Long-Chain Polyunsaturated Fatty Acids in Early Life: Effects on Multiple Health Outcomes
A Critical Review of Current Status, Gaps and Knowledge

Mary S. Fewtrell

MRC Childhood Nutrition Research Centre, Institute of Child Health, London, UK

The efficacy and safety of long-chain polyunsaturated fatty acid (LC-PUFA) supplementation of infant formula has become one of the major and most controversial areas of infant nutrition research over the past 15 years. This paper reviews the current status of research into the effects of LC-PUFA supplementation during early life on functional outcome, and identifies the major areas that contribute to ongoing uncertainties in the field. A brief review of LC-PUFA biochemistry, function and status during fetal life and infancy is first provided as background.

LC-PUFA Biochemistry and Functions

The long-chain fatty acids, docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (AA; 20:4n-6) are synthesized from their parent 18-carbon precursors, α-linolenic acid (ALA; 18:3n-3) and linoleic acid (LA; 18:2n-6), respectively, by a process of desaturation and chain elongation (fig. 1). The two families of essential fatty acids, n-3 and n-6, are defined by the position of the double bond closest to the methyl terminal of the fatty acid. Mammalian cells are unable to insert double-bonds more proximal to the methyl terminal than the seventh carbon atom, hence ALA and LA are nutritionally essential. Once ingested, n-3 and n-6 fatty acids are not interconvertible, but the different families compete for the same enzymes in the metabolic cascade to their respective LC-PUFAs with n-3 having greater conversion efficiency, emphasizing the importance of the dietary ratio of 18:n-3 to 18:n-6.

The importance of LC-PUFAs as constituents of cell membranes has long been appreciated. LC-PUFAs of the n-3 family, especially DHA, are particularly
important in the nervous system and retina where they are essential for normal functioning. In contrast, AA is more ubiquitous and found in cell membranes in a number of organs. It is increasingly recognized, however, that LC-PUFAs are not simply structural lipids, but highly bioactive substances with multiple actions [1]. For example, they affect membrane fluidity, influencing insulin sensitivity and, potentially, transmembrane signaling. Both families of LC-PUFAs are also precursors for the formation of prostaglandins and eicosanoids. In general, those derived from the n-6 family are both more potent and often antagonistic to those derived from the n-3 family. Thus n-6-derived eicosanoids are pro-inflammatory and adipogenic whereas n-3 eicosanoids are anti-inflammatory and may reduce fat deposition, again emphasizing the importance of balance between the two families. Polyunsaturated fatty acids also affect gene transcription, influencing the abundance or activity of transcription factors that play a major role in hepatic carbohydrate, fatty acid, triglyceride, cholesterol and bile acid metabolism. This is a rapidly evolving field in LC-PUFA research. Although currently the significance of these different actions of LC-PUFAs for functional outcomes may be unclear, the increasing appreciation of the bioactive nature of LC-PUFAs has served to highlight the multiple potential effects of supplementation. Thus, although historically studies of LC-PUFA supplementation in infants were directed at improving cognitive and visual outcomes, there is now increasing interest in plausible effects on other aspects of health.

Fig. 1. Formation of LC-PUFA from essential fatty acid precursors.
LC-PUFA Accretion in the Fetus and Infant

LC-PUFA accretion is maximal during late fetal life and infancy. The total amount of DHA in the brain increases approximately 30-fold during this period, coinciding with the most rapid period of brain growth. DHA accumulation also occurs in liver and adipose tissue during the last trimester and may be used to fuel rapid postnatal brain growth. It is thought that most LC-PUFA is obtained from the mother, possibly by selective placental transfer, rather than being synthesized by the fetus.

Breastfed infants receive a preformed supply of LC-PUFAs since both DHA and AA and their 18-carbon precursor fatty acids are present in human milk. Concentrations of AA are fairly constant across and within populations. In contrast, DHA concentrations show more marked variability depending on maternal diet, in particular the intake of fish and marine products. Thus, breast milk DHA concentrations in North America tend to fall between 0.2 and 0.4% whilst mean concentrations in Inuit and Japanese women may reach 1.4% [2]. The effect of ingested fish on breast milk DHA is acute and lasts 1–2 days.

Historically, infant formulas did not contain preformed LC-PUFAs, leaving the infant to synthesize them from ALA and LA. Both term and preterm infants possess the necessary enzymes to synthesize LC-PUFAs and initial concerns that these enzymes might be functionally immature in preterm infants appear to be unfounded. However, both ALA and LA are readily oxidized to provide energy, and if an infant is in negative energy balance it is unlikely that they will be used to synthesize LC-PUFAs.

The issue of whether preformed LC-PUFAs should be added to infant formulas has been extensively investigated over the past 15 years, whilst there has been more limited research into the effects of supplementing the mother during pregnancy or lactation. The following section summarizes the current status of this research.

LC-PUFA Supplementation and Outcome

LC-PUFA Supplementation during Pregnancy

Randomized trials show that supplementing women with fish oil during pregnancy generally improves their LC-PUFA status and results in higher n-3 in cord blood. However, the evidence that improved biochemical status has functional benefits for the infant is less persuasive. Malcolm et al. [3] found no effect of maternal fish oil supplementation on infant visual outcomes up to 6 months post-term, although DHA status in cord blood itself predicted visual outcome. Helland et al. [4] randomized mothers to fish oil versus corn oil during pregnancy and lactation (up to 3 months postpartum) and showed no effect on infant growth or development. However, in a 4-year follow-up of a group of these children, those whose mothers had received fish oil during
pregnancy had significantly higher scores on the Kaufmann ABC test of mental ability than those from the corn oil group. This finding emphasizes the importance of longer-term follow-up, both to detect ‘emerging’ effects and also to permit the use of more specific and discriminating tests of cognition.

There has been some interest in the possibility that maternal supplementation with n-3 might reduce the development of atopy in the infant, based on the hypothesis that a relative increase in proinflammatory n-6 fatty acids and a decrease in anti-inflammatory n-3 fatty acids might be involved in pathogenesis. In the large, prospective, population-based Avon Longitudinal Study of Pregnancy and Childhood cohort, maternal and cord blood fatty acid concentrations were not significantly related to the frequency of atopic symptoms in the offspring during early childhood [5]. However, in a randomized trial of fish oil supplementation during pregnancy in atopic mothers, fish oil resulted in increased n-3 status of the infant at birth and lower concentrations of some cytokines [6]. These infants were 3 times less likely to show a positive skin-prick reaction to egg at 12 months of age, and, although the frequency of atopic dermatitis was not significantly different between randomized groups, the fish oil group had less severe disease. These findings suggest a potential protective effect of n-3 supplementation on the development of atopy in high-risk infants.

**LC-PUFA Supplementation during Lactation**

Breast milk n-3 concentrations are related to infant plasma and erythrocyte phospholipid DHA concentrations in a saturable curvilinear manner. Above a breast milk DHA content of approximately 0.8% fatty acids, no further increase in infant DHA status is seen [7]. Supplementation of lactating mothers with fish oil has also been shown to result in higher breast milk DHA content, but to date there is no evidence that this results in improved functional outcome in the infant. In Danish breastfeeding mothers, fish oil supplementation resulted in increased breast milk DHA content (a 3-fold increase at 4 months). However, although visual outcome was significantly related to breast milk DHA content, the fish oil group did not show improved visual outcome at 2 or 4 months [8].

**LC-PUFA and Infant Nutrition**

Several observational studies in both term and preterm infants have shown that infants who are breastfed have higher LC-PUFA concentrations in blood [9, 10] and tissue [11, 12] than infants fed unsupplemented formulas. Furthermore, infants who receive LC-PUFA-supplemented formulas show improved blood and tissue LC-PUFA status [13]. However, it has been more difficult to establish that these undoubted biochemical effects are accompanied by beneficial effects on clinical and functional outcome in either group of infants.

Most research in this field has focused on adding preformed LC-PUFAs to infant formulas. The alternative strategy of increasing the concentration of
the precursors, particularly ALA, has not been adequately investigated as no studies have compared formulas with enough difference in ALA content. Current recommendations for the levels and ratio of LA and ALA in infant formulas are based essentially on the ranges observed in samples of human milk, and were made in the absence of data on functional or clinical outcomes. In a randomized trial of healthy term infants assigned formulas with LA:ALA ratios of either 10:1 or 5:1, those fed the lower ratio formula had higher plasma and red blood cell DHA concentrations than those fed the higher ratio formula; however, concentrations did not reach those seen in a parallel group of breastfed infants [14]. No differences were seen in visual acuity or growth up to 34 weeks of age. These data, together with those from two other studies [15, 16] suggest that the LA:ALA ratio must be lowered to <6:1 to improve the DHA status of formula-fed infants.

Randomized Trials of LC-PUFA Supplementation and Clinical Outcome

Preterm Infants

A systematic review for the Cochrane Library, last updated in November 2003, summarized the result of randomized trials of LC-PUFA supplementation and outcome in preterm infants [17]. Based on 11 identified trials, the authors concluded that no long-term effects of LC-PUFA supplementation were demonstrated on either developmental outcome or growth. However, they commented that infants enrolled in the trials were relatively mature and healthy preterm infants, and that marked differences in assessment schedules and methodology, dose and source of supplementation and fatty acid composition of the control formula between trials made direct comparisons difficult. Indeed, individual trials showed a range of beneficial, neutral or even apparently detrimental effects of supplementation on visual outcome, cognitive development and/or growth.

In a separate meta-analysis, SanGiovanni et al. [18] focused on short-term visual outcome and concluded that LC-PUFA supplementation was associated with short-term benefits for visual resolution acuity at 2 and 4 months, but whether supplementation confers lasting advantages remained to be determined.

The inconsistent results observed in LC-PUFA supplementation trials are well demonstrated by the results of three large studies that our own center has conducted or participated in. In the first study, supplementation was associated with improved cognitive development in the sub-group of infants with birth weights of <1,250 g, but had no effect on growth [19]. In a second trial, no effect of supplementation was seen on developmental outcome but supplemented infants were shorter than controls at 18 months post-term [20]. In the third study, published since the most recent update of the
Cochrane review, we showed that LC-PUFA supplementation was associated with greater weight and length gain up to 9 months [21]. Benefits were greater in boys, who showed greater length gain and higher Bayley Mental Development Index (MDI) at 18 months in boys. A further study published recently in abstract form [22] also reported significantly higher Bayley MDI and Psychomotor Developmental Index at 18 months post-term in preterm infants supplemented with LC-PUFAs during the first 12 months.

**Term Infants**

The Cochrane Systematic Review on LC-PUFA supplementation and outcome in term infants was last updated in June 2001 [23]. Based on 10 identified trials, it concluded that there was little evidence that supplementation produced benefits for visual or general developmental outcome, or influenced growth. However, as in preterm infants, the results of individual trials varied considerably. For example, a small randomized trial (approximately 20 infants per group) reported a 7-point higher Bayley MDI in supplemented infants [24] whereas our larger trial (approximately 150 infants per group) found no significant difference between groups using the same developmental test [25]. When the results of these two trials were combined, no overall effect on developmental outcome was observed. Another large trial (approximately 110 infants per group), reported in abstract form [26], also found no significant effect of LC-PUFA supplementation on developmental outcome at 12 months of age.

Since the last update of the Cochrane Systematic Review, data from an 18-month follow-up of infants randomized to unsupplemented versus LC-PUFA-supplemented formulas for the first 2 months of life have been published [27], showing no effect of supplementation on Bayley developmental scores, or on neurological status assessed using an examination specifically designed to detect minor abnormalities. A separate meta-analysis of trials evaluating effects on visual resolution acuity was also performed, and concluded there was some evidence of better visual outcome in LC-PUFA-supplemented infants at 2 months, but that longer-term effects remained to be determined [28].

Importantly, longer-term follow-up data are also now starting to appear. Auestad et al. [29] reported a 39-month follow-up of infants from one randomized trial in which no effect of either DHA or combined DHA/AA supplementation had been seen during the first year. At 14 months of age, infants supplemented with DHA alone had shown lower scores on tests of vocabulary production and comprehension than infants fed unsupplemented formula or those who were breastfed. At 39 months of age, about half of the infants were tested using a more comprehensive range of developmental tests (assessing overall IQ but also receptive and expressive vocabulary and visuomotor function). Scores were not significantly different between control and supplemented groups: in fact the 2 supplemented groups had both raw and adjusted IQ scores that were 3–4 points lower than those of the control and
breastfed groups. The study had approximately 35 infants per randomized group, and was not powered to detect this effect size (approximately 0.3 SD).

Our own (unpublished) data from a 5- to 6-year follow-up of term infants randomized to LC-PUFA-supplemented versus unsupplemented formula during infancy shows a significant 5-point deficit in IQ for previously supplemented infants using the WISC-IQ (approximately 90 infants per group); no difference was seen between groups at the 18-month follow-up using the Bayley scales of infant development. It is not clear whether this finding reflects the emergence of an effect since the previous follow-up, or whether the global developmental test used during infancy was too insensitive to detect differences. It is possible that effects of LC-PUFA supplementation on cognitive outcome are subtle, perhaps affecting information processing. Willatts et al. [30] previously showed that term infants randomized to LC-PUFA-supplemented formula showed better performance on a specific test of problem-solving at age 10 months, and a more recent (unpublished) follow-up of this cohort shows that the supplemented group performs better on a test of information processing at age 5 years.

All of the studies discussed so far involved LC-PUFA supplementation of infant formula fed from birth. More recent studies have also addressed the effect of LC-PUFA supplementation later in infancy. In two separate trials, infants randomized to LC-PUFA-supplemented formulas when they stopped breastfeeding at either 6 weeks [31] of age or 4–6 months [32] of age had significantly better visual acuity up to 1 year of age than those weaned onto unsupplemented formula. These findings suggest that term infants may benefit from a supply of LC-PUFAs beyond the first few weeks or months of life, at least for visual outcome.

Two studies have examined the effects of providing additional DHA in weaning foods. Breast- and formula-fed infants who received DHA-enriched egg yolks four times per week from 6–12 months had higher red cell DHA levels at 12 months than those fed standard egg yolks or no egg [33]. Hoffman et al. [34] randomized breastfed infants to receive either 1 jar/day of weaning foods containing DHA-enriched egg yolk, or control baby food, between 6 and 12 months. Although many infants in both groups continued to breastfeed for a mean of 9 months, by 12 months those receiving the DHA-enriched weaning foods showed an increase in red cell DHA levels (compared to a fall in the control group). They also showed a greater increase in visual acuity resolution over the trial period.

Current Status and Limitations of LC-PUFA Supplementation Trials

It is apparent from the preceding discussion that, despite good theoretical reasons to anticipate beneficial effects of LC-PUFA supplementation, and
often despite clear evidence of biochemical effects, it has been difficult to establish clinical efficacy. A number of factors that may contribute to the inconsistent findings are discussed below.

(1) The minimum requirement for LC-PUFA is unknown, making it difficult to determine an optimal level for supplementation. This difficulty is compounded by the variable concentrations of LC-PUFAs, particular DHA, in breast milk.

(2) LC-PUFA supplementation trials are heterogeneous in terms of the intervention used. Although described generically as ‘LC-PUFA-supplemented formulas’, these products vary in so many respects that the value of directly comparing or pooling trials in a conventional meta-analyses is questionable. Thus ‘LC-PUFA-supplemented formulas’ differ in:

(a) The content of precursor 18-carbon fatty acid and ratio of 18:n-3/18:n-6
(b) The ‘dose’ of DHA and AA
(c) The source of LC-PUFAs

The concentration of LC-PUFA in formulas used in intervention trials has varied considerably. The potential contribution of this factor to the inconsistent trial results has recently been considered by 2 groups, using a novel approach relating functional outcomes to the relative ‘dose’ of DHA [2, 35]. This necessitates making assumptions, for example, about the conversion efficiency of ALA to DHA, and the DHA content and intake of breast milk. Nevertheless, both analyses concluded that trials with the greatest difference in ‘DHA equivalents’ between control and intervention groups, or between breast- and formula-fed groups, showed the greatest difference in outcome. This provides some support for the concept of a ‘dose response’ between DHA intake (whether as ALA or DHA) and outcome.

A further issue relates to the positional distribution of LC-PUFAs. For example, in single-cell oils, triglycerides may be present with LC-PUFA esterified in the sn-1 position, or with two or three DHA or AA molecules. Such triglycerides are unphysiological and not seen in human milk. The extent to which the positional distribution of LC-PUFAs influences digestion, absorption or the subsequent actions of LC-PUFAs is unclear.
LC-PUFA supplementation trials are heterogeneous in terms of the outcome measures and testing methods used. Lauritzen et al. [2] found that those trials using an objective ‘electrical’ measure of visual function such as visual evoked response or electro-retinogram were more likely to report a positive effect of supplementation than trials that used behaviorally based tests such as Teller acuity cards. Similarly, the few studies using a more specific test of cognitive function, such as problem-solving ability, were more likely to see positive effects of LC-PUFA supplementation than those using a global test of infant development such as the Bayley.

Small sample size was undoubtedly an issue in the early supplementation trials which were generally powered to detect differences in biochemical outcomes, not clinical or functional endpoints. However, this criticism does not apply to many more recent studies which have shown similarly inconsistent results.

In addition to these problems, two further issues deserve mention. Firstly, there is currently a lack of long-term data on the efficacy and safety of LC-PUFA interventions. The limited longer-term cognitive data have in fact highlighted the potential for unexpected adverse effects that were either not present or undetectable during infancy. Such late emergence of programmed effects has previously been reported for other health outcomes in both animals and in humans.

Secondly, there are few data on the effects of LC-PUFAs on other aspects of health. Such effects are entirely plausible given the bioactive nature of LC-PUFAs. Areas that have received limited attention include the following.

**Cardiovascular Risk**

LC-PUFA supplementation was associated with lower low-density lipoprotein cholesterol concentrations at age 4 months in one study [36]. LC-PUFA intake may also influence the fatty acid composition of skeletal muscle membranes, which in turn affects insulin resistance. Baur et al. [37] found higher LC-PUFA concentrations in the skeletal muscle of breastfed infants, and this was associated with lower fasting blood glucose concentrations. Whether this has longer term consequences for glucose and insulin metabolism is unknown.

Forsyth et al. [38] reported lower blood pressure at age 6 years in children randomized to LC-PUFA-supplemented versus control formula during infancy. The magnitude of the effect (approximately 3-mm Hg) was similar to that reported in individuals who were breastfed rather than formula-fed. In contrast to these findings, in a randomized trial of maternal fish oil supplementation during lactation, offspring blood pressure at 2.5 years of age was not influenced by the assignment [39]. The studies differed not only in the age at follow-up, but also the study population; the Forsyth study enrolled formula-fed infants, and the control group received no LC-PUFAs, whereas in
the second study, all infants were breastfed so the difference between groups in total LC-PUFA intake was less.

**Type-1 Diabetes**

A case-control study in Norway [40] with 545 cases of childhood-onset type-1 diabetes and 1,668 controls reported a significantly lower risk of diabetes associated with taking cod liver oil supplements during the first year of life (OR 0.74; 95% CI 0.56, 0.99). This could represent an anti-inflammatory effect of n-3 LC-PUFAs present in cod liver oil.

**Atopy**

The Childhood Asthma Prevention Study [41] involved 616 children at high risk of atopy who were enrolled antenatally in a two-by-two factorial randomized trial to examine the effect of both n-3 fatty acid supplementation and house dust mite allergen avoidance. There was a significant (10%) reduction in cough at 3 years of age in atopic children in the active diet group, but no difference in non-atopic children.

**Conclusions**

There are good theoretical reasons why LC-PUFA supplementation should have beneficial effects on cognitive and visual outcome and, given the bioactive nature of LC-PUFAs, on other outcomes. Despite this, clinical efficacy and safety have not been established. Research into LC-PUFA supplementation has highlighted issues of more general relevance to the design and testing of infant formula. Firstly, there may be potential pitfalls in generically grouping ‘supplemented’ products, when the supplementation strategy and process may itself influence outcome. Secondly, the current situation in which LC-PUFAs are added to most infant formulas despite inconsistent effects on outcome in clinical trials raises the more difficult issue of what constitutes evidence of acceptable efficacy and safety for the addition of novel ingredients to formulas, as well as the potential role of post-marketing surveillance.

Differences in intervention, design and outcome measures between LC-PUFA supplementation trials make conventional meta-analyses difficult to interpret. Approaches relating the dose of LC-PUFAs to outcome across trials may be more useful, but currently it may be best to evaluate efficacy and safety for individual formulations. A final consideration is that, whilst formulas have been supplemented with LC-PUFAs with the objective of improving visual and cognitive outcome, the bioactive nature of LC-PUFAs and the effects on other health outcomes now being observed in longer-term follow-up studies raise the possibility that LC-PUFA supplementation may one day be recommended for other reasons entirely.
References

26 Carlson SE, Mehra S, Nagey WJ, et al: Growth and development of term infants fed formulas with docosahexaenoic acid (DHA) from algal oil or fish oil and arachidonic acid (ARA) from fungal oil. Pediatr Res 1999;45:278A.
Discussion

Dr. Björkstén: Thank you for a very elegant presentation. I have some comments because we have been interested not in mental development but rather in immunology and allergy. As you may have noticed there is actually a difference in the composition of breast milk that affects the outcome of allergy in the babies, not only that the composition differs between allergic and non-allergic mothers but in fully breastfed babies we could identify certain associations with allergy development independent of maternal allergy. But it wasn’t really the levels that we were interested in, it was the ratios between the longest n-3 and the 2–6 series rather than the absolute levels, and it seems quite clear that there is some sort of abnormality but again it is not the total levels. I was wondering to what extent some of these discrepancies that you are reporting are not necessarily the breast milk in different societies, and the other thing is it is the ratio rather than the levels?

Dr. Fewtrell: I talked about the approach of DHA equivalents to explain some of the inconsistent results, but another alternative would be to do the same thing with respect to the n-6/n-3 ratio. I suppose the problem is that if you wanted to compare formula-fed and breastfed infants you would not necessarily have the data on the n-6/n-3 ratios in the breastfed infants unless milk samples were collected prospectively.

Dr. Björkstén: Yes, but my suggestion is that perhaps in order to really sort things out one would have to go there and have to do that in a sort of multi-dose way.

Dr. Fewtrell: I agree and I think it could well be important. I deliberately didn’t discuss any of the allergy data in my presentation so as not to overlap with Dr. Hanson’s talk.

Dr. Laron: Head circumference is measured in babies as a measure of brain growth. Has any analysis been made to find out whether additions of LCPUFA influenced head circumference?

Dr. Fewtrell: In our own trials the head circumference hasn’t been different between groups even when we have seen differences in weight and length. I am not aware of differences in head growth in other studies.

Dr. Jensen: I am not aware of a trial in which a difference in head circumference was observed between a LCPUFA-supplemented and a non-supplemented group.

Dr. Bernardo: Would you please comment on the addition of preformed LCPUFA among follow-on formula?

Dr. Fewtrell: I am not aware of any randomized trials looking at the effects of LCPUFA supplementation of follow-on formulas – that is, those designed for use beyond 6 months of age. The Birch group performed trials looking at supplementation when mothers stopped breastfeeding at either 6 weeks or 4–6 months and showed apparent benefits of LCPUFA supplementation for visual outcomes during infancy, but I think these trials used standard term formula. There have been two studies looking at LCPUFA supplementation of weaning foods with DHA-enriched eggs, again showing some benefits for DHA status and/or visual function.

Dr. Suavedra: Another trend has also arisen with regard to what to do with mothers either during pregnancy or during lactation. As we know there are very big differences between DHA contents in mothers which is particularly dependent on their diet and in many cases on their geographic location. From that point of view, and again with relatively few data, would you care to speculate on whether we should be looking at content in geographic areas and recommending, as we do for some salts and minerals, that mothers should either consume fish or DHA supplementation during pregnancy, lactation or both depending on the area they live in?

Dr. Fewtrell: I guess if you wanted to do that you would first need evidence, ideally from randomized trials, that supplementing mothers during pregnancy and lactation improves infant outcome and that the effect differed according to the mother’s
breast milk DHA content. Perhaps Dr. Jensen would like to comment on his soon-to-be-published trial that provides some evidence that supplementing mothers during lactation may improve their offspring's outcome?

**Dr. Jensen:** We actually used an analogy derived source of DHA. It was a very modest supplementation, 200 mg DHA, from shortly after delivery through 4 months postpartum. We administered some tests of neural development at 12 months of age and saw no differences between the two groups, but at 30 months of age those infants whose mothers had received the DHA supplement had about an 8.5 higher Bailey PDI than those infants whose mothers had received the placebo. What we did as supplementation was basically increase the amount of DHA in human milk from 0.2 to about 0.35% of total fatty acids. We also found that at 5–5.5 years of age those children whose mothers had received the DHA supplement performed better on a test of sustained attention than those children whose mothers received the placebo.

**Dr. Fewtrell:** With regard to pregnancy supplementation with fish or fish oil, ideally you would need to have trials in both low and high fish-consuming populations to see if habitual intake influenced the response to supplementation.

**Dr. Jensen:** I might ask, and this is an open-ended question, I am fishing for your comments, that in trials conducted in term infants the arachidonic acid to DHA ratio varied dramatically. In your large trial it was approximately 1, maybe a little under 1, in the Australian trial it was 1, in two large North American trials it was 3.2–3.5. Willets has pointed out that term trials in which the ratio was between 1.4 and 2 have shown some benefit on some measure of neurodevelopmental or neuropsychological status at least in trials in which the ratio falls outside that range either higher or lower. Any comments about that?

**Dr. Fewtrell:** Although I certainly would advocate looking at potential explanations for inconsistent results, it seems to me that we have to bear in mind the risk of identifying possible spurious explanations, especially when we are talking about one or two trials showing a particular effect, as is the case with the term studies showing positive neurodevelopmental or neuropsychological effects.

**Dr. Chad:** There seems to be an increased epidemic of attention deficit disorders in North America. A lot of children are on drugs to treat this and I was wondering if there are any trials under way to look at the association of DHA versus this as a possible outcome problem?

**Dr. Fewtrell:** Yes, there are certainly trials, and in the UK giving fish oil supplements to children with the aim of improving behavior and school performance is currently quite fashionable. This area wasn’t one that I reviewed for the purpose of this presentation. I don’t know if anyone else would like to expand on this.

**Dr. Jensen:** I will make a brief comment. We actually conducted a study of DHA supplementation in children with attention-deficit/hyperactivity disorder (ADHD) [1]. These were children between 6 and 11 years of age at strict DSM for our criteria for ADHD diagnosed by one of our developmental pediatricians. They were supplemented with 325 mg DHA/day for several months. The rating scale was given to both the parents and the teachers. They were administered the TOVA which is a computerized test of attention and impulse control, and we found no differences on any measure between the groups, granted this supplementation was started late in their lives, and it was several months of supplementation. There is study from the UK on a group of children with both ADHD and dyslexia as I recall and on a couple of measures, I believe reading ability, I don’t recall the details. They did see some benefits perhaps not on attention per se, but I know no more about that.

**Dr. Lucas:** This is a very difficult area, the full-term infant. But it is interesting that advisory groups, for instance the group that advise the FDA, have not come to the conclusion that LCPUFA is established and certainly in term formulas. I think there is more a general agreement that it may be useful in the high-risk preterm infant, and I
think that the evidence weighs slightly in favor of visual effects rather than not, but weighs slightly more in favor of adverse and neurodevelopmental effects than positive ones. Then there are two long-term trials showing adverse effects, 5- to 6-point deficit, and I think that the case is not proven. So the issue is whether we think there is enough evidence to make an ad hoc management decision that formula should contain LCPUFA in the present state of knowledge. I don’t think that this group should be influenced by vested interests or anything else that should come to dispatch their view on that.

Dr. Van Dael: Based on the evidence that you have presented what would be your recommendation for regulations which, as you know, are currently quite a bit under revision. The European Union is revising its regulation for infant formula and follow-on formula and they may make recommendations for LCPUFA fortification. The same will happen at the CODEX level, which shows it to have a global implication. So based on the evidence you presented what would be your recommendation with respect to LCPUFA?

Dr. Fewtrell: As I understand it, the SCF, who advise on potential changes to the EU Directive are currently of the opinion that there isn’t sufficient evidence to recommend the compulsory addition of LCPUFA to infant formulas, and they have recommended that addition should remain optional. My current reading of the literature is that the evidence for beneficial effects of LCPUFA supplementation in preterm infants may be growing but this is not the case for term infants. If I were evaluating the evidence from scratch, I don’t think I would be recommending that LCPUFA be added to term formula, but possibly to preterm formula. I think there is a further generic issue here, and that is how we decide if and when something should be added to a formula. For example, we could specify in scientific terms that we require a certain number of trials in a minimum number of infants to show a desired effect, as was discussed in the context of hypoallergenic formulas yesterday. However, we are talking about evaluating specific products and performing long-term follow-ups. This may not be feasible in the real world. This seems to be a dilemma.

Dr. Hamburger: Are you answering that question in terms of required content or permissive content, the addition of LCPUFA?

Dr. Fewtrell: I certainly would not say they were required, and I’m not sure I would say they were permissive in term infants based on current evidence. That is my personal opinion.

Dr. Haschke: I would like to make a comment, which has probably nothing to do with science but which reflects the real world as it is. There is a parallel development which probably has not too much to do with science, it is marketing of infant formulas, and the train has left the station. Very clever, the producers of LCPUFA, the suppliers have informed the public directly about the benefits, and after a certain while this consistent message has reached the public. So when the consumers were asked, they said, ‘yes, this is a perceived benefit, I am ready to pay extra for this perceived added value’. And from that moment onwards, when the first company started to add it, all the other companies, at least in the premium segment of infant formulas, followed. It is interesting, the differences between continents, how sensitive people are to this message. Here in the US it is definitely an issue, in the European Union it is mixed, in Asia it is definitely an issue, it is the main topic in infant formula. So in the near future we probably will not have the chance to make trials without LCPUFA supplementation because they will be everywhere.

Dr. Fewtrell: I totally agree that the train has left the station. When I gave a similar presentation recently, someone asked me why we couldn’t just take LCPUFA out of infant formulas. In the real world, in the absence of a major demonstrable adverse effect, that is probably not going to happen and I was answering the earlier question as though LCPUFA had not yet been added to formulas. However, they have and I suspect they will stay there.
Dr. Zeiger: I am wondering why that train can’t be derailed. Certainly faulty information have gone out with many other therapies such as hormonal replacement therapy for women has shown various detrimental effects, attitudes have changed, they are no longer being used to the degree they were used before. If some of the information that you presented today went into the public’s hands I think they would be re-educated and I don’t think the women would be using these supplemented formulas to feed their children due to great fears associated with it.

Dr. Fewtrell: I think it’s a very difficult issue in practice because the data are not consistent. For example, some of the longer term effects may be beneficial whereas effects on other outcomes could be adverse. It is very difficult to distill the current data into a clear but simple message for parents.

Dr. Haschke: I think there is no reason to discuss this in an emotional way anyway; marketing and medical sciences do not always go hand in hand. I would agree with you, the train can be stopped if there are severe adverse effects of LCPUFA supplementation, then it will be stopped next day, but otherwise it will be very difficult even to deviate the train in a different area.

Dr. Fewtrell: It seems to me that in the meantime we should make sure we know what is happening in terms of longer term outcome in these trials. If we have raised some concerns about effects on certain outcomes we must investigate them further in the follow-up of other established studies, and we should also look at the potential benefits for other outcomes.

Dr. Lucas: Just to make a comment here, I think what happens here is that a number of things in the past were added to infant formulas such as taurine and nucleotides without any research because evidence-based practice was not the common thing at that time. LCPUFA occurred exactly on the cast of the development of evidence-based practices so most of the work that was done before it then became rather criticized. But nevertheless as has been said, the train left the station at that point and we ended up with that. In fact I think that if the LCPUFA field had just started 5 years later then it would have been caught up in a current of much greater scrutiny of evidence basis.

Dr. Fewtrell: I am not sure that even that would have prevented the current situation with LCPUFA supplementation because even if we had specified that we require a certain number of trials in a certain number of infants looking at a particular outcome, we would probably still be left with the same uncertainty. I’m not sure that specifying a certain number of trials is going to be a solution, particularly with something like LCPUFA where many of the issues have to do with the strategy of supplementation. Perhaps that brings us back to the issue of considering different formulations more and not grouping formulas generically.

Dr. Lucas: I think we would have been more critical. For instance we have one trial that has been particularly influential in the addition of infant formulas in the United States, it has got about 17–20 subjects in each follow-up and I think we would have been more critical about that now.

Dr. Jensen: Speaking of formulations the one large trial in which an adverse long-term effect was observed of course is your trial, and Dr. Lucas in his comments on Wednesday eluded to the fact that there may have been a source issue but no mechanism was tossed out. We do have another European trial and the results have yet to be published with respect to the neuropsychological outcome data but arguably there was what most of us would regard as a positive blood pressure effect at 6 years of age and if you look at the performance of the 6-year-olds on a test that theoretically assesses the speed of central nervous system information processing in a very targeted test, there was a benefit. So I am curious to know about the source, those of you who were involved in the trial feel it was a potential problem.

Dr. Fewtrell: Do you want to comment on that Dr. Lucas?
Dr. Lucas: We are talking about isolated trials so it is very difficult to have a statistical approach to what sources are good or bad. The problem is that everybody uses a slightly different strategy, this was an egg phospholipid source. We have another trial with a phospholipid source that showed reduced growth and a beneficial effect on neurodevelopment, and there was a trend in preterm infants. So you might say that on the basis of that phospholipids don't look like a very good way to go as far as LCPUFA supplementation is concerned. But really that is not a very well defensible view given that we don't have a very large experience of anything other than probably single-chain oils that are probably used now more than most.

Dr. Moukarzel: You answered almost all my questions. You know that the FDA has considered one source of LCPUFA as grass which is generally considered as safe, and this is a particular source. So of course when you talk to us about two studies showing some long-term potential side effects of adding LCPUFA we need really to think about just said about the source of this LCPUFA. I wonder about this controversy, if we gather all the studies from the same source of LCPUFA, would this controversy still exist? I mean I have the feeling that if we collect all the studies using the same source of LCPUFA, one of these sources will be beneficial whereas the others won't be, and this is probably as you just said in your talk, one of the key questions that we need to look at, not only on how much we are adding but from which source we are adding it, what is the component we are adding, how much EPA we are adding? What would be your comment, and do you still consider the LCPUFA as safe?

Dr. Fewtrell: I agree that it is a sensible approach to look at the outcome of trials in relation to the LCPUFA source. In fact, the Gibson group has done a meta-analysis of the effects of LCPUFA supplementation on growth and they did consider the source. But unfortunately when you pool trials for such an analysis you end up looking at the short-term outcomes that are available for most trials, such as growth during the first year. Very few trials have followed the subjects beyond infancy so far, so I don't think it's possible to relate LCPUFA source to longer term outcomes which may potentially be of greater concern.

Dr. Björkstén: I have a comment about what Dr. Haschke said regarding this interaction between science and research. If it is indeed the case that the train has left the station and if indeed it is difficult to derail, which I am not sure I agree, then I think I would like to go back to our discussion of yesterday, and that is that probiotics are now on the way of being put into the products and getting on the train and the train is about to start. So I would actually urge people who are involved in these product developments to be quite sure of what they are doing because we don't know if they are entirely safe in early infancy for everyone under every circumstance, and certainly documentation is not there yet. So I would hate to hear in 2 or 3 years the same comment from Dr. Haschke that the train has left the station and we can't do anything about it, you can.

Dr. Saavedra: I agree totally with what Dr. Björkstén has just said. I think in part, we need to control the kind of work that we ultimately have to do because the interesting thing here is that if we had all the studies that we would have to do for LCPUFA in terms of doses, ratios, sources, we would end up with a table of permutations of clinical trials that would have essentially no ending. Half of it I do believe has to do with what you communicate and there is no question that when you go to buy food in a store there are foods with benefits and there are those with risks, and they both have benefits and risks, as a matter of fact probably all of them have some benefit and some risk. The way to solve the dilemma when something has already been out in the public versus how to solve dilemmas that may happen as we come to providing and making these foods and different new ingredients available to the public is to continue being as clear as possible as to what the degree of evidence there is, and ultimately that depends on the kind of claims and benefits that both clinicians as well as consumers
are educated in. I don't think anybody buying chocolate needs to be educated on the benefits and the risks; would it be any different than what we do with any food and that certainly includes infant foods. The possibility of educating consumers and physicians to the point that both benefits and risks known to that point are there and the possibility of providing adequate choices for both, clinicians and consumers, so that they can decide if they want to take that benefit and know what the potential risks are is what we need to do. And having said that of course it is very complicated to educate everybody.

**Dr. Lucas:** If you were to take for instance the treatment of high blood pressure, it doesn't matter whether it is a nice inhibitor or a β-blocker or whatever, we would all agree that dropping blood pressure in people with very raised blood pressure is going to reduce the incidence of strokes, and that is such an important outcome that we are prepared to take some risks and most drugs carry risks. But in infant nutrition risks are not terribly acceptable unless there really is a massive benefit and it is not obvious in the way that the reduction in blood pressure reduces strokes that the incorporation of LCPUFA in the formula produces cognitive benefits. There are data going in both directions, so is any risk acceptable in a situation where you don't have a very obvious benefit in a global public health situation like this, and I just pose that as a rhetorical question.

**Dr. Saavedra:** We should say no risk is acceptable if we know it. The problem is that we are living in a world where we don't know all the benefits and all the risks.

**Dr. Zeiger:** Dr. Saavedra, I would agree there are certainly risks and benefits associated with foods but the analogy with formula I don’t think is as strong. With respect to formulas a real trust is expected by mothers that the formula is safe and not associated with risks. Before new nutrients/supplements are added to formulas, one would expect that strong evidence would exist for both safety and efficacy.

**Dr. Chad:** Is that the challenge, to identify if there are target groups that would benefit from this? In other words, as you presented the data globally in terms of meta-analyses there are pros and cons, whatever, but you can tease out certain groups that might benefit and I am not saying they do benefit but they might benefit, and is it the challenge now, if there is a role for this at all, to find which groups would benefit from this and use the educational initiatives to say that it would work for that and not for everybody else, and that is where you could possibly change the direction of the train.

**Dr. Fewtrell:** In an ideal world, yes. A lot of the studies have done sub-group analyses and some have shown for example that you get a greater effect of LCPUFA supplementation in lower birth weight infants, lower gestation infants, or in males. But the findings aren't consistent, so with the current state of knowledge I think it would be difficult to select particular groups of infants who should receive LCPUFA.

**Dr. Hursting:** I was wondering if a reverse translation approach might be useful here. We have had a couple of cases in the cancer field where a couple of trials had unusual findings. Going back to the animal models it has been very important and complex in the number of permutations you have got here, it depends on your animal models for the outcomes you are looking at. I don’t know that, it is just a naïve question.

**Dr. Fewtrell:** I don’t have particular knowledge of animal models in this field but in principle it sounds like a reasonable approach.

**Dr. Laron:** I would like to ask if reverse experiments have been done because there are no long-term studies on students who are excellent in their university studies and going back to see what their early nutrition was. In this country there are many Asian students with excellent records and I doubt that they had enriched formulas.

**Dr. Fewtrell:** I am not aware.

**Dr. Jensen:** Obviously you are talking about hundreds of potentially confounding factors in such a study.

220
Dr. Saavedra: Just one final comment. I think it is important to go back to the fact that we always want to know risk. We should be absolutely certain that what we try to do has no risk, and there is no question of that in the infant nutrition field, whether it is complementary foods, whether it is infant formula, that an even bigger precedent and almost anything else that we do particularly because of the reason for this meeting, that we are probably making differences that may have extremely long-lasting effects. Unfortunately, on the other hand, we have to accept that when we have taken that not so good path of not breastfeeding we have already accepted a risk, and from that point of view we need to do whatever is best to minimize that risk, and I think that is what we need to do.

Dr. Fewtrell: I think an important issue is whether there should be some requirement for long-term follow-up of intervention trials. As this currently isn't the case, it is entirely dependent on individuals to get funding and do the study.

Reference
