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"LCPUFAs as conditionally essential nutrients for very low birth weight and low birth weight infants: metabolism, functional and clinical outcomes - how much is enough?"

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KEY POINTS:

Preterm infants have a high requirement for preformed dietary DHA, approximately 3 times the concentration in mature human milk and infant formula, if they are to meet the *in utero* rapid accumulation of DHA as it normally occurs in late pregnancy.

LCPUFA intervention trials prior to 2000 largely assessed whether infant formulas that lacked LCPUFA should be supplemented to the equivalent concentrations of DHA and other LCPUFA found in typical human milk of women from Westernized societies.

Trials of LCPUFA supplemented formulas demonstrate that supplementation with at least 0.3% total fatty acids as n-3 LCPUFA improved visual development, especially in infants born <30 weeks' gestation or with birth weights <1500g.

Attention is now focused on determining whether there is added advantage to meeting the *in utero* accumulation rate of DHA.

The largest intervention trial to date indicates that higher dose DHA may improve cognitive scores, reduce the risk of developmental delay and reduce the risk of broncopulmonary dysplasia in the smallest and most immature infants.

SYNOPSIS

Infants born preterm are denied the rapid accumulation of docosahexaenoic acid (DHA) that occurs during the third trimester *in utero.* The benefit of long chain polyunsaturated fatty acids (LCPUFA) for preterm infants has generated much interest over the last 3 decades. Early intervention trials involving preterm infants were designed to assess the benefits of supplementing infant formulas that lacked DHA with equivalent concentrations of LCPUFA found in typical human milk of women from westernized societies. These studies led to the inclusion of LCPUFA in all preterm infant formula by the year 2000. Over the last decade, attention has moved towards determining the optimal dose of DHA required by preterm infants and whether there is advantage in using higher doses of DHA (approximately 3 times the concentration in most human milk and formulas) to match the *in utero* accumulation rate of late pregnancy.

INTRODUCTION

In examining the effects of long chain polyunsaturated fatty acids (LCPUFAs) on the clinical and developmental outcomes of preterm children we have considered it logical to separately evaluate the early trials of formula feeding in relatively "healthy" low birth weight and very low birth weight infants from the more recent controlled trials which assessed higher doses of LCPUFA, particularly considering the role of long chain n-3 fatty acids [EPA (eicosapentaenoic acid 20:5 n-3) and DHA (docosahexaenoic acid 22:6 n-3)] in more immature, sicker preterm infants.

The early randomized controlled trials of LCPUFA interventions were designed to assess whether the infant formula for preterm infants required supplementation with n-3 and n-6 LCPUFA as formulas were devoid of all LCPUFA and contained only the precursor essential fatty acid (EFA), n-3 alpha-linolenic acid (ALA, 18:3n-3), in small amounts and much larger quantities of the n-6 EFA, linoleic acid (LA, 18:2 n-6) (Figure 1).

Such trials were limited to preterm infants who were exclusively fed formula from the time enteral feeding began, comparing formulas containing only precursor EFAs with those supplemented with LCPUFA. Initial studies focused only on n-3 LCPUFA supplementation through fish oils but later studies also included the n-6 LCPUFA, arachidonic acid (AA, 20:4n-6) to try and mimic the concentrations of LCPUFA in the human milk of women from westernized societies. The infants studied were selected from those relatively healthy enough to receive enteral feeds; few of these infants had birth weights <1000 g. Almost none of these intervention trials of formula feeding intervened with DHA concentrations that exceeded ~ 0.3% of total fatty acids. However, the first set of studies used deodorized menhaden oil as a source of DHA, this oil contained ~ 0.3-0.4% DHA but also provided ~ 0.6% EPA, thus the total

amount of ≥ C 20 n-3 fatty acids was close to 1%. These early studies focused on the effects on biochemical endpoints and sensory or cortical neuro-development, were of small sample size and thus were not powered to examine relevant clinical outcomes. Collectively, these studies led to the gradual inclusion of both n-6 and n-3 LCPUFAs to premature and later to full term infant formula, so that by the year 2000 infant formula for preterm infants in developed countries was universally supplemented with LCPUFAs equivalent to the concentration found in mature human milk of Westernized societies. Attention since then has focused on determining the optimal dose of DHA required by preterm infants.

LCPUFA fetal accretion rate and metabolism in preterm infants

The measurement of fetal accretion and early *ex utero* accretion rates represent a relatively common approach to estimate a minimum dietary requirement. The amount of nutrient required to match accretion at the corresponding post conceptional age represents the absolute minimum for the specific nutrient required by preterm infants. In addition this amount needs to be corrected by relative absorption of the nutrient from human milk or infant formulas and by the oxidative losses, since not all that is ingested is absorbed and some of what enters the body is used as fuel and cannot be considered available for tissue deposition. Thus, the recommendation can be derived by considering the minimum amount that needs to be taken to compensate the absorptive losses that will result in a net retention rate similar to the intrauterine accretion rate. Most attention has focused on DHA accumulation in the central nervous system. Whether the brain is preferentially protected when availability of DHA is limited is not known, but the ease with which fetal brain DHA is altered by maternal dietary n-3 fatty acid intake suggests that the membrane lipid composition of the fetal brain is sensitive to changes in DHA supply

[1]. Because most LCPUFAs accumulate in white adipose tissue and, to a lesser

extent, in lean mass and the liver [2], it is important to consider accumulation of DHA and other LCPUFAs in all relevant organs.

Analyses of fetal autopsy tissue yield estimates of intrauterine accretion of LCPUFAs during the last trimester. They are: 106 mg/kg/d, 4 mg/kg/d, 212 mg/kg/d and 43 mg/kg/d for LA, ALA, AA, and DHA, respectively [2]. It is likely that the accumulation of LCPUFAs is not linear over time during the last trimester. Using these numbers to calculate average daily rates of fatty acid accumulation will overestimate or underestimate tissue requirements during specific periods of growth. A more precise estimate of the fetal accretion rate cannot be determined until more data become available. However these recent data based on post-mortem tissue analyses of stillbirths suggest that during the third trimester *in utero* whole body accumulation of DHA is of the order of 60mg/kg/day [2].

Based on this information we have estimated that preterm infants, who are born early and denied the rapid accumulation of DHA occurring predominantly during the last trimester of pregnancy, require DHA in $\geq$1% total fatty acids [3]. Present research is now focusing on supplementation strategies to increase the LCPUFA concentration in both human milk and infant formula from ~ 0.3% total fatty acids as DHA to 1 % in order to match *ex utero* intakes to *in utero* accretion during the third trimester. Thus in our discussion of the clinical outcomes associated to DHA supplementation we will examine the relevant trials in two separate sections, those relating to the effects of LCPUFA supplementation of infant formula (comparing no LCPUFA vs LCPUFA equivalent to human milk levels) and those trials reporting the effects of LCPUFA supplementation that assessed higher doses (comparing LCPUFA concentrations equivalent to human milk vs measured *in utero* accretion levels).

Effect of LCPUFA supplementation of infant formula on visual development of preterm infants

The role of LCPUFA, particularly that of DHA, has been a point of intense investigation since the early 1990's when the first published clinical study showed that electroretinographic function and cortical processing of visual stimuli, as measured by visual evoked potentials of preterm human infants born weighing <1500g, was improved following supplementation of formula with marine oil rich in n-3 LCPUFA (0.36% of total fatty acids), compared with a control formula high in LA (n-6 FA present in corn oil) without n-3 LCPUFA and with only trace amounts of ALA the metabolic precursor of DHA [4, 5]. A third group in the intervention study included infants who were fed formula containing soy oil as a source of ALA. The retinal and cortical function of the infants in the soy-oil formula group were intermediate between the control and marine oil group indicating that preformed n-3 LCPUFA was needed for optimal function (matching the performance of human milk fed neonates) (Figure 2) [4, 5]. Importantly the visual function of the n-3 LCPUFA supplemented infants at 36 weeks post-conceptual age did not differ from a reference group of infants fed human milk, which contains LCPUFA or from a group of neonates born at the equivalent post- conceptional age studied soon after birth [4]. The poignancy of these early observations stem from the fact that the control formula used in this clinical study derived most of its PUFA from corn oil [4] and had a fatty acid composition not dissimilar to the n-3 fatty acid deficient diet used by Neuringer et al [6, 7] who showed that infant rhesus monkeys fed n-3 fatty acid deficient formula experienced visual loss that was associated with reductions in brain DHA concentration, compared with infant monkeys fed their mothers' milk or the n-3 fatty acid sufficient diet based on soy oil.

The follow-up assessments of this study also showed similar effect on visual acuity at 4 months corrected age using electrophysiological assessments [5]. The 1990s and the early 2000 saw a number of randomised intervention trials of formula supplemented with LCPUFA and many of these studies focused their efficacy assessment on visual function during infancy. The relevant trials are summarised in the most recent Cochrane systematic review and although the review concludes that there is no consistent benefit of LCPUFA supplementation of infant formulas for preterm infants on visual development, it acknowledges that major differences in assessment methods between studies does not allow for a meta-analysis to be performed [8]. It is therefore interesting to consider the differences between the trials that did report some improvement in visual maturity with LCPUFA supplementation, compared with the trials reporting no effects. It appears that two factors may be influential, first, the dose of n-3 LCPUFA or DHA supplied and second, the maturity of the infants included in the trials. Trials of n-3 LCPUFA supplementation are more likely to report a beneficial effect on visual development if the majority of infants included were < 30 weeks' gestation or <1500g and the dietary intervention contained at least 0.3% total fatty acids as n-3 LCPUFA [4, 9, 10]. Further analysis to explain the heterogeneity in responses across different studies has considered not only the preformed DHA consumed but the potential contribution to the DHA pool from the endogenous conversion of ALA to DHA. Measurements of DHA formation from deuterium labelled ALA have revealed low levels of conversion for preterm infants (3-5%), this is further compromised by intrauterine growth retardation [11].Thus only a small fraction of the ALA fed to a group of growth retarded infants and or low birth weight infants is converted to DHA. A meta-regression dose response analysis of the effect of DHA supply on visual acuity measures in term infants across multiple

studies considering not only the preformed DHA consumed but the total DHA equivalents formed from ALA desaturation and elongation considering a potential 1, 5 and 10 % conversion revealed a progressively stronger correlation reaching 0.7 when a 10 % endogenous formation from ALA was considered [12]. A similar approach with trials involving preterm infants may be useful to better understand the differences between individual trials.

Effects of LCPUFA supplementation of infant formula on global indices of development

Beyond visual function, 7 different randomized trials of formula feeding with LCPUFA have assessed global indices of neurodevelopment, generally using the Bayley Scales of Infant and Toddler Development (BSITD) [10, 13-18]. While some have criticized the use of these global indices as being blunt measures of specific developmental domains, they nevertheless provide standardized measures that are useful to clinicians and families alike. The outcomes of these 7 trials with Bayley developmental quotients (DQs) from either the first or second version of the BSITD have been summarized in two relatively recent systematic reviews [8, 19]. The two reviews had somewhat different approaches to combining the data in meta-analyses and as a result have differing outcomes. Schulzke et al [8] separately reported DQs of preterm children at 12 and 18 months corrected age despite the fact that the BSITD is age standardized. They showed no significant difference in cognitive DQ between groups at either age (weighted mean difference, WMD, 0.96 points, 95% CI -1.42 to 3.34 at 12 months corrected age, 4 trials including 364 preterm infants; WMD 2.4 points, 95% CI -0.33 to 5.12 at 18 months corrected age, 3 trials with 494 preterm infants) [8]. On the other hand, Smithers et al [19] combined the 12 and 18 month data, because all of the DQ scores are age standardized, and conducted a sub-group

analysis according to BSITD version as the second version of the BSITD included more language and problem solving items for 12 to 18 month old children compared with the first version as well as having differences in scoring and administration. Smithers et al [19] found that in the meta-analysis of all 7 trials the cognitive DQ of LCPUFA treated preterm formula fed children did not differ from control (WMD 2.13 points, 95% CI -0.87 to 5.14, 976 preterm infants), however, the meta-analysis of data from the BSITD version II demonstrated an advantage of LCPUFA treatment (WMD, 3.4 points, 95% CI 0.56 to 6.31, 5 trials with 976 infants). These 5 trials included the majority of infants and were less likely than other trials to be subject to biases.

Beyond 18 months, only one published study has followed children into childhood to determine cognitive effects of LCPUFA supplementation in infancy [20]. They found no difference in intelligence quotient (IQ) but did report that girls who received LCPUFA supplemented formula performed significantly better at single word reading accuracy and spelling than girls who received unsupplemented formula [20]. However, the study was limited by large losses to follow-up (55%) making interpretation and generalization difficult. It therefore seems that the question of whether LCPUFA supplementation of preterm infant formula results in long term neurodevelopmental benefit remains open, and may be difficult to definitively answer as formulas for preterm infants are now all supplemented with LCPUFA.

LCPUFA needs for LBW and VLBW affected by common diseases of prematurity

Considering the potential beneficial effect of dietary LCPUFA on the diseases of prematurity demands due consideration of the importance of n-3 and n-6 LCPUFA in

modulating the inflammatory immune response as well as the effects of these FA on endothelial function, coagulation, inflammation, and neural tissue recovery after ischemic/hypoxic injury. These processes define the severity or potential recovery from hypoxic/ischemic injury [21].

Many of the randomized controlled trials comparing the outcomes of preterm infants receiving supplemented formulas with either DHA or both DHA and AA with infants receiving unsupplemented formula have reported a range of clinical outcomes including necrotizing enterocolitis, sepsis, retinopathy or prematurity, intraventricular haemorrhage and bronchopulmonary dysplasia (BPD). The relevant trials have been summarized in a systematic review and meta-analysis [19]. As the clinical signs and symptoms used to diagnose these diseases may differ between neonatal units and may change with improvements in clinical practice over time, two sensitivity analyses were conducted. Apart from combining all data, sensitivity analyses included trials only using internationally accepted definitions of the relevant diseases, or trials with a low risk of bias based on reporting adequate concealment of randomization and analysis according to the intention to treat principle. In meta-analyses of data from about 1,500 preterm infants, the risk of necrotizing enterocolitis and sepsis did not differ between infants fed LCPUFA supplemented or control formula when all available data were included, when necrotizing enterocolitis or sepsis were confirmed according to international standards or in the trial quality sensitivity analysis [19]. There were also no clear differences in retinopathy of prematurity, intraventricular haemorrhage, or BPD between preterm infants fed LCPUFA or control supplemented formula in overall analyses, or when trials reported diseases according to the pre-specified definitions or in the trial quality sensitivity analysis.

However, the data were limited by small sample sizes and potential biases associated with the studies and the definitions of the diseases [19].

Effects of LCPUFA supplementation designed to mimic *in utero* accumulation

Recent attention has turned to assess whether dietary DHA supplementation to match *in utero* supply results in measurable benefits to the growth, development or clinical outcomes of children born preterm with the publication of two relevant intervention trials in the last 5 years [22, 23]. One trial focused on human milk fed preterm infants [22] while the other was inclusive of all infants regardless of whether they were human milk fed, formula fed or a mixture of both [23]. Henriksen et al randomly allocated 141 very low birth weight infants (<1500 g) who were human milk fed to 6.9% DHA and 6.7% AA (% total fatty acids) and demonstrated an improvement in problem solving at 6 months corrected age [22]. In a further follow-up at 20 months of age, they showed no difference in cognitive DQ but reported a significant improvement in sustained attention in free play activities [24]. No other differences in clinical outcomes were reported [22, 24]. The relatively small sample size and losses to follow-up make interpretation difficult.

The single largest trial, involving over 650 infants born <33 weeks', was designed to assess the delivery of approximately 1% total fatty acids with DHA compared with approximately 0.3% DHA supplied either through human milk, infant formula or a combination of both in order to mimic typical feeding practices in neonatal units [23]. All milks contained 0.4-0.5% total fatty acids as AA. The trial was powered for neurodevelopmental outcomes and also reported on outcomes related to visual development, growth and the typical diseases of prematurity. Although there were no significant differences between groups in overall cognitive DQ at 18 months

corrected age (MD 1.9; 95% CI −1.0 to 4.7), severe cognitive delay (score <70) was reduced from 10.5% in the control group to 5% in the higher DHA group (RR 0.50; 95% CI 0.26 to 0.93) [23]. Furthermore there were significant treatment interactions indicating that higher DHA treatment had differential responses by infant sex and birth weight category. Girls had a significant improvement in cognitive DQ with high-DHA treatment, while boys did not differ between groups. For infants born weighing <1250 g, the cognitive DQ in the high-DHA group was higher than with standard DHA and there were no group difference in infants born weighing at least 1250 g (Figure 3). In secondary analyses relating to the clinical outcomes of infants, there were no group differences relating to the incidence of sepsis, necrotising enterocolitis or intra- ventricular haemorrhage, but high-DHA treatment may in fact result in lower rates of BPD particularly in infants born weighing <1250g and male infants [23, 25]. Other secondary analyses indicated that the high-DHA group had better visual acuity at 4 months of age compared with the standard-DHA group [26] and that infants fed higher DHA were 0·7 cm (95% CI 0·1, 1·4 cm; P=0·02) longer at 18 months corrected age [27]. There was an interaction effect between treatment and birth weight strata for weight and length. Higher DHA resulted in increased length in infants born weighing ≥1250 g, at 4 months corrected age and in both weight and length at 12 and 18 months corrected age [27]. While complex, these data indicate that DHA up to 1% total dietary fatty acids is safe, does not adversely affect growth and may in fact have other clinical advantages in relation to BPD and early childhood neurodevelopmental outcomes for important subgroups of infants. The relatively consistent benefit of higher dietary DHA, designed to emulate *in utero* accretion, in the smallest and most immature infants is consistent with the hypothesis that suboptimal DHA availability during the critical neonatal period results in disturbed

DHA accumulation and has consequences on development. Current large-scale trials, such as N3RO (N-3 LCPUFA for Respiratory Outcomes in infants born <29 weeks'), should provide conclusive and contemporary data for higher-dose DHA supplementation to the most vulnerable infants as well as offer some new insights into the mechanisms by which higher-dose dietary DHA may work to dampen inflammatory immune responses.

Future directions of relevance to neonatal and perinatal medicine

Over the past three decades knowledge on DHA effects on gene expression and on the production of n-3 derived eicosanoids has expanded significantly beyond the areas we have covered in this short review. Animal studies using a genetic modification have produced a rat that over expresses delta-6 desaturase (fat-1 rat), allowing significant experimentation in animals that have increased DHA content of all tissues, as well as the unique models of stroke as a hypoxic injury that can be treated with DHA-derived compounds capable of resolving the associated inflammatory insult. Due to their potential relevance to neonatal health and/or amelioration of neonatal conditions affecting very low birth weight infants we suggest some areas where further research may reveal significant benefits:

(1) Preventing excessive inflammation, especially in the gut and lung and understanding some of the mechanisms by which dietary n-3 LCPUFA may alter the onset and progression of NEC and BPD

(2) Protecting from hypoxic/ischemic organ damage as demonstrated by the use of DHA derivatives in ischemic brain infarctions and may have relevance to the hypoxic/ischemic brain injury experienced with IVH.

(3) Some of the latest trials suggesting that higher dose DHA administered in pregnancy reduces the risk of early preterm birth may offer additional treatment modalities with potential to administer DHA to mothers who

potentially will deliver preterm or growth retarded infants in order to prevent or ameliorate the later consequences of these conditions.

SUMMARY

The essentiality of LCPUFA, particularly DHA, for preterm infants has been a point of discussion in the literature for some 25 years. While most of the biochemical studies clearly show the insufficiency of DHA in the diet of preterm infants, the picture has not been so clear from the intervention trials with clinical and developmental outcomes. The early intervention trials, and in fact the majority of the controlled trials, were designed to assess whether infant formulas that were devoid of LCPUFA should be supplemented to the equivalent concentrations of DHA and other LCPUFA found in typical human milk. Intervention studies involving exclusively formula fed preterm infants have demonstrated improved visual development using neurosensory and behavioral techniques and the trials showing the most consistent benefit included those in which the majority of infants were born <30 weeks' gestation or had birth weights <1500g and the dietary intervention contained at least 0.3% total fatty acids as n-3 LCPUFA. With the universal supplementation of all preterm infant formula with LCPUFA since the year 2000, attention has since focused on determining the specific dietary requirement of DHA and whether there is added advantage to meeting the *in utero* accumulation rate of DHA which is approximately 3 times the concentration in most human milks and infant formula. The largest intervention trial addressing this question to date indicates that higher dose DHA may improve cognitive scores, reduce the risk of developmental delay and reduce the risk of broncopulmonary dysplasia in the smallest and most immature infants.

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