Long-chain polyunsaturated fatty acids and the preterm infant: a case study in developmentally sensitive nutrient needs in the United States

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ABSTRACT
The vast majority of infant formulas in the United States contain the long-chain polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (22:6n–3) and arachidonic acid (20:4n–6), which were first permitted by the US Food and Drug Administration in 2001. As a scientific case study, preclinical animal studies of these nutrients definitively influenced the design and interpretation of human clinical studies. Early studies were tied to the availability of test substances, and in hindsight suggest re-evaluation of the essential fatty acid concept in light of the totality of available evidence. Research in the 1950s established the essentiality of n–6 PUFAs for skin integrity; however, widespread recognition of the essentiality of n–3 PUFAs came decades later despite compelling evidence of their significance. Barriers to an understanding of the essentiality of n–3 PUFAs were as follows: 1) their role is in neural function, which is measured only with difficulty compared with skin lesions and growth faltering that are apparent for n–6 PUFAs; 2) the experimental use of vegetable oils as PUFAs sources that contain the inefficiently used C18 PUFAs rather than the operative C20 and C22 PUFAs; 3) the shift from reliance on high-quality animal studies to define mechanisms that established the required nutrients in the first part of the 20th century to inherently challenging human studies. Advances in nutrition of premature infants require the best practices and opinions available, taking into account the totality of preclinical and clinical evidence. Am J Clin Nutr doi: 10.3945/ajcn.114.103994.

Keywords: LC-PUFA, preterm infants, docosahexaenoic acid, arachidonic acid, essential fatty acids

INTRODUCTION
Nutrition in premature infants has been a major research topic in clinical nutrition for a quarter century. Human infants born at 27 wk of gestation or earlier have survived to reproductive age in appreciable numbers for only the past 40 y, approximately since the recognition that adequate lung function could be achieved with a combination of corticosteroids (1), studies first conducted in sheep (2), as well as the routine availability of neonatal respirators. Very premature infants are not fetuses nor are they term newborns, and thus they represent a new and metabolically unique population of humans. The customary and well-justified assumption that breast milk evolved to meet the needs of normal term newborns cannot be extended to this population in the same way it can for term infants; moreover, placental transfer, even if its composition could be monitored in detail through one-third of normal gestation, would similarly not apply strictly to preterm infants, who have the challenges of high oxygen tension, very different skin barrier functions and buoyancy issues required for life in air compared with amniotic fluid, and myriad other issues of life outside the womb. As a medical issue, the first goal of feeding is survival with basic functions preserved, with optimization of later function a secondary consideration.

PUFA nutrition in preterm infants has a long and complex history that has been successful by most standards, with numerous lessons that point to the art of medicine as much to the science of rigidly applied evidence-based medicine (EBM). The story applies to all infants regardless of postconceptional age at birth, although the current state of understanding indicates that it is more critical for preterm than for term infants. Clinical reviews appear regularly that discuss the current medical practice and issues around preterm infant intake of long-chain PUFAs (LC-PUFAs) (3, 4). The purpose here is to review the historical development of fatty acid nutrition leading to the recognition of PUFA requirements, with an emphasis on animal and preclinical data that informed the ongoing development of clinical evidence.

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3 This article’s contents are solely the responsibility of the author and do not necessarily represent the official views of the NCCAM, the ODS, or the NIH.
4 Supplemental material is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.
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5 Abbreviations used: AA, arachidonic acid (20:4n–6); AIN, American Institute of Nutrition; ALA, α-linolenic acid (18:3n–3); CNS, central nervous system; EBM, evidence-based medicine; EFA, essential fatty acid; EFSA, European Food Safety Agency; FADS2, fatty acid desaturase 2; FDA, Food and Drug Administration; LA, linoleic acid (18:2n–6); LC-PUFA, long-chain PUFA;RCT, randomized controlled trial.
PUFA STRUCTURE AND FUNCTION

In medicine and pharmacology, LC-PUFAs are known as precursors of highly active signaling molecules, particularly eicosanoids, for which a large array of drugs that modulate their synthesis have been available for a century, starting with the general cyclooxygenase inhibitor aspirin and continuing with nonsteroidal anti-inflammatory drugs and lipooxygenase inhibitors; more recent developments are modulators of docosanoids. These moderately water-soluble signaling molecules modulate clotting, inflammation, and a wide variety of specific functions such as parturition signals, all of which are easily shown by acute changes in physiology on dosing in experimental (e.g., in vitro) or clinical studies and usually at low concentrations.

Remarkably, a feature of LC-PUFAs that is less well appreciated is their role in modulating membrane function, even when present at high concentrations in specific membrane types, universally across mammalian species. Compared with signaling by water-soluble molecules, intramembrane protein-lipid interactions by fat-soluble LC-PUFAs are notoriously difficult to study simply because the tools available are relatively crude. Moreover, outcomes in studies of human neural function, in which structural LC-PUFAs are likely to be of great importance, are slow and imprecise compared with, for instance, the drama of a myocardial infarct or stroke. Neural functions such as cognition, mood, behavior, and impulse control are, however, of no less priority and by many measures no less a source of morbidity and mortality than vessel occlusion.

HISTORICAL DEVELOPMENT OF LINOLEIC ACID AND α-LINOLENIC ACID AS ESSENTIAL FATTY ACIDS

PUFAs are widely referred to as "essential fatty acids" (EFAs), but for various reasons this term is an anachronism of understanding of the 1960s (5, 6). The term "essential fatty acid" is used in a variety of ways and deserves revisiting (see reference 5 and Supplemental Discussion A). For our purposes, EFAs refer specifically to linoleic acid [LA; 18:2n-6 in the International Union of Pure and Applied Chemistry-International Union of Biochemistry (IUPAC-IUB) notation or 18:2ω6 in the Holman notation (7)] and α-linolenic acid (ALA; 18:3ω-3), primarily because they are the most abundant food PUFAs, available in various seed and fruit oils. These oils contain no other PUFAs and therefore are exactly the EFAs in experimental diets in which they are the only source of fat, not because they play any unique metabolic role in and of themselves or are the most efficacious at preventing skin lesions, the most obvious phenotype of EFA deficiency; as noted below, arachidonic acid (AA; 20:4n-6) and DHA are now known to uniquely fulfill these definitions. Before the availability of oils with diverse compositions, experimental diets could be made up with common vegetable oils to test the effects of these PUFAs as sources of LA and ALA alone. Detailed studies in animals (7, 8) were undertaken that showed that the competition between the n-6 and n-3 PUFAs, using only LA and ALA, led to predictable changes in tissue PUFAs (9, 10) based on intake that applied to humans (11). Among the principles established in these studies is the transition from competition to antagonism when the dietary intake of one PUFA is far greater than that of another, most commonly LA dominating ALA. Figure 1 shows the structures and a simplified biochemical pathway for n-6 and n-3 PUFAs.

Molecular studies have now shown definitively that the 2 PUFA families compete for the same enzymes that convert them to more unsaturated and longer-chain PUFAs, although many details have yet to be worked out. AA and EPA (see Supplemental Discussion B) are synthesized at similar rates when precursors are at similar concentrations, although AA accumulates at higher concentrations in various tissues than does EPA—for instance, in neural tissue and the capillary endothelium. DHA is the n-3 PUFA that accumulates in tissue, notably in the central nervous system (CNS), including the retina and some reproductive tissue. Unfortunately, animal studies reporting basal fatty acid profiles of tissues use routine feed pellet diets that are very low in fat by human standards (e.g., 7% by weight, 15% of energy) and are typically dominated by LA-rich oils [e.g., American Institute of Nutrition (AIN)-76 with inherently n-3-deficient corn oil or AIN-93 with commodity soy oil], leading to overwhelmingly high n-6 PUFA concentrations (Supplemental Discussion C). Diets containing PUFAs that are considered normal for the preindustrial food era—for instance, those formulated with the new lines of high-oleic acid oils and foods—lead to much different tissue PUFA composition, largely because LA is much lower in these oils.

No evidence exists to uniquely assign the term “EFA” to LA; on the contrary, much evidence shows that LA does not uniquely prevent EFA-deficiency symptoms. The water-deprived rat bioassay of the 1950s and 1960s, based on growth limitation due to
skin lesions and impaired water barrier function, was used to establish EFA activity of various PUFAs. In retrospect, focus on skin barrier properties of PUFAs led to a description of “EFA potency” that focused exclusively on skin and not on any other biological property. Because ALA poorly supports the skin permeability property, the n–6 configuration was deemed essential by the early 1960s (13) and investigated and verified in human studies (14) insofar as possible with LA. It is likely that this contributed to the emphasis on vegetable oils devoid of ALA in the food supply soon afterward, such as corn oil, which we now know when used as the sole source of fat for pregnant animals supports excellent growth in terms of body weight gain but frank deficiency in neural function (more on this later).

In 1976, Wene et al. (15) argued that LA is the n–6 “EFA” because AA can be synthesized from LA. Viewed alternatively, AA is the n–6 EFA because it has the greatest efficacy for restarting growth in water-deprived fat-free–fed rats (16). In an early original study, AA was the only fat of a dozen tested that had a greater EFA activity (termed “vitamin F activity”) than LA, the reference fatty acid: “Arachidonic acid has turned out to be the most active...[suggesting] that this acid is the active form of the essential fatty acids in the body” (16). At that time, AA was thought to be an exclusively animal product, rather than an environmentally abundant fatty acid comprising >25% of fatty acids in many plants—for instance, bryophytes (e.g., mosses)—suggesting that animals alone could make AA (17). More-specific studies in the early 1960s found AA to be 3-fold more effective than LA in eliminating EFA deficiency as assessed by dermal symptoms (18). In fact, new data show that diets formulated with AA and DHA as the sole dietary PUFAs support growth and development of mice through 6 generations and, compared with LA-fed mice, maintain reproductive viability to a much greater age, long delaying the phenomenon known as “mouseopause” (19); despite the absence of LA and ALA in this diet, the triene to tetrane ratio, the accepted biochemical marker for EFA deficiency, does not increase (20). Neural testing on mice raised through 10 generations fed AA- and DHA-only diets showed no abnormalities (M Puder, personal communication, 2014). From the perspective of greatest efficacy, neither LA nor ALA is “essential.” AA and DHA are the essential fatty acids, the EFAs, if that term is understood to mean those fatty acids that are most active in avoiding deficiency symptoms as well as in supporting optimal health.

**CASE HISTORY LEADING TO THE HYPOTHESIS THAT DHA IS KEY TO PRETERM FEEDING**

Observations that led to the recognition of DHA’s importance for preterm neural development are linked to the brain’s normal composition, the timing of human brain development, and concerns over whether preterm infants who receive no source of preformed DHA could endogenously synthesize all needed DHA from precursor ALA, a situation unlike the comparable postconceptional age fetus. DHA and brain growth have been linked since its initial characterization from hog brain in 1953 (21) on the basis of previous publications showing that the brain is rich in at least one hexaene. DHA increases in parallel with brain weight, and this increase is not from myelin lipids, which were later shown to be very saturated. Rather, DHA is very high in CNS gray matter (12), and there is some evidence that its concentration from brain region to region tracks metabolic rate (22).

**CNS DHA accretion in all species is similar**

Analyses of the fatty acid profiles of brains and livers of 32 diverse species showed a remarkable dichotomy in DHA concentration in the 2 organs, as shown in Figure 2. The observed range of liver DHA was from 0.2% to 32% of fatty acids, or 160-fold, whereas brain DHA was from 13% to 29% of fatty acids, or <2.5-fold. Variation in liver DHA was attributable to environmental and dietary niches: herbivores (e.g., zebras) that inherently ingest negligible amounts of DHA and must synthesize it from precursor ALA had the lowest liver DHA content, whereas carnivores (e.g., cats) have high liver DHA concentrations (23). It should not escape notice that vertebrate conservation of brain DHA implies an indispensable function in that organ. DHA’s dominant concentration in the brain also implies that a limitation in the acquisition of DHA could constrain final evolved brain size for each particular species. Unsurprisingly, human fetal brain from an autopsy was reported to be similarly high in DHA, reaching 18% in a phosphatidylethanolamine, a major brain lipid class (24). The brain growth spurt, starting at approximately week 27 of gestation and continuing to 2 y of age when the cranial

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**FIGURE 2** DHA concentrations in livers and brains of 32 diverse species, arranged from low to high liver DHA concentrations, as a percent of total fatty acids. Liver concentrations range from 0.4% to 32% (wt:wt) and brain DHA concentrations range from 13% to 29%, showing that brain DHA concentrations are maintained across a wide range of sizes and ecological niches. Can, Canadian; CARN, Carnivore; LNR, large nonruminant; LR large ruminant; MAR, marine mammal; P, primate; SM, small mammal. Adapted from reference 23 with permission.
n–3 Deficiency and neural function

The functional importance of DHA was first elucidated in studies of the retina, considered an extension of the brain. Retinal intracellular membranes hosting rhodopsin are the richest in DHA of any known human membranes, reaching concentrations approaching 50% in some phospholipids, and are finely tuned to support light signal amplification (28). Pregnant rats fed n–3-deficient diets had retinal photoreceptor (rod outer segment) DHA concentrations decrease precipitously in the offspring with substitution by the docosapentaenoic acid n–6 isomer (22:5n–6), the n–6 fatty acid synthesized from LA that has the closest structural similarity to DHA, differing in only 1 double bond. Electroretinogram responses generated from a flash of light were attenuated in both the initial retinal response (“a” wave) and the more vigorous neural response (“b” wave) (29). These data have been recapitulated in studies of neonatal rhesus monkeys whose dams were fed n–3-deficient diets (30, 31).

The general paradigm for n–3 deficiency is a feeding of specific seed oils replete in LA but deficient in ALA to pregnant females as a sole source of PUFAs through breeding to at least parturition and usually through lactation. All kinds of neural impairments are found, including abnormal electrophysiology, poor maze performance, poor balance, low tolerance to neurotoxins, and true in a restricted sense. More recent work recapitulating those experimental results are the origin of the widespread assumption that it is the “ratio” of 18:2 to 18:3, or of n–6 to n–3, that controls LC-PUFA concentrations, but this is only partially true in a restricted sense. More recent work recapitulating those data, and others, fed 54 diets containing only 18:2 and 18:3 in varying proportions, and varying total fat, to mice and reported plasma phospholipid fatty acids as the measure of PUFA status. DHA status is optimal when total 18:2n–6+18:3n–3 is a low 2–4% of calories. The average American diet delivering in excess of 10% 18:2n–6 (37) strongly suppresses DHA status. Importantly, contours of constant ratio (18:2 to 18:3) do not determine DHA synthesis efficiency from precursors

Qualitatively, the biosynthesis of DHA from precursors is reasonably well understood from molecular and biochemical perspectives. The biochemical pathways for DHA synthesis from ALA (Figure 1) have been under active investigation in a variety of organisms since the 1980s. Each step in the pathway either increments the chain length by 2, increasing the first number of the designation, termed elongation (e.g., 18:4n–3 → 20:4n–3) or adds a double bond, termed desaturation (e.g., 18:3n–3 → 20:3n–3). The first step in the pathway, conversion of 18:3 to 22:6 is 6-desaturation (18:3n–3 → 18:4n–3) and is mediated by the protein product of the fatty acid desaturase 2 gene (FADS2). This step is commonly referred to as the “rate limiting” step in LC-PUFA synthesis because it is the slow step in conventional in vitro biochemical preparations of rat liver (33). More recent data suggest that elongation can be rate limiting (34). The overall synthetic pathway to 22:6 requires 5 (35) or possibly 7 (36) steps and is thus considered inefficient compared with the n–6 pathway, 3 steps, from 18:2 to 20:4 (AA). Early animal data that established the concept of the competitive nature of n–6 and n–3 PUFAs are shown in Figure 3. Here, livers of rats fed diets with variable amounts of 18:2 and constant 18:3 showed that n–3 LC-PUFA concentrations are strongly dependent on the amount of the n–6 PUFA and vice-versa. Dietary LA much above 4% of energy as LA inhibits the accumulation of most 22:6 and 20:5. These experimental results are the origin of the widespread assumption that it is the “ratio” of 18:2 to 18:3, or of n–6 to n–3, that controls LC-PUFA concentrations, but this is only partially true in a restricted sense. More recent work recapitulating those data, and others, fed 54 diets containing only 18:2 and 18:3 in varying proportions, and varying total fat, to mice and reported plasma phospholipid fatty acids as the measure of PUFA status.

![FIGURE 3](image_url) Liver PUFA composition in rats fed diets with various proportions of LA and ALA (n = 6/group). The left panel shows the suppression of AA (“20:4α6”) and intermediates derived from LA (0.6% of energy) by increasing ALA; the right panel shows the suppression of n–3 metabolites of ALA (at 1% of energy) including DHA (“22:6α3”) by increasing LA. Note the strong dependence of liver DHA at LA concentrations below ~4% of calories. American diets average >7.2% of calories as LA and 0.7% of calories [1999 data (37)]. Symbols refer to the labeled fatty acids. AA, arachidonic acid; ALA, α-linolenic acid; LA, linoleic acid. Reproduced from reference 8 with permission and drawn from data in reference 18.
22:6 status but are directly related to 22:6 concentrations only at constant PUFA. These data are consistent with current knowledge about the molecular details of LC-PUFA synthesis as well as data in adult humans showing that changes in dietary ratio by increasing 18:3 do not result in changes in circulating 22:6 (38). The human dietary pattern that contains only 18:2 and 18:3 and no LC-PUFAs would be described as “vegan” because LC-PUFAs are only present in animal-derived foods.

In vivo studies in many experimental species and in humans with isotonically labeled 18:3 typically show synthesis of all of the intermediates and 22:6n–3 (39). However, all of the studies in humans showed that conversion to 18:3n–3 → 22:6n–3 is <5%, as measured in the circulation, which is neither the site of conversion nor of most relevant functions (40–42). These results are consistent with studies in the 1990s of human supplementation with 18:3n–3, typically from flax or flax oil, and measurement of circulating LC-PUFAs. Although most studies showed that all intermediates increased in plasma, serum, or platelets, it was surprising as late as 1999 that 22:6n–3 (DHA) never increased when supplemented with 18:3n–3 (43). Subsequent studies have unequivocally established that general circulating 22:6n–3 status does not increase with dietary supplementation of any intermediate—18:3n–3, 18:4n–3, or 20:5n–3—in men or nonpregnant/nonlactating women (38). Moreover, the concentration of 22:6n–3 does not increase in the breast milk of women supplemented with 18:3n–3 (44), despite upregulation of synthesis detected from labeling studies (39), although circulating 22:6n–3 does increase in transgenic individuals (45). Some suggestive evidence indicates that bloodborne 22:6n–3 does increase in infants provided with 18:3n–3 (46, 47). In contrast, lowering of dietary 18:2n–6 (LA) in adults increases circulating 22:6n–3 concentrations (48), consistent with animal studies of the 1960s (8) and many since then (e.g., reference 9).

In contrast, the consumption of 22:6n–3 causes a rapid increase in tissue and fluid 22:6n–3 (49). A rapid and stunningly linear increase (45) was found in breast-milk 22:6n–3 and improvement in a nursing infant’s 22:6n–3 status in a trial of DHA supplementation of the mother (50).

**Recognition of importance of n–3 in infant formula**

The absence of DHA in infant formulas was an unplanned experiment that showed differences in tissue composition, leading to the hypothesis that a source of preformed DHA is necessary to normalize preterm DHA to breastfed term concentrations. Infant formulas in the United States before the 1990s contained only the precursor fatty acids LA and ALA, and no LC-PUFAs, mostly because of the low cost, stability, and safety of vegetable oils and the challenges of sourcing DHA before the 1990s. Generations of infants fed DHA-free formulas were presumed to be able to biosynthesize all of the DHA needed from ALA in the formula, if ALA was present at all. The Infant Formula Act of 1980, prompted by the overzealous reduction of salt by one formula maker leading to chloride-deficiency–induced mental retardation, identifies minimal required amounts of nutrients for infant formulas: the PUFA entry reads “Essential fatty acids (linoleate): percent cal 2.7, mg...300.0” (51). Remarkably, the current Code of Federal Regulations, based on this legislation and amendments since, recognizes no need for n–3 PUFAs, even the precursor ALA, as required for infant feeding (51). It is little short of breathtaking that n–3-deficient oils long known to support growth but to compromise neural development are legally acceptable despite the overwhelming evidence of n–3 requirement for neural development generated in scores of animal studies (32).

Evidence that formulas containing LA and ALA only did not support circulating LC-PUFA in preterm human infants at concentrations similar to preterm infants fed breast milk began to appear ~1990 (52–56) and followed earlier work indicating that plasma phospholipid DHA was half that of breastfed term infants, and AA was also lower (57). Later in the era in which all US formulas contained ALA as the sole n–3 PUFA, autopsy studies showed that infant brains did not achieve similar concentrations of brain DHA as breastfed infants (58, 59), results that are fully consistent with experimental PUFA feeding studies. Research on omnivorous baboons showed that the addition of DHA to infant formulas resulted in higher brain DHA than in formulas containing ALA as the only source of n–3 (60), supported by stable isotope studies (61), and these studies included preterm neonates that were taken by cesarean delivery 3.5 wk early (of a 26-wk normal baboon term gestation). DHA inclusion in formula led to retinal DHA concentrations that were similar to those in randomly assigned breastfed baboons. Importantly, electroretinogram studies showed that DHA-fed baboons had improved retinal response compared with non-DHA-fed baboons, and nearly as high as breastfed baboons (62), thus showing not just an increase in concentration but a practical improvement in function, as shown in Figure 4. These data contribute detailed mechanistic information that parallel numerous studies in human infants that showed consistently improved visual measures in DHA-fed preterms (63–66).

**HUMAN RANDOMIZED CONTROLLED TRIALS FROM ~1995 TO ~2005 ON DHA AND AA AND US FORMULA**

The majority of studies on DHA also included AA and usually a DHA source with no EPA (e.g., algal oil) or minimal EPA (tuna oil, egg phospholipid). More than 10 randomized controlled trials (RCTs) were conducted in preterm infants, and a similar number in term infants, to establish clinically relevant data showing a need to include DHA and AA. A serious controversy over “mixed” results hinged on the amount of DHA delivered. Studies of experimental formulas with near the global mean of breast-milk DHA, slightly

![Figure 4](Image 362x151 to 483x253) Comparison of 2 electroretinogram variables measured in preterm baboons fed commercial infant formula (P-) with no DHA/AA (long-chain PUFAs) and with DHA (P+DHA). "a" represents the response related to initial amplification of the light pulse and is greater in the P+DHA group; "a wave implicit time" is the latency from flash to the initial retinal signal peak and is shorter (improved) in the P+LCP group. *P < 0.05. Mean ± SD retinal DHA concentrations were 14.4% ± 0.6% and 18.4% ± 1.2% (wt: wt) in the P- and P+DHA groups, respectively (P < 0.05, 1-factor ANOVA/ Tukey’s honest significant difference test); n = 4/group. Adapted from data in reference 62. AA, arachidonic acid; arb, arbitrary; LCP, LC-PUFA.
above 0.3% wt:wt (weight percentage of fatty acids), showed positive effects, whereas studies that included ~0.2%, similar to breast-milk concentrations in urban populations, were null. By 2001, 11 preterm studies were available; systematic reviews concluded "mostly positive effects" (65) and "[RCTs] involving preterm infants have shown a clear requirement for DHA for full visual and neural development" (66). There was (and is) less consensus for term infants. Later reviews concluded that a significant relation exists between DHA and effectiveness in supporting visual acuity at 4 mo of age in term studies (67).

A summary of visual acuity studies, a meta-regression of sorts, is presented in Figure 5 (64). Here data from ~10 infant groups reported in 4 articles (1998–2005) that used sweep visually evoked potentials for assessing visual acuity were summarized. All of the measurements were made in infants at 1 y of age; the x axis reflects only the duration of LC-PUFA feeding, from either DHA/AA formula or breast milk. A trend is evident that shows that greater duration of LC-PUFA intake supports better visual acuity at 12 mo of age. This visual acuity improvement over time reflects neural maturation and not refractive maturity correctable with eyeglasses, indicating that the entire CNS matures more rapidly with DHA exposure in a dose-dependent manner.

There is now direct support for improvement in neurocognitive function. Infants who consumed DHA formula performed better on the Willatts test of executive function than did those who consumed formulas with no LC-PUFAs (68, 69). Even with the strength of consensus, a 2011 Cochrane review of relatively mature and healthy preterm infants on pooling of results concluded no net benefit despite 3 of 7 studies that found neurodevelopmental benefit, presumably due to heterogeneity of experimental designs (70). More recent data show effects of LC-PUFAs that emerge at ages 3–5 y on rule learning, inhibition, and Peabody picture vocabulary tests (71). Bayley scales are widely recognized as being insensitive to DHA supplementation in healthy infants, and tests that are age appropriate and probe other cognitive domains are needed to show effects (72).

DHA and AA inclusion in US formulas was first permitted by the US Food and Drug Administration (FDA) (73), with formulas appearing in retail stores in 2002, although they were available in many other countries as early as 1995 (73). Within 1 decade in the United States, nearly 100% of all formulas contained DHA and AA, whereas at this writing only approximately half of the formulas purchased in Canada have DHA and AA.

**AA AND INFANT FEEDING**

AA deserves a separate section because of an ongoing controversy about whether it should be considered optional in formula despite the presence of DHA (74, 75). Nutrition and metabolic effects of AA are linked to the metabolism of EPA, AA’s n–3 analog, as well as to DHA with which AA metabolism also interacts.

The motivation for the inclusion of AA in infant formula and as a required nutrient perinatally is, like DHA, rooted in the older formulations. DHA and its fetal and postnatal accretion in the brain and retina were the driving scientific principles that led to the recognition that breast-milk DHA is required for human health. All early sources of DHA also contained EPA because they were prepared from fish oils, and when the first clinical studies were undertaken in the late 1980s no sources of enriched AA were available. The first clinical studies in preterm infants were therefore conducted with DHA and EPA and no AA (52, 76, 77). Because of the known breast-milk composition and the importance of growth as a variable to indicate health, attention turned to what at the time was assumed to be AA-mediated growth. Basic science supports a role for AA support of growth, although not as a definitive limiting nutrient for growth. Observational (78) data led one of the pioneering LC-PUFA groups to propose that AA was key to growth, and soon afterward analysis of correlative data from an RCT studying DHA and its influence on neural development in preterm infants indicated that preterm AA status was correlated with growth (76). These observations led to widespread concern that substantial amounts of EPA carried along with DHA in the fish oils of the day were inhibiting AA-mediated growth. A 1999 GRAS (Generally Recognized As Safe) panel FDA report showed 32 studies of DHA/AA in term (17 studies) and preterm (15 studies) infants (79), and of those published since 1994 only 5 of 17 did not intentionally include AA. The FDA eventually ruled as acceptable the use of the single-cell oils in late 2001 (73), leading to the introduction of DHA and AA into US infant formulas in 2002. Notably, they had been introduced into Europe by 1995, in part on the basis of recommendations of several years earlier (79).

Despite >20 y of routine human feeding, the issue of the inclusion of AA in infant formulas remains open and a matter of current debate. The European Food Safety Agency (EFSA) in mid-2014 issued a scientific opinion on the composition of infant formulas that includes recommendations on LC-PUFAs (74), partially reversing EFSA guidance since 2004 on AA. The 2014 report concluded that sufficient evidence is available to support Adequate Intake values for LA, ALA, and DHA and carried forward a reversal of a recommendation for AA for infants, citing a 2013 EFSA report (80), which, in turn, cited a 2010 EFSA report that declined to set a Dietary Reference Value for AA for any age group (81). The reasoning (82) appears to be a misunderstanding about LA as the "EFA" and apparently a lack of support by an RCT for the role of AA in growth (76), which was noted previously (83).

This author is aware of only 3 cohorts of infants (84–86) in studies that compared formulas with DHA only (algal oil excluding EPA, or predominantly DHA from tuna oil) with a formula with

![Figure 5](image-url)
DHA and AA, noting that they were in term infants, not in preterm infants. The one study with the highest AA amount (0.72% wt:wt) reported improved improvement compared with the control in the Bayley Mental Development Index (MDI) in the DHA+AA group; the DHA-only group was not significantly different from control (87). No significant improvements compared with placebo were found for the Bayley scales or visual acuity in the tuna-oil study in which the amount of AA was 0.34% wt:wt (85); a third study found no significant effects, possibly because it used low DHA and AA amounts relative to global breast-milk concentrations (86). The weight of evidence now is that the Bayley scales, which are best at detecting suboptimal development, are not very sensitive to dietary LC-PUFAs in infants; measures of executive function more consistently show effects of DHA+AA (72). Clearly, the clinical evidence for deleting AA from formula is thin, and reliance on growth as the sole arbiter dismisses a wealth of knowledge about the effects of AA. Breast-milk AA concentration conserved throughout the world (88). AA itself is a precursor for perhaps 100 eicosanoids that are, collectively, bioactive paracrine signaling compounds with well-characterized mechanistic roles in a diverse array of physiological functions at all life stages. Medical issues particular to preterm infants include vascular (e.g., retinopathy of prematurity, periventricular hemorrhage, necrotizing enterocolitis) and immune (general infection, necrotizing enterocolitis) functions, both closely associated with AA-mediated signaling. These outcomes have not been addressed in the presence, or absence, of AA alone. The present author concludes that the evidence for overruling breast milk and eliminating the AA as a required component of infant formula when DHA is included in formula is minimal.

LESSONS LEARNED

LA was considered the sole “EFA” despite clear and convincing evidence that some source of ALA was required for proper development. This fact, although known since the early 1970s, appears to be refractory to recall among some researchers (8) who continue to measure weight gain as a sole definitive proxy for health. Human health is first and foremost linked to brain, not brawn, and all mammalian brains have approximately the same amount of DHA. The slow recognition of n–3 ALA by nutritionists testifies to the overemphasis on body weight gain. As such, it is remarkable, at least in hindsight, to realize that the most prominent, standardized, open-formula, purified experimental animal feed, AIN-76A, was formulated with n–3-deficient corn oil (89). It was certainly known by 1976, the year of release of the diet formulation, that n–3 deficiency results in alterations in neural PUFAs that are never observed in free-living mammals, and that visual deficiency is the result. Still, it was not until 1993 that the standardized feed AIN-93 was formulated with soy oil, which, although still containing very high amounts of LA (>50%), contains ALA (8%), which avoids the most severe deficiency symptoms (90). Studies of n–3 deficiency typically use feeds formulated with oils with LA and ALA that are similar to corn oil, and controls are typically soy oil (32). Thus, AIN-76A-type oils lead to normal growth and very reproducible neural deficiency. Nevertheless, the food system produced oils with overwhelming amounts of LA and minuscule amounts of ALA, concerned more with adjusting serum cholesterol concentrations presumed to be related to the heart health of middle-aged individuals than the development of brain capacity of the next generation (91). An additional and possibly definitive driver is the generally greater shelf and frying life of low n–3 (= low ALA) oils, which lowers costs, a benefit for everyone. The appearance of high-oleic (= low LA) sunflower and, more importantly, soy oil (92) holds hope for a return to balanced n–6 and n–3 and a reduction in the excess LA +ALA that inhibits conversion and incorporation of DHA and EPA into tissue.

CLINICAL (EBM) AND PRECLINICAL EVIDENCE—PRACTICE AND FOUNDATION IN NUTRITION RESEARCH AND DECISION MAKING

EBM is generally outlined in terms that put meta-analyses and systematic reviews of exclusively human studies as the highest level of clinical evidence, followed by RCTs, prospective cohort studies, and other studies, and generally considers animal studies at the lowest rung of Evidence (proper name), when listed at all. A formal definition of “evidence” in such a hierarchy is reasonable for drugs and procedures novel to therapeutics, with specific indications. In high-signal-to-noise medical situations—that is, cases that are obvious from clinical practice—insistence on EBM support is counterproductive (93). As purely clinical examples, consider, for instance, why blood transfusions for severe hemorrhagic shock, tracheotomy for tracheal obstruction, and insulin for diabetes are standard-of-care despite the absence of RCTs to verify their efficacy (94). The former 2 derive their legitimacy from purely clinical evidence (little “e”), whereas the latter combines preclinical and clinical evidence, and all stand as sufficiently compelling evidence to guide clinical practice. As a purely preclinical, relevant example, the author is not aware of any RCT that randomly assigned women to diets supplying 100% of fat from edible oils with trace n–3 PUFAs, similar to commodity (high-linoleic) corn, sunflower, safflower, or peanut oils. On the basis of the >60 extant animal studies, or even one-tenth this number, such a study would be judged unethical because of the outcomes of animal studies alone (32). This body of preclinical work along with conserved neural composition and related metabolic data constitutes the wide breadth of evidence supporting the robust understanding that obviates RCTs.

Nutrients, as opposed to pharmacologics (xenobiotics), are endogenous biochemicals that have wide-ranging physiologic effects. It is a common error to ascribe a single target to nutrients (e.g., growth for AA) in the same way that a xenobiotic is assigned a “target” with perhaps attendant “side effects.” Nutrients are not designed and cannot be deleted from the body as can a chemically synthesized drug or biological that is intended to treat a particular indication. Moreover, nutrients are delivered in highly heterogeneous forms, with most not subjected to nearly the manufacturing or compositional control of conventional patented pharmaceuticals. Statistically combining studies that use various amounts and proportions of PUFAs from various sources, and using presumed intake rather than biomarkers (e.g., circulating DHA) as the independent variable, is debatable. Extending results leads to unwarranted recommendations to delete endogenous compounds (nutrients) from the diet because of the “absence of evidence” that they are required in the diet judged by a limited outcome measure. And these limited outcome measures are typically driven by process-based design to combine studies (i.e., meta-analyses) for which combination is questionable. It is with these consideration caveats in mind that the implications of Cochrane and similar treatments should be judged, particularly when they fail to find significant effects.
LESSONS SPECIFIC TO LC-PUFAs

PUFAs

The term “PUFA” and similar terms (LC-PUFA) are descriptive of chemical structure. Overwhelming evidence on nutritional and biological effects shows that each particular fatty acid has specific biochemical, molecular, and nutritional properties. As noted here, many pairs of PUFAs have competitive and antagonistic effects. To avoid confusion, amounts of each relevant fatty acid should be reported and they should be combined rarely and only with justification for doing so.

Breast-milk composition guides, experiment decides

Neonatologists and other pediatricians considering diet for the very young are in the unique position to have a model, breast milk, to consider. As noted, a premature infant is not a term infant and the premature breast milk is not evolutionarily optimized for the very preterm. However, it remains a guide to the formulation of breast-milk substitutes and formulas for preterm nutritional support. Formula composition should prudently but not slavishly mimic breast milk, with specific notice to conserved components. For instance, whereas all breast milks contain DHA and AA, all breast milks also contain dioxins (95) and nitro musks (96). The question of which of these conserved components should be included in infant formula feeds, and which should not, turns on theory. Theory is consistent with the recognition that dioxins are mannmade compound of industrial use and that nitro musks are fragrances. No RCTs are necessary to decide whether they should not be purposefully added to formula. The question of whether AA should be included in infant formulas should similarly be influenced by theoretical considerations confirmed by RCTs that isolate the effects of AA and report outcomes likely to be limited by AA supply. The conservation of AA in breast milks globally as well as the base of metabolic science around AA compellingly show that AA is a component of normally functioning tissue. Basic metabolic and biochemical evidence to undergird the inherently imprecise RCTs should be required to delete AA as a routine component, thereby overruling breast milk.

Composition of the available food supply matters

The fat composition of the food supply affects health and thereby affects the efficacy of any particular treatment compared with a placebo control. The current high amount of dietary LA overwhelms the metabolism of all PUFAs and especially of n–3 PUFAs. As LA amounts decrease because of the planned acceleration in availability of olive oil–like high-oleic (= low LA) soy and other oils (92), LA antagonism toward all PUFAs in the body will decrease and ALA will be more efficiently converted to LC-PUFAs, including DHA. Among others, this is an unequivocal reason why combining studies done in various places and at various times in meta-analyses is questionable, in the language of meta-analysis, highly heterogeneous, although not necessarily in a statistical sense.

Improvement in one key variable is sufficient

Broad consensus holds that the supply of LC-PUFAs in the form of DHA and AA is required for optimal development of visual acuity in premature infants. The wide array of tissue composition, biochemical, metabolic, and related basic biology strongly supports a role for DHA and probably AA in the nutrition of infants. No negative side effects have been established or even consistently suggested for LC-PUFAs, despite dozens of studies and many millions of infants fed LC-PUFA oils, not to mention the evolution of breastfed humans with a steady supply of these nutrients.

Continuing studies on other outcomes, e.g., Bayley scales, are relevant for those specific outcomes and should not be overextended to imply that support for other outcomes is diminished. For instance, studies of DHA and outcomes such as atopy symptoms might indicate that formula DHA amounts above those currently in use would support improvements in atopy. Such studies might conceivably also raise red flags (although none have been raised). Unfortunately, null results are often discussed in isolation as if they were evidence against the use of DHA, rather than that DHA is simply not relevant to the outcomes at hand within the particular experimental constraints. To use a concrete example, the absence of LC-PUFA influence on Bayley scales performance in healthy children (72) does not make less compelling the visual acuity data that support LC-PUFAs. It merely means that DHA has no effect on performance on Bayley scales in healthy children.

ONGOING RESEARCH

Research on longer-term outcomes from DHA, including neurocognitive performance, success in school, positive mood, and a host of health indicators, continues in children whose parents participated in DHA studies in pregnancy and in infancy. New studies are in various stages of planning and implementation. Among the goals of newer studies are to consider the best intake amounts and whether subtle effects emerge in later life, and how genetics may modulate responses to intake. Serious research may well be prompted by the current controversy about AA. The basic scientific facts around EFAs, however defined, remain well established and the anchors for clinical science.

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