Essential fatty acids in infant nutrition: lessons and limitations from animal studies in relation to studies on infant fatty acid requirements¹,²

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ABSTRACT Animal studies have been of pivotal importance in advancing knowledge of the metabolism and roles of n-6 and n-3 fatty acids and the effects of specific dietary intakes on membrane composition and related functions. Advantages of animal studies include the rigid control of fatty acid and other nutrient intakes and the degree, timing, and duration of deficiency or excess, the absence of confounding environmental and clinical variables, and the tissue analysis and testing procedures that cannot be performed in human studies. However, differences among species in nutrient requirements and metabolism and the severity and duration of the dietary treatment must be considered before extrapolating results to humans. Studies in rodents and nonhuman primates fed diets severely deficient in α-linolenic acid (18:3n-3) showed altered visual function and behavioral problems, and played a fundamental role by identifying neural systems that may be sensitive to dietary n-3 fatty acid intakes; this information has assisted researchers in planning clinical studies. However, whereas animal studies have focused mainly on 18:3n-3 deficiency, there is considerable clinical interest in docosahexaenoic acid (22:6n-3) and arachidonic acid (20:4n-6) supplementation. Information from animal studies suggests that brain and retinal concentrations of 22:6n-3 plateau with 18:3n-3 intakes of ~0.7% of energy, but this requirement is influenced by dietary 18:2n-6 intake. Blood and tissue concentrations of 22:6n-3 increase as 22:6n-3 intake increases, with adverse effects on growth and function at high intakes. Animal studies can provide important information on the mechanisms of both beneficial and adverse effects and the pathways of brain 22:6n-3 uptake. Am J Clin Nutr 2000;71(suppl):238S–44S.

KEY WORDS Essential fatty acids, n-6 fatty acids, n-3 fatty acids, arachidonic acid, docosahexaenoic acid, α-linolenic acid, animal studies, animal models, infant development, neurologic development, retina, brain, fatty acid requirements, visual function

INTRODUCTION Two series of polyunsaturated fatty acids, the n-6 and n-3 fatty acids, are essential in the human diet (1). There is uncertainty, however, about how much of and which of the n-6 and n-3 fatty acids are needed in the diets of young infants. The scientific literature includes many studies that suggest that as a group, term infants who are breast-fed have a developmental advantage and subsequently a higher intelligence quotient (IQ) than do infants who are not breast-fed (2–7). These observations have generated considerable research into the possible roles of breast-milk components in providing for optimum infant development. The presence of high concentrations of the n-6 fatty acid arachidonic acid (20:4n-6) and the n-3 fatty acid docosahexaenoic acid (22:6n-3) in specific membranes of the brain and retina (8, 9) and of small amounts of these fatty acids in breast milk (10) has raised questions about the potential role of dietary sources of 20:4n-6 and 22:6n-3 in facilitating optimal development. Animal studies have led to considerable understanding of the dietary requirements for and the metabolism and physiologic roles of n-6 and n-3 fatty acids during growth and development. However, there is still much to be learned about the roles of various n-6 and n-3 fatty acids in visual and neural function and other areas, ie, the regulation of gene expression. Animal studies can focus on a range of dietary conditions including extreme deficiencies, nutrient interactions, and excessive intakes. Thus, animal research still has great potential for advancing our knowledge of the roles of specific fatty acids in biochemical processes and for defining the safe and adequate ranges of intakes at different stages of life (Table 1).

One of the advantages of animal studies is that they are able to overcome many of the complexities and limitations of studies of nutrition and outcome in young human infants. These limitations and complexities include bias in socioeconomic class and other environmental and genetic factors that are difficult to control, particularly in small studies. When relating the results of animal studies to human infants, important factors to consider include the choice of animal species for study, the stage of development (eg, prenatal or postnatal), and the duration and severity of deficiency or excessive intake. There is considerable interest

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ESSENTIAL FATTY ACID METABOLISM

Studies conducted over a half century ago established that the fatty acid composition of dietary lipids influences the fatty acid composition of tissue lipids, and that certain fatty acids that cannot be formed in animal cells are needed for normal growth, reproduction, and cell function (1). Researchers then discovered the importance of 18:2n−6 and 18:3n−3, found primarily in polyunsaturated vegetable oils, as precursors of synthesis of 20:4n−6 and 22:6n−3 in mammalian cells, and research turned toward elucidating the pathways of conversion.

Studies in animals have shown that all of the enzymes needed for synthesis of 20:4n−6 and 22:6n−3 are present in the liver, brain, and eye—specifically the retina (13–16). The final steps in the pathway of 22:6n−3 and 22:5n−6 formation were only recently elucidated, and were discovered during in vitro studies with animal cells. It is now known that the synthesis of 22:6n−3 from 20:5n−3 proceeds via elongation to 22:5n−3 and then to 24:5n−3 with a Δ6 desaturation to form 24:6n−3, followed by a partial β-oxidation to 22:6n−3 (17). The steps in the synthesis of 22:5n−6 from 20:4n−6 appear analogous to those of the n−3 series: 20:4n−6 to 22:4n−6 to 24:4n−6 to 24:5n−6, with chain shortening to 22:5n−6 (17). It is not yet clear whether these pathways involve more than one Δ6 desaturase for the 18- and 20-carbon chains and for n−6 and n−3 fatty acids, or if different elongation enzymes are involved. Further elucidation of these important points will be fundamental to understanding both dietary fatty acid requirements and the regulation of fatty acid metabolism.

An unusual feature of the brain and retina is that concentrations of 18:2n−6 and 18:3n−3 are low (generally <2% of total fatty acids), whereas concentrations of the products 20:4n−6 and 22:6n−3 are high (8, 9). In other tissues, 18:2n−6 can exceed 20% of total fatty acids and those concentrations increase with increasing dietary intake. Large amounts of 20:4n−6 and 22:6n−3 are incorporated into the central nervous system during development (18–20) in a membrane- and lipid-class–specific pattern (9). The relevant question is, do humans need to consume 20:4n−6 and 22:6n−3 from the diet, or can dietary 18:2n−6 and 18:3n−3 be converted to 20:4n−6 and 22:6n−3, thereby fulfilling tissue requirements for n−6 and n−3 fatty acids during growth and development?

Desaturation and elongation of 18:2n−6 and 18:3n−3 to 20:4n−6 and 22:6n−3, respectively, depends on the dietary content of 18:2n−6 and 18:3n−3 and the ratio of one to the other. In addition, 20:4n−6, 20:5n−3, and 22:6n−3 appear to inhibit desaturation of the 18-carbon precursors and are actively incorporated into membrane lipids. The concentrations of 20:4n−6 and 22:6n−3 found in tissue glycerophospholipids are therefore the net result of the rates of endogenous synthesis from 18:2n−6 and 18:3n−3; respectively, the amounts of preformed 20:4n−6 and 22:6n−3 in the diet; and the activity of the acyl transferases involved in glycerophospholipid synthesis and remodeling (17, 21). It seems unlikely that 20:4n−6 and 22:6n−3 are synthesized from 18:2n−6 and 18:3n−3 in vivo in excess of the need for membrane synthesis, yet many studies have shown that increasing dietary 20:4n−6 and 22:6n−3 intake results in increased phospholipid concentrations of 20:4n−6 and 22:6n−3. This might be explained by the relatively low mitochondrial Δ6-oxida- tion of 20- and 22-carbon polyunsaturated fatty acids. This difference in metabolism and tissue fatty acid response to increasing intake suggests that the appropriate ranges of intake for 20:4n−6 and 22:6n−3 may be much lower and narrower than those for 18:2n−6 and 18:3n−3 (eg, 6–20% of dietary fatty acids as 18:2n−6 compared with 0.5–1.0% as 20:4n−6).

Unfortunately, no definitive information is as yet available on the preferred pathways by which the brain and eye normally acquire 20:4n−6 and 22:6n−3 (ie, by uptake and further metabolism of 18:2n−6 and 18:3n−3 or by uptake of 20:4n−6 and 22:6n−3 from the circulation) (1). Unlike the brain and eye, tissues such as the heart and kidney may lack some desaturase enzyme activities (22, 23). Similarly, in vitro evidence suggests that intestinal cells may not be able to form 22:6n−3, although synthesis of 20:4n−6 does occur (24, 25). Tissues without desaturase activity may therefore depend on uptake of 20:4n−6 and
22:6n−3 from the circulation to maintain membrane phospholipid 20:4n−6 and 22:6n−3 concentrations.

Animal studies have provided evidence that the brain synthesizes most, if not all, of the 16:0 and cholesterol incorporated into brain lipids (26, 27). This suggests that the brain has a high degree of selectivity and specificity for fatty acid uptake and may not utilize pathways of receptor-mediated lipoprotein uptake or lipoprotein-lipase–mediated hydrolysis of lipoprotein triacylglycerols in a manner similar to the liver, adipose tissue, or muscle. Further investigation of the pathways of n−6 and n−3 fatty acid metabolism and accretion in the brain will undoubtedly be of major importance in reaching a better understanding of the relations among diet, blood lipids, and brain accretion of n−6 and n−3 fatty acids.

Early studies of the n−6 and n−3 fatty acid requirements of newborn infants proposed that the activity of enzymes involved in desaturation, particularly that of a putative Δ3 desaturase (22:5n−3 to 22:6n−3), was low during the newborn period (28). More recent studies using stable isotopes, however, have shown that preterm and term infants are able to convert 18:2n−6 to 20:4n−6 and 18:3n−3 to 22:6n−3 via pathways similar to those in animals (29–31). However, the data derived from studies with stable isotopes have involved analyses of plasma, which may or may not reflect desaturation and elongation processes in organs such as the brain. Moreover, stable isotope studies are not yet quantitative. Thus, it remains unclear whether rates of 20:4n−6 and 22:6n−3 synthesis in young infants are sufficient to meet the needs of developing tissues.

**STUDIES WITH ANIMALS FED DIETS DEFICIENT IN n−3 FATTY ACIDS**

Studies in animals have shown that the brain and retina tenaciously retain 20:4n−6 and 22:6n−3, even during prolonged dietary deficiency of n−6 and n−3 fatty acids (1). Thus, severe experimental conditions of diet composition and duration have been used to generate 22:6n−3 deficiency in the developing central nervous system to explore the functional effects. Research in the 1970s identified reduced a- and b-wave amplitudes and reduced brain and retinal concentrations of 22:6n−3 in rats fed diets essentially devoid of 18:3n−3 (32, 33). Subsequently, studies with rhesus monkeys fed diets containing <0.04% and 0.1% of energy, respectively, from 18:3n−3 caused 4% and 8.6% 22:6n−3 compared with 15% and 17.3% 22:6n−3 in the control group, respectively (34). For comparison, 22:6n−3 usually represents 4–5% of fatty acids in plasma phospholipids of 12-wk-old, breast-fed, term infants and ≈2% of plasma phospholipid fatty acids of term infants bottle-fed with formula lacking 22:6n−3 (46–48). Autopsy analyses from studies conducted in Australia reported mean values of 7.5% 22:6n−3 in the brain cortex of term infants who had been fed formula compared with 8.5% 22:6n−3 (P < 0.05) in term infants who had been breast-fed (49). In this study, retinal concentrations of 22:6n−3 in the formula-fed and breast-fed infants were not significantly different. Animal studies have not yet reported functional differences resulting from differences in central nervous system 22:6n−3 concentrations within the range identified in human infant autopsy studies.

It is important to note that a fundamental contribution of studies in animals fed diets extremely low in 18:3n−3 has been the identification of some of the functional roles of 22:6n−3. Animal studies have been extremely valuable in identifying the neural systems (eg, visual function) that may be sensitive to dietary n−3 fatty acids so that clinical studies could focus on these systems. Future work with animals offers the potential to gain more specific information about the biochemical roles of 20:4n−6 and 22:6n−3 in these and other as yet unknown aspects of neural or other tissue functions.

**STUDIES OF n−6 AND n−3 FATTY ACID REQUIREMENTS IN ANIMALS**

Several studies have used the classic approach of increasing dietary intakes of 18:2n−6 and 18:3n−3 to assess the dietary...
requirements for n−6 and n−3 fatty acids (38, 50–53). As the intakes of 18:2n−6 and 18:3n−3 increase from zero, tissue concentrations of 20:4n−6 and 22:6n−3, respectively, increase rapidly and then plateau. This plateau is reached in brain, synaptic terminal, and retinal lipid 20:4n−6 and 22:6n−3 at intakes of ≈2.4% of energy from 18:2n−6 and ≈0.7% of energy from 18:3n−3. The plateau of 22:6n−3 concentrations in other organs, such as kidney and muscle, is attained at somewhat lower dietary intakes of 18:3n−3 (38).

Studies in neonatal piglets have evaluated the adequacy of dietary n−3 fatty acids for the developing central nervous system within the range potentially found in infant diets (50, 54–56). Corn oil contains ≈55% of its fatty acids as 18:2n−6 and 0.8% as 18:3n−3 and has been used in the past as the sole source of polyunsaturated fatty acids in some infant formulas. When blended with a source of saturated fatty acids (eg, coconut oil), formula made with corn oil typically provided ≈22% of energy as 18:2n−6 and 0.3% as 18:3n−3. Newborn piglets are useful animals in which to evaluate neonatal n−6 and n−3 fatty acid requirements because lipid metabolism and the composition of lipids in the brain and milk of pigs are similar to those of humans (57). Piglets fed from birth with formula providing 0.4% of energy as 18:3n−3 (with percentages of total fatty acids of 29% 18:2n−6 and 0.8% 18:3n−3) had significantly lower 22:6n−3 concentrations in the brain, brain synaptic terminal membranes, retina, liver, and blood lipids than did piglets fed sow milk (54–56). Of interest is that studies in premature and term infants fed formula with similar concentrations of 18:3n−3 from corn oil found reduced measures of visual function (58, 59). The lower brain and retinal concentrations of 22:6n−3 in piglets fed formula with 0.4% of energy as 18:3n−3 were accompanied by higher n−6 fatty acids, particularly 22:5n−6 (54–56). As in rodents and monkeys fed 18:3n−3-deficient diets (34, 35, 38–40), the higher 22:5n−6 concentrations resulted in central nervous system total n−6 plus n−3 fatty acid concentrations similar to those in the control animals. This pattern of reciprocal change in n−6 and n−3 fatty acids suggests an inadequate intake of 18:3n−3 or excessive n−6 fatty acid intake. Alternatively, the results could be explained by a requirement for dietary 22:6n−3, assuming that there is a difference between the pathways of n−6 and n−3 fatty acid metabolism.

Subsequent studies in piglets therefore addressed the effect of feeding formula with 18:3n−3 concentrations increased to 4% of fatty acids. The importance of the 18:2n−6 to 18:3n−3 ratio was addressed at the same time by feeding 4% 18:3n−3 with 16% or 35% 18:2n−6, or 1% 18:3n−3 with 16% or 30% 18:2n−6 (18:2n−6 to 18:3n−3 ratios of 4:1, 9:1, 16:1, and 30:1, respectively) (50, 60, 61). The results of these studies showed that piglets fed 4% 18:3n−3, but not those fed 1% 18:3n−3, had synaptic plasma membrane and retinal phospholipid 22:6n−3 concentrations similar to those fed sow milk. High dietary 18:2n−6 to 18:3n−3 ratios (30:1 compared with 16:1) exacerbated the 22:6n−3 deficiency imposed by the 1% 18:3n−3 diets (50). However, cerebrum weight, synaptic plasma membrane and retinal 20:4n−6 concentrations, and liver and brain concentrations of saturated fatty acids were reduced by the higher concentration of 18:3n−3 in the formula (50, 60, 61).

Studies in piglets have also evaluated the efficacy of providing preformed 22:6n−3 in formula as a source of 22:6n−3 for the developing central nervous system as well as the effects on 22:6n−3 and 20:4n−6 in the liver and other organs (60, 62, 63). Addition of 22:6n−3 (from fish oil; ≈0.3% of fatty acids) to formula without 20:4n−6 resulted in concentrations of 22:6n−3 in central nervous system tissues that were similar to those of piglets fed sow milk, with no apparent adverse effect on 20:4n−6 concentrations (50, 62). However, concentrations of 20:4n−6 in the plasma, liver, and kidney were significantly reduced (61, 62). When formula without 20:4n−6 was supplemented with 6 g fish oil/L formula, providing 0.9% 22:6n−3, the brain weights of piglets were also reduced (62).

STUDIES OF THE TRANSFER OF MATERNAL DIETARY FATTY ACIDS THROUGH BREAST MILK TO INFANTS

Breast-milk fatty acids usually contain 8–30% 18:2n−6, 0.5–2.0% 18:3n−3, 0.5–0.8% 20:4n−6, 0.1–0.4% 22:6n−3, and small amounts of other n−6 and n−3 fatty acids (10). It is now known that the variability in breast-milk n−6 and n−3 fatty acid concentrations is largely explained by variations in the fat composition of the mother’s diet (10, 64–66). Several studies have shown that the increases in breast-milk 22:6n−3 concentrations that follow supplementation of the maternal diet with 22:6n−3 result in increased 22:6n−3 concentrations in the blood lipids of breast-fed infants (64, 65). Thus, there is considerable interest in the effect of varying breast-milk fatty acid concentrations on tissue fatty acid accretion in breast-fed infants. Studies in piglets have shown that feeding milk with 1.5% 22:6n−3, similar to the upper end of the range of concentrations found in breast milk (10), results in significantly higher concentrations of 22:6n−3 in the plasma, liver, and brain of nursing piglets than are found in piglets fed milk with 0.1% 22:6n−3 (67). However, whereas 22:6n−3 concentrations changed from 2.6 ± 0.2 to 7.9 ± 0.2% in plasma and from 5.5 ± 0.5 to 15.8 ± 0.6% in liver, brain 22:6n−3 concentrations were 9.7 ± 0.2 and 10.9 ± 0.2% in piglets receiving milk with 0.1% compared with 1.5% 22:6n−3, respectively. Clearly, the wide variations in plasma 22:6n−3 concentrations in response to diet were not accompanied by similar changes in the brain (67). However, feeding the milk with the higher 22:6n−3 concentration (1.5% of fatty acids) was associated with a lower ratio of cerebrum to body weight than was found in piglets fed milk with low 22:6n−3 concentrations (67).

STUDIES IN ANIMALS FED DIETARY SOURCES OF ARACHIDONIC AND DOCOSAHEXAENOIC ACIDS

It is well established that addition of 20:4n−6 and 22:6n−3 to formulas results in increased concentrations of 20:4n−6 and 22:6n−3, respectively, in the blood lipids of formula-fed infants (68–70). In adults, inclusion of 20:4n−6 and 22:6n−3 in the diet similarly influences the concentrations of these fatty acids in plasma and red blood cell lipids (71, 72). Several sources of 20:4n−6 and 22:6n−3 are currently available for supplementation of infant or other diets. These include fish oils, egg total lipid or phospholipid, and oils derived from microalgal and fungal sources (single-cell triacylglycerols). Animal studies clearly provide the opportunity to explore whether potential sources of 20:4n−6 and 22:6n−3 for humans have advantageous or untoward effects at the tissue level, including the effects of other fatty acids or components in the oils. Animal research can also investigate whether the pathways of digestion, absorption, and tissue assimilation with these dietary sources are comparable to those with 20:4n−6 and 22:6n−3 from breast milk.
Little is known about the effects in human nutrition of single-cell-derived triacylglycerol containing ≥40% of its fatty acids as 20:4n-6 or 22:6n-3. A study of the acute effects of 20:4n-6 and 22:6n-3 at intakes similar to or ≤5 times the intakes provided to infants by breast milk found dose-dependent increases in plasma lipid 20:4n-6, 22:6n-3, and cholesterol concentrations and a dose-dependent decrease in plasma triacylglycerol concentrations in adult men (71). Several animal studies have shown that these oils are also efficacious sources of 20:4n-6 and 22:6n-3 for the developing brain (73–75). The addition of some fish oils to formula to provide 22:6n-3 has been found to reduce growth and alter neurodevelopment either positively or negatively in premature infants (76–79) and to possibly reduce language development in term infants (80). These effects seem strikingly similar to the reduced brain growth seen in piglets fed formula with high 18:3n-3 or 22:6n-3 concentrations. As with fish oils, high intakes of 22:6n-3 from single-cell triacylglycerol also lowered brain weight and 20:4n-6 concentration in mice (75). Recent information suggests that this effect of dietary n-3 fatty acids may be offset by including 20:4n-6 in the diet (75). The metabolic explanation, however, is not known. In another animal study, high intakes of 20:5n-3 and 22:6n-3 resulted in increased concentrations of 20:5n-3 and 22:6n-3 and reduced concentrations of 20:4n-6 in some lipid classes in the brain and retina; these fatty acid compositional changes were accompanied by reduced visual function (81). A biochemical explanation for the adverse effect of high tissue 22:6n-3 concentration on visual function is not available.

**SUMMARY AND CONCLUSIONS**

In summary, studies in animals fed well-controlled diets have played an important role in establishing the dietary essentiality of 18:2n-6 and 18:3n-3 and in elucidating the biochemical and physiologic roles of 20:4n-6 and 22:6n-3. Animal studies can provide much important information to further our understanding of the importance of the types and amounts of the different n-6 and n-3 fatty acids in the diet during various stages of fetal and infant development. If we extrapolate this information to humans, however, we must consider possible species differences in growth, development, nutrient metabolism and requirements, and the severity and timing of any experimental conditions. Of particular importance is that animal studies offer the opportunity to explore the reasons for any adverse effects of some oils high in n-3 fatty acids and the potential for avoiding these effects by cosupplementation with 20:4n-6. Animal research can also further our understanding of the biochemical pathways in which these fatty acids participate.

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