

Polyunsaturated Fatty Acids in Human Milk and Neurological Development in Breastfed Infants

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Abstract: Long-chain polyunsaturated fatty acids (LCPUFA) are bioactive compounds that include docosahexaenoic acid (DHA) and arachidonic acid (AA). DHA seems to be necessary to optimize growth and development of infant's neural functions. For preterm infants there is a wide agreement on LCPUFA benefits, while for term infants results are more controversial. There is presently a growing interest in supplementing pregnant and breastfeeding women with DHA. LCPUFA may act as coadjuvants in conditions of functional and organic brain impairments, as well as some inflammatory states, but caution is urged to prevent false expectations. Therefore, pediatricians should be aware of the present scientific evidence concerning dietary LCPUFA supplementations.

INTRODUCTION

Repeated observations on the improved neurodevelopmental achievement among infants who have been breastfed compared to the formula-fed ones [1] have prompted researchers to identify the components of human milk that are possibly implicated. Long-chain polyunsaturated fatty acids (LCPUFA), in particular arachidonic acid (AA, 20:4 n-6) and docosahexaenoic acid (DHA, 22:6n-3) have been identified as the possible effectors [2]. LCPUFA include eicosapentaenoic acid (EPA, 20:5n-3), DHA and AA deriving from the essential fatty acids, alpha-linolenic acid (ALA, 18:3 n-3) and linoleic acid (LA, 18:2 n-6), respectively. Of these, DHA has received the greatest attention, since it appears necessary for normal growth and normal development of infant's neural functions. Indeed, DHA is the predominant n-3 fatty acid both in the developing brain and the retinal photoreceptor membrane. AA is the predominant n-6 fatty acid in the developing brain and is a precursor of biologically important eicosanoids.

METABOLIC INTERRELATIONSHIPS OF POLYUNSATURATED FATTY ACIDS

Ralph Holman in the 60's described how the n-6 series, the n-3 series, and n-9 series (with oleic acid) compete for the same desaturating and elongating enzymatic pathway [3]. Accordingly, a higher intake of n-6 PUFA should depress the metabolic synthesis of n-3 PUFA. On the other hand, a high availability of n-3 PUFA modulates the synthesis of n-6 downstream products from LA. Notably, the rate-limiting enzymatic step of n-6 long-chain PUFA synthesis is also inhibited by an excess of LA (substrate-induced inhibition) [4]. More recently, it has been shown that DHA synthesis involves a complex series of reactions involving a double delta-6 desaturase step and a beta-oxidation in peroxisomes [5]. The issue on an optimal ratio of LA and ALA for

formula-fed infants, in absence of dietary long-chain PUFA, has been object of intense debate in the 90's [6]. According to Gibson, the DHA status maybe improved lowering the LA/ALA down to values close to 4:1/3:1, as observed both in humans and animals [7]. Lower ratios seem not to be of any advantage [8].

LONG CHAIN POLYUNSATURATED FATTY ACIDS AND HUMAN MILK LIPIDS

Human milk does contain both AA and DHA, and their quantitative levels are kept within an homogeneous range. Their levels in Western populations seem to be quite stable from colostrum through 12 months of life [9], ranging around 12-16 mg/dL for AA and 6-8 mg/dL for DHA (Tables 1-3). Most LCPUFA are supplied as triglycerides, representing 98-99% of human milk fats, but a relatively high fraction is represented in the limited phospholipid fraction, that is 1-2% of human milk fats. This distribution may have functional consequences, since it has been speculated that fatty acids from the phospholipid fraction could be driven directly to target tissues and membranes. An estimated 30% of human milk fatty acids (as estimated particularly for LA) are derived directly from the current maternal diet, whereas the major portion derives from mother's body stores [10]. Human milk DHA content may be influenced by maternal dietary intakes more than AA content.

Human milk lipids are mostly represented by saturated and monounsaturated fatty acids, accounting for more than 80% fat. Saturated fats are involved in the production of energy, and the preferred 2-position for palmitic acid (25% of the total fatty acids in human milk) in the triglyceride skeleton, allows for maximum fat and calcium absorption [11]. Oleic acid (30% fatty acids in the average) shares a similar role in energy production, and may affect lipoprotein metabolism [12]. On the whole, the human milk fatty acid profile is a "concerto", reflecting the quality of the exogenous fat intake, the endogenous synthesis and the individual genetic "disposition" towards utilizing, storing and/or synthesizing specific fatty acids [13]. Human milk

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Table 1. Fatty acid concentration (%) through 12-month lactation in 10 Italian mothers [ref. 9].

age	1 day	1 month	3 months	6 months	9 months	12 months
Saturated (%)	38	39	38	43	42	41
Monounsaturated (%)	45	47	46	42	42	41
Polyunsaturated (%)	16	13	14	14	15	16

Table 2. Polyunsaturated fatty acid concentrations (%) through 12-month lactation in 10 Italian mothers [ref 9].

age	1 day	1 month	3 months	6 months	9 months	12 months
18:2n-6 (%)	11,9	10,1	12,0	11,6	12,6	12,9
18:3n-3 (%)	0,6	0,6	0,7	0,6	0,7	0,9
20:4n-6 (%)	1,0	0,6	0,5	0,5	0,5	0,5
22:6n-3 (%)	0,5	0,3	0,2	0,3	0,2	0,3

Table 3. Polyunsaturated fatty acid concentrations (mg/dL) through 12-month lactation in 10 Italian mothers [ref 9].

age	1 day	1 month	3 months	6 months	9 months	12 months
18:2n-6 (%)	179	347	390	350	395	398
18:3n-3 (%)	9	18	23	18	24	26
20:4n-6 (%)	14	16	15	12	14	12
22:6n-3 (%)	8	9	8	7	8	7

samples largely differ also as far as their absolute fat content [14]. As a final result, each breastfed infant has a unique fat intake, both qualitatively and quantitatively, differing by many factors such as time postpartum, gestational age, parity, mothers' genetic inheritance, culture and countryside, and, above all, maternal dietary habits.

The variability in the essential fatty acid content of breast milk within and among different populations is mainly due to different diets, but few information is available on the role of the ethnic background [15]. Indeed, geographically distinct populations have different diets. Studies published in the last 5 years show that human milk from women following Western diets generally contains 10% to 17% LA, 0.8% to 1.4% ALA, 0.3% to 0.7% AA, and 0.1% to 0.5% DHA [16]. Studies from other areas of the world show concentrations of DHA as high as 2.8% in human milk in Zhangzi, China [17], and 1% AA and 1.1% DHA in the milk of women in Japan [18], probably explained by a higher intake of DHA from fish and seafood among these populations than in North America. Some discrepancies are also explained by higher intakes of carbohydrates of women eating non-Western diets, which are known to increase the mammary gland *de novo* synthesis of 12:0 and 14:0. These dietary habits are also apparently associated with lower milk 18:2n-6 contents [15].

The presence of LCPUFA in human milk makes to some extent useless any discussion on the ideal ratios between the two precursor fatty acids, LA and ALA. Indeed, as a general feature, human milk always contains LCPUFA, and even in strictly vegan women both DHA and AA are represented [19]. Thiombiano-Coulibaly *et al.* [20] have shown a characteristic PUFA pattern among two groups of rural and urban lactating women from Burkina Faso in SubSaharian Africa, during two distinct periods, lean and post-harvest seasons. In fact, a general trend towards high levels of LA and consequent high LA:ALA and n-6:n-3 LCPUFA ratios are clearly present. Values are particularly striking for urban women during the lean season, when the LA:ALA ratio approaches the value of 53. The findings are consistent with a high-carbohydrate, low-fat diet of these populations, constituted by 80% carbohydrates and 4% fats through all the year with only minor changes of the composition of food items [21].

In a previous paper from Burkina Faso and Congo, Rocquelin *et al.* have reported a negative, non-linear association between the LA:ALA ratio in milk and infants' weight gain [22]. This relationship contrasts with the observation in a smaller group of formula-fed infants, living in a completely different setting, that lower than recommended LA:ALA ratios, due to high intakes of ALA,

results in a decline of the body weight [23]. Thus, as this question is controversial, it has to be tested in further studies.

POLYUNSATURATED FATTY ACIDS IN INFANT FORMULAS

Most authorities have based their recommendations for the composition of infant formulae on mature human milk. Possibly because of the lack of additional information, these "standards" are mostly based on milk obtained from mothers with Caucasian ethnicity, consuming typically Western diets. For example the ESPGHAN recommendations are based on human milk data derived from Sweden, Germany and UK. Furthermore there is no scientific evidence that the breast milk from Caucasian mothers is superior to that from other ethnic populations [15].

According to an European expert Committee [24], "the minimum level of ALA is now proposed to be set at 12 [24] mg/100 kJ or 50 [100] mg/100 kcal corresponding to 1 [2] percent of total fatty acids". Indeed, some trials indicate that 2% of fatty acids, which is 1% of energy, as ALA, in absence of dietary DHA, may be safe and effective for the functional outcome of formula-fed infants [25-27]. "The concentration of ALA is further regulated by a proposed ratio between LA to ALA of 5-15 [20] to ascertain a proper balance between the precursors of the respective n-6 and n-3 fatty acid series, which is justified by the use of common enzymes for their metabolism to LCPUFA of the respective n-6 and n-3 series. The higher minimum level should be used in infant formulas to which no long-chain PUFA has been added, while the lower level is appropriate in formulas containing at least 0.2% of the fatty acids as DHA. In such formulas the more generous ratio of 5-20 between LA and ALA should be adequate, while it is proposed that a ratio of 5-15 should be applied for formulas containing less than 0.2% of the fatty acids as DHA" [24].

Maybe institutions will refine indications, as far as limits of composition and ingredients, and formula companies will patent new compounds, to improve bottle-fed infants' functional outcomes, not just to copy human milk composition. But the easiest way to give all infants the best dietary and genetic balanced variety of human milk fats is to give them their own mother's milk. Lawrence Weaver has recently commented in a suggestive fashion: "The Holy Grail in the quest for perfect infant formula is of course something that already exists→human milk. It is logically possible and desirable that refinement of the perfect breast milk substitute and achievement of universal breastfeeding will coincide, and the holy grails of both industry and child public health will be realized at once and in one, when all babies are fed by their mothers on the breast" [28].

DIETARY LCPUFA, VISUAL FUNCTION AND MENTAL DEVELOPMENT IN ANIMAL STUDIES

A number of brain processes are thought to depend on the presence of adequate concentrations of DHA and AA as well as on balanced interactions between n-3 and n-6 LCPUFAs. These processes include apoptosis, gene transcription, neurite outgrowth, membrane excitability, prostaglandin formation, desaturation-elongation, cerebral

ischemia, inflammatory and immunological events and membrane fluidity and elasticity [29].

DHA is avidly incorporated and retained in brain cerebral phospholipids and the high concentrations of DHA in the retina and of DHA and AA in brain gray matter suggests that these fatty acids have important roles in neural function that could result in reduced visual function and learning deficits.

LCPUFA have a central place in the structure of fluid, excitable, metabolically active membranes such as photoreceptor outer segments and synaptic membranes [30]. Animal studies on the effects of n-3 fatty acids and visual function have shown that dietary n-3 deficiency is associated with reduced maximal electroretinogram (ERG) amplitudes in rodents, suggesting as a possible causal factor a lower number of photoreceptors or a reduced potentiality "to capture" (mV/photon) light. While transient reductions in ERG amplitude were reported in n-3-deficient monkey infants, [31] no changes have been seen in monkey infants fed DHA versus ALA [32].

Several studies have shown that in n-3 fatty acid-depleted rodents the performance in learning tests is reduced [27], but in any case the association between dietary n-3 fatty acids and learning ability does not seem to be straightforward. These effects might be explained by changes in the membrane bilayer that alter membrane-associated receptors and signal transduction systems with ion channel activities, or even directly affecting gene expression [16].

Dietary LCPUFA have also been shown to affect other behavioral domains, such as sensory, motivation and motor areas, that, in turn, might also influence the development of cognitive functions [33]. For instance, the supplementation of the murine fetus with high levels LCPUFA *in utero* reduces the immobility in the swim test in the offspring [34]. In rhesus monkeys, a n-3 fatty acid deficiency has been shown to prolong the duration of a visual attention test, suggesting a slower speed of information processing [35]. These animal studies indicate that infancy is a critical period in the visual and neurological development and that a deficiency in the n-3 fatty acid supply early in life can have long-term effects.

LONG-CHAIN POLYUNSATURATED FATTY ACIDS AND INFANT NUTRITION: HEALTH EFFECTS

Although infants can synthesize DHA and AA from ALA and LA, respectively, formula-fed infants, not receiving LCPUFA from diet, have lower plasma and erythrocyte levels of DHA and AA than breast-fed infants and infants fed formulas supplemented with these LCPUFA. The definition of the dietary role of LCPUFA (essentiality? Conditional essentiality? Semiessentiality?) in infancy has been considered through systematic analyses by several experts, and in terms of dietary recommendations by Scientific Committees. The question mainly arises on the evidence of any measurable functional outcome, since the biochemical evidence clearly shows that the exogenous LCPUFA supply raises plasma circulating levels. Functional data have been considered in terms of neural development, since few other functional domains have been thoroughly explored.

For preterm infants there is a wide agreement on the neural functional benefits, considering the short-term outcome, while the persistence of the results at both medium and long term would require further assessments [36-37]. For term infants results are more controversial, depending also on the large differences of the study designs, therefore conclusions and recommendations differ by some aspects. A Cochrane research concludes that *“at present there is little evidence from randomized trials of LCP supplementation to support the hypothesis that LCP confer any benefit on visual or cognitive development”* [38]. On the other hand, considering a larger set of studies, recommendations of an expert Meeting [39] conclude that *“infant formulas for term infants should contain at least 0.2% of total fatty acids as DHA and 0.35% as AA, while formulas for preterm infants should include at least 0.35% DHA and 0.4% AA”*. Reviews and recommendations always underline the lack of adverse effects deriving from LCPUFA supplementations.

Yet, it is not convincingly proved that LCPUFA supplementation in full-term infants confers a benefit for visual, motor, and cognitive development extending beyond the first year of age. However, studies that following children up to school age, have not been carried out or, at most, are presently in progress. One of the major bias in interpreting the results on infants is the poor predictivity of the most widely used developmental scales. Most studies dealing with LCPUFA supplementation's effect on infant motor and cognitive development have included in the methodology of assessment the Bayley Scales of Infant Development or the Fagan test. The Bayley Scales are a frequently used, but relatively gross instruments, to investigate infants' motor and cognitive abilities. The Fagan test is supposed to be a specific cognitive test evaluating infants' interest in novelty. Putative positive effects of LCPUFA have been found when more specific assessment techniques have been adopted to test infant development [40].

Evaluation of general movements has been shown to be a possibly more accurate tool to test the quality of brain function in young infants [41]. The quality of general movements may be a strong predictor of neural development, in particular if the quality of movements is tested at 2-4 months of age after term. Indeed, the occurrence of mildly abnormal general movements at 2-4 months of age is associated with a significant increase in the risk of development of minor neural dysfunction, attention problems and aggressive behavior at school age [42]. In a prospective, double blind, randomized controlled trial, the supplementation of healthy infants with LCPUFA during the first two months of life has reduced the occurrence of mildly abnormal general movements [43].

In the meantime, further data are accumulating on the possible functional role of DHA for structural development of young infants. During pregnancy, DHA is preferentially transferred from the mother to the foetus, particularly in the last trimester, and impairments in this passage are associated with intrauterine growth retardation [44]. Recently, DHA intake in early infancy has been associated also to lower blood pressure values at 5 years of age [45].

LONG-CHAIN POLYUNSATURATED FATTY ACIDS AND MATERNAL NUTRITION IN PREGNANCY AND LACTATION

The supplementation of pregnant and breastfeeding women with DHA has been hypothesized to improve the early developmental outcome of the offspring. Few scientific data concerning this issue are published. On the other hand, mercury, particularly methylmercury, is an established worldwide environmental pollutant, toxic for humans. Recent data suggest that fetal exposure to methylmercury at high levels results in subtle decrements in several measures of neurological development at seven years of age [46]. Both DHA and methylmercury have a unique dietary source for humans, that is, fish, in which they accumulate deriving from the food chain. DHA is stored mainly in deep ocean fish species as result of the biologic adaptation of living in waters, while mercurials in the seafood mainly derive from pollutants. What is the net benefit on infants' neural development if mothers increase their fish intake? A dietary enrichment of maternal diet with DHA sources has been supposed to have beneficial effects for fetal and infant development, in spite of the lack of direct proves [39]. Conversely, at the same time, a consumer advisory from the Food and Drug Administration, has recommended that pregnant women avoid fish species with the highest average amounts of methylmercury [47].

In a recent paper, a developmental advantage has been shown, for the first time, at 4 years of age for children born to mothers supplemented with cod liver oil from the 18th week of pregnancy up to 3 months after delivery through lactation, compared with a placebo-supplemented group [48]. These data show an effect for a dose of more than 1 gram DHA as dietary supplement per day, a limit almost impossible to reach by just eating fish, unless assumed in quite large and unusual daily amounts.

Therefore, while the safety of eating fish during pregnancy is not firmly established, there is now evidence that DHA supplementation during pregnancy may be beneficial to infants.

LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN CHRONIC CHILDHOOD DISORDERS

Infants and/or children affected by foetal alcohol syndrome, attention deficit hyperactivity disorder (ADHD), cystic fibrosis (CF), phenylketonuria (PKU), unipolar depression, aggressive hostility, and congenital peroxisomal disorders, particularly adrenoleukodystrophy, have low plasma and/or erythrocyte levels of DHA, suggesting a relationship between low DHA status and some symptoms of these disorders. In addition, EPA and DHA are thought to play an anti-inflammatory role and their use in a variety of such disorders, in both adults and children, has been proposed. To date, it is difficult to justify supplementations of DHA in patients with most of these disorders. The effects of DHA, alone or together with other LCPUFA, have been investigated only in a few appropriate trials. Accordingly, preformed LCPUFA may have advantageous effects in some chronic childhood disorders involving brain (e.g., PKU) and/or inflammatory diseases (e.g., CF).

Dietary treatment of patients affected by PKU includes use of manufactured products with little or no phenylalanine, but it precludes the major source of DHA and AA, and also of their precursors, especially ALA. It has been observed that the addition of DHA and AA [49] or DHA and EPA [50] to diets of older children affected by PKU raises plasma levels of these fatty acids and improves the visual response of treated children. However, 3 years after supplementation, no biochemical or functional differences between supplemented and unsupplemented children are apparent [51]. Early supplementation with preformed LCPUFA could be an important addition to the nutrition of these infants, because the most vulnerable period for brain development is represented by the first few months of life.

A supplementation of LCPUFA has been proposed to increase their blood levels and possibly decrease the symptoms of ADHD. In fact, many supplements are readily available and are marketed for the treatment of children affected by ADHD. Nevertheless, a single double-blind, placebo controlled study showed that DHA supplementation for 4 months increased dramatically plasma phospholipid DHA but had no effect on symptoms of ADHD [52].

Specific disorders in which this combination might have a role include different inborn errors of amino acid and organic acid metabolism, as well as rare disorders of peroxisomes. At present, data from randomized controlled trials in these conditions are not available.

CF is an example of inflammatory disorder that may potentially benefit from preformed n-3 LCPUFA supplementation [53]. A recent Cochrane review concluded that, "Regular n-3 supplements may provide some benefits for CF patients with relatively few adverse effects" but cautioned that "the evidence, so far, is insufficient to draw firm conclusions" [54]. It has been suggested that such supplementation may also be beneficial in intestinal inflammatory disorders [55] but further information is required. Interestingly, it is likely that a combination of EPA and DHA (e.g., fish oil) provides optimal anti-inflammatory effects but the potential negative effect of EPA on growth must be resolved before this can be thoroughly evaluated in infants and children.

Although a prudent use of these highly bioactive fatty acids on a purely deductive basis appears reasonably safe, caution is urged to prevent false expectations and potential harm.

CONCLUSIONS

Human milk fats include both the main long chain polyunsaturated fatty acids, that is, arachidonic acid and docosahexaenoic acid, in structural forms that are highly available for absorption and metabolism. Maternal dietary habits may influence fatty acid composition of human milk. In spite of years of intensive research, few firm evidence is accepted on the role of dietary LCPUFA. They seem dietary essential for preterms and may act as coadjuvant, in a drug-like effect, in conditions of functional and/or organic brain impairments, as well as some inflammatory states. Their benefits to pregnant and lactating women need larger confirmations. Since LCPUFA are proposed for many

conditions without any clinical evidence yet, pediatricians should be aware of the present knowledge about these dietary compounds to prevent an unjustified overuse.

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