including enzymes, thus affecting their activity level. In many cases, the nutrient-induced changes in gene expression are part of the adaptive response to a given level of nutrient exposure (43,44). Thus, nutrients may affect the uptake, metabolism, storage, or excretion of the nutrient that triggered the gene regulatory response.

LC-PUFA are involved in the regulation of cell growth and differentiation by modulation of gene expression. For example, the effect of DHA on the functional maturation of the retina observed in several animal species including primates may now be potentially related to a direct effect on photoreceptor differentiation. Studies using primary culture of retinal neuronal cells have revealed a potential mechanism by which this conditionally essential nutrient affects gene expression critical to retinal function and survival (46). In cultured cells from rats, DHA significantly increased the differentiation of apical processes in rod outer segments, the locus for rhodopsin and opsin-dependent light transduction. This was paralleled by an increase in opsin expression and content in the rod photoreceptor apical processes (46). The molecular mechanisms underlying these effects have not been fully clarified, but these data suggest an effect of DHA on opsin gene expression and possibly on other proteins required for the assembly of disc membranes. Recent studies from Bazan's group (47) have demonstrated that the transport of opsin and rhodopsin to the apical process *via* post-Golgi membranes is coupled to DHA transport. The close molecular interaction between these key photoreceptor proteins and DHA suggests that DHA influences retinal photoreceptor structural development as well as function.

Regulation of gene expression by LC-PUFA also occurs at the transcriptional level and is mediated by transcription factors that bind *cis*-regulatory elements found in target genes. These transcription factors, which are activated by FA, have a structure similar to that of the steroid-thyroid supergene family of nuclear receptors, which includes the steroid hormone receptors, glucocorticoid receptor, vitamin D receptor, thyroxine receptor (TR), and the retinoic acid receptor (RxR) (48–50). PUFA-responsive transcription factors recently have been characterized; for example, PPAR can be activated by clofibric acid and other peroxisome proliferators (45,48,50). Recent studies have identified a number of proteins and co-activators that in-

teract with nuclear receptors involved in the regulation of transcriptional activity. The formation of the binding site for the co-activator in the nuclear receptor is ligand dependent.

The activation of PPAR by FA was first characterized in *Xenopus laevis*; α-, β-, and γ-isoforms were able to respond to FA with overlapping specificity (45). However, few studies have systematically explored the differential activation of PPAR by FA of different chain length or unsaturation. Yu *et al.* (49) compared the ability of FA to activate the different PPAR isoforms using chimeric constructs. The tetR/PPARα chimeric receptor was activated to almost the same extent by LA and DHA, whereas the γ-isoform was activated by DHA but not by LA and the β-isoform was responsive to DHA > LA. PPARα is apparently also activated by medium- and long-chain unsaturated FA. This evidence has been used to support the notion that reduction of hepatic expression of lipogenic enzymes induced by dietary n-3 and n-6 PUFA is mediated by PUFA-activated PPARα (45,48,50). In this chimeric PPAR expression model, DHA was the most potent activator, whereas saturated myristic acid (14:0) was a considerably poorer inducing agent (48). The net effects of PPAR on cellular processes and metabolism include enhanced peroxisomal proliferation, increased FA oxidation, decreased FA synthesis, and enhanced glucose oxidation (50). Additional work will be necessary to better characterize the intracellular FA metabolites that regulate transcriptional activation and responsiveness of target genes critical for retinal and brain development.

EFA effects on neural structures and functional properties. Lipids, such as various phospholipids and cholesterol, serve as components of specialized cell membranes and organelles. The overall quantity and relative composition of these lipid species may affect membrane fluidity and protein/lipid interactions, resulting in changes in overall cell function. The FA composition of structural membrane lipids can affect membrane function by modifying overall membrane fluidity (order parameter), membrane thickness, lipid phase properties, membrane microenvironment, or by interaction of FA with membrane proteins (51–53). These effects may modulate receptor activity, transport of metabolites in and out of cells, and hormonal or other signal transduction processes. Most dietary n-3 FA–induced membrane changes are not reflected by an overall change in membrane fluidity, but result in selective changes in specific domains of membrane microenvironment (54). The replacement of DHA by docosapentaenoic acid (22:5n-6) observed in n-3 deficiency results in a very similar overall lipid unsaturation level because only one double bond has been lost. Thus, on average, membrane fluidity as measured by fluorescent probe polarization remains unchanged. Furthermore, the major changes in the physical state induced by the FA composition of lipid bilayers occur when mono- or diunsaturated moieties are introduced; namely, when a saturated FA such as stearic acid (18:0) is replaced by oleic acid (18:1n-9) or by LA (18:2n-6) (52,53). Others have suggested that the DHA supply modifies the phospholipid molecular species present in neural tissues, thus affecting overall function (55).

One of the most significant membrane effects of DHA is its role in photoreceptor signal transduction processing. Recently, Litman and Mitchell (56) reported that LC-PUFA present in membrane phospholipid molecular species have profound effects on rhodopsin activation and related structural modifications. Rhodopsin is a membrane protein present in rod outer segment disk membranes, accounting for 90% of the protein content. It functions as a photon receptor coupled to a G protein. The light-induced conformational change of rhodopsin triggers a biochemical cascade finally leading to an increase in phosphodiesterase activity and a decrease in cGMP that closes sodium ion channels in the photoreceptor disk membrane. The result is a hyperpolarization that is reflected in the ERG as the leading edge of the a-wave and that ultimately leads to the release of synaptic neurotransmitters. Membrane FA composition affects the ability of photons to transform rhodopsin to the activated state (56–58). The rhodopsin activation in response to light involves a transformation of metarhodopsin I (M I) to metarhodopsin II (M II).

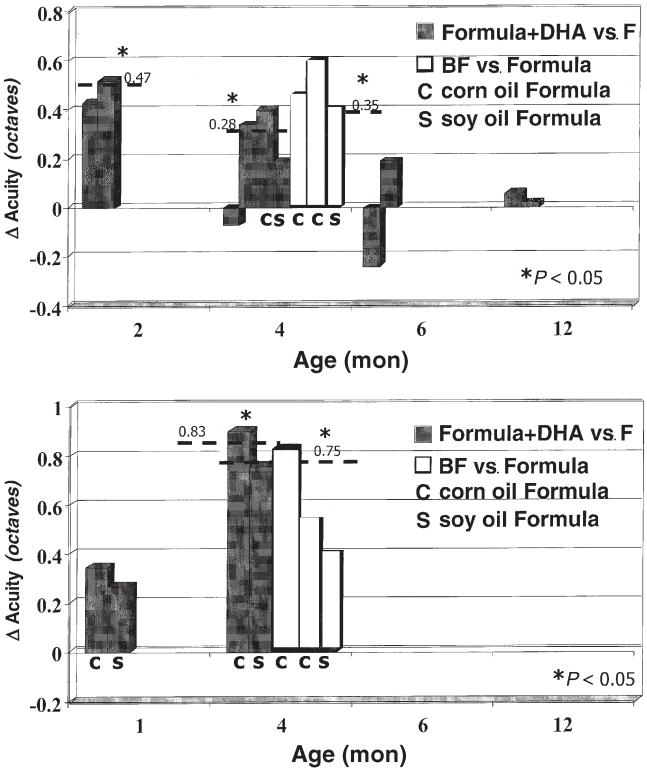

FIG. 1. Meta-analysis of visual acuity differences in preterm infants; solid bars represent randomized comparisons [group fed formula with long-chain polyunsaturated fatty acids (LC-PUFA) compared with group fed formula without LC-PUFA]. Open bars represent nonrandomized comparisons (group fed human milk compared with formula without LC-PUFA). Dashed line represents mean of estimate from combined data for various studies for given month. C and S refer to comparison formulas containing corn and soy oil, respectively. (A) Acuity differences measured by behavioral tests (acuity cards); (B) acuity differences measured by visual-evoked potentials; all values expressed in octaves (one-octave difference is a 50% reduction in width of stimulus element). Data obtained from Reference 41. DHA, docosahexaenoic acid; F, formula; BF, breast feeding.

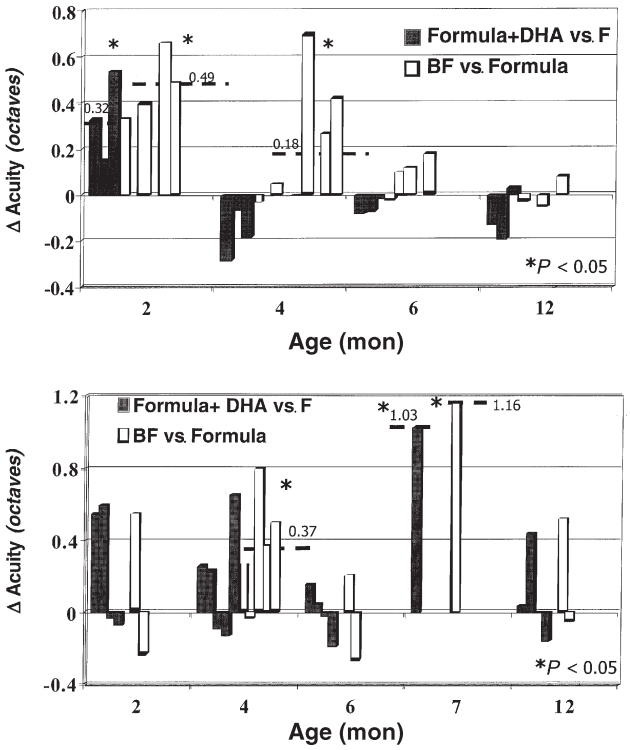

FIG. 2. Meta-analysis of visual acuity differences in term infants; solid bars represent randomized comparisons (group fed formula with LC-PUFA compared with group fed formula without LC-PUFA). Open bars represent nonrandomized comparisons (group fed human milk compared with formula without LC-PUFA). Dashed line represents mean of estimate from combined data for various studies for given month. (A) Acuity differences measured by behavioral tests (acuity cards); (B) acuity differences measured by visual evoked potentials; all values expressed in octaves (one-octave difference is a 50% reduction in width of stimulus element). Data obtained from Reference 42. See Figure 1 for abbreviations.

The MI → MII equilibrium constant is six times higher with di-DHA acylated PC than with di-myristoyl (14:0) PC. The di-DHA PC has an equilibrium constant that is almost identical to that of phospholipids extracted from native rod disks. The effect is explained mainly by the increase in membrane free volume. This greater mobility of rhodopsin within the lipid microenvironment most likely explains the change in G protein activation and the corresponding enhanced signal transduction to photon stimuli (58). The corresponding physiologic phenomenon is an increase in retinal sensitivity to light, such as we observe by providing DHA in the infant diet.

EFA effects on electrophysiologic responses. The role of membrane lipid composition in determining the electrical properties of cultured neuronal cells exposed to exogenous FA has also been investigated (59). Both n-3 and n-6 FA reduce the rate of rise and lower the amplitude of Na^+ action potentials. The opposite effects were observed when saturated or *trans* monoenoic FA were added to the culture (60). These effects are likely mediated by a change in the number of active Na^+ channels. A change in membrane composition or altered FA availability to the cells may explain this effect. Free LC-PUFA have been shown to modulate the inactivation of calcium and sodium channels in rat neural cells (61). There are also changes in cation currents in hippocampal neurons (60) and a higher seizure threshold in rat cortex (62). These effects appear to depend on free extracellular LC-PUFA concentrations and not on membrane phospholipid composition (61). The responsiveness of free LC-PUFA to dietary interventions, which alter tissue composition, remains unclear. The release of free LC-PUFA from membranes could have widespread effects on neurosensory function.

At the CNS level, interest in the effect of EFA on the maturation of visual function is based on their role as key structural components of cell membranes and their accumulation in visual and neural structures. Data from breast-fed infants who received DHA indicate that a higher content of this FA is present in brain cortex relative to infants fed formula (63). Neuringer and colleagues (64) established the need for n-3 FA in the diet, utilizing infant rhesus monkeys as a model system for n-3 FA deficiency. Following prenatal (maternal) and

TABLE 3
Critical Aspects in Experimental Design of Studies of Long-Chain Polyunsaturated Fatty Acid (LC-PUFA) Supplementation on Visual and Neurodevelopment in Infants[a]

Design	Diet
Prospective randomized, controlled, double blind study	Content and balance of EFA: LA and ALA
Nonrandomized, formula compared with breast-fed reference group	Content and balance of each LC-PUFA
Descriptive, nonrandomized trial	Source of LC-PUFA: egg, single cell, marine
Preplanned or *post-hoc* analysis	Energy balance and content of critical nutrients (I, Fe, Zn, vitamin A, folate)
Sample size to detect effect	Digestibility and utilization
	Duration of the dietary intervention
Subjects	**Outcome**
Term or preterm infants	Sensitivity of selected outcome
Birth weight and gestational age	Variability in measurement of outcome
Special groups or representative population	Age of evaluation, time of day of evaluation
Socioeconomic and ethnic group	Time of effect relative to critical period
Maternal education	Biological and clinical significance
Home environment	Duration and reversibility of effects
Pre- and postnatal growth	Safety of intervention and side effects

[a]Abbreviation: EFA, essential fatty acids. For other abbreviations see Table 1.

postnatal diets deficient in n-3 FA, the DHA concentrations in both the occipital cortex and the retina were reduced to 20% of that in control monkeys. The n-3 FA deficiency also impaired visual acuity as measured by preferential looking techniques. By 12 wk of age, the deficient monkeys presented Snellen acuities of ~20/125 vs. 20/50 in controls (20/20 is the average adult acuity; 20/50 is the average for 12 wk of age in monkeys). In addition, the b-wave amplitudes of ERG were reduced by 30% in the n-3 FA–deficient rhesus monkeys (64).

Several potential mechanisms by which early dietary EFA supply may affect visual and brain maturation and long-term function can be outlined on the basis of the available experimental data. The potential role of DHA as a modulator of membrane properties is supported by the *in vitro* studies of membrane fluidity and transport in neural cells modified in their membrane FA. The putative role of DHA in amplifying the phototransduction cascade is supported by the electrophysiologic findings in animals and humans. Dietary n-3 supplementation in these studies resulted in decreased retinal rod threshold and higher maximum amplitude, meaning that less light is required to trigger a retinal response and that more signal is being transmitted to the visual pathway, respectively. Moreover, the discovery of biochemical differences in phosphorylated microtubular-associated proteins in neurons from the visual cortex of light-deprived kittens during early development provides a mechanism for the classical observations by Hubel and Wiesel (65,66). The expression of microtubular proteins in the visual cortex induced by light plays a key role in establishing the dendritic arborization and interconnections necessary for visual perception; darkness inhibits the expression of this gene product (67,68). Thus the role of light-mediated stimuli in triggering cortical differentiation offers a plausible explanation for the phenomenon of a critical period for ocular dominance that has a biochemical basis as well as structural and functional correlates (65–70). We speculate that the effect of DHA on light transduction in the retina early in life may have lasting effects (i.e., imprint) on the organization and function of the visual cortex. The alternative possibility is that DHA has an effect on photoreceptors as well as an independent effect on cortical maturation itself. No definitive answer can be established from the available data. As previously indicated, the fact that human milk-fed infants exhibited more mature stereo acuity at 3 yr relative to formula-fed infants suggests that this phenomenon may be relevant to humans (16). Additional supportive evidence on the possible long-term effect of early DHA supply on maturation of the visual cortex comes from a large population-based prospective study of determinants of stereo acuity development conducted in Bristol, United Kingdom in collaboration with one of us (E.B.). The results indicate that a maternal antenatal diet rich in DHA is associated with enhanced stereopsis of infants at age 3.5 yr after adjusting for other environmental as well as maternal and infant factors (71).

In conclusion, evidence for a beneficial effect of AA + DHA supplementation on CNS development is strong. The followup of supplemented infants beyond infancy should help to address the question concerning the persistence of effects beyond early life. Studies summarized in this review provide evidence supporting the view that dietary EFA supply affects visual development of preterm and term infants. The preliminary information on cognitive development is insufficient to fully establish a claim for an LC-PUFA effect on mental development. Human infants require dietary AA and DHA to maintain normal FA composition of plasma and RBC membrane lipids, and presumably of brain and retina. Differential gene expression induced by EFA and changes in membrane biophysical properties are likely responsible for the observed functional effects. Further work should lead to a better understanding of the mechanisms by which EFA affect visual and brain development and allow for a better definition of optimal nutrition in early life. Optimal in this context implies the right amount and balance of nutrients to obtain a desirable outcome (i.e., enhanced cognitive development or decrease in burden of disease in adult life) considering that there are inherent risks from excesses as well as deficits.

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