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Review Article

Impact of fatty acid status on growth and neurobehavioural development in humans

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Abstract

Many studies have been conducted to investigate the effect of n-3 long-chain polyunsaturated fatty acid (LCPUFA) supplementation during the perinatal period on the growth and neurobehavioural development of young children. Most of these intervention trials have involved infants from high-income countries, and a significant proportion have investigated supplementation of infant formulas. Generally, supplementation of infant formula for preterm rather than term infants has demonstrated more consistent, positive effects on aspects of neurobehavioural development, while the growth of both term and preterm infants appears unaffected by LCPUFA supplementation. Maternal n-3 LCPUFA supplementation during pregnancy has consistently resulted in modest increases in birth size, and the most recent study suggests that this is also true from women from low-income environments. The effect of maternal supplementation on global neurobehavioural outcomes for children born at term remains unclear, although n-3 LCPUFA supplementation of women expressing milk for their preterm infants does improve their performance on tests of global neurodevelopment. Further work is required to determine whether dietary n-3 LCPUFA is neuroprotective for children from disadvantaged or low-income backgrounds.

Keywords: long-chain polyunsaturated fatty acids, docosahexaenoic acid, perinatal, neonatal, neurodevelopment, growth.

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Introduction

The synaptosomal membranes of the central nervous system contain high concentrations of the n-3 long-chain polyunsaturated fatty acid (LCPUFA), docosahexaenoic acid (DHA, 22:6 n-3). Animal studies show that diets deficient in n-3 fatty acids are associated with reductions in brain DHA concentrations, decreased dopamine and serotonin, reduced neuronal cell size as well as decreased visual function, impaired visual recognition memory and compromised learning behaviour (Innis 2009). The earliest publications in human infants were from the early 1990s and involved infants born preterm (Uauy *et al*. 1990; Birch *et al*. 1992a; Carlson *et al*. 1993). Their results were consistent with the findings from animal studies and showed that preterm infants fed a formula supplemented with n-3 LCPUFA, mainly as DHA, had improved retinal sensitivity and visual acuity compared with preterm infants fed the standard unsupplemented formulas of the day, which were low in n-3 fatty acids and rich in n-6 fatty acids (Uauy *et al*.

1990; Birch *et al*. 1992a; Carlson *et al*. 1993). Since then, there has been an explosion of interest in the role of LCPUFA during the perinatal period in the growth and development of all infants. Today, there are in excess of 40 perinatal randomized controlled trials involving LCPUFA interventions assessing different aspects of early childhood development and/or growth. This paper draws on this rich evidence base to review the effect of fatty acid status on the more global indices of neurobehavioural development and growth, attempting to separate the potential effects of prenatal and post-natal interventions with a particular focus on low-income countries. We focus on global indices of neurobehavioural development because of their high clinical utility. We have only reviewed randomized controlled trials, or systematic reviews of randomized controlled trials, as only randomized controlled trials can establish a cause and effect relationship not possible from other study designs. Although the majority of trials involving perinatal LCPUFA interventions have been conducted in high-income countries, where possible, we highlight the results of trials conducted in low-income areas or subgroups within trials from low-income backgrounds.

Prenatal supplementation – growth

The earliest studies on n-3 LCPUFA supplementation during pregnancy were designed to assess the effect of high-dose fish oil interventions on pregnancy duration and the prevention of preterm birth. It was hypothesized that fish oil fatty acids could delay initiation of labour and cervical ripening by inhibiting the production of prostaglandins F2α and E2. This biochemical plausibility taken together with the observational studies showing an association between high fish consumption and increased duration of pregnancy and higher birthweight (Olsen & Joensen 1985; Olsen *et al*. 1986) resulted in a number of randomized controlled trials to assess the cause and effect relationship between fish oil supplementation in pregnancy and improving major pregnancy outcomes.

Three systematic reviews have aggregated the results of the relevant randomized controlled trials (Makrides *et al*. 2006; Szajewska *et al*. 2006; Horvath *et al*. 2007). The meta-analyses showed remarkably consistent results despite the fact that these reviews had differing inclusion criteria. In brief, supplementation with marine oil (usually 3 g of n-3 LCPUFA) in the second half of pregnancy resulted in higher mean birthweights (approximately 50 g) and higher mean birth lengths (0.48cm) in the marine oil groups compared with control (Makrides *et al*. 2006; Szajewska *et al*. 2006; Horvath *et al*. 2007). However, it is important to note that there was also a modest increase in the length of gestation (approximately 2.5 days) with marine oil treatment. The small increases in birthweight and length with n-3 LCPUFA treatment were commensurate with the small increase in gestation length. Furthermore, there were no overall differences between the groups in the proportion of small-for-gestational age babies (Makrides *et al*. 2006). It is therefore entirely feasible to suggest that the observed increases in birthweight and birth length with n-3 LCPUFA supplementation are a function of the increased duration of gestation.

It is noteworthy that two intervention trials have been conducted in low-income countries. Tofail *et al*. (2006) investigated the effect of fish oil (1.2 g DHA and 1.8 g eicosapentaenoic acid, EPA 20:5 n-3, per

Key messages

- Maternal n-3 long-chain polyunsaturated fatty acid (LCPUFA) supplementation during pregnancy has consistently resulted in modest increases in birth size in both low-income and high-income populations, but post-natal supplementation with LCPUFA does not appear to influence infant growth.
- The effect of maternal prenatal and post-natal LCPUFA supplementation on global neurobehavioural outcomes for children born at term remains unclear, although n-3 LCPUFA supplementation of women expressing milk for their preterm infants does improve their performance on tests of global neurodevelopment.
- Further work is required to determine whether dietary n-3 LCPUFA is neuroprotective for children from disadvantaged or low-income backgrounds.

day) from 25 weeks gestation until birth on growth and development in an area of Dhaka City, Bangladesh, where illiteracy, poverty, overcrowding, poor housing and poor hygiene were common. Although 400 women were recruited for the trial, data for only 249 mother–infant pairs are reported. The percentage of women with a preterm birth was high (22–24%), and mean birthweight in the fish oil and control group was 2.7 kg. Birth length, birth head circumference and ponderal index also did not differ between groups. Similarly, follow-up of children at 10 months did not show differences between groups for weight, length or weight for length (Tofail *et al*. 2006).

Ramakrishnan *et al*. (2010b) studied the effect of only DHA supplementation (400 mg day^{-1}) compared with placebo in 1094 pregnant women in Cuernavaca, Mexico. Supplementation was from 18 to 22 weeks of gestation until birth. Women were known to have a low background dietary intake of DHA (median 55 mg day^{-1}). Mean gestational age at birth did not differ between groups [39.1 standard deviation (SD), 1.7 weeks vs. 39.0 SD, 1.9 weeks] nor did birthweight (3.20 SD, 0.47 kg vs. 3.21 SD, 0.45 kg), birth length or birth head circumference (Ramakrishnan *et al*. 2010b). However, the babies of women experiencing their first pregnancy were heavier [mean difference (MD) 99 g, 95% confidence interval (CI) 5–193 g] and had larger head circumferences (MD 0.5cm, 95% CI 0.1–0.9 cm) at birth compared with controls (Ramakrishnan *et al*. 2010b). There were no differences in birth size for the offspring of multigravida women (Ramakrishnan *et al*. 2010b). While these data suggest that DHA supplementation of primagravida women may increase birth size, further studies are required to determine the clinical significance of these observations and their impact on subsequent growth and development.

Prenatal supplementation – neurobehavioural development

The trial of Tofail *et al*. (2006) from Bangladesh and the trial of Ramakrishnan *et al*. (2010b) from Mexico are relevant and the only two available that investigate the effect of n-3 LCPUFA supplementation during pregnancy on neurobehavioural development of the resultant infants. The degree of social and nutritional disadvantage in the study from Bangladesh appears to be greater than that in the Mexican trial, and this is reflected in the birthweights of the control groups in the two studies. It is interesting to note that no differences were shown between treatment and control groups in either the Bangladesh trial at 10 months of age (Tofail *et al*. 2006) or the Mexican trial at 18 months of age (Ramakrishnan *et al*. 2010a) using the Bayley Scales of Infant Development (BSID). The further follow-up planned by Ramakrishnan's group will be important because global neurodevelopmental measures during the first year of life are known to lack sensitivity in predicting later cognitive development. Additionally, assessments at later ages will allow different developmental domains to emerge and be reliably assessed.

In high-income environments, some cohort studies show that dietary intake of fish and seafood, and/or n-3 LCPUFA are positively associated with developmental and behavioural outcomes during childhood (Hibbeln *et al*. 2007; Oken *et al*. 2008), but there are relatively few well-conducted randomized controlled trials of n-3 LCPUFA supplementation during pregnancy that robustly assess neurobehavioural development. To date, there are three published trials in which supplementation is limited only to pregnancy (Dunstan *et al*. 2006; Judge *et al*. 2007; Makrides *et al*. 2010a). Judge *et al*. (2007) report improved problem solving at 9 months with DHA supplementation at 300 mg day^{-1}, while Dunstan *et al*. (2006) report improved hand–eye coordination at 30 months following high-dose EPA and DHA supplementation in pregnancy. However, both trials had methodological limitations. Both had relatively small sample sizes, making it difficult to exclude random error. Furthermore, there was also significant attrition and/or selective loss from the fish oil-supplemented group (Dunstan *et al*. 2006). Significant attrition or selective loss can both interfere with the integrity of the original randomization and hence increase the likelihood of bias. The most recent and largest trial conducted in Australia showed that supplementation with high-DHA fish oil from mid-pregnancy until birth did not result in any significant benefit to mean cognitive and language scores of children at 18 months of age

(Makrides *et al*. 2010a). Secondary analyses indicated a significant treatment by sex interaction so that girls treated with DHA *in utero* had lower language scores than girls from the control group (Makrides *et al*. 2010a). Clearly, further follow-up is required, as language development is difficult to robustly assess at 18 months of age.

Prenatal and post-natal supplementation

One trial conducted in Norway compared supplementing women with cod liver oil (1.2 g day^{-1} DHA and 0.8 g day^{-1} EPA) or corn oil from 18 weeks gestation until 3 months post-partum and reported cognitive and growth outcomes in a subgroup of the children (Helland *et al*. 2001, 2003, 2008). Results from this trial have been inconsistent, with no group differences being reported in early cognitive development at 6 or 9 months (Helland *et al*. 2001), a positive effect of DHA supplementation on IQ at 4 years (Helland *et al*. 2003) but no effect on IQ at 7 years (Helland *et al*. 2008). In addition, no differences in growth or body mass index have been reported at any age. As with other trials from high-income countries involving prenatal supplementation, the study of Helland *et al*. (2001, 2003, 2008) was also plagued by large attrition and post-randomization exclusions that could have contributed to random error or bias, making it difficult to have a high degree of confidence in the reported outcomes.

Post-natal supplementation – term infants

Most infants are born at term and breastfed at least for some period of time. However, the majority of LCPUFA intervention trials involving term infants has been conducted with exclusively formula-fed infants in high-income environments. The rationale has been to add LCPUFA to infant formulas that were previously devoid of LCPUFA at concentrations equivalent to those observed in the human milk of women from high-income, Westernized countries in an attempt to mimic the biochemical, physiological and developmental outcomes of breastfed infants. Although many studies have successfully demonstrated that adding LCPUFA (DHA and arachidonic acid, AA, 20:4 n-6) to infant formulas increases the concentration of these fatty acids in plasma and red cells of formula-fed infants to be equivalent to the profiles observed in breastfed infants, the data from studies relating to developmental outcomes have been more complex.

While many more LCPUFA interventions trials with formula-fed infants have focused their attention on visual acuity outcomes, six trials have assessed global developmental indices using the BSID and have been summarized in a recent meta-analysis (Makrides *et al*. 2010b). Individually, only one of the six trials suggests a positive effect of LCPUFA supplementation on mental development index (MDI), while the meta-analysis demonstrated that LCPUFA supplementation of infant formula resulted in no effect, positive or negative, on MDI scores compared with control [weighted MD (WMD) 0.18, 95% CI –1.84, 2.19, $n = 960, P = 0.86$]. Further subgroup analyses indicated no effect of the type of LCPUFA supplementation on MDI scores, suggesting that there were no differential effects on infant development if DHA is used alone or in combination with AA. However, both subgroup analyses had moderate to significant heterogeneity, indicating that the differences between trials limit the degree of confidence in the result. The differences between trials may arise from the sample population studied, the way the intervention was applied, trial quality or trial methodology.

The aggregation of trials involving term formula-fed infants to assess the effect of LCPUFA supplementation on growth during the first year of life included 14 eligible trials with longitudinal data from 1846 infants. This meta-analysis showed no positive or negative effect of LCPUFA supplementation on infant weight, length or head circumference at any assessment age (Makrides *et al*. 2005). Importantly, subgroup analyses showed that supplementation with only n-3 LCPUFA (no AA) had no effect on infant weight, length or head circumference despite reductions in the plasma and erythrocyte AA status of infants involved in these trials (Makrides *et al*. 2005).

Collectively, there are few consistent data to support the hypothesis that LCPUFA supplementa-

tion of infant formula for term infants in high-income environments will have a measurable effect on the growth or neurobehavioural development in early childhood. Contrary to the numbers of studies assessing supplementation of infant formula, relatively few studies have focused their attention on n-3 LCPUFA supplementation of term breastfed infants, who already receive a supply of LCPUFA through breast milk. These supplementation studies have been modestly sized and primarily designed to assess biochemical and physiological outcomes. The three key supplementation studies of breastfeeding women during the first 3–4 months of lactation showed no growth difference during infancy (Gibson *et al*. 1997; Lauritzen *et al*. 2005b), although Lauritzen *et al*. (2005b) suggest that DHA-supplemented children had a higher body mass index at 2.5 years that was not evident at 7 years (Asserhoj *et al*. 2009). Jensen *et al*. (2005) suggested that supplementation during the first 4 months of lactation improved the Bayley psychomotor development index at 30 months and enhanced attention at 5 years compared with control (Jensen *et al*. 2010), although several other tests of neurobehavioural domains at these ages showed no differences. Gibson *et al*. (1997) showed no effect of DHA supplementation during lactation on Bayley scores, and Lauritzen *et al*. (2005a) reported a reduction in vocabulary comprehension with fish oil supplementation during lactation compared with olive oil. Caution is required in interpreting these effects because of the relatively modest sample sizes, high attrition and multiple comparisons, which mean that random error cannot be excluded.

The apparent lack of consistency in the global neurobehavioural results of LCPUFA supplementation trials involving breastfed and formula-fed infants is somewhat unexpected and may indicate substantial variation in the conduct and quality of the studies, or small, inconsistent effects. If the latter is true, it is perhaps not surprising as most term infants in high-income environments are born with a fat reserve and having had the benefit of a supply of LCPUFA across the placenta during the time that brain growth was at its maximum velocity. The effect of post-natal LCPUFA supplementation in infants who had been growth restricted *in utero* has not been well studied and deserves further investigation.

Post-natal supplementation – preterm infants

However, infants born preterm are well recognized as being at risk of LCPUFA dietary insufficiency. Newborn preterm infants have lower plasma and red cell concentrations of LCPUFA compared with newborn term infants (Smithers *et al*. 2008a), and their often complex feeding regimens and feeding intolerances limit intake of a consistent supply of LCPUFA whether through expressed human milk or infant formula.

Formula feeding studies

The first generation of studies in preterm infants were conducted in stable, formula-fed infants comparing formula supplemented with LCPUFA to formula with no LCPUFA. While several studies reported that preterm infants fed LCPUFA-enriched formulas had enhanced visual development, including improved retinal sensitivity and visual acuity, compared with those fed unsupplemented formulas (Birch *et al*. 1992a,b; Carlson *et al*. 1993, 1996; Carlson 2001), the effect on neurodevelopment and growth has been less clear. Two recent systematic reviews concluded that supplemented formula had no significant effect on neurodevelopment (Simmer *et al*. 2008; Smithers *et al*. 2008b). However, trials included in the reviews used either the first or the second version of the BSID. When these different versions of BSID were considered as separate subgroups, the mental development of LCPUFA-supplemented infants assessed using the BSID version II was 3.4 points higher than that of control infants (WMD 3.4, 95% CI 0.6, 6.3; $P = 0.02$; $n = 879$) (Smithers *et al*. 2008b). No differences were found with version I. The revision of the mental scale of version II included more items to test language and problem solving along with changes to the administration and scoring of the test. This may have introduced systematic differences in the evaluation of the underlying cognitive domains.

Table 1. Impact of maternal social variables on the effectiveness of high DHA (~1% of total fatty acids) relative to standard DHA (~0.3%) during the preterm period on the mental development index (MDI) of preterm children at 18 months corrected age

Social variable (*n* high DHA/*n* standard DHA)	MDI of high DHA (mean, SE)	MDI of standard DHA (mean, SE)	Mean difference, SE	*P*-value
Maternal education				
Degree or higher degree (62/79)	98.0, 2.5	97.2, 2.1	0.9, 3.3	0.79
Certificate/diploma or completed secondary school (150/144)	95.0, 1.2	94.5, 1.6	0.5, 2.0	0.79
Secondary school incomplete (84/93)	93.8, 1.5	88.5, 2.2	5.3, 2.7	0.04
Maternal occupation				
Professional or managerial (94/119)	96.8, 1.9	97.9, 1.6	–1.1, 2.5	0.65
Semi-skilled, trade or unskilled (119/111)	95.9, 1.4	90.3, 2.1	5.6, 2.5	0.02
Other, including home duties, unemployed, student, pension (84/86)	92.6, 1.6	91.1, 1.8	1.5, 2.4	0.52

Interaction terms: treatment group × maternal education $P = 0.33$; treatment group × occupation $P = 0.16$.
DHA, docosahexaenoic acid; SE, standard error.

The effect of LCPUFA-supplemented formula on growth of preterm infants has also been systematically reviewed (Simmer *et al*. 2008). While some trials show a beneficial effect on growth (Innis *et al*. 2002; Fewtrell *et al*. 2004; Clandinin *et al*. 2005), others suggested a detrimental effect (Carlson *et al*. 1992, 1996). When n-3 LCPUFA supplements were used without AA, there was a reduction in plasma AA, which was postulated to be the reason for the poorer growth and which prompted the addition of both AA and DHA to formula. However, meta-analyses of studies assessing growth at 2, 12 and/or 18 months post-term showed no evidence of impaired growth with or without the addition of AA to infant formula (Simmer *et al*. 2008).

Studies based on human milk feeding

The second generation of studies compared two different doses of DHA and included both human milk- and formula-fed preterm infants (Henriksen *et al*. 2008; Makrides *et al*. 2009). Additionally, infants included in these studies were representative of the usual clinical profile of preterm infants. Both studies found positive effects of higher dose DHA on cognitive outcome. The DINO (DHA to Improve the Neurodevelopmental Outcome of preterm infants) trial (Makrides *et al*. 2009) is the largest trial to date, and while no overall difference in mental development was found, severe mental delay (MDI < 70) was halved in the higher DHA group (Relative Risk (RR) 0.50, 95% CI 0.26–0.93; $P = 0.03$). In *a priori* subgroup analyses based on the randomization strata, there were interactions between dietary treatment and sex, and between dietary treatment and birthweight, indicating differential responses of higher DHA diets by sex and birthweight. Higher DHA significantly improved the mental development of girls (RR 4.5, 95% CI 0.5–8.5; $P = 0.03$) and reduced mild (MDI < 85) and severe mental delay (RR 0.43, 95% CI 0.23–0.80; $P = 0.01$ and RR 0.17, 95% CI 0.04–0.72; $P = 0.02$, respectively), while no effect of higher DHA was noted in boys. In infants born <1250 g, mild mental delay was decreased (RR 0.57, 95% CI 0.36–0.91; $P = 0.02$). Henriksen *et al*. (2008) showed that higher DHA was associated with better recognition memory and higher problem-solving scores at 6 months of age. Both trials were conducted in high-income countries, limiting generalizabilty to low-income countries.

With the large DINO data set, we conducted exploratory analyses to determine the impact of maternal social variables on the effectiveness of higher DHA on the mental development scores of preterm children. We tested whether there was evidence of effect modification by a high-DHA diet in the preterm period on mental development score using linear GEE regression models. The GEE (Generalized Estimation Equations) models were used to adjust for clustering at the mother level and thus account for twins. The data are shown in Table 1 and need to be interpreted with caution as there were

no overall interaction effects between dietary treatment and education or occupation, indicating that any statistically significant differences may be because of chance. In children born <33 weeks whose mothers did not complete secondary education, higher DHA afforded a 5-point advantage ($P = 0.047$) compared with control, while no differences in mental development scores were noted in the children of women who completed secondary education or had further education. Similarly, children of women in semi-skilled, trade or unskilled occupations had a 6-point ($P = 0.02$) advantage if they received higher DHA compared with control (Table 1). This effect was most pronounced for infants born <1250 g receiving higher DHA, with an 11-point advantage ($P = 0.003$). In the same group of children (from women in semi-skilled, trade or unskilled occupations), significantly fewer infants in the higher DHA group were severely delayed (RR 0.15, 95% CI 0.04–0.49; $P = 0.002$). Although these data are limited by the lack of an interaction effect between treatment group and maternal education ($P = 0.33$), and between treatment group and maternal occupation ($P = 0.16$), the *post hoc* comparisons consistently raise the hypothesis that a higher DHA diet in the preterm period may offer some protection to the cognitive development of children from disadvantaged families. Clearly, further intervention trials are required to test this hypothesis.

Summary

Relatively few studies have been specifically designed to assess the effects of dietary LCPUFA during the perinatal period on the growth and neurobehavioural development of children in low-income environments. Only two trials have attempted to address the issue of prenatal supplementation, and robust data should be available from the trial of Ramakrishnan *et al*. (2010b) once further follow-up assessments are published. However, there are no published trials of post-natal LCPUFA supplementation in low-income environments, and potentially relevant subgroups such as growth-restricted infants are also poorly studied, highlighting a major gap in the scientific literature. Our preliminary observations involving preterm children raise the hypothesis that n-3 LCPUFA, particularly DHA, may be neuroprotective for children from disadvantaged families and add support to the need for detailed investigations assessing the effects of dietary strategies designed to increase n-3 LCPUFA status of young children from disadvantaged or low-income backgrounds.

Conflicts of interest

Dr Makrides serves on scientific advisory boards for Nestle, Fonterra and Nutricia. Dr Gibson serves on scientific advisory boards for Nestle and Fonterra. Associated honoraria for Drs Makrides and Gibson are paid to their institutions to support conference travel and continuing education for post-graduate students and early career researchers. The authors have no financial interest in the sales or marketing of infant formula products or maternal supplements.

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