The Proper Balance of Essential Fatty Acids for Life

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Abstract: It has been known for more than 70 years that some fatty acids are essential in the diet, but quantitative requirements for n-3 and n-6 fatty acids have been difficult to define. Recent research has established the importance of n-3 and n-6 long chain polyunsaturated fatty acids (LCPs) during infancy. Since n-6 and n-3 fatty acids compete for elongation and desaturation to biologically active LCPs that may have opposing biological effects, awareness of the n-3/n-6 balance is critical.

Requirements for LCPs in infancy have been empirically determined by comparing formula-fed to breast fed infants. The composition of human milk, and the biochemical, metabolic and behavioral responses of the breast-fed infant serve as guides to the requirements for LCPs. While n-6 LCP levels in human milk are rather constant on a global basis, the concentrations of n-3 LCPs in human milk vary widely. A balanced addition of LCPs to infant formula improves visual acuity, cognitive development, modifies the immune response, and modulates cholesterol metabolism all to more closely resemble breast-fed infants. Unbalanced additions (n-3 LCPs with no n-6 LCPs), however, have been associated with impaired language development in males and decreased growth in low body weight (LBW) infants. Benefits of LCP supplementation extend beyond the first 6 months of life.

Little is known about the optimal n-3/n-6 balance after weaning and in puberty, when nutrient demands dramatically increase to support growth and development. However, efforts to modify total and saturated fat intake, to prevent the beginnings of some adult diseases, have drawn attention to the importance of fatty acid balance in childhood.

Unbalanced consumption of n-3 and n-6 fatty acids is associated with many different diseases in adulthood; n-3 LCP supplementation is linked to a decrease in the risk of hypertension, mental diseases, immune hyper-reactivity, and heart disease. Even small amounts of n-3 LCPs in the diet have been associated with reduced risk of sudden cardiac death, probably due to their anti-arrhythmic effects. In vivo studies have shown that LCPs prevent arrhythmias and modify heart rate variability. In cultured cardiac myocytes LCPs interact directly with ion channels to protect against arrhythmic responses.

Prospective, properly controlled studies need to be conducted to further define the role of the n-3/n-6 ratio in adolescent and adult life. By integrating our knowledge of LCP balance in infancy with current and future information concerning requirements later in life, we will be able to establish appropriate balance recommendations for optimal balance throughout a lifetime.


Key words: n-3/n-6 ratio, n-3 fatty acid, n-6 fatty acid, long chain polyunsaturated fatty acid

1 Introduction
The essentiality of polyunsaturated fatty acids has been recognized for more than seventy years. Early work focused on the n-6 family, namely linoleic acid (LA) and its C20 homologue, arachidonic acid (AA).

More recently, the essentiality of n-3 fatty acids, alpha linolenic acid (ALA) and its longer desaturated derivatives, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have also been recognized. LA and ALA compete for the same chain elongation/desaturation enzymes, and the balance of
these two precursors determines the proportions of n-3 and n-6 long chain polyunsaturated fatty acids (LCPs) which are synthesized. Chain elongation/desaturation is down regulated if final product from either family is present. For example, AA synthesis is down regulated in individuals consuming n-3 LCPs. The long chain derivatives of the C18 parent fatty acids serve as substrates for the generation of bioactive eicosanoids; for example, PGE2 and PGE3 are generated from AA and EPA, respectively.

The requirements for both n-3 and n-6 fatty acids have been extensively evaluated in infants, and are relatively high because of rapid growth and development during the neonatal period. Recommendations for adults are primarily focused on disease prevention, since fatty acid deficiency in healthy adults is relatively rare. Requirements in children have not been clearly defined, and are assumed to lay intermediate between infant and adults requirements. This paper explores the interactions between n-6 and n-3 fatty acids in terms of the requirements for growth and development in infants and for maintenance of health in adults.

2 Infancy

Human milk is assumed to meet all essential fatty acid requirements of the neonate, containing the C18 parent fatty acids as well as LCPs. Plasma and red blood cell levels of both AA and DHA are higher in breast-fed infants than in formula-fed infants (1). In addition, autopsy studies demonstrated that brain DHA and liver DHA and AA are higher in infants who were breast fed than in formula-fed infants (2,3), suggesting that infant formula C18 polyunsaturates may not meet all LCP requirements. An attractive explanation for the differences in tissue LCP concentrations of breast-fed and formula-fed infants is that human milk contains LCPs and traditionally infant formulas do not.

We considered human milk composition to be an important guide to the LCP requirements of infants. We conducted an analysis of human milk fatty acids in samples collected from women in nine countries (n > 50 per country), including sites in Asia, North America, South America, Australia and the UK. AA levels, expressed as a percent of total fatty acids, vary little among countries (Table 1). The lowest level was found in Chile (0.36 weight percent) and the highest level was found in China (0.49 weight percent). In contrast, the DHA levels varied widely, ranging from 0.17 weight percent in Canada to 0.99 weight percent in Japan. It therefore appears that AA is highly protected while DHA is readily influenced by diet. The response in breast milk content of DHA to maternal dietary DHA is clearly seen in controlled studies in which lactating women consumed n-3 LCP dietary supplements (4,5).

The LCP requirements of formula-fed infants could conceivably be met by either C18 essential fatty acids (and their subsequent chain elongation and desaturation by the infant) or by direct addition of LCPs to formula. Bearing in mind the competition between n-6 and n-3 fatty acids for elongation and desaturation, the LA : ALA ratio of infant formulas has been modified in attempts to enhance DHA synthesis (6). Infants consuming formulas with relatively low LA : ALA ratios (4 : 1-5 : 1) did not have plasma DHA (or AA) levels equivalent to breast fed infants (6,7). Experimental results suggest that no level or ratio of LA and ALA will permit formula-fed infants to have plasma concentrations of LCPs that match those of breast-fed infants.

Another way to assess adequacy of dietary fatty acids in infancy is to measure fatty acid accumulation over time. Cunnane et al. used autopsy data to calculate DHA accretion of infants (8). Total requirements (including oxidation and carbon recycling) appear to be approximately 20 mg/day. Human milk, with even low levels of DHA shown earlier for Canada and the US (0.2 weight percent) provides about 60 mg per day. How much DHA can infants synthesize from dietary ALA? The conversion rate of ALA to DHA in infants has not been measured directly in humans (and is not currently technically possible) but some data were reported for conversion in other species. In rats

<table>
<thead>
<tr>
<th>Country</th>
<th>AA Weight%</th>
<th>DHA Weight%</th>
</tr>
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<tbody>
<tr>
<td>Canada</td>
<td>0.37 ± 0.01</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>United States</td>
<td>0.45 ± 0.12</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>Australia</td>
<td>0.38 ± 0.01</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>0.36 ± 0.01</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>Mexico</td>
<td>0.42 ± 0.01</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>China</td>
<td>0.49 ± 0.02</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>Chile</td>
<td>0.42 ± 0.02</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td>Philippines</td>
<td>0.39 ± 0.01</td>
<td>0.74 ± 0.05</td>
</tr>
<tr>
<td>Japan</td>
<td>0.40 ± 0.01</td>
<td>0.99 ± 0.09</td>
</tr>
</tbody>
</table>
only 1.4% of dietary ALA is converted to DHA. In infant baboons 0.23% of ALA is converted to DHA (8). These low rates of DHA formation have long been thought to be a consequence of oxidation of dietary ALA. Recently a novel, non-oxidative route of metabolism was described that may also utilize a significant amount of dietary ALA (9). The amount of DHA synthesized from formula ALA, therefore, is between 1 and 5 mg per day. Even when added to the modest total body stores of DHA present at birth, conversion rates this low cannot meet the brain and peripheral tissue DHA accretion needs of the infant. This explains why the brain of unsupplemented formula-fed infants accumulates only one half the DHA of breast-fed infants, and the total DHA level in the rest of the body actually declines over the first six months of life (2,3). The case to supplement infant formula with LCPs is strong.

To match the amounts of AA and DHA afforded the breast fed infant, a balanced addition of both AA and DHA to infant formula is essential. Addition of n-3 LCPs (whether as a combination of EPA and DHA or as just DHA) to infant formula without AA supplementation resulted in a reduction in growth in preterm infants (10), and a suppression of plasma AA in preterm (10) or term infants (11). Therefore, addition of both AA and DHA to formula is warranted.

Maintaining appropriate levels and ratios of AA and DHA are of great importance, and can only be accomplished by supplementation with both. From the human milk data presented above, it appears that AA levels of 0.3-0.5 percent of fatty acids would match the human milk level. DHA is more difficult to address due to the wide variation in human milk DHA levels. Cunnane et al. calculated that addition of 0.2% DHA would result in brain DHA accretion similar to that of breast-fed infants (8). Clinical validation of such a recommendation would be extremely useful. Gibson et al. conducted an evaluation of LCP addition to term infant formula, with AA levels ranging from 0.2 weight percent to 0.4 weight percent and with DHA levels at either 0.2 weight percent or 0.25 percent (12). An unfortified formula group and a breast-fed group were also included. The groups receiving the higher two LCP levels (0.3% AA/0.2% DHA and 0.4% AA/0.25% DHA) had plasma phospholipid LCP levels similar to the group receiving human milk.

A number of beneficial outcomes have been demonstrated in infants receiving formula supplemented with LCPs. Term infants fed formula without LCP supplementation have a less mature immune system than breast-fed infants, as demonstrated by a higher mononuclear lymphocyte CD4/CD8 ratio (13). A similar pattern was observed in preterm infants receiving either human milk or formula, which was not supplemented with LCPs (14). Interestingly, infants fed LCP-supplemented formula had CD4/CD8 profiles identical to the group receiving human milk. In addition, IL-10 levels and IL-2 receptor levels were normalized to the human milk-fed level in the LCP-supplemented group. Other outcome benefits have been identified in infants fed formula fortified with LCPs. Meta analyses in term (15) and preterm (16) infants have documented the advantages of LCP supplementation in visual acuity. Others have reported improvements in cognitive function (17-19).

LCP status in the latter half of infancy may be further decreased (20) as weaning foods such as cereal grains, vegetables and fruits, typically have a low content of EFAs and little or no LCPs (21). Therefore, supplementation of follow-on formulas has the potential to provide continuing benefits.

3 Childhood

Fatty acid balance has been thoroughly studied in infancy, but few data are available on fatty acid balance in childhood, a time of continued rapid growth and development. In children consuming a mixed diet, overt essential fatty acid deficiency is rare. However, subtle endpoints, like those studied in infants, have not yet been studied in children and there is no agreed reference intake, like breast milk provides in earlier life. At the same time, data continue to accumulate that indicate the adult diseases associated with n-6: n-3 imbalance have their antecedents in childhood. It appears appropriate to consider the fatty acid requirements during childhood to be a combination of requirements for growth and development as in infancy and the requirements for prevention of disease as in adults.

The infant diet, whether breast milk or formula, is very high in total fat (50 energy percent) and relatively low in polyunsaturated fatty acids (generally less than 20 percent of total fat derived from polyunsaturated fatty acids). Recommendations vary on the timing and rate of change in intake of total fat and proportion of polyunsaturated fat from levels in infant diets to levels
recommended for adults (30 percent of energy from fat, 10 percent of energy from polyunsaturated fatty acids). The American Academy of Pediatrics, Committee on Nutrition suggests that this transition occurring during the period from age two to age four years, while Canadian recommendations suggest a much longer time frame for this transition, starting at age two and completing the transition during adolescence. Interestingly, the total amount of polyunsaturated fatty acids as a percent of dietary energy is approximately equivalent in infants and in adults (10 energy percent). Current transition recommendations do not distinguish between n-3 and n-6 fatty acids.

Childhood may be a time to consider initial prevention of adult disease. For example, the Bogalusa Heart study clearly noted determinants of cardiovascular disease were present even during the first decade of life. Neither Canadian or US pediatric associations are persuaded that possible long term consequences from early-life high fat diets outweigh concerns that adequate caloric intake be met during childhood. However, in Finland, a country concerned about CHD prevalence in adults, investigators evaluated very early and sustained dietary fat intervention on outcomes during the first five years of life (22). At seven months of age, infants were assigned to an intervention group, in which families were counseled to reduce both total fat intake and saturated fatty acid intake. The levels of dietary LA increased and LA/ALA levels decreased in this group compared to controls. Growth and cognitive development were similar between groups, but the intervention group had lower total cholesterol and LDL cholesterol levels, and marginally lower HDL cholesterol levels. Much more information is required concerning the appropriate levels of n-3 fatty acids and ratios of n-6 to n-3 fatty acids during this time of life.

4 Adult Life

During infancy the primary concerns dealing with fatty acid balance are related to ensuring appropriate growth and development. In adults the recommendations for dietary fat and balance between n-6 and n-3 fatty acids arise from efforts to reduce the prevalence of chronic diseases (Fig. 1). LCP balance is implicated as important in inflammatory diseases, rheumatologic diseases, lung disorders, immune response disorders, and prevention of CHD. The most prominent focus of research has been on cardiovascular disease, following evidence linking dietary fish (as a source of n-3 fatty acids) to reduced CHD.

Within the arena of research on n-3 in CHD, studies have measured the effects of n-3 intake on all cause mortality, CHD mortality, and subsets of CHD mortality such as sudden death, and secondary prevention of myocardial infarction (MI). Possible mechanisms of CHD risk reduction include effects on initiation of atherosclerosis, factors that affect progression like blood lipoprotein cholesterol, particle buoyancy and susceptibility to oxidation; vascular hemodynamic and metabolic responsiveness, thrombosis and vascular occlusion, mitigation of re-perfusion injury, and prevention of restenosis.

The relationship between fish oil consumption and sudden cardiac death has recently been evaluated in a large prospective study (23). Albert and her colleagues found that consumption of fish at least once a week was associated with a significantly reduced risk of sudden cardiac death in men. Cardiac arrhythmias are frequently associated with sudden cardiac death, leading to investigations of n-3 LCPs and arrhythmias. Fish oil supplementation of MI survivors increased heart rate variability (24), a measure linked directly to survival post infarct (25). Similar effects were seen in a trial of healthy adults. Platelet n-3 content was strongly linked to the increase in heart rate variability (26), and can be utilized as a measure of dietary compliance.

The manner in which n-3 LCPs are incorporated into cardiac membranes and how their presence in the membrane may affect membrane excitability, resulting in anti-arrhythmic effects, has been a subject of several studies. Studies in several species showed that fish oils could prevent ventricular fibrillation caused by coronary artery occlusion (27-29). Billman et al. (29) found that fish oil administration prevented ventricular fibrillation in dogs following experimental
coronary ischemia. Animals given soybean oil, rich in LA but also containing ALA, had no change from control animals. As a result of these in vivo observations, studies in isolated cardiac myocytes have been used to elucidate the cellular and molecular mechanism(s) responsible for these protective effects.

Cultured neonatal rat cardiac myocytes aggregate and beat synchronously, providing an excellent model system in which to study the effects of fatty acids at the cellular level (30). When these cells are exposed to known cardiac arrhythmic agents such as isoproterenol, tachyarrhythmias are produced. Addition of free n-3 LCPs prevented these tachyarrhythmias. Protection was produced rapidly and ethyl esters of these acids were ineffective in providing protection, suggesting metabolism of the fatty acids was not required for the effect. Finally, the effects were reversed by addition of albumin which binds free fatty acids, indicating that the fatty acids had not become covalently associated with the cells.

Further studies determined that n-3 LCPs can affect ion flux through myocyte calcium, potassium and sodium channels, which offers a possible explanation for their antiarrhythmic effects. Arrhythmic protection may be related to effects on sodium channels, the major class of channels determining cardiac excitability. Based on data showing n-3 LCP displacement by radiolabeled ligand that binds specifically to the transmembrane region of the sodium channel, Leaf and his colleagues hypothesize that the n-3 fatty acids insert into the membrane with the long chain unsaturated regions binding to the non-polar transmembrane regions of the sodium channel (31). The negatively charge carboxyl group of the fatty acid would then be in close proximity to the positively charged amino acids of the channel’s voltage sensor. The effect of this interaction would be to reduce the electrical excitability of the membrane and protect against arrhythmias.

Similarly, n-3 LCP intercalation in the calcium channels prevents increased cytosolic calcium. Excessive cytosolic calcium is known to play a role in the induction of ventricular tachycardia by a variety of arrhythmogenic agents.

Some Western diets may be relatively high in n-6 fatty acids. What level of intake of n-3 fatty acids is needed to restore balance? What is the appropriate ratio of LA to ALA? What effect does dietary LCP intake have on recommendations for dietary LA : ALA ratio?

WHO recommends a ratio of LA : ALA be from 10 : 1 to 5 : 1 (32). Other recommendations for infants that specify both LA and ALA have ratios in this same range. Many regulatory bodies, including the U.S. government have no recommendations for n-6 : n-3 ratios. The most recent recommendations for adults are from ISSFAL and recommends an LA : ALA ratio of 2 : 1, the lowest ratio to date (33). ISSFAL also recommends a relatively high intake of DHA EPA (650 mg/d) and includes specific AA and DHA recommendations that give a 3 : 2 ratio. Unfortunately, the rationale and documentation for the recommendations was not included in the publication, so these should be viewed with some caution. How closely do the recommendations of WHO and others for the balance of n-6 and n-3 fatty acids compare to current dietary intakes? And more importantly, how well do all these recommendations align with evidence of protection against disease?

US dietary recommendations and AHA Step 1 diet recommendations are to have 30 % or less of calories from fat. The 30 % is evenly divided between saturated fat, monounsaturated fat and polyunsaturated fat. At a caloric intake of 2000 kcal, the daily intake of polyunsaturated fat is calculated to be approximately 22 grams. Applying the WHO recommendations, a ratio of 10 : 1 corresponds to 20 g LA and 2 g ALA, and a ratio of 5 : 1 corresponds to 18.4 g LA and 3.6 g ALA. Thus, the approximate recommended intake of ALA is from 2-3.6 g/d.

The recommended intake of LA is not difficult to achieve given the high content of LA in common vegetable oils. But what about ALA? Current estimates of dietary intake of ALA in the US are 1.1 to 1.6 g (34), although estimated intake as reported in studies on CHD risk suggest intake may be even lower. At the conversion rate of ALA to DHA of 1.4 % (a liberal estimate, based on data from rats), diets that contain ALA in the range of current consumption but which contain no DHA (i.e. among humans who eat no fish in the studies cited above), approximately 10 mg DHA would be formed.

The consumption of modest amounts of n-3 LCPs has been shown to be protective against CHD. Secondary prevention trials with fish (35) fish oil (36) or a diet that shifted the LA:ALA intake from a ratio of 20 :1 to 4.5 : 1 (37) find a reduction in risk from sudden death after increased n-3 fatty acid intake. Prospective epidemiologic studies (refs. 23, 38, 39) and a case control study (40) also find a 20-30 %
Table 2 DHA (mg/day) Primary Prevention.

<table>
<thead>
<tr>
<th>Lowest Intake Group</th>
<th>DHA from ALA</th>
<th>Dietary DHA</th>
<th>DHA Associated with Reduced Risk of Sudden Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Electric</td>
<td>10*</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Physicians’ Health</td>
<td>14’</td>
<td>&lt;10</td>
<td>90</td>
</tr>
<tr>
<td>Health Professionals</td>
<td>14’</td>
<td>&lt;70</td>
<td>150</td>
</tr>
<tr>
<td>Siscovick et al.</td>
<td>14’</td>
<td>32</td>
<td>100</td>
</tr>
</tbody>
</table>

*At a conversion rate of 1.4% (Cunnane et al. 2000)
1 Assuming ALA intake of 1 g/day

A reduction in risk of sudden death associated with modest n-3 or fish intakes (Table 2), but generally have not found that higher intake of n-3 LCPs further reduces the risk.

The modest DHA consumption shown to be protective against sudden death is considerably more than would be made from dietary ALA (Table 2). Expressed as n-3 LCP intake per day, the protective level of DHA is about 100 mg/d, about an order of magnitude greater than what would be formed from ALA in the absence of any dietary DHA. In the absence of dietary DHA, the recommendations for dietary ALA intake, calculated from recommended ratios of LA:ALA, may be inadequate to produce enough DHA to ensure normal ion channel in the heart.

What balance is needed to retard atherosclerosis? Recent data suggest a diet containing 175-350 mg EPA + DHA/d can slow progression and enhance regression of atherosclerotic coronary segments (41). What balance is needed to prevent arthritis or Crohn’s disease? We don’t know yet. Our studies in infants have shown benefits of addition of modest DHA to a liberal supply of ALA. The fatty acid balance in infants may be a useful guide in our search for balance in adults.

In conclusion, we have shown that infants require a balanced addition of AA and DHA for growth and development. Requirements for LCPs in older infants and children are not yet known, and need to take into account the prevention of the antecedents of adult disease as well as meeting needs for continued growth and development. In adults, the proper balance of LCPs may attenuate or prevent chronic diseases. In fact, it is possible that small amounts of n-3 LCPs may be effective in reducing the risk of sudden cardiac death. There is still much to learn in order to provide the proper balance of fatty acids at the many stages of life.

References