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THE ROLE OF ESSENTIAL FATTY ACIDS IN DEVELOPMENT

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Key Words docosahexaenoic acid (DHA), arachidonic acid (ARA), neurodevelopmental outcome, visual acuity, long-chain polyunsaturated fatty acid (LC-PUFA)

■ **Abstract** The presence of docosahexaenoic acid (DHA) and arachidonic acid (ARA) in human milk but not in infant formula, coupled with lower plasma and brain lipid contents of DHA in formula-fed than in breast-fed infants and reports of higher IQ in individuals who were breast-fed versus formula-fed as infants, suggest that exogenous DHA (and ARA) may be essential for optimal development. Thus, since 1990, several studies have examined the impact of formulas containing DHA or DHA plus ARA on visual function and neurodevelopmental outcome. Some of these studies have shown benefits but others have not. These results leave largely unanswered the question of whether these fatty acids are beneficial for either the term or preterm infant. However, evidence that preterm infants might benefit is somewhat more convincing than that for term infants. Despite the limited evidence for efficacy, formulas supplemented with DHA and ARA are now available and appear to be safe.

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INTRODUCTION

Fatty acids are aliphatic monocarboxylicacids that may have multiple, but usually no more than six, double bonds (polyunsaturated fatty acids), one double bond (monosaturated fatty acids), or no double bonds (saturated fatty acids). Fatty acids other than the essential fatty acids (see below) can be synthesized endogenously; however, the major source is from dietary fat, which accounts for 25%–50% of the energy content of most diets. Triglycerides, which have three, usually different, fatty acid molecules esterified to a molecule of glycerol, are the major components of dietary fat. These are hydrolyzed in the intestinal lumen, the bulk of released fatty acids are reassembled within the enterocyte, and the reassembled triglycerides along with phospholipids, monoglycerides, diglycerides, and sterol esters are absorbed into the thoracic duct, eventually reaching the bloodstream, where they circulate primarily as components of the various lipoproteins. The remaining free fatty acids circulate bound to albumen.

Fatty acids have common names but also are identified by a "shorthand" system indicating their number of carbon atoms, their number of double bonds, and the site of the first double bond from the terminal methyl group of the molecule. For example, palmitic acid, a 16-carbon saturated fatty acid, is designated 16:0, and oleic acid, an 18-carbon monounsaturated fatty acid with its single double bond located between the ninth and tenth carbon from the methyl terminal, is designated 18:1ω9. Linoleic acid (18:2ω6) and α-linolenic acid (18:3ω3), both of which are 18-carbon polyunsaturated fatty acids, are designated 18:2ω6 and 18:3ω3, respectively. 18:2ω6 has two double bonds, the first between the sixth and seventh carbon from the methyl terminal, and 18:3ω3 has three double bonds, the first between the third and fourth carbon from the methyl terminal. Table 1 shows the common names as well as the shorthand designations of several common fatty acids. In this chapter, only the shorthand designations are used.

ESSENTIAL FATTY ACIDS

Fatty acids with double bonds at the ω6 and ω3 positions (e.g., 18:2ω6 and 18:3ω3) cannot be synthesized endogenously by the human species and, hence, must be provided in the diet. Both 18:2ω6 and 18:3ω3 are metabolized by the same series of desaturases and elongases to longer-chain, more unsaturated fatty acids. This pathway is outlined in Figure 1. As indicated, two desaturation-elongation cycles result in conversion of 18:2ω6 and 18:3ω3 to 22:4ω6 and 22:5ω3, respectively. Another elongation step converts these fatty acids to 24:4ω6 and 24:5ω3, which are then desaturated by Δ^6-desaturase to 24:5ω6 and 24:6ω3 and undergo partial β-oxidation, forming 22:5ω6 and 22:6ω3. This pathway was described in the early 1990s by Voss et al. (94). Whether there also is a Δ^4-desaturase step in which 22:4ω6 and 22:5ω3 are converted directly to 22:5ω6 and 22:6ω3, as formerly thought, is not clear. Involvement of the pathway described by Voss in formation

TABLE 1 Common names and numerical nomenclature of selected fatty acids

Common name	Numerical nomenclature
Caprylic acid	8:0
Capric acid	10:0
Lauric acid	12:0
Myristic acid	14:0
Palmitic acid	16:0
Stearic acid	18:0
Oleic acid	18:1ω9*
Linoleic acid	18:2ω6*
γ-Linolenic acid	18:3ω6
Dihomogamma-linolenic acid	20:3ω6
Arachidonic acid	20:4ω6
α-Linolenic acid	18:3ω3*
Eicosapentaenoic acid	20:5ω3
Docosahexaenoic acid	22:6ω3

*ω9, ω6, and ω3 are used interchangeably with n-9, n-6, and n-3.

of 20:4ω6 and 22:6ω3 from labeled 18:2ω6 and 18:3ω3, respectively, has been demonstrated in both infants (85) and adults (36).

Collectively, the longer-chain, more unsaturated fatty acids synthesized from 18:2ω6 and 18:3ω3 are referred to as long-chain (i.e., more than 18 carbons) polyunsaturated fatty acids (LC-PUFAs). Important metabolites of 18:2ω6 include 18:3ω6, 20:3ω6, and 20:4ω6. 20:5ω3 and 22:6ω3 are the most important metabolites of 18:3ω3. The parent fatty acids, 18:2ω6 and 18:3ω3, are found in storage lipids, cell membrane phospholipids, intracellular cholesterol esters, and plasma lipids. LC-PUFAs synthesized from these precursors, on the other hand, are found primarily in specific cell membrane phospholipids. 20:3ω6, 20:4ω6, and 20:5ω3 are immediate precursors of eicosanoids (42, 73) and compete with each other for the enzymes involved in eicosanoid synthesis. Each is converted to a different series of eicosanoids with different biological activities and/or functions.

18:2ω6 has been recognized as an essential nutrient for the human species for almost 75 years (12, 32). Symptoms of deficiency include poor growth and scaly skin lesions. Although the essentiality of 18:3ω3 was suspected for some time, it was not recognized as an essential nutrient until approximately 20 years ago. In animals, including primates, deficiency of this fatty acid results in visual and neurological abnormalities (3, 69, 70, 95). Neurological abnormalities also were observed in a human infant who had been maintained for several weeks on a parenteral nutrition regimen lacking 18:3ω3 (40) and in elderly nursing

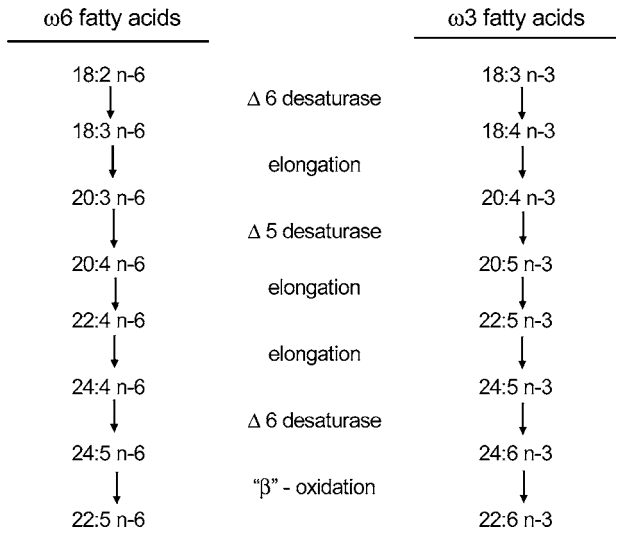

Figure 1 Metabolic pathways of ω6 and ω3 fatty acids.

home residents who were receiving intragastric feedings of an elemental formula with no 18:3ω3 (9). These symptoms were reversed by administration of 18:3ω3.

The clinical symptoms of ω6 fatty acid deficiency can be corrected by 18:2ω6, 18:3ω6, 20:3ω6, or 20:4ω6, and those of ω3 fatty acid deficiency can be corrected by 18:3ω3, 20:5ω3, or 22:6ω3. Whether 18:2ω6 and 18:3ω3 have specific functions that cannot be met by their metabolites is not clear.

The same series of enzymes that catalyze desaturation and elongation of ω6 and ω3 fatty acids also catalyze desaturation and elongation of ω9 fatty acids. The substrate preference of these enzymes is ω3, followed by ω6 and, finally, ω9; thus, competition between the ω9 fatty acids and either the ω6 or ω3 fatty acids is rare. However, if the concentrations of 18:2ω6 and 18:3ω3 are low, 18:1ω9 is readily desaturated and elongated to 20:3ω9 (eicosatrienoic acid). An elevated plasma ratio of this fatty acid to 20:4ω6, the triene/tetraene ratio, is a diagnostic index of ω6 fatty acid deficiency. A ratio of $>$0.4 was initially considered indicative of deficiency (38). However, since this ratio usually is $<$0.1, a ratio of $>$0.2 is now thought to be a more reasonable indicator. In the few documented cases of isolated 18:3ω3 deficiency in which it was measured, the triene/tetraene ratio was not elevated.

The minimum requirement for 18:2ω6 is thought to be from 2% to 4% of total energy intake (39). The minimum requirement for 18:3ω3 is less well defined but is thought to be about 1% of total energy intake (43). 18:2ω6 comprises about 15% to 20% of the total fatty acids of infant formulas available in the United States, and 18:3ω3 comprises 1.5% to 2%. Human milk contents of 18:2ω6 and 18:3ω3 are more variable than the contents in formulas. On average, 18:2ω6 comprises $\sim$16%

of the total fatty acid content of the milk of U.S. women and 18:3ω3 comprises about 1% (51). Human milk also contains small amounts of a number of longer-chain, more unsaturated metabolites of both 18:2ω6 and 18:3ω3, including 20:4ω6 and 22:6ω3.

Maternal diet has a marked impact on the concentration of all fatty acids in human milk, particularly that of 22:6ω3. The concentration of this fatty acid in the milk of women consuming a typical North American diet is generally in the range of 0.1% to 0.3% of total fatty acids and the concentration of 20:4ω6 ranges from 0.4% to 0.6% (51). The milk of vegetarian women contains less 22:6ω3 than the milk of omnivorous women (81), whereas the milk of women whose dietary fish consumption is high, or who take ω3 fatty acid supplements, is higher than that of the typical North American woman (34, 48, 59). The 20:4ω6 content of human milk is less variable and appears to be less dependent on maternal intake of 20:4ω6, perhaps because the 18:2ω6 intake of most populations is relatively high.

Recent recommendations for the minimal content of 18:2ω6 in infant formulas range from 2.7% to 8% of total fatty acids, and those for the maximum content range from 21% to 35% of total fatty acids (26, 76). The recommendations for the minimum and maximum contents of 18:3ω3 in infant formulas are 1.75% and 4% of total fatty acids, respectively (76). To maintain a reasonable balance between the two fatty acids, it is further recommended that the 18:2ω6/18:3ω3 ratio should not be less than 5–6 nor more than 15–16 (26, 76). The term and preterm infant formulas currently available in the United States have 18:2ω6/18:3ω3 ratios of $\sim$10 (see above). Formulas containing the LC-PUFAs 22:6ω3 and 20:4ω6 also are available for both term and preterm infants.

LONG-CHAIN POLYUNSATURATED FATTY ACIDS

The current interest in the role of LC-PUFAs in infant nutrition was precipitated by observations that plasma and erythrocyte lipid contents of these fatty acids, primarily 22:6ω3 and 20:4ω6, were lower in formula-fed than in breast-fed infants (14, 45, 52, 75). Since these fatty acids are present in human milk but not in formulas, the lower plasma and erythrocyte lipid content of these fatty acids in formula-fed infants was interpreted as indicating that infants cannot synthesize sufficient 22:6ω3 and 20:4ω6 to meet ongoing needs. Concurrent and earlier observations of better cognitive function of breast-fed versus formula-fed infants (55, 56, 67, 78) focused attention on the possibility that the lower cognitive function of formula-fed infants also might be related to inadequate intake of LC-PUFA. This, of course, ignores the many differences in education, socioeconomic status, etc., between women who choose to breast-feed versus formula-feed their infants that may have a greater impact on development than what the infant is fed. Further, of necessity, none of these observations are based on data from randomized, controlled trials, and attempts to control for differences in characteristics of mothers who choose to breast-feed versus formula-feed their infants either decreases or

abolishes differences in cognitive function between breast-fed versus formula-fed infants.

The possibility that LC-PUFA intake may be necessary for optimal development is supported by the facts that 22:6ω3 and 20:4ω6 are the major ω3 and ω6 fatty acids, respectively, of neural tissues (18, 19, 62) and 22:6ω3 is the major fatty acid of retinal photoreceptor membranes (62). In addition, postmortem studies of infants who died suddenly during the first year of life (29, 47, 55) show that the cerebral content of 22:6ω3, but not 20:4ω6, is lower in formula-fed than in breast-fed term infants. In contrast, the 22:6ω3 content of the retina of breast-fed and formula-fed term infants does not differ (55), perhaps because the retinal content of 22:6ω3 reaches adult levels at approximately term, whereas the adult level of 22:6ω3 in cerebrum is not reached until approximately 2 years post-term (62). One of the postmortem studies showed that the cerebral 22:6ω3 content of infants fed a formula with a relatively high content of 18:3ω3 was higher than that of infants fed a formula with less 18:3ω3 (47). This finding is consistent with studies in piglets showing that an 18:3ω3 intake of at least 1.75% of total fatty acids is necessary to maintain normal brain levels of 22:6ω3 (1) and studies in infants showing a positive relationship between 18:3ω3 intake and the appearance of labeled 22:6ω3 in plasma following administration of labeled 18:3ω3 (84).

During pregnancy, LC-PUFAs are transported actively to the fetus from maternal plasma (4, 25). Thus, the infant who is born early in the third trimester of pregnancy receives less LC-PUFA prior to birth than does the infant who is born at term and is thought to have a higher LC-PUFA requirement. However, the daily accumulation of LC-PUFA in the developing central nervous system changes minimally between mid-gestation and 18–24 months of age (62), which suggests that the total daily LC-PUFA needs of preterm and term infants are likely to be similar. On the other hand, since preterm infants are smaller, their daily needs per kilogram, or per 100 kcal, are undoubtedly greater, particularly during early life.

Both term and preterm infants can convert 18:2ω6 to 20:4ω6 and 18:3ω3 to 22:6ω3 (17, 22, 80, 84, 92). This has been established by administering the precursor fatty acids labeled with stable isotopes of either carbon (^{13}C) or hydrogen (^{2}H) and serially measuring blood levels of the labeled precursors as well as labeled metabolites of each by gas chromatography/mass spectrometry. The studies of Sauerwald et al. (84, 85) and Uauy et al. (92), which included both term and preterm infants, suggest that the overall ability of preterm infants to synthesize LC-PUFA is at least equal to that of term infants. However, the amount of LC-PUFA synthesized is a function of both the rate of synthesis and the pool size of the precursor. Hence, the preterm infant's greater apparent rate of conversion of 18:2ω6 and 18:3ω3 to 20:4ω6 and 22:6ω3, respectively, may not be great enough to compensate for its smaller precursor pool. Additionally, there is considerable variability in apparent conversion among both preterm and term infants, including infants fed the same formulas. Further, measurement of the enrichment of 22:6ω3 and 20:4ω6 following administration of labeled precursors has been limited to plasma, which is only a fraction of the body pool of both the precursor and product

fatty acids. Thus, since conversion as estimated from plasma enrichments may not be representative of conversion rates of other tissues, the amounts of LC-PUFA that either preterm or term infants can synthesize are not known.

The higher LC-PUFA content of the plasma lipids of breast-fed infants and infants fed formulas supplemented with LC-PUFA versus infants fed unsupplemented formulas support the concept that the amounts of LC-PUFA formed endogenously are less than the amounts provided by human milk or supplemented formulas. However, the extent to which the concentration of individual LC-PUFAs in plasma reflects the content of these fatty acids in tissues, particularly the brain, is not known. In piglets, the brain content of LC-PUFA is not correlated as highly with the content in plasma as is the content in erythrocytes, skeletal muscle, and liver (77). In contrast, one of the postmortem studies showed a weak, but statistically significant, correlation between brain and erythrocyte contents of 22:6ω3 (55). The correlation between the erythrocyte content of this fatty acid and its content in other tissues was not reported.

Studies in isolated cell systems suggest that 20:5ω3 and 22:5ω3 are transferred from plasma to astrocytes where they are converted to 22:6ω3, which, in turn, is transferred to neurons (5, 65, 66). Whether direct synthesis of 22:6ω3 within the central nervous system occurs in humans is not known, but at least one in vivo study in animals is compatible with this possibility (74).

Importance of Long-Chain Polyunsaturated Fatty Acids in Development

The findings discussed above, although far from definitive, are compatible with the possibility that failure to provide preformed LC-PUFA during early infancy, and perhaps longer, may compromise development of tissues/organs with a high content of these fatty acids, particularly 22:6ω3. However, the specific role(s) of LC-PUFA in normal development is (are) not clear (91, 54). It is known that these fatty acids affect gene transcription. They also may produce post-translational modifications. Moreover, many are precursors of eicosanoids that, in turn, modify a number of processes. These fatty acids also have other effects on signal transduction. Finally, the amount of these fatty acids in cell membranes can modify membrane fluidity, membrane thickness, and the microenvironment of the membrane as well as interactions between the fatty acid and membrane proteins. Such changes, in turn, can affect receptor function; in addition, the fatty acids may have direct effects on receptor function. Although the degree of unsaturation of membrane fatty acids affects fluidity, this effect is most marked by substituting a monounsaturated fatty acid or a fatty acid such as 18:2ω6 for a saturated fatty acid. In 22:6ω3 deficiency, 22:5ω6 replaces 22:6ω3 with little effect on fluidity. Possible mechanisms for the effects of LC-PUFAs have been reviewed recently by Uauy et al. (91) and by Lauritzen et al. (54).

Despite the lack of a clear mechanism of the role of LC-PUFAs in development, a number of studies over the past several years have focused on differences in visual

acuity and neurodevelopmental indices between breast-fed and formula-fed infants as well as between infants fed LC-PUFA-supplemented and unsupplemented formulas. Since human milk contains a number of factors other than LC-PUFA that might affect visual acuity and/or neurodevelopmental indices, studies comparing breast-fed with formula-fed infants cannot help resolve the role of LC-PUFA in infant development. On the other hand, studies taking advantage of the natural variability in milk contents of LC-PUFA or enhanced variability secondary to maternal supplementation and, hence, differences in LC-PUFA intake of the recipient infants (31, 33, 46, 61) provide important insights. The following discussions of the effect of LC-PUFA intake on visual function and on cognitive/behavioral development are limited to findings from these types of studies and findings from studies in which LC-PUFA-supplemented formulas were compared with unsupplemented formulas.

Long-Chain Polyunsaturated Fatty Acid Intake and Visual Function

Early studies in rodents established the importance of ω3 fatty acids for normal retinal function (3, 95) and subsequent studies established this in primates (69, 70). More recently, studies have focused on the effect of ω3 fatty acids on retinal function and/or overall visual function of human infants. However, whereas the abnormal retinal/visual function of ω3 fatty acid–deficient animals clearly resulted from an inadequate intake of 18:3ω3 and were reversed by adding this fatty acid, the more recent studies in human infants have focused primarily on the effects of 22:6ω3 intake on retinal and/or visual function. Studies have been conducted in both term and preterm infants and have utilized both behaviorally based and electrophysiologically based methods for assessing visual function.

The most commonly utilized behaviorally based method for assessing visual acuity, the Teller Acuity Card procedure, is based on the innate tendency to look toward a discernible pattern rather than a blank field (23, 24, 64). This rapid measure of resolution acuity combines forced-choice and operant preferential looking procedures. The subject is shown a series of cards with stripes (gratings) of different widths on one side and a blank field on the other and his/her looking behavior is observed through a peephole in the center of the card. Cards with wider stripes are shown initially, followed by cards with progressively decreasing stripe widths. The subject's visual acuity is assumed to be the finest grating toward which he/she clearly looks preferentially (i.e., the finest grating that he/she is able to resolve).

The electrophysiologically based tests utilize visual evoked potentials (VEPs), which measure the activation of the visual cortex in response to visual information that is processed by the retina and transmitted to the visual cortex (88, 89). The presence of a reliable evoked response indicates that the stimulus information was resolved up to the visual cortex, where the response is processed. Use of VEPs to assess visual acuity requires measuring the electrical potentials of the visual cortex in response to patterns of contrast reversal with vertical square wave gratings

or checkerboards. The frequency of the gratings or checkerboards is decreased from low (large) to high (small), and the visual acuity threshold is estimated by linear regression of the VEP amplitudes versus the frequency, or size, of the grating or checkerboard stimulus (71, 88). Data are recorded as the $\log_{10}$ of the minimum angle of resolution (logMAR), which is the smallest grating that results in a measurable amplitude. Thus, smaller logMAR values indicate better visual acuity. A rapid VEP method (sweep VEP) has been developed for use in infant populations (71).

The standard VEP also allows assessment of latency, or the time between presentation of a stimulus and the peak of the electrical potential. This reflects the rate of transmission of the stimulus and, hence, should be useful for assessing the effects of LC-PUFA (or other intervention). However, it has been used for this purpose by only a few investigators (10, 28, 49, 61, 93), perhaps because it does not provide a direct assessment of acuity.

Electroretinography, unlike the above procedures that measure the response of the entire visual system, measures only the activity of the retina (41, 68). However, this methodology is somewhat more invasive and time consuming than the other methods and has been used to assess effects of LC-PUFA in only a few studies (6, 89). The primary components of the electroretinogram generated in response to a flash of light are the a-wave, which is produced by hyperpolarization of the photoreceptor, and the b-wave, which reflects the subsequent activation of retinal neurons. Performance is quantified by parameters (41, 68) such as the threshold (the minimal intensity of light necessary to elicit a small amplitude), the implicit time or peak latency (the time from the presentation of a brief flash of light to the response peak), the maximal amplitude, and the sensitivity (the intensity of light that elicits a response of half the maximal amplitude).

Meta-analyses of data from studies using both behavioral and electrophysiological methods of assessment in both term and preterm infants have been reported (82, 83). Data from behaviorally based tests of visual acuity obtained in randomized studies of term infants fed 22:6ω3-supplemented versus unsupplemented formula show a statistically significant advantage of the supplemented formula at 2 months of age but not at other ages. Data from randomized studies utilizing electrophysiological methods of assessment in term infants fed 22:6ω3-supplemented versus unsupplemented formula showed no statistically significant advantages at any age (82).

A recently reported relatively large multicenter study (2), which was not included in the meta-analysis, showed no advantages of 22:6ω3 (0.14% of total fatty acids) plus 20:4ω6 (0.46% of total fatty acids) supplementation on visual acuity as assessed by the Teller Acuity Card procedure at 2, 4, 6, or 12 months of age. Whether inclusion of these additional data in a future meta-analysis will change the conclusions is not clear.

Recent studies of different designs also have shown an apparent relationship between 22:6ω3 intake and visual acuity measured by the Teller Acuity Card procedure. In one such study (46), term infants were breast-fed exclusively for

at least three months following birth and then weaned to a standard formula. Visual acuity at 2 and 12 months of age was significantly correlated with the 22:6ω3 content of erythrocyte phosphatidylethanolamine at 2 months of age, but acuity at 4 and 6 months of age was not. In addition, infants with an erythrocyte phosphatidylethanolamine 22:6ω3 concentration $>$10.78 g/100 g at 2 months of age (i.e., the upper tertile) had significantly better acuity at 2 and 12 but not at 4 and 6 months of age than those with an erythrocyte phosphatidylethanolamine 22:6ω3 concentration $<$8.53 g/100 g at 2 months of age (i.e., the lowest tertile).

Birch et al. (8) recently reported results of a randomized controlled trial of supplemented (0.36% of total fatty acids as 22:6ω3 and 0.72% 20:4ω6) versus unsupplemented formula following near-exclusive breast-feeding (one feeding/day of formula allowed) for the first six weeks of life. Visual acuity of the two groups, measured by sweep VEP, was similar at enrollment, but that of the supplemented group was better at 17, 26, and 52 weeks of age. Random dot stereoacuity of the supplemented group also was better at 17 weeks of age but not at 26 and 52 weeks of age. Random dot stereopsis, which reflects processing in the visual cortex, is not present before 3 months of age and develops rapidly between 3 and 5 months of age. It is thought to be particularly sensitive to differences in maturation of the visual cortex between 3 and 5 months of age. If so and if adequate 22:6ω3 is necessary for optimal maturation, a dietary source of this fatty acid between 3 and 5 months of age ($\sim$12 and $\sim$20 weeks) should enhance development of random dot stereopsis.

In a similar study, Hoffman et al. (35) assigned infants to the same formulas after 4 to 6 months ($\sim$16 to $\sim$24 weeks) rather than 6 weeks of near-exclusive breast-feeding. Visual acuity of the supplemented group, assessed by sweep VEP, was better at 12 months of age but there was no difference in random dot stereoacuity between the two groups, presumably because the infants were still breast-fed during the period of rapid development of the visual cortex required for optimal stereopsis. Nonetheless, these findings suggest a possible need for 22:6ω3 beyond 4 to 6 months of age, the maximum duration of supplementation in most previous studies.

This possibility is further supported by another recent study by the same group of investigators (37). In this study, 6-month-old infants who had been primarily breast-fed were randomly assigned to either complementary foods that contained no 22:6ω3 or complementary foods with 22:6ω3-enriched egg yolk. Many in both groups continued breast-feeding, but this was not quantitated. The primary outcome variable was visual acuity assessed by sweep VEP at 12 months of age. Acuity of the control group improved from 0.49 log MAR at 6 months of age to 0.29 log MAR at 12 months of age, and that of the 22:6ω3-supplemented group improved from 0.48 log MAR at 6 months to 0.14 log MAR at 12 months of age, indicating a more rapid rate of retrieval and/or visual cortex maturation in the supplemented group. 22:6ω3 intake of the supplemented group from complementary foods was 83 mg/d and that of the control group was 0 mg/d. The 22:6ω3 content of erythrocyte lipids of the control group decreased by 21% between 6 and 12 months of age, whereas that of the supplemented group increased by 34%.

As observed in other studies (see above), VEP acuity of the total population at 12 months of age was significantly correlated with erythrocyte lipid 22:6ω3 content, further supporting the potential benefit of 22:6ω3 beyond 6 months of age.

Finally, a retrospective study of 435 children showed that stereoacuity was better at 3.5 years of age in children who had been breast-fed for at least four months than in those who had been formula-fed (97). Further, stereoacuity of the breast-fed infants at 3.5 years of age was greater in those whose mothers ate oily fish during pregnancy than in those whose mothers did not.

The aforementioned meta-analysis of data from randomized studies in preterm infants showed an advantage of 22:6ω3-supplemented versus unsupplemented formulas on both behaviorally based and electrophysiologically based measurements of visual acuity (83). Advantages with behaviorally based tests were apparent at 2 and 4 months post-term and advantages with electrophysiologically based measurements were apparent at 4 months post-term. A recent randomized, controlled trial in preterm infants (72), when analyzed according to intent to treat, showed no advantage of 22:6ω3 and 20:4ω6 supplementation (0.26% and 0.42% of total fatty acids, respectively, from birth to term, and then 0.16% and 0.42%, respectively, through the first year of life) on visual acuity at 2, 4, or 6 months of age as assessed by the Teller Acuity Card procedure or VEP. However, posthoc analysis showed that, in a subset of infants, there was an advantage of supplementation on acuity as assessed by sweep VEP at 6 but not at 4 months post-term. Another recent multicenter study in which supplemented (0.36% of total fatty acids as 22:6ω3 and 0.72% as 20:4ω6) versus unsupplemented formulas were fed to preterm infants for an average of $\sim$28 days during hospitalization showed no effect on visual acuity at either 48 or 57 weeks postmenstrual age as assessed by the Teller Acuity Card procedure (44). Electrophysiological assessments were not performed. In yet another study (93) in which preterm infants were assigned randomly to receive a supplemented (0.34% and 0.70% of total fatty acids as 22:6ω3 and 20:4ω6, respectively, both from single cell oils) versus an unsupplemented formula until a corrected age of 6 months, visual acuity as assessed by the Teller Acuity Card procedure did not differ between groups at 3, 6, 12, or 24 months of age. VEP latency and amplitude of the two groups, assessed at 3 and 12 months of age, also did not differ (see below).

In apparent contrast to some of the findings discussed above, including the findings of the meta analyses (82, 83), recent Cochrane reviews of much, but not all, of the same data conclude that there are no consistent effects of LC-PUFA on visual acuity of either term (87) or preterm infants (86). In a review of most, but again not all, reported randomized controlled trials of supplemented versus unsupplemented formulas in both term and preterm infants in 2001, Gibson et al. (30) concluded that the evidence for a beneficial effect of LC-PUFA supplementation on visual function of preterm infants was "reasonably compelling," whereas the evidence of a beneficial effect on visual function of term infants was less so. Clearly, there is no consensus concerning the effect of LC-PUFA supplementation of either term or preterm infants on visual function. In part, this may be related to the fact that the

studies reported to date have utilized different types as well as different durations of supplementation, making it difficult to reach a conclusion. An additional problem is the possibility that some infants might benefit from supplementation, whereas others receiving the same supplement may not.

Long-Chain Polyunsaturated Fatty Acids and Cognitive/Behavioral Development

Most studies addressing the cognitive/behavioral development of infants fed LC-PUFA-supplemented versus unsupplemented formulas have utilized the Bayley Scales of Infant Development, which are considered the gold standard for assessing global abilities of infants from birth to 42 months of age. They provide standardized indices of both mental (MDI) and psychomotor development (PDI). However, they are intended to distinguish between "normal" and "abnormal," not degrees of either. Thus, unless cognitive and/or psychomotor function as assessed by the Bayley Scales early in life is definitely abnormal, the relationship between these early scores and later function is poor (63).

The Fagan Test of Infant Intelligence (FTII) also has been used in several studies, either alone or in addition to the Bayley Scales. This test assesses novelty preference (27). The infant is shown a single stimulus (usually a face) for a standardized, age-based period, and then is shown this stimulus along with a novel one. If the infant has "learned" the original stimulus prior to the novelty test, the typical response is to look selectively toward the novel rather than the familiar image. Scores on this test during infancy are somewhat more predictive of later cognitive function than the Bayley MDI; however, its internal consistency (reproducibility), unlike that of the Bayley Scales, is relatively poor (21). Look duration during the familiarization and the paired comparison phases of the test also is a modest predictor of both concurrent performance on other tests during infancy and later tests of intelligence (21); shorter look durations during the familiarization phase predict better concurrent as well as later cognitive performance.

One or both of these tests has been used to evaluate the effect of LC-PUFA supplementation on cognitive/behavioral development. Some of these studies have shown advantages of LC-PUFA supplementation with both tests, some with one but not the other, and still others with neither. Available studies in term infants were reviewed in 1998 by an expert panel appointed by the Life Sciences Research Organization to assess the nutrient requirements for term infant formulas (76). The studies reviewed were criticized by consultants to the panel for including too few infants, failing to control adequately for confounding factors, failing to assess function at more than one age, failing to examine individual differences in development, and failing to follow the infants for a sufficiently long period (e.g., none of the studies available at that time included data beyond 1 year of age). Based partially on this critique, the panel did not recommend addition of LC-PUFA to term infant formulas but suggested that the issue be re-evaluated in about five years.

The randomized trials in term infants published since 1998 (2, 7, 57, 60) have not resolved many of these difficulties. The trials have differed with respect to the source of LC-PUFA supplementation, the duration of supplementation, the amounts of 22:6ω3 and 20:4ω6 supplementation, and the 22:6ω3/20:4ω6 ratio. There also were some differences in the 18:2ω6 and 18:3ω3 contents of the control and experimental formulas. The variance in Bayley MDI and PDI scores also varied among studies, being smallest in the one study that showed an advantage of 22:6ω3 and 20:4ω6 supplementation for the first 4 months of life on the Bayley MDI score at 18 months of age (7).

Fewer studies are available in preterm infants fed LC-PUFA-supplemented versus unsupplemented formulas, and these are subject to many of the same criticisms levied against the studies in term infants. The available data, including those from recently reported large, multicenter studies (20, 72), suggest that preterm infants may be more likely than term infants to benefit from supplementation. However, it is important to note that this conclusion is based on posthoc analyses of data from selected groups of the study populations (e.g., infants who received only the assigned formula rather than the assigned formula plus breast milk; infants who weighed less than 1250 g at birth; infants whose parents were English speaking). Analysis of the data according to how the infants were randomized shows few differences in any outcome variable among groups. The study of van Wezel-Meijler et al. (93) showed no difference in scores on the Dutch version of the Bayley Scales of Infant Development between preterm infants who were assigned to receive supplemented (0.34% and 0.70% 22:6ω3 and 20:4ω6, respectively) versus unsupplemented formulas through 6 months corrected age (see below). This was true at 12 and 24 as well as at 3 and 6 months of age.

A few methods other than the Bayley Scales and the FTII have been used to assess the effects of LC-PUFA on cognitive/behavioral development. For example, Willats et al. (96) found that term infants assigned to a formula supplemented with both 22:6ω3 and 20:4ω6 versus an unsupplemented formula had better visual habituation scores at 4 months of age and performed better on a means-end problem-solving test at 10 months of age. The supplemented group not only had more intentional solutions to items of this test but also scored higher than those assigned to the control formula, findings that have been related to higher intelligence quotient scores later in childhood.

Innis et al. (46), studying breast-fed term infants with a range of 22:6ω3 and 20:4ω6 as well as 18:2ω6 and 18:3ω3 intakes and, hence, a range of plasma and erythrocyte lipid 22:6ω3 and 20:4ω6 contents, found no statistically significant relationships between infant 22:6ω3 and 20:4ω6 status at 2 months, when all infants were exclusively breast-fed, and scores on an object-search test at either 6 or 12 months of age. There also was no statistically significant relationship at either 6 or 12 months of age between infant 22:6ω3 or 20:4ω6 status at 2 months of age and novelty preference, Bayley MDI, or Bayley PDI scores. However, there was a positive relationship between ability to discriminate non-native retroflex and phonetic contrasts at 9 months of age and the 22:6ω3 content of plasma

phospholipid as well as erythrocyte phosphatidylethanolamine at 2 months of age. This finding was interpreted by the investigators to indicate more rapid language development in those with higher plasma and erythrocyte lipid levels of 22:6ω3 at 2 months of age.

Two other studies of the effect of maternal 22:6ω3 supplementation and, hence, intake of 22:6ω3 by the breast-feeding infant are relevant. Gibson et al. (31) supplemented breast-feeding mothers with varying amounts of 22:6ω3 and achieved breast milk 22:6ω3 concentrations ranging from 0.1%–1.7% of total fatty acids and, hence, a wide range in 22:6ω3 content of infant plasma lipids. Although there was no relationship between the 22:6ω3 content of milk or infant plasma lipids and VEP acuity at either 12 or 16 weeks of age, Bayley MDI scores were weakly correlated with milk 22:6ω3 content at 12 but not at 24 months of age.

In a somewhat similar study, Jensen et al. (50) assigned breast-feeding mothers to receive 22:6ω3 ($\sim$250 mg/d as an algal-derived triglyceride) or a control oil containing no 22:6ω3 for the first 4 months postpartum. At 4 months of age, plasma phospholipid 22:6ω3 content of infants whose mothers had received 22:6ω3 was approximately 50% higher than that of infants whose mothers received the control oil, but there was no difference in visual acuity between the two groups at either 4 or 8 months of age, whether assessed by sweep VEP or the Teller Acuity Card procedure. There also was no difference between groups in scores on a variety of neurodevelopmental tests at either 12 or 18 months of age. However, at 30 months of age, the mean Bayley PDI of the group whose mothers received 22:6ω3 was eight points higher than that of the group whose mothers received the control oil ($p < 0.01$). There was no statistically significant difference in mean Bayley MDI between the two groups at 30 months of age. Nor was there a statistically significant relationship between either PDI or MDI scores at 30 months of age and plasma phospholipid 22:6ω3 content at 4 months of age.

Helland et al. (33), examining the effect of supplementing women with either cod liver oil ($\sim$1.2 g/d of 22:6ω3 and 0.8 mg/d of 20:5ω3) or corn oil from week 18 of pregnancy until 3 months after delivery, found that children whose mothers received cod liver oil scored higher at 4 years of age on the Mental Processing Composite of the Kaufman Assessment Battery for Children (106.4 ± 7.4; $n = 48$) than children whose mothers received corn oil (102.3 ± 11.3; $n = 36$). In a multiple regression model, maternal 22:6ω3 intake during pregnancy was the only variable related significantly to the mental processing scores of the children at 4 years of age.

In a somewhat similar study, Malcolm et al. (61) assigned pregnant women to fish oil ($n = 50$) or control oil capsules ($n = 50$) from week 15 of pregnancy through term. The fish oil capsules provided about 200 mg 22:6ω3 per day. Although the 22:6ω3 content of maternal erythrocytes at birth was $\sim$50% higher in the supplemented group, the 22:6ω3 content of umbilical cord erythrocytes did not differ between the two groups and there was no difference in erythrocyte 22:6ω3 contents between groups at either 50 or 66 weeks postconceptional age. However, infants with higher erythrocyte 22:6ω3 content at birth had shorter pattern-evoked VEP latencies at 50 and 66 weeks postconceptional age than those with

lower erythrocyte 22:6ω3 content at birth. This finding suggests more rapid nerve conduction.

Long-Chain Polyunsaturated Fatty Acids and Other Aspects of Central Nervous System Development

The effects of LC-PUFA supplementation of infant formulas on other aspects of brain function and/or development also have been examined. Three studies showed no effects of 22:6ω3-supplemented versus unsupplemented formulas on brain auditory evoked potentials of preterm infants (10, 28, 91). In one of these studies (10), supplemented infants had peripheral nerve conduction that was slower than that of infants fed human milk but not slower than that of infants fed the control formula.

Another recent study (11) investigated the effect of supplemented (0.3% of total fatty acids, by weight, as 22:6ω3 and 0.45% as 20:4ω6 from a mixture of egg yolk, tuna oil, and a fungal oil) versus unsupplemented formula during the first 2 months of life on the quality of the infants' general movements at 3 months of age as assessed from videotapes made at that time. The quality of general movements is thought to be useful for evaluating the overall quality of brain function in young infants. The unsupplemented group had mildly abnormal general movements significantly more often than did either the supplemented group or a breast-fed group that was studied concurrently (31%, 19%, and 20%, respectively); however, the frequency of "normal optimal" movements did not differ between supplemented and unsupplemented groups (18% and 21%, respectively) but was less in both than was the frequency observed in the breast-fed group (34%).

Only one study has examined the effect of LC-PUFA supplementation on structural brain development (93). In this study, preterm infants were assigned randomly to receive either a standard formula ($n = 20$) or a formula supplemented with 0.34% and 0.70% of total fatty acids as 22:6ω3 and 20:4ω6, respectively ($n = 22$), both from single cell oils, until a corrected age of 6 months (preterm formula until weight reached 3000 g and a term formula thereafter). Brain structural development, assessed by magnetic resonance imaging at 3 and 12 months corrected age, did not differ between groups at either age. This method essentially assesses the degree of myelination and, in this study, neither global myelination nor myelination of the cerebral visual system differed between groups. This may not be surprising since the LC-PUFA content of myelin is low. However, myelin deposition is dependent on close interaction among neurons, their axons, and their oligodendrocytes, all of which are rich in LC-PUFA. Thus, myelination is thought to reflect the functional maturity of all these components.

Effects of Long-Chain Polyunsaturated Fatty Acids on Growth

The observation in the early 1990s that preterm infants assigned to a formula supplemented with fish oil (0.3% of total fatty acids as 20:5ω3 and 0.2% as 22:6ω3) weighed less and had a lower weight-for-length at various times during the first year

of life than did infants assigned to an unsupplemented formula (13) has generated considerable concern. In this study, weight at 12 months corrected age correlated with plasma phospholipid 20:4ω6 content at various times during the first year of life (15). A less marked effect on growth was observed subsequently by the same investigators in preterm infants fed a formula supplemented with low-20:5ω3 fish oil versus an unsupplemented formula (16). In this study, there was no correlation between 20:4ω6 status and growth, but there was a correlation between weight at some ages and the plasma phospholipid ratio of 20:4ω6/22:6ω3.

Interestingly, a smaller study, also conducted in the early 1990s, did not show differences in growth between supplemented and unsupplemented preterm infants, although the supplemented group received even more of a similar fish oil (90). However, the duration of this study may not have been sufficient to permit detection of weight differences.

Ryan et al. (79) observed lower rates of growth in preterm male, but not female, infants fed a formula supplemented with low-20:5ω3 fish oil (0.2% of total fatty acids as 22:6ω3) versus a control formula from shortly before hospital discharge until 59 weeks postmenstrual age (PMA). In this study, as in most studies in which 20:4ω6 is not supplemented, plasma phospholipid 20:4ω6 content of the supplemented group was lower through 59 weeks PMA, but there was no statistically significant correlation between plasma phospholipid 20:4ω6 content and any aspect of growth. Rather, rates of increase in both weight and length of male infants were inversely correlated with plasma phospholipid 22:6ω3 content.

A lower weight at 4 months of age also was observed in term infants fed formulas with a 18:2ω6:18:3ω3 ratio of $\sim$4 versus $\sim$40 and, in this study, weight at 4 months of age was correlated with plasma phospholipid 20:4ω6 content (49).

More recently, Innis et al. (46), studying infants who were breast-fed exclusively for the first 3 months of life, reported a statistically significant inverse correlation between erythrocyte phosphatidyl choline and phosphatidyl ethanolamine contents of 22:6ω3 and weight at 6 but not at 12 months of age. There was no statistically significant relationship between 22:6ω3 or 20:4ω6 contents of plasma lipids and size at any age.

In contrast to these observations of a possible adverse effect of ω3 fatty acids on growth, Innis et al. (44) reported a more rapid rate of growth in preterm infants fed a formula supplemented with both 22:6ω3 (0.33% of fat as an algal oil) and 20:4ω6 (0.6% of fat as a fungal oil) versus a formula supplemented with only 22:6ω3 (0.34% of fat as an algal oil) or infants fed an unsupplemented formula for at least 28 days prior to hospital discharge and followed until 57 weeks PMA. Another recent study [so far reported only as an abstract (20)] also showed a positive growth effect of formulas supplemented with both 22:6ω3 and 20:4ω6 either as single cell oils or a mixture of fish and fungal oils (20). In this study, weight of preterm infants fed the formula supplemented with single cell oils was greater than that of the control infants from 66 through 118 weeks PMA and equal to that of breast-fed term infants at 118 weeks PMA. Length of this group also was greater at 79 and 92 weeks PMA than that of either the control group or the

group supplemented with fish and fungal oil and equal to that of the breast-fed term infants by 79 weeks PMA.

The reason(s) for an inhibitory effect of ω3 fatty acids or, perhaps, a stimulatory effect of ω6 fatty acids on growth are not clear (53). Possibilities that have been suggested include inhibition of desaturation and elongation of 18:2ω6 to 20:4ω6 by the ω3 fatty acids, inhibition of eicosanoid synthesis from 20:4ω6 by the intake of preformed 20:5ω3 or endogenous synthesis of 20:5ω3 from a moderately high intake of 18:3ω3, and effects of ω3 and ω6 fatty acids on transcription of genes controlling lipolysis and lipogenesis.

SUMMARY

The LC-PUFAs 22:6ω3 and 20:4ω6 are the major ω3 and ω6 fatty acids, respectively, in the developing central nervous system, and 22:6ω3 comprises 30%–40% of the fatty acid content of photoreceptor membranes. Hence, both are thought to be needed for optimal development of the brain and retina. Both fatty acids are present in human milk and the content of both, particularly 22:6ω3, are lower in the plasma and erythrocyte membranes as well as the brain of formula-fed in comparison with breast-fed infants. Moreover, some but by no means all studies of the effects of these fatty acids on neurodevelopmental as well as visual outcomes suggest that provision of preformed 22:6ω3 and 20:4ω6, particularly the former, may be beneficial. Hence, these fatty acids are often considered conditionally indispensable nutrients.

Both term and preterm infants can synthesize 22:6ω3 and 20:4ω6 from their respective precursors, 18:3ω3 and 18:2ω6; however, the amounts synthesized are difficult to quantitate. Furthermore, although intake of 18:2ω6 (which is usually high) has little effect on plasma lipid content of 20:4ω6, even a relatively high intake of 18:3ω3 does not result in a plasma lipid content of 22:6ω3 equal to that of breast-fed infants. Many studies, therefore, have examined visual and neurodevelopmental outcomes of infants fed unsupplemented formulas versus formulas supplemented with either 22:6ω3 or both 22:6ω3 and 20:4ω6. Some of these studies have shown advantages of supplementation, particularly for preterm infants, but roughly an equal number have not. An intriguing and logical explanation for these discrepant findings is that the rates of endogenous synthesis and, hence, the requirements of 22:6ω3 vary considerably from infant to infant. However, until rates of endogenous synthesis can be more accurately quantitated than is currently possible, this potential reason for the discrepancies among studies must remain theoretical. Finally, considering the generally satisfactory outcomes of infants fed unsupplemented formulas, any advantage of 22:6ω3 (or 22:6ω3 plus 20:4ω6) supplementation is likely to be subtle and possibly not detectable by currently available methods of assessment.

Formulas containing 22:6ω3 and 20:4ω6 are now available throughout the world and, regardless of their efficacy, appear to be safe. Although some studies have

suggested that ω3 fatty acids may inhibit growth, most have shown no effects on growth, and recent studies suggest that supplementation of formulas with 22:6ω3 and 20:4ω6 may enhance growth of preterm infants. Current studies focus on possible long-term effects of supplementation during early life as well as possible benefits of supplementation during pregnancy and lactation and beyond the first 4 to 6 months of life, when infants are either breast-fed or fed supplemented formulas.

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