n−3 Polyunsaturated fatty acid requirements of term infants[1–3]

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ABSTRACT The benchmarks for human nutrient requirements are the recommended dietary intakes (RDIs). However, the RDIs are set to prevent a clinical deficiency state in an otherwise healthy population and there are few nutrient recommendations set with the goal of achieving an optimal or maximal state of nutrition and health. This is becoming an increasing challenge with the introduction of many nutraceuticals and functional foods, a prime example being the debate surrounding the introduction of long-chain polyunsaturated fatty acids (LCPUFAs) into infant formulas. Most expert nutrition committees have used the fatty acid composition of breast milk as a basis for recommendations for infant formulas, with little information on the minimum absolute requirement for essential PUFAs. It has been difficult to determine a minimum requirement for fatty acids because *1*) LCPUFAs can be synthesized from precursor fatty acids, *2*) plasma n−3 LCPUFA concentrations representing deficiency and sufficiency are not clearly defined, and *3*) there are no recognized clinical tests for n−3 LCPUFA deficiency and sufficiency. Therefore, there is a clear need to associate a measure of LCPUFA status with a specific functional outcome before any recommendations can be made for achieving optimal or maximal LCPUFA status. *Am J Clin Nutr* 2000;71(suppl):251S–5S.

KEY WORDS n−3 Polyunsaturated fatty acids, long-chain polyunsaturated fatty acids, infant formula, neural development, neurologic development, term infants, docosahexaenoic acid, DHA, breast milk, human milk

INTRODUCTION

For some vitamins and minerals, there are well-defined dietary requirements. For example, there are separate recommended dietary intakes (RDIs) of iron for infants, adult males, females, and pregnant women (1). These RDIs have been determined from measures of intake that correlate with markers of iron status, such as blood hemoglobin and plasma ferritin concentrations. Individuals who consume iron-deficient diets are at risk of anemia and have reduced hemoglobin concentrations. In addition, excessive dietary iron consumption may be toxic. Between these 2 extremes is the range of adequate dietary iron intakes. At the low end of the adequate range, individuals may be classified as nonanemic iron-deficient (marginal status) with reduced plasma ferritin concentrations; such individuals are considered at risk of clinical deficiency. Although much research is still focused on defining the subtleties of nutrient requirements in specific circumstances, there is general agreement, which is reflected in a similarity of dietary requirements among various countries.

Nutritional requirements for polyunsaturated fatty acids (PUFAs), on the other hand, are not clearly defined for either adults or infants and few known dietary intake values have been equated with blood concentrations that in turn can be related to function. This is further complicated by the fact that, although we are dependent on our diets for the 2 known essential fatty acids, linoleic acid (LA, 18:2n−6) and α-linolenic acid (ALA, 18:3n−3), they can also be converted to 20- and 22-carbon metabolites [long-chain PUFAs (LCPUFAs)], which have profound biological activity. The need for dietary essential fatty acids may be obviated if LCPUFAs are included in the diet.

The nutritional requirement for LA is the best known. An absence of LA from the diet results in growth retardation and dermatologic abnormalities. Although reduced dietary intake of LA can be correlated with reduced plasma concentrations of LA and elevated concentrations of mead acid (eicosatrienoic acid, 20:3n−9), no studies have associated plasma concentrations of LA or mead acid with a biological function or clinical state. Furthermore, mead acid is a product of the n−9 pathway and its concentrations can also be influenced by high oleic acid (18:1n−9) intakes (2). Dietary LA intakes of 0.6% energy as LA are thought to be adequate for avoiding essential fatty acid deficiency as indicated by the triene to tetraene ratio [ratio of mead acid to arachidonic acid (AA)], but currently most regulatory authorities recommend the provision of ≥7.06 μg/J (300 mg/100 kcal) or 2.7% energy as LA to infants (3).

The amount of dietary LA that is toxic is not known and infant formulas with as much as 70% total fat as LA have been marketed in the past with no reported adverse clinical effects (4). High-LA formulas are currently not recommended because of concerns

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about increased risk of adverse effects from LA peroxidation products and because of the known inhibition by LA of n−3 LCPUFA incorporation into cells. Interestingly, the optimum dietary intake of the long-chain derivative of LA, AA (20:4n−6), is not known, although estimates have been made (5).

In contrast to LA deficiency, ALA deficiency in humans has not been extensively documented and most clues have come from animal studies (6). For example, ALA deficiency produced alopecia and scaly dermatitis in capuchin monkeys (7) and loss of visual acuity in young rhesus monkeys (8). In humans, ALA deficiency has been described in only one 6-y-old girl who presented with neurologic and visual symptoms (9) and 5 adult patients who presented with cutaneous symptoms after either prolonged intravenous nutrition (10) or long-term gastric tube feeding (11). However, in all the human cases the symptoms attributed to ALA deficiency may have been partly accounted for by other nutritional deficiencies, such as low concentrations of vitamin E or total essential fatty acid deficiency. In rhesus monkey trials of ALA deficiency in which high concentrations of dietary LA were maintained, no cutaneous symptoms or growth failure were reported (8). Researchers have estimated that the daily ALA requirement to avoid deficiency symptoms is ≈0.3–0.5% of energy (9–11).

The biological benefit of ALA may be dependent on its conversion to LCPUFAs. The neurologic and visual deficits found in monkeys deficient in n−3 fatty acids have been ascribed to deficiency of docosahexaenoic acid (DHA, 22:6n−3), the longer-chain metabolite of ALA, because neural deficits in the monkeys were correlated with loss of DHA from the brain and retina (8, 12). Similarly, more recent reports stated that human infants incapable of synthesizing DHA (Zellweger syndrome) had reduced brain and retinal DHA concentrations and suffered from blindness and general atonia (13). Some of the symptoms were reversed with DHA supplementation (13).

The theory that human infants may be at risk of n−3 fatty acid or DHA deficiency arose primarily from the rhesus monkey studies, because the n−3 fatty acid–deficient diets used in these studies were similar in fatty acid composition to many commercially available infant formulas of the time (14). Subsequent clinical studies with both preterm and term infants attempted to address the issue of dietary n−3 PUFA requirements for the development of visual and neural function. Our review is based on randomized clinical trials only, because these studies limit the bias and confounding influences associated with comparisons between breast- and formula-fed infants.

RANDOMIZED CONTROLLED TRIALS

Nine placebo-controlled, randomized studies of formula feeding with different amounts of n−3 PUFA supplementation in term infants have been published (15–22) or reported in an unpublished abstract (U Clausen, A Damli, UV Schenck, B Koletzko, unpublished observations, 1996) (**Tables 1** and **2**) and many more are in progress. These trials have proved difficult to interpret because different research groups have used diverse dietary sources of PUFAs and the developmental assessments have also differed. Not surprisingly, therefore, some studies have found a beneficial effect of n−3 PUFA supplementation (17, 18, 20, 22), whereas others reported no effect (15, 16, 19, and Clausen et al, unpublished observations, 1996). Before we can decide whether these studies actually help us determine the n−3 PUFA requirements of term infants, we need to examine several issues critically.

Dietary treatments

The fatty acid compositions of the standard formulas, particularly the LA to ALA ratio and the absolute amounts of LA and ALA, are important because these determine the rates at which n−6 and n−3 LCPUFAs are synthesized by the infants (24). This issue has important implications for a critical examination of the literature. First, there is the basic question of whether the n−3 requirements can be met by providing ALA alone rather than adding LCPUFAs. Second, how do we interpret the results of various LCPUFA-supplementation studies that used different LA to ALA ratios in the standard formula?

Several studies have examined the effects of various LA-to-ALA ratios by using biochemical endpoints (2, 4) and it is clear that increasing the concentration and proportion of ALA in formula fats improves the DHA status of infants, yet tissue concentrations of DHA do not reach those found in breast-fed infants. Nevertheless, the possibility exists that the highest concentrations of DHA attained may be enough to meet all the nutritional needs of infants. However, few studies have addressed the potential physiologic effects of altering ALA concentrations in formula-fed infants (Table 1). Jensen et al (15) tested LA to ALA ratios ranging from 5:1 to 45:1. Although they measured higher plasma and erythrocyte DHA concentrations in infants fed the lowest-ratio formula (25), the only visual test attempted was VEP latency, which has not previously been reported to be sensitive to dietary manipulation. Innis et al (16) used a more widely accepted test of visual performance (acuity), but the range of LA-to-ALA ratios was small (7–9:1). Both formulas had ALA concentrations >1.9% of total fats and no differences in infant fatty acid profiles or visual acuity were detected.

The ratios of LA to ALA and the concentrations of ALA in standard (control) formulas used in LCPUFA intervention studies have also varied (Table 2). Whether this helps to explain the variation in reported neurologic responses is difficult to ascertain because few groups have used identical testing procedures. In addition, the base formula composition is not always under the control of the investigators. In our own studies, the LA to ALA ratios of reference or control formulas have become lower over the years because of the general trend of manufacturers to increase the concentration of n−3 PUFAs in formulas that has undoubtedly influenced the concentrations of DHA in infant tissues.

The alternative strategy for improving the PUFA blends of infant formulas has been to add LCPUFAs directly. The type of LCPUFA added has largely been governed by the availability of different oils and LCPUFA supplements. For example, at the beginning of our trials the only feasible DHA source was a fish oil high in eicosapentaenoic acid (EPA, 20:5n−3) (Table 2). More recent studies have avoided the addition of EPA by using fish oils high in DHA, such as tuna oil (19), or by supplementing with fractions of egg phospholipids (18–20). Egg phospholipids can be used to mimic the concentrations of DHA and AA found in the breast milk of Western women (18–20), although they provide these fatty acids in a form different from that of breast milk and can contain extra phosphate, choline, and cholesterol (26). Other trials currently underway are using oils from unicellular organisms such as fungi and algae. The toxicology of all LCPUFA sources is rarely reported; therefore,

TABLE 1
Neurodevelopmental outcomes of term infants fed breast milk or n−3 fatty acid–supplemented formula in randomized clinical trials[1]

Study and diet group	Subject characteristics	Test	Results
Jensen et al, 1997 (15)	*n* = 63; age 4 mo	Visual evoked potential latency	No differences between diet groups
Breast milk			
Formula with LA-to-ALA ratio of			
18:0.4			
17:1			
17:1.7			
16:3.2			
Innis et al, 1997 (16)	*n* = 172; age 3 mo	Acuity cards	No differences between diet groups
Breast milk			
Formula with LA-to-ALA ratio of			
34:4.7			
18:1.9			

[1] LA, linoleic acid; ALA, α-linolenic acid.

further investigations are needed to evaluate the safety of these substances for consumption by infants.

Power of the study

Calculations of statistical power are used to estimate the sample size that is required to show an effect in a trial. Most randomized trials have enrolled and followed 20–30 infants per treatment group (Tables 1 and 2). Although these numbers are ample for detecting changes in the fatty acid profiles of plasma and erythrocyte membranes and some visual outcomes, they may be inadequate for determining true differences in developmental scores and growth. Studies of adequate power are necessary to determine where differences due to dietary treatments exist and hence to be able to state with confidence which n−3 PUFA concentration is associated with a particular functional outcome. The issue of insufficient sample size in clinical trials has been reviewed elsewhere (27).

Visual and cognitive assessments

There has been some confusion among nutritionists regarding the neural assessments used to determine the potential benefits of n−3 PUFAs during infancy. Many authors have focused on visual outcome measures because these can be objective or easy to use, whereas other researchers have chosen more global assessments of development. The relevance and use of different tests for the assessment of visual and cognitive function in relation to dietary PUFAs have been reviewed extensively by Carlson et al (28). The variety of tests used has added to the complexity of determining specific dietary n−3 fatty acid thresholds for various visual and cognitive functions. For example, visual acuity may be tested by electrophysiologic [visual evoked potential (VEP)] or behavioral methods. Even for just these 2 types of methods, different research groups extrapolated the results differently to determine visual acuity. Some investigators have used VEP to measure latency, which cannot be translated to acuity (15). Similarly, research groups that have opted for more global assessments of development have used tests that measure different aspects of mental, language, and motor development (Table 2). Not surprisingly, all have shown different effects. Studies with similar assessment protocols will be necessary to elucidate the dietary requirement for n−3 PUFAs by using indexes of visual and cognitive development. Because of the use of different assessment methods, caution should be exercised if such studies are combined for meta-analyses.

Confounding influences and effect modifiers

All neural processes are complex and multifactorial. Assessments are subject to various influences despite efforts to exclude bias and confounding through randomization. For example, the requirement may be different for boys compared with girls, or for babies of smoking compared with nonsmoking mothers. This may hold true even for electrophysiologically determined responses. For example, we have detected poorer VEP acuity scores in infants whose mothers smoked than in a comparable group of infants whose mothers did not smoke; there was also a trend toward better acuity scores in female infants (M Neumann, M Makrides, R Gibson, unpublished observations, 1996).

When randomized clinical trials use more global measures of neural development, such as the Bayley's Scales of Infant Development, there are even more potential effect modifiers and confounders that require consideration. To illustrate this point, an examination of the literature relating to comparisons between breast-feeding and formula feeding is instructive and gives some idea of the range of factors that can impinge upon the outcome measure (29–32). Although differences in social class, education, and parenting styles between mothers who choose to breast-feed and those who choose to formula feed would be reduced in a randomized clinical trial by comparing the outcomes of formula-fed infants randomly assigned to receive either a control or an n−3-PUFA-modified formula, noteworthy confounding environmental and biological variables still remain. For example, factors that have all been implicated as affecting developmental scores in populations of infants include sex; race; gestational age; birth weight; incidence of infection or illness; bilirubin levels in the first week of life; alcohol use in pregnancy; strenuous exercise during pregnancy; and maternal parity, smoking, nutrition, weight, age, and marital status (32–34). To control for all known variables, and perhaps some that have yet to be discovered, will require large numbers of subjects, stringent inclusion and exclusion criteria, or both.

SUMMARY

The human need for dietary PUFAs is unquestioned, but we must accurately define the PUFA requirements of not only infants but also groups that we suspect may have special needs, such as pregnant women and the aged. For example, even for LA there is no known plasma concentration that corresponds to a specific clinical condition in the manner that has been documented for

TABLE 2
Neurodevelopmental outcomes of term infants given long-chain–fatty acid supplementation in randomized clinical trials[1]

Study and age of testing	Diets	Tests	Results
Makrides et al, 1995 (17) (n = 55)	BM, SF (LA:ALA = 17:1.5), and SF plus FO and EPO	Transient VEP	
16 wk			Subjects fed BM and SF plus FO and EPO tested better than those fed SF alone.
30 wk			Subjects fed BM and SF plus FO and EPO tested better than those fed SF alone.
Agostoni et al, 1995 (20) (n = 86)	BM, SF (LA:ALA = 11:0.7), and SF plus egg PL and EPO	Developmental quotient	
4 mo			Subjects fed BM and SF plus egg PL and EPO tested better than those fed SF alone.
Carlson et al, 1995 (18) (n = 58)	BM ≤3 mo, SF (LA:ALA = 22:2), and SF plus egg PL	Acuity cards	
2 mo			Subjects fed BM and SF plus egg PL tested better than those fed SF alone.
4, 6, 9, and 12 mo			No differences among diet groups
Auestad et al, 1995(19) and Jarowsky et al, 1995 (23) (n = 120–200)	BM ≤3 mo, SF (LA:ALA = 22.2), SF plus egg PL, and SF plus TO		
2, 4, 6, 9, and 12 mo		Sweep VEP	No differences among diet groups
2, 4, 6, 9, and 12 mo		Acuity cards	No differences among diet groups
12 mo		Bayley's test	No differences among diet groups
14 mo		McArthur CDI	RBC DHA and CDI correlated negatively
Jorgensen et al, 1998 (21) (n = 62)	BM, SF (LA:ALA = 12:1.2), SF plus FO, and SF plus FO and BO	Sweep VEP	
4 mo			Subjects fed BM tested better than those fed SF alone.
Birch et al, 1998 (22) (n = 112)	SF (LA:ALA = 15:1.5), SF plus DHA, and SF plus DHA and AA		
6 and 17 wk		Sweep VEP	Subjects fed SF plus DHA and SF plus DHA and AA tested better than those fed SF alone.
26 wk		Sweep VEP	No differences among diet groups.
52 wk		Sweep VEP	Subjects fed SF plus DHA and SF plus DHA and AA tested better than those fed SF alone.
6, 17, 26, and 52 wk		Acuity cards	No differences among diet groups.
Clausen et al, 1996 (23) (n = 97)	BM, SF (LA:ALA not reported), SF plus DHA and AA	Acuity cards	
3 mo			No differences among diet groups.

[1] BM, breast milk; SF, standard formula; LA, linoleic acid; ALA, α-linolenic acid; PL, phospholipid; EPO, evening primrose oil; FO, fish oil; TO, tuna oil; BO, borage oil; VEP, visual evoked potential; CDI, communicative developmental inventory; RBC, red blood cell; DHA, docosahexaenoic acid; AA, arachidonic acid.

many vitamins and minerals. Instead, a low intake of LA is estimated from the presence of a surrogate marker, mead acid. The absolute requirement for ALA is also unclear, and although dietary recommendations have been made, they have not been correlated with plasma concentrations of either ALA or any of its metabolites. Furthermore, there is no plasma concentration of n−3 LCPUFAs that can be unequivocally related to a physiologic or developmental response in humans.

It seems reasonable to suggest that the PUFA requirements of some groups (eg, pregnant or nonpregnant women, the young or old) could be framed in such a way that dietary intakes would be related to plasma concentrations which in turn would be related to a desired outcome. In some areas, such as vascular disease, there may be sufficient data to be able to relate plasma PUFA concentrations to specific plasma cholesterol concentrations (LDL or HDL subfractions) or a physiologic test such as blood flow, blood pressure, or whole blood or platelet aggregation. However, almost no data are available currently to support such recommendations for infants. Until we have a specific outcome measure (eg, visual acuity, cognitive function scores, insulin sensitivity index, or growth) that we can relate to blood PUFA concentrations (with all dietary fat indexes, ie, the ratios of LA to ALA and of polyunsaturated to saturated fatty acids and intakes of n−6 and n−3 LCPUFAs, defined to reach that concentration), we may need to rely on the composition of breast milk from well-nourished mothers as a guide to dietary recommendations for infants. Because the LCPUFAs of breast milk appear to be dependent on maternal dietary LCPUFAs, it also seems prudent to ensure that breast milk from mothers who include some fish in their diets is used to guide dietary recommendations for infants.

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