Essential fatty acids in infancy: nutritional requirements, problems and practicalities

M Makrides

Department of Obstetrics & Gynaecology, University of Adelaide, Women's & Children's Hospital, 72 King William Road, North Adelaide SA 5006

Summary

One of the current controversies in infant nutrition is whether dietary docosahexaenoic acid (DHA, 22:6n-3) is required by term infants to achieve their full developmental potential. Studies of brain fatty acid composition have demonstrated that infants who were breast fed have greater levels of cerebral cortex DHA than infants who were formula fed, suggesting that DHA in the cerebrum is dependent on a supply in the diet. Some physiological studies report that electrophysiological and behavioural assessments of visual function are improved in breast fed compared with formula fed infants. However the data from trials where formula fed infants were randomly allocated to a placebo or DHA supplemented formula are equivocal. Some trials demonstrate a beneficial effect of supplementation on various measures of neural maturity, while others show no effect. Variations in dietary treatments, the formulation of background fat blends, the contribution of confounding variables and differing methods of assessment have all been put forward as possible explanations to inconsistent study findings. Further work is necessary to rigorously establish if there are long term benefits of dietary DHA to the term infant.

Introduction

The central nervous system has the second largest concentration of lipids after adipose tissue and these are not involved in energy metabolism (1). Lipids in the nervous system are an integral part of the morphology of neural cells. Saturated and monounsaturated fatty acids are components of the myelin sheaths which surround axons and long chain polyunsaturated fatty acids (LCPUFA) are avidly incorporated into the gray matter of the cerebral cortex (1). A number of authors have consequently postulated an important functional role for LCPUFA as brain gray matter is a hub of synapses and neurotransmission, and much research attention has surrounded the role of LCPUFA in the development of brain function.

There are two groups or families of LCPUFA (the n-3 and the n-6 families) and these can be derived directly from the diet or from synthesis from precursor fatty acids. Foods such as eggs, offal meats and fish are abundant sources of LCPUFA, but for those who avoid eating such food items there is a reliance on synthesis from precursors. Both α-linolenic acid (18:3n-3, ALA; n-3 precursor) and linoleic acid (18:2n-6, LA; n-6 precursor) are primarily found in vegetable oils. Thus omnivorous adults would be expected to have an abundant dietary supply of LCPUFA whereas vegans, who only consume foods of vegetable origin, would only have a supply of ALA and LA. This categorisation also applies to breast and formula fed infants as breast milk contains a full complement of all PUFA including precursors and metabolites, whereas infant formulas only contain ALA and LA (2). Thus, an average Australian infant of 7 kg drinking 800 ml breast milk per day would consume about 130 mg each of docosahexaenoic acid (22:6n-3, DHA) and arachidonic acid (20:4n-6, AA) (a dose of about 19 mg/kg/d). For a 70 kg adult this would translate to 1300 mg each of DHA and AA/day. It has been calculated that the average intake of DHA and AA by Australian and American adults is only of the order of 100-200 mg each per day (3, 4). In other words, breast fed infants are consuming LCPUFA at over four times the rate of omnivorous adults whereas formula fed infants consume no LCPUFA and must synthesise their whole LCPUFA requirement from ALA and LA.

Biochemical studies have demonstrated that formula fed preterm infants have less DHA and less AA in their erythrocyte lipids relative to those fed breast milk (5). This suggests that a supply of

just 18 carbon precursors may be only partially effective at meeting the LCPUFA requirements of preterm infants. Indeed subsequent physiological studies with preterm infants have shown neural effects associated with fatty acid nutrition. Two randomised controlled feeding trials have demonstrated that preterm infants fed fish oil (FO) supplemented formula (+n-3 LCPUFA) have improved visual acuity and higher erythrocyte DHA relative to those fed standard unsupplemented formula (-n-3 LCPUFA) (6,7). Visual acuity and erythrocyte DHA of supplemented formula infants were also similar to those of infants fed breast milk (6), suggesting that n-3 LCPUFA in breast milk are important for the optimal development of the visual pathway. Other workers have also reported a positive association between infant DHA status and Bayley's developmental indices in preterm infants at one year corrected age in both an observational (8) and randomised trial (9).

This raises questions as to whether similar effects are also seen in healthy, term infants, particularly as most infants are born at term. In Australia, the incidence of breast feeding is about 80% at discharge from postnatal wards and this rapidly declines with time. Most Australian infants therefore consume much more formula than breast milk during their first 12 months. The goal of our research program was to establish the adequacy of fat supply in the diets of healthy, term infants.

i. Biochemical studies in term infants

Studies comparing breast and formula feeding indicate that formula fed term infants have ≈50% less DHA and ≈15% less AA in their erythrocytes relative to term breast fed infants (10, 11). This was shown to be due in part to an imbalance in the ratio of ALA and LA. However, studies that have tried to increase the availability of ALA to formula fed infants demonstrate that it is not possible to match erythrocyte DHA levels to those of breast fed infants (10, 12). These results suggest that adding LCPUFA directly to infant formula may be necessary to match fatty acid profiles of breast fed infants.

It is perhaps not surprising that dietary fat composition has an effect on the fatty acids that are accumulated in brain tissue of human infants during the first year of life as the weight of the brain of infants is reported to increase by 750 g (13). Although much of this weight gain is thought to be because of myelination (thus chiefly involving the accumulation of saturated and monounsaturated fatty acids), a large degree of nerve cell proliferation also occurs involving cell membranes rich in LCPUFA. Our work has demonstrated that the lipids of the cerebral cortex taken from breast fed infants were higher in DHA than infants fed formula (14). These results have been confirmed by other workers (13). We have continued to analyse samples of tissue as they became available and the results confirm that accumulation of DHA in the cerebral cortex of breast fed infants continues for at least 40 weeks of life, increasing from a value of about 7.5% to about 10% of total fatty acids (15). Although our earlier results (14) indicated no change in birth levels of formula fed infants, the increased sample size makes it clear that DHA also accumulates in formula fed infants but at a much reduced rate (from about 7% to about 8% total fatty acids in 40 weeks) relative to breast fed infants (15).

Levels of AA also change dramatically after birth increasing in a curvilinear manner from approximately 9% to 12% of total fatty acids in the first 20 weeks of life (14,15). Interestingly, these changes are independent of diet indicating that either AA is aggressively accumulated or that synthesis of AA from its 18 carbon precursor, LA, is sufficient to meet the requirement of the growing infant brain. Another way of phrasing this is that the dietary AA provided in breast milk may be redundant as far as the biochemistry of the brain is concerned and that the lower AA levels in the blood of formula fed infants may have limited relevance.

The percentage of LCPUFA does not increase in all areas of the brain in the first year of life. In the brain stem there is a continuous depletion in the proportions of both DHA and AA which is also independent of diet (15). These results are consistent with the myelination that is known to occur in the brain stem during this period, and reflected by the increasing levels of monounsaturated fatty acids ($r = 0.73$, $P<0.001$).

It is important to stress that the infants from which brain samples were taken in both our studies and that reported by Farquharson and coworkers (13) were all term infants. In particular the infants in our studies all died suddenly and were apparently well immediately prior death. Thus the spectrum of fatty acids observed in these subjects is likely to reflect the pattern of the brains of living infants of equivalent ages. Even though term infants have presumably benefited from a full placental supply of LCPUFA, brain growth continues postnatally and the brain remains plastic to compositional change for at least 40 weeks.

The fundamental importance of these results is that the level of DHA of the cerebrum is dependent on a supply of DHA in the diet. Providing only ALA in the diet, at least at the levels provided in current infant formulas, appears to be inadequate to support maximum deposition of DHA in the brain of the developing term infant. It is reasonable to postulate that these diet-induced changes in the brain cortex composition may have some physiological sequelae.

ii. Breast vs formula feeding

Our initial work suggested that there was a measurable functional difference between breast and formula fed infants (16). In this study (16), visual evoked potential (VEP) acuity was examined as a measure of neural maturity of the visual pathway of healthy term infants fed either breast milk or formula along with their PUFA status. We observed that breast fed infants had better VEP acuity than formula fed infants and that there was also a correlation ($r = 0.65$, $P<0.01$) between VEP acuity and erythrocyte DHA. Thus formula fed infants had low DHA levels and reduced visual acuity while breast fed infants had high DHA levels and increased acuity.

Some other studies comparing breast and formula feeding have demonstrated comparable results (17, 18). In contrast, Innis and coworkers did not detect any differences in visual acuity, measured using a preferential looking technique, between breast and formula fed term infants at three or nine months of age (19, 20). However, while the reports are interesting in terms of making comparisons between breast and formula feeding, no conclusions can be drawn about the dietary supply on n-3 fatty acids and visual development as there are many other differences between breast milk and infant formula other than fatty acid supply. For example, breast milk contains growth factors and hormones that may be trophic for neural maturation. The association between acuity development and mode of feeding provides no evidence of causation. Randomised studies comparing the visual and neural development of infants fed formula supplemented with and without DHA are necessary for a causative link between dietary DHA and visual development in healthy, term infants to be established.

iii. Randomised trials of formula feeding

We hypothesised that infants randomly allocated to a formula supplemented with DHA, at levels found in breast milk, would have higher erythrocyte DHA and better VEP function than infants fed a placebo formula. Furthermore, if DHA was the active component in breast milk, the outcomes of the DHA supplemented infants would be expected to be similar to those who were breast fed. The supplement chosen for the trial was a blend of FO and evening primrose oil (EPO) in a 1:1 ratio. The EPO was added in an attempt provide a metabolic source of AA since low plasma AA in preterm infants receiving FO supplemented formula had been associated with poorer growth relative to those fed standard formula (21). At the time of the study there were no commercial sources of AA. Mothers who chose to formula feed their infants from birth were randomised to receive either a supplemented or placebo formula. All infants were followed at six, 16 and 30 weeks of age. Anthropometrics were assessed and erythrocyte fatty acids analysed by capillary gas chromatography. VEPs were performed under transient conditions at 16 and 30 weeks and VEP acuity determined (11).

Breast fed infants had significantly better acuities than infants fed placebo formula at both 16 ($P<0.001$) and 30 ($P<0.01$) weeks. Supplementation with 0.36% DHA (in the form of a FO/EPO

blend) resulted in an improvement in VEP acuity to match that of fully breast fed infants. Comparable studies with preterm infants have drawn similar conclusions (6, 7, 22). Differences of approximately 0.3 log units (one octave) were apparent between infants that received n-3 LCPUFA (breast fed and supplemented formula groups) and those fed standard, placebo formula at both 16 and 30 weeks of age. The results provide no evidence of 'catch-up' with age and are consistent with the visual loss observed in the rhesus monkey model of n-3 fatty acid deficiency (23).

There were no differences between dietary groups for normalised weight and length (z-scores). Although the number of infants in this study was relatively modest in terms of growth assessment, the availability of paired data on each infant allowed for the detection of one z-score unit differences between dietary groups with a $P<0.05$ level of significance and 90% power. Furthermore, all mean weight-for-age and length-for-age z-scores were between the 25th and 75th percentiles, supporting good, normal growth. These results are in accordance with previously published data on the standardised growth of infants showing that breast fed infants are leaner than those fed formula (24).

In a subsequent and larger trial we aimed to determine the effect of formulas supplemented with either DHA alone or together with AA on growth and VEP acuity in term infants (25). A total of 67 healthy term infants were randomly assigned to one of three formulas containing either 0% DHA, 0.3% DHA or 0.3% DHA with 0.3% AA. The infants consumed the assigned formula for at least 34 weeks. At 16 and 34 weeks of age, VEP acuity was assessed, growth was determined by measuring weight, length and head circumference and a 200μL blood sample was collected by heel prick for the determination of erythrocyte phospholipid fatty acids.

There were no differences in growth patterns among any of the formula treatments. Levels of DHA in the supplemented groups exceeded the range seen in breast fed infants. When AA was absent from formula, erythrocyte AA levels were below values seen in unsupplemented infants (25). VEP acuity scores were not significantly different between dietary groups although all were within the normal expected values (25). This was an unexpected finding and contradicts our earlier work (11). Since the publication of our initial trial in 1995 (11), numerous other trials of DHA supplementation in term formula fed infants have also been published. Some demonstrate a beneficial effect of LCPUFA supplementation (26-28), while others show no effect (29, 30). There are no adequate explanations as to the discrepancy in study results. Numerous postulates have been put forward as possible explanations although neither one on its own adequately accounts for all trials without exception.

Firstly, it has been postulated that the background levels of ALA in the formula fat blend may be an important determinant of study outcome (31). Therefore, infants fed placebo formulas with high ALA will be able to synthesise and incorporate enough DHA to meet their needs for neural function, but infants fed placebo formulas with low ALA will be DHA depleted. However, there are now trials that demonstrate a beneficial effect of DHA supplementation in a background of high ALA (26) and also trials that show no effect of DHA supplementation in a background of low ALA (25). Secondly, the issue of DHA dose has been raised. It is suggested that supplementation with at least 0.2% DHA as total fatty acids may be necessary to show an effect. However, Carlson et al (26) and Auestad et al (29) using two identical formulas with 0.1% DHA yielded different results; Carlson demonstrating a beneficial effect and Auestad showing no effect. Furthermore, two other trials with 0.3% DHA also demonstrated no effect of DHA supplementation (25, 29). Thirdly, we have advocated the need to account for potential confounding and effect modifying variables (32). Although it could be argued that this is unnecessary since the purpose of randomisation is to avoid the introduction of bias, it remains possible that other factors that influence infant DHA status or neural outcome, such as parity, gestational age, maternal smoking, social class and parental education, will modify the results of trials with relatively small numbers of subjects. There are probably may other tangible explanations and the design of future trials should take account of as many possibilities as practical.

iv. Randomised trials of breast feeding

The issue of dietary DHA dose required to attain maximal tissue incorporation and function has not been fully addressed during infancy. Infants can be exposed to a wide range of DHA levels in breast milk that are largely dependent on the dietary intake of the mother (33-35). For example, breast fed Korean or Malaysian infants would receive up to five times the amount of DHA of their Western cousins (36, 37).

We undertook a study to determine the effect of increasing DHA in breast milk on infant fatty acid profiles. A secondary aim was to examine aspects of neural development. Lactating mothers were randomised on day five post-partum to groups consuming equal numbers of capsules but containing either placebo or an oil with DHA as its only polyunsaturate to receive 0, 0.2, 0.4, 0.9, 1.3 g DHA/day for 12 weeks. This resulted in breast milk with DHA concentrations that ranged from 0.1 to 1.7% of total fatty acids. Infants who were still exclusively breast fed at 12 weeks (n=52) were assessed. VEP acuity was measured at 12 and 16 weeks and Bayley's Scales of infant development were conducted at one and two years.

Breast milk DHA was related to infant plasma ($r = 0.89$, $P<0.001$) and erythrocyte ($r = 0.88$, $P<0.001$) phospholipids in a saturable curvilinear manner so that breast milk DHA above 0.8% of total fatty acids resulted in little further increase in infant plasma or erythrocyte DHA levels. The rise in plasma and erythrocyte DHA was approximated by a fall in total n-6 PUFA. We could detect no relationship between VEP acuity (measured at 12 and 16 weeks) of infants by either the dietary grouping or the DHA status of individuals. A stepwise multiple regression showed that infant erythrocyte DHA at 12 weeks and home stimulation were the only independent factors associated with Bayley's mental developmental index (MDI) at one year (adjusted model $r = 0.42$, $P<0.005$); while at two years gender and social score of the spouse were the only significant predictors of Bayley's MDI (adjusted model $r = 0.47$, $P<0.005$). Finally, all infants had appropriate growth for age and no association were noted between breast milk DHA levels and any infant health measures. From this select group of breast fed infants fed a range of DHA levels that covers all reported ranges, our data suggest that the effect of a single dietary factor, such as DHA, will be small compared with social and environmental influences.

v. Is there a requirement for dietary dha for a specific health benefit in term infants?

We are a long way from being able to answer the question of whether dietary DHA provides a specific health benefit for term infants. It is hoped that the many trials that are in progress world-wide will yield enough information to answer this question. The only point of commonality between all the trials with LCPUFA supplementation in term infants is that none have reported any hazardous effects.

Acknowledgments

This work has been supported by grants from Channel 7 Children's Research Foundation, the National Health & Medical Research Council, The Sudden Infant Death Syndrome Association of South Australia, Nestec Ltd, Switzerland, Nestle Australia and Martek Biosciences, USA.

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