Effects of supplementation with ω3 long-chain polyunsaturated fatty acids on retinal and cortical development in premature infants1–3

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ABSTRACT Deficiency of ω3 long-chain polyunsaturated fatty acids (LCPUFAs) in vertebrates produces subtle adverse effects on visual and neural function. Preterm infants 1) are deprived of vital intratrophic fat accretion during late pregnancy, 2) must rely solely on formula for fatty acid supplies if not breastfed. and 3) may have limited postnatal desaturase activity. In a study to evaluate the necessity of dietary ω3 LCPUFAs, preterm infants were fed human milk, corn-oil-based formula (ω3 fatty acid deficient), soy-oil-based formula (rich in precursor fatty acids), or marine-oil–supplemented formula (containing docosahexaenoic acid). At 36 and 57 wk postconception, the LCPUFA profiles in red blood cell lipids were nearly equivalent in the human-milk and marine-oil groups whereas the corn-oil group had markedly lower values for ω3 fatty acids. Rod photoreceptor function was significantly more mature in the corn-oil group compared with the human-milk and marine-oil-enriched groups in early postnatal development (36 wk). The corn-oil group also had impaired visual acuity at both 36 and 57 wk. The potential benefit of ω3 LCPUFA–enriched full-term formula is discussed. The study supports a role for ω3 LCPUFAs as required nutrients for the optimal maturation of visual and cortical function in preterm infants. Am J Clin Nutr 1993:57(suppl): 807S–12S.

KEY WORDS Omega-3 fatty acids, dietary fat, infants, visual function, lipids, marine oil

Introduction

The inability of vertebrates to synthesize linoleic acid (18:2ω6) and α-linolenic acid (18:3ω3) leads to the essential requirement for these fatty acids (FAs) in the diet (1). The essential fatty acids (EFAs) were considered of marginal nutritional importance until the 1960s, when clinical signs of EFA deficiency became apparent in infants fed skim-milk–based formula and in those given lipid-free parenteral nutrition (2–4). Lipids traditionally have been considered a key energy source for growth, metabolism, and muscle activity during infant development. The structural role of long-chain polyunsaturated fatty acids (LCPUFAs) and the functional correlates of specific FAs are being increasingly recognized (5). The LCPUFA arachidonic acid (20:4ω6) is an important membrane component and precursor to prostaglandins, leukotrienes, and epoxides (6, 7). The requirement for bioactive lipids in numerous physiologic processes is reflected in the myriad of clinical complications accompanying deficiency of the ω6 EFA linoleic acid (5). More subtle clinical symptoms that appear in ω3 EFA deficiency include abnormal visual function and peripheral neuropathy (8). These complications may occur more in response to an insufficiency of the specific metabolic derivative of α-linolenic acid, namely docosahexaenoic acid (DHA, or cervonacid, 22:6ω3). Indeed, the high concentrations of DHA in cerebral cortex and retina would suggest that it participates in neural and visual function (9, 10). DHA reaches levels of up to 40% of total FAs in the phospholipids of these tissues in humans (9), yet a specific role for DHA in the physiological or biochemical function of neural tissues remains to be defined. The highly unsaturated nature of DHA may be responsible for the observed effects of ω3 FA deficiency on the function of developing brain and retina (10, 11). Membrane physiologists have suggested that the altered FA composition of structural lipids can effect function by modifying membrane fluidity or membrane thickness, by changing properties of the liquid phase, or by specific interactions with vital membrane proteins (10–14). In the retina, the outer segment of rod photoreceptors consists of thousands of plasma-membrane invaginations forming discs of phospholipid bilayers. Imbedded in this membrane is the photosensitive protein rhodopsin and other enzymes of the visual cascade. The fluid microenvironment of these proteins may significantly influence their ability to transmit visual signals. Thus, the high concentration of DHA in photoreceptors may reflect the need for a highly fluid membrane that permits rapid enzyme action and ion transport (11, 14). Indeed, the lateral

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diffusion coefficient for rhodopsin as determined by fluorescence photobleaching and recovery measurements has been reported to result in a diffusion rate an order of magnitude faster than that of most other cell-surface proteins (15).

We recently evaluated the concepts that relate DHA content, membrane fluidity, and biological function to each other by use of a relevant human retinal cell line (16). Y79 retinoblastoma cells in culture were supplemented with DHA to increase their cellular-membrane content fivefold compared with that of untreated cells (Fig 1). Rotational membrane fluidity, as measured by the diphenylhexatriene fluoroprobe, was unchanged in DHA-enriched cells; however, lateral membrane fluidity as reported by the pyrene fluoroprobe was significantly increased compared with that of the controls. Concomitant with these increases in both DHA content and lateral fluidity was a significant increase in transmembrane choline transport. These results tend to support membrane fluidity as a mediator of retinal function; however, to our knowledge, no studies have specifically addressed the role of DHA in biochemical reactions occurring in the phototransduction cascade of rod and cone photoreceptors.

Electrophysiological functions of neural tissues can be influenced by the dietary FA supply. Anderson and colleagues (17) first observed changes in the electroretinograms (ERGs) of rats fed diets devoid of fat or supplemented with specific FAs. The ERG-response components measured were the amplitudes of the a and b waves. Dietary ω9 FA did not alter the ERG amplitudes compared with those of rats on fat-free diets. Diets containing ω6 FA increased the responses by 20–30% whereas dietary ω3 FAs alone resulted in 40–70% increases in both the a- and b-wave amplitudes.

Neuringer, Connor, and colleagues (18–20) have made significant contributions toward establishing the need for ω3 FAs in the diet by using infant rhesus monkeys as a model system for ω3 FA deficiency. After a period when prenatal (maternal) and postnatal diets were deficient in ω3 FAs, the DHA concentrations in both the occipital cortex and the retina were reduced to 20% of those in control monkeys. A common metabolic response found in ω3 FA deficiency is a compensatory rise in ω6 FAs, particularly the end product, docosapentaenoic acid (DPA, 22:5ω6). In the ω3 FA-deficient animals, the DPA content in these neural tissues was 45-fold higher than that of controls. Similar FA profiles were found in plasma phospholipids; DHA was reduced by 94% in deficient monkeys. These studies provide a basis for utilizing the FA profiles in blood lipids as an index of the FA status in neural tissues. The ω3 FA deficiency also impaired visual acuity as measured by preferential looking techniques. By age 12 wk, the deficient monkeys presented Snellen acuities of 20/125 whereas the value for controls was 20/50 (20/20 is the average adult acuity; 20/50 is average for age 12 wk). In addition, the a-wave amplitudes of ERGs were reduced in the ω3 FA–deficient rhesus monkeys.

In humans, Clandinin and colleagues (21–23) showed that there are significant increases in the DHA content of brain tissue during the last trimester of gestation. This becomes very significant to preterm infants who do not receive this intrauterine source of fat. These investigators also showed that there is a lag

FIG 1. Membrane docosahexaenoic acid (DHA) composition, membrane fluidity, and biological function in ω3 fatty acid–supplemented Y79 retinoblastoma cells. Membrane rotational mobility was measured by the fluoroprobe diphenylhexatriene. Lateral fluidity was reported by the ability of the monomeric fluoroprobe pyrene to collide and form dimers (eximers). Biological function was assessed by transmembrane transport of radiolabeled choline into cellular lipids. *Significantly different from control, P < 0.001 (Student’s t test).
phase in the accretion rate of ω3 FAs in the brain tissue of human neonates. Data from both animals and humans suggest that the desaturation of parent EFAs in full-term newborns may be limited.

Mothers of premature infants who choose to breast-feed will provide both ω6 and ω3 LCPUFAs to the infant. However, preterm infants who are not breast-fed must rely on formulas for fat supplies. Because no infant formula in the United States provides the LCPUFAs found in human milk, the preterm infant is at risk for ω3 and perhaps ω6 LCPUFA deficiency. Two factors that contribute to this ω3 FA deficiency include the loss of a period of EFA accretion during the last trimester of gestation and, possibly, a limited desaturase activity.

Early studies of reconstituting DHA levels in preterm infants utilized a bolus of marine oil containing DHA (24, 25). Carlson et al (26) demonstrated that the blood lipid levels of DHA could be maintained for at least 60 wk after birth by marine-oil supplementation.

In our studies, we have evaluated the effects of dietary ω3 FA supplementation, incorporated into formula, on the development of visual function in preterm infants (5, 27–29). These visual-function measures offer a powerful index for evaluating the effects of early ω3 FA nutrition on eye and brain development in humans. In addition, we have determined the FA profiles in blood lipids as an index of the FA composition of neural and retinal tissues.

Preterm-infant study

We recently completed a study of 83 infants with birth weights between 1000 and 1500 g and born at 27–33 wk gestation. Infants whose mothers chose to breast-feed served as a “gold standard” group. The remaining infants were randomly assigned to three groups receiving formulas varying in EFA content. Healthy very-low-birth-weight infants entered the study by day 10 of life and received the diets until 57 wk postconceptional age (ie, equivalent to 4 mo postterm). Further description of inclusion and exclusion criteria related to the study cohorts and additional methodological details were previously described (5). Blood samples were taken at entry, at 36 wk postconception after ≈6 wk on the diets, and at 57 wk after 27 wk on the diet. FA compositions were determined in red blood cell (RBC) lipids by capillary-column gas chromatography (5, 27) of methyl esters. The retinal function and visual acuities of the infants were also measured at 36 and 57 wk postconception (5, 28, 29). The study protocol was approved by the University of Texas Southwestern Medical Center human-experimentation ethics committee.

The EFA composition of human milk and study formulas is presented in Table 1. The corn oil-based formula corresponded to commercial powdered premature formula and contained a high concentration of linoleic acid (18:2ω6) as EFA, an extremely low concentration of n-linoleic acid (18:3w3), and no ω3 LCPUFA. The soy-oil–based formula was equivalent to a ready-to-feed premature diet and supplied 18:2 and elevated 18:3 as precursors of LCPUFAs. A third experimental formula was also soy-oil based but was enriched with marine oil to give an ω3 LCPUFA content equivalent to that found in human milk (30). Statistical treatment of the data eliminated all infants for which visual-function tests were not obtained.

The DHA compositions of RBC lipids for all diet groups were similar on entry into the study (5, 27, 29). However, at 36 wk postconception, marked differences in DHA content were evident in the RBC lipids of all diet groups (Fig 2). Infants fed the corn-oil formula had significant reductions in DHA compared with the other formula groups. The marine-oil group had the highest DHA concentrations of all the groups. The DPA profile found at 36 wk in RBC lipids was inversely proportional to the DHA profile. The corn-oil group was significantly elevated compared with the human-milk group. By 57 wk, the levels of the end products of ω3 and ω6 FA metabolism, namely DHA and DPA, were greatly accentuated compared with 36-wk values. The corn-oil group was significantly different in both DHA and

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**FIG 2.** Docosahexaenoic acid (DHA) and ω6 docosapentaenoic acid (DPA) distribution of total red blood cell lipids in diet-study preterm infants. Group values with different letters are significantly different (P ≤ 0.0017) by multiple comparison with Bonferroni adjustment.

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**TABLE 1**

EFA composition of preterm-study diets

<table>
<thead>
<tr>
<th></th>
<th>Human milk†</th>
<th>Corn oil‡</th>
<th>Soy oil†‡</th>
<th>Soy ‡ + marine†‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFAs 100 g lip</td>
<td>44.4 ± 1.2</td>
<td>62.3 ± 1.2</td>
<td>63.0 ± 3.3</td>
<td>64.2 ± 4.3</td>
</tr>
<tr>
<td>MUFA S 100 g lip</td>
<td>35.5 ± 0.9</td>
<td>11.8 ± 0.3</td>
<td>10.3 ± 0.7</td>
<td>10.7 ± 1.2</td>
</tr>
<tr>
<td>18:2ω6 100 g lip</td>
<td>12.7 ± 0.8</td>
<td>24.2 ± 0.8</td>
<td>20.8 ± 1.6</td>
<td>20.4 ± 2.7</td>
</tr>
<tr>
<td>18:3ω3 100 g lip</td>
<td>0.8 ± 0.2</td>
<td>0 ± 0.0</td>
<td>2.7 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>20:5ω3 100 g lip</td>
<td>0.2 ± 0.1</td>
<td>0 ± 0.0</td>
<td>0.65 ± 0.07</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>22:6ω3 100 g lip</td>
<td>0.29 ± 0.1</td>
<td>0 ± 0.0</td>
<td>0 ± 0.0</td>
<td>0 ± 0.0</td>
</tr>
</tbody>
</table>

* SD ±. SFAs, saturated fatty acids; MUFA s, monounsaturated fatty acids.
† Representative of a day 20 breast-milk sample (mean of two samples).
‡ n = 3.
DPA content compared with all other diet groups. RBC lipids of the human milk and marine-oil groups were statistically different in DHA composition but showed a similar pattern across total ω3 and ω6 LCPUFAs. The soy-oil group had intermediate DHA and DPA values.

Retinal-function tests demonstrated significantly higher rod threshold and lower maximum amplitude (Vmax) values than did the corn-oil group at 36 wk relative to infants receiving dietary ω3 FAs (Table 2). The lower Vmax values indicate that the gain of the rod photoreceptors of the corn-oil–fed infants was significantly reduced compared with those of the other groups. The retinal illuminance constant (k) at half maximum amplitude was significantly elevated in the corn-oil-fed group, consistent with a reduced quantal catch of the rod photoreceptors. Thus, a greater illumination was necessary for the rod response to reach a specific amplitude in the infants receiving the corn-oil–based formula. Cone function was not significantly affected by diet although the trends were similar to those found in rods.

Visual-acuity tests measure the integrity of the neural pathway from the retina to the occipital cortex. Effects of dietary FA modification on the function of the visual cortex as measured by pattern-reversal visual evoked potentials (VEPs) and behavioral preferential-looking visual-acuity response are presented in Table 3. The infants in the human-milk and marine-oil groups (both receiving DHA) had improved visual function relative to infants fed formulas devoid of DHA at the follow-up check (57 wk).

Safety evaluations of the experimental formula containing up to 1% of total fat as ω3 LCPUFA (EPA + DHA) did not reveal problems of clinical significance. Utilizing a specially designed Surgitcut (International Technidyne, Edison, NJ) apparatus to determine bleeding time in infants, no significant differences were found relative to diet assignment (31). Oxidative status in the RBCs of the infants was evaluated by two techniques (27, 32). No difference in the diet groups was evident by malonyldialdehyde production (thiobarbituric acid–reactive substances) or by fragility determination of peroxidation-stressed RBCs. Similarly, using diphenylhexatriene fluorescence polarization, we found no changes in the membrane fluidity of intact RBCs from infants on the four diets. In addition, feeding tolerance and other clinical parameters were not influenced by the ω3 FA dietary intervention (5, 27, 32). Our results (5, 27–29) and similar preliminary studies reported by Carlson et al (24–26) support an essential role for ω3 FAs in normal eye and brain development.

| **TABLE 2** | Rod ERG results of preterm-infant study: 36 wk postconception (x̄ ± SD) |
|-------------|-------------------|----------------|----------------|----------------|----------------|
|             | Human milk (n = 10) | Corn oil (n = 19) | Soy oil (n = 24) | Soy oil + marine (n = 28) |
| log Threshold* | 0.41 ± 0.59 | 1.08 ± 0.37 | 0.71 ± 0.59 | 0.41 ± 0.61 |
| log Vmax‡ | 1.20 ± 0.14 | 1.05 ± 0.11 | 1.08 ± 0.20 | 1.22 ± 0.20 |
| log k* | 1.25 ± 0.54 | 1.73 ± 0.28 | 1.39 ± 0.55 | 1.24 ± 0.47 |

* Scotopic troland-seconds.
‡ Significantly different from human milk and soy + marine groups: TP < 0.001, SP < 0.005.

To date, the data suggest that DHA, as present in human milk and in supplemented formula, is required for optimal visual development in the human. Further studies will serve to delineate the best and safest form of LCPUFA supplementation for infants.

**Term-infant studies**

The concentration of DHA in the RBCs of full-term infants fed formula is lower than that of breast-fed infants (25, 33, 34). This suggests that current commercial formulas provide insufficient 18:3ω2 or that chain-elongation and/or desaturation enzymes are not sufficiently active during initial postnatal life to support tissue accretion of DHA. This may have functional significance for full-term infants who may also be dependent on a dietary source of DHA for optimal functional maturation of the retina and visual cortex.

We conducted preliminary studies (28, 35–37) in full-term infants fed either human milk (n = 18) or cow-milk formula (n = 12), with 12–18% linoleic acid and 0.5–1.0% α-linolenic acid, in order to obtain data on the effect of postnatal age on visual-acuity maturation. These studies, also approved by the Medical Center’s ethics committee, indicated that conceptional age, not postnatal age, determined visual maturation. In addition, we found that both VEP and forced-choice preferential looking (FPL) acuities were higher in 4-mo-old exclusively breast-fed infants compared with formula-fed infants. The visual acuities, expressed as log of minimum angle of resolution (logMAR) and Snellen equivalents, for VEP were significantly worse for the formula-fed infants [0.62 ± 0.20 logMAR (x̄ ± SD), or 20/85 (x̄ Snellen value) compared with the human-milk-fed group (0.51 ± 0.18 logMAR, or 20/65; p < 0.05). Similarly, FPL values were worse in the formula-fed group (0.81 ± 0.12 logMAR, or 20/130) than in the human-milk-fed infants (0.73 ± 0.07 logMAR, 20/110; p < 0.025), indicating improved vision in the term infants receiving dietary LCPUFAs.

In a long-term follow-up comparison of mental development of breast-fed and formula-fed children, better picture-recognition intelligence at age 8 y and better scores in mathematics, nonverbal abilities, and sentence completion at age 15 y could be associated with early breast-feeding (38). The study, based on
children living in Great Britain in 1946, used confirmed records of infants known to have been entirely bottle-fed (n = 1133) or never bottle-fed (n = 1291). The functional benefits were proportional to the duration of breast-feeding and remained statistically significant after social, cultural, and demographic variables were controlled for by multivariate analysis. However, along with reduced ω3 FA content, these formulas were based on unmodified, diluted cow milk with the sole addition of sugar and the protein supply may have been excessive. Recent studies have associated a neurodevelopmental advantage in children aged 7–8 y with consumption of mother’s milk during the first 31 d of life compared with preterm formula-fed infants (39). The IQ scores (103.7 ± 1.1, n ± SE; n = 193) for children that successfully received breast milk were significantly (P < 0.001) better than for children provided formula (92.8 ± 1.6; n = 90). In addition, children whose mothers chose to provide breast milk but were unsuccessful were also disadvantaged (IQ = 94.8 ± 4.6; n = 17) compared with those receiving human-milk nutrition. These studies reinforce the need to further investigate the specific nutritive value of minor components in human breast milk. Although present-day preterm and term formulations have been adapted to meet all known nutritional requirements of infants, continued evolution of the diet is necessary to meet the more subtle developmental requirements of infants.

We conclude that the early dietary fat regimen can influence the development of brain and visual function. An essential role for both ω3 and ω6 LCPUFAs in preterm and term infant formulas is currently being addressed.

We thank Mead-Johnson Nutritional Division for providing the infant formulas used in this study.

References

Discussion

David F Horrobin: What is the EPA-DHA ratio in the formula?

Dennis R Hoffman: It was about 2 to 1, EPA to DHA. It was a relatively common fish-oil preparation. It was a highly purified, deodorized, decolorized preparation of stabilized menhaden oil.

Michael A Crawford: You gave us the ratios of your w6 to w3 fatty acids in the different milks, and I was puzzled because you mentioned that the soy milk ratio was 7.7. Yet the ratio of the marine-oil-enriched soy milk was 8.5. One would have expected it to be a lower ratio if one adds more w3 fatty acids. What happened there?

Hoffman: There was a little less linolenic acid in that formula to accommodate the added long-chain w3 fatty acids, yet the final product had the 8.5 ratio according to gas chromatography.

Crawford: Another point about Δ-4-desaturation. Howard Sprecher quite recently conceded that he can’t find any Δ-4-desaturation for eicosapentaenoic acid of the n-3 series. [Voss A, Reinhart M, Sankarappa S, Sprecher H. The metabolism of 7, 10, 13, 16, 19-docosahexaenoic acid to 4, 7, 10, 13, 16, 19-docosahexaenoic acid in rat liver independent of a 4-desaturase. J Biol Chem 1991:266:19995-20000.] Because he is often considered the father of fatty acid enzymology, one listens to what he has to say. This doesn’t necessarily imply that there is no upward conversion to DHA, but if there is, he postulates that it goes around a very long route of further chain elongation to 24 carbons, then desaturation by the Δ-5-desaturase, and retroconversion back to DHA. Whatever the pathway is, the conversion of eicosapentaenoic acid into DHA is a pretty difficult process. I think that your data support that view to some extent.

Hoffman: Yes, I think that’s right. The EPA concentrations in the marine-oil group are similarly increased, just as the DHA concentrations are. We didn’t really see a selective enrichment of DHA vs EPA in the RBCs, but it appears to make quite a difference when you try to compare RBC values to retinal values. We have relied on the work of Neuringer and use the fatty acid index of RBCs as reflecting the fatty acid status in the retina [Neuringer M, Connor WE, Lin DS, Barstad L, Luck S. Biochemical and functional effects of prenatal and postnatal w3 fatty acid deficiency on retina and brain in rhesus monkeys. Proc Natl Acad Sci USA 1986;4021–45]. However, the retina has a tremendous mechanism for accumulating and concentrating DHA, whereas, apparently the RBCs don’t have this mechanism. We suggest that there may be a discrepancy there as far as the fatty acid compositions in the two tissues are concerned.

Crawford: Would you like to comment just very briefly on whether the soy milk induced a significant increase in eicosapentaenoic acid?

Hoffman: No, it did not.

Kristian S Bjerve: Have you any idea about the connection between visual acuity and arachidonic acid as reported by Dr Carlson [Carlson SE. Are n-3 polyunsaturated fatty acids essential for growth and development? In: Nelson GJ. Ed. Health effects of dietary fatty acids. Champaign, IL: American Oil Chemists’ Society, 1991:42–9]. She indicated that getting a sufficient supply of arachidonic acid was probably also an important issue in infant growth development. We don’t find any association between the Bayley development scores and arachidonic acid. Do you have an indication on arachidonic acid?

Hoffman: We find a very poor correlation between visual acuity and arachidonic acid content. The correlation that she is talking about has to do with body length of the infants. We don’t see this in our data but our infants have not been on formulas as long as Dr Carlson’s infants have.

Frits Muskiet: I was very much impressed by the fact that you could still pick up at 3 years of age, with your test, differences between human-milk-fed and formula-fed children. Would you suggest that what happens during development is irreversible? Would you, for instance, be able to pick up formula- and human-milk-fed participants here in this audience? How long does it linger? Are the visual acuity changes permanent?

Hoffman: Unfortunately, a long-term follow up of these preterm infants is not very likely. The fatty acid profiles probably return to normal. We don’t have any data for older children. With the preterm infants, the ERGs returned to normal by 57 weeks but the visual acuity remained affected at that time. The results of the preterm study and the 3-year-old children would suggest that the disability is permanent, particularly with feeding of an omega-3-insufficient formula. I think this is reflected in the work of Lucas [Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C. Breast milk and subsequent intelligence quotient in children born preterm. Lancet 1992;339:261–4], where he found a neurodevelopmental advantage for breast-milk-fed infants compared with formula-fed infants at 8 years of age.