***...higher DHA doses than currently found in infant formulas
or the breast milk of women with Westernized diets
may be needed for preterm infants***

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Is Early Nutrition Related to Short-Term Health and Long-Term Outcome?
by Hania Szajewska and Maria Makrides

Key insights

This article provides a thorough review of clinically relevant publications regarding the three common types of supplements to preterm infant formula: long-chain polyunsaturated fatty acids (LCPUFAs), probiotics and prebiotics. The focus is on whether these supplements can reduce the risk of complications, e.g. necrotizing enterocolitis (NEC), and improve key outcomes in preterm infants.

Current knowledge

A positive relationship has been established between LCPUFA administration and development in preterm infants. New evidence suggests that disturbances in gut flora during early life may have both short- and long-term consequences, extending into adulthood. However, the use of probiotics/prebiotics in infant formula is not clear-cut.

Practical implications

A dietary source of LCPUFA, particularly docosahexaenoic acid (DHA), is important to support neurodevelopment. Certain bacteria appear better than others in reducing the incidence of NEC.

Recommended reading

Szajewska H, Guandalini S, Morelli L, Van Goudoever JB, Walker A: Effect of *Bifidobacterium animalis subsp lactis* supplementation in preterm infants: a systematic review of randomized controlled trials. *J Pediatr Gastroenterol Nutr* 2010;51:203–209.

Effects of supplements.

Is Early Nutrition Related to Short-Term Health and Long-Term Outcome?

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Key Messages

- Docosahexaenoic acid (DHA) supplementation is important in supporting the preterm infant's neurodevelopment.
- Prevention of necrotizing enterocolitis (NEC) is possible with probiotics; other suggested benefits of probiotics are not yet established.
- Further research is needed to establish the optimal DHA doses as well as the safety and efficacy of probiotics/prebiotics in preterm infant formulas.

Key Words

Docosahexaenoic acid • Growth • Long-chain polyunsaturated fatty acids • Microbiota • Neurodevelopment • Prebiotics • Preterm infant • Probiotics • Synbiotics

Abstract

This paper summarizes the literature concerning the effects of administering (1) long-chain polyunsaturated fatty acids (LCPUFA), (2) probiotics and/or (3) prebiotics to preterm infants. Clinically relevant, short- and long-term efficacy outcomes, such as those related to a reduced risk of disease, as well as outcomes related to safety, were sought. MEDLINE and the Cochrane Library literature searches performed in September 2010 were limited to randomized controlled tri-

als, their systematic reviews or meta-analyses. LCPUFA supplementation, particularly docosahexaenoic acid (DHA), of infant formula for preterm infants has consistently demonstrated better visual development of preterm infants compared with unsupplemented formulas. There is increasing evidence to suggest that LCPUFA supplementation for preterm infants is also related to improvements in more global measures of development, without any adverse effects. It is, however, important to note that the DHA doses tested in the infant formula interventions for preterm infants have been rather conservative. Newer studies comparing dietary DHA concentrations that match in utero accumulation rates with dietary DHA concentrations typical in the milk of women consuming little fish or in supplemented infant formulas demonstrate that these higher DHA doses are related to improvements in domains of cognitive development. Although further work is needed to better understand the optimal DHA requirements of preterm infants, it is clear that a dietary source of DHA is important to support neurodevelopment. To date, the most promising application of probiotics in preterm infants is the prevention of necrotizing enterocolitis by the administration of certain probiotics. Many other benefits of administering probiotics and/or prebiotics to preterm infants are, however, largely unproven. Efficacy and safety should be established for each probiotic and/or prebiotic product. Further research should specify strain-specific outcomes and determine optimal dosing schedules. Safety and long-term follow-up studies are of particular interest.

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Introduction

The magnitude of the problem of prematurity worldwide justifies any attempt aiming at the prevention of morbidity and mortality in preterm infants. Recently, the Committee on Nutrition of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) commented on enteral nutrient supply for preterm infants [1]. The Committee concluded that the preferred food for premature infants is fortified human milk from the infant's own mother, or, alternatively, formula designed for premature infants. The Committee also provided proposed advisable ranges for nutrient intakes.

In addition to the interest in major nutrients, research has also focused on the role of non-traditional nutrients/dietary supplements for the preterm infant. The purpose of this paper is to summarize the literature concerning the effects of administering (1) long-chain polyunsaturated fatty acids (LCPUFA), (2) probiotics and/or (3) prebiotics to preterm infants. The interest was in clinically relevant, short-term and long-term efficacy outcomes, such as those related to a reduced risk of disease, as well as in outcomes related to safety. MEDLINE and the Cochrane Library were searched in September 2010. The literature searches were limited to randomized controlled trials (RCTs) or their systematic reviews or meta-analyses. Regarding outcomes of interest that have previously been reviewed systematically, a summary of the findings from those reviews is presented in table 1. In addition, data from primary studies published subsequent to those reviews are assessed.

Long-Chain Polyunsaturated Fatty Acids

There has been a long-standing research interest into the health effects of LCPUFA. LCPUFA are present in every cell membrane and are the 20- and 22-carbon derivatives of the essential fatty acids α -linolenic acid (18:3n-3) and linoleic acid (18:2n-6). The key n-3 LCPUFA are eicosapentaenoic acid (20:5n-3, EPA) and DHA and the key n-6 LCPUFA is arachidonic acid (20:4n-6, AA; fig. 1). Although they can be synthesized from their respective precursor fatty acids, there is general agreement that synthesis is slow, especially for DHA. It is for this reason that preformed LCPUFA are thought to be important during the perinatal period, a time when there is rapid growth and development of new tissues and organ systems [2]. Fish, seafood, marine oils and human milk are a rich source of n-3 LCPUFA, especially DHA. n-3 LCPUFA are bioactive but their activity is difficult to

Table 1. Summary of findings from systematic reviews

Diseases of prematurity [12]:

- Focused on core clinical measures of infant health and development with LCPUFA supplementation; as such, the neurodevelopmental outcomes were assessed with global measures.
- A limitation of this approach is that it did not include other outcome measures relevant to the developing visual system, behavior or specific domains of cognition.
- Thus, any benefit to neurodevelopmental outcome may be important to this group of vulnerable infants, as the mean scores of the preterm infants included in these analyses were approximately 1 SD lower than the standardized norm.
- Most trials included in this review were restricted to exclusively formula-fed infants or only followed-up infants who were predominantly fed the trial formula.
- In clinical practice, however, a large proportion of preterm infants are fed both human milk and formula [24–26].
- Newer trials have been designed to incorporate the typical feeding patterns of preterm infants; in addition, they are specifically designed to address the dose of DHA.

Prevention of NEC [38–41]:

- Certain probiotics show potential in reducing the risk of NEC.
- Whether probiotic supplementation should become the standard of care is still under discussion. Before the routine use of probiotics in preterm infants, data regarding which products should be administered, at what dose, and for how long, are needed.
- While awaiting new studies and consensus among specialists, it seems reasonable to discuss the current evidence regarding probiotics with the parents and let them decide whether the intervention might be beneficial.

Prebiotics [63]/synbiotics [70]:

- The quantity and quality of the evidence regarding the effectiveness of the use of specific prebiotics in preterm infants is limited and does not allow one to formulate conclusions regarding the use of prebiotics in clinical practice.
- The relationship between the administration of synbiotics and health outcomes in preterm infants remains unclear because of the limited data in this area.

consider in isolation from the proportions of other dietary fatty acids. DHA is highly concentrated in retinal and neural cell membranes and is associated with signal transduction, neurotransmission and neurogenesis [3]. EPA is the precursor to the 3-series eicosanoids (prostaglandins/leukotrienes) that generally have an opposing action to the 2-series eicosanoids derived from AA. DHA also gives rise to resolvin D1 and protectin D1 (neuroprotectin D1) that are newly identified anti-inflammatory lipid mediators [4]. Leukotrienes and lipoxins also derived from EPA and AA are additional lipid mediators

that are involved in regulating acute inflammatory responses, although acute inflammation normally resolves by mechanisms that have remained somewhat elusive [4].

Fig. 1. LCPUFA: EPA, DHA, and AA. Most infant formula products contain a combination of DHA and AA.

Preterm infants are recognized as being at risk of LCPUFA dietary insufficiency. These infants are born before the last trimester is completed, prematurely ending the supply of LCPUFA across the placenta. Postmortem studies indicate that average whole body accretion of DHA during late pregnancy is in excess of 50 mg/kg/day [5], which for a preterm infant would be equivalent to a dietary DHA content of approximately 1% of total fatty acids. However, most preterm infants have dietary intakes which are significantly lower. It is therefore not surprising that newborn preterm infants have lower plasma and red cell concentrations of LCPUFA compared with newborn term infants [6]. Furthermore, the complex feeding regimens and feeding intolerances of these vulnerable infants often limit a consistent supply of LCPUFA whether through expressed human milk or infant formula.

The consequence of having a low or absent dietary supply of LCPUFA for preterm infants has been an important area of investigation for the last two decades. Initial studies investigated the effect of adding LCPUFA to infant formulas for preterm infants at concentrations that approximate the concentrations observed in the average

breast milk of women consuming Westernized diets. However, the choice of supplementation was limited to EPA-rich fish oils so that the earliest intervention studies tested the effect of adding EPA and DHA without AA. Although these early trials showed that fish oil supplementation of infant formula improved visual development during infancy [7, 8], some observed poorer growth when preterm infants were fed formulas containing only n-3 LCPUFA that resulted in a depression of plasma AA [9]. It was hypothesized that n-3 LCPUFA supplementation may have been a factor contributing to the growth deficit. However, this hypothesis has not been substantiated by systematic reviews of RCTs of LCPUFA supplementation [10, 11]. Further analyses using raw data from individual infants support these findings and show no growth deviations in infants supplemented with n-3 LCPUFA alone or in combination with AA [11].

Nevertheless, based on the composition of breast milk, most infant formula products contain a combination of DHA and AA and this is indeed the most commonly studied combination for infants with regard to neurodevelopmental and other clinical outcomes. We recently evaluated the effect of feeding LCPUFA-enriched compared with control formula to preterm infants on neurodevelopment and on the risk of NEC, bronchopulmonary dysplasia, sepsis, retinopathy of prematurity and intraventricular hemorrhage through a systematic review and meta-analysis of published RCTs [12]. We focused on these outcomes because they are associated with long-term health and well-being of infants born before term.

Data from 11 trials were included in this review [13–23]. The interventions of all included trials used a standard cow's milk-based formula for preterm infants.

Furthermore, the complex feeding regimens and feeding intolerances of these vulnerable infants often limit a consistent supply of LCPUFA whether through expressed human milk or infant formula.

LCPUFA were sourced from fish oils, egg triacylglycerol or phospholipids, and algal and fungal oils. All trials included at least 0.2% total fatty acids as n-3 LCPUFA and 7 of the trials tested n-6 as well as n-3 LCPUFA in formulas. Doses of n-3 LCPUFA ranged from 0.2 to 0.6% of total fatty acids

and from 0 to 0.7% of total fatty acids for AA. The supplementation periods varied from 1 month to over 1 year. When intervention periods extended beyond the neonatal period, infants were usually fed a transitional or term formula. Three trials permitted breast milk feeding in addition to the allocated trial formula [14, 15, 19]. Partici-

pant characteristics differed between trials, with some trials including relatively healthy infants born <37 weeks of gestation [18], while others included extremely low-birth-weight infants with concomitant morbidities typical of preterm infants [14]. Attrition varied between 11 and 50%.

Neurodevelopment

Neurodevelopment was assessed in 7 trials and in all cases with the Bayley Scales of Infant Development (BSID; version I or II). As the different versions of the BSID involve different procedures for administration and scoring, the data generated from these tests were considered as separate subgroups in the meta-analysis. The meta-analysis showed that infants fed LCPUFA-supplemented formula and tested with the BSID-II had a Mental Developmental Index (MDI) that was 3 points higher than infants fed control formula [weighted mean difference (WMD) 3.44, 95% confidence interval (CI) 0.56–6.31; $n = 879$, $p = 0.02$] [12]. Fewer MDI data were available for infants tested with BSID-I and the control and treatment groups did not differ (WMD –4.09, 95% CI –9.85 to 1.67; $n = 97$, $p = 0.16$). Overall, no significant difference in MDI was observed between infants fed control or LCPUFA-supplemented formula when MDI data from both BSID-I and BSID-II assessments were combined (WMD 2.13, 95% CI –0.87 to 5.14; $n = 976$, $p = 0.16$) [12]. The incongruence observed between the MDI scores and the version of the BSID was surprising and added to the heterogeneity between trials, contributing to the need to apply random effect models. It was not possible to combine the BSID-I and -II data in a meaningful way because the differences between trials contributed to a greater diversity in responses than expected. The differences between trials may arise from the sample population studied, the way the intervention was applied, the types of outcomes or trial methodology. We have limited confidence in the BSID-I outcome, as these data were generated from two trials with small sample sizes and methodological limitations [16, 21].

Diseases of Prematurity

In the published systematic review of trials investigating the effect of LCPUFA-supplemented compared with control formula on the incidence of diseases of prematurity, we noted no effect of LCPUFA on the risk of NEC and sepsis (table 2) [12]. These data contribute to the growing body of knowledge regarding the safety of LCPUFA-supplemented formulas. Too few data were available to investigate the outcomes of retinopathy of

prematurity, intraventricular hemorrhage and bronchopulmonary dysplasia and further studies are necessary to confirm the safety of LCPUFA-enriched formulas in trials with a low risk of bias and with sufficient statistical rigor. However, collecting further data to compare incidences of disease between infants fed LCPUFA-supplemented and control formulas may be unlikely as LCPUFA-supplemented formulas are already widely used in clinical practice.

Table 2. Effect of LCPUFA on the risk of NEC and sepsis

	Relative risk	95% CI
NEC ($n = 1,488$)	1.12	0.64–1.97
Sepsis ($n = 1,519$)	1.09	0.81–1.48

What Is the Optimal Dose of DHA for Preterm Infants?

The new phase of LCPUFA research for preterm infants is focusing on dose. Postmortem tissue analyses of stillbirths suggest that in utero whole body accumulation of DHA is >50 mg/kg/day [5], whereas the estimated intake is ~20 mg/kg/day in preterm infants consuming commercially available LCPUFA-supplemented preterm formula or expressed human milk of women who do not regularly eat more than 2 fish meals per week. The DHA for the Improvement in Neurodevelopmental Outcome of Preterm Infants (DINO) trial tested whether increasing the amount of dietary DHA from ~20 mg/kg/day to levels calculated to provide the fetal accumulation rate (~60 mg/kg/day) would improve neurodevelopment in infants born <33 weeks of gestation [27]. DHA enrichment of breast milk fed to infants was achieved through maternal supplementation with tuna oil or direct addition to infant formula. This large and inclusive trial showed that infants fed the DHA-enriched diet had better visual development in infancy [28]. The DINO trial also demonstrated an improvement in mean MDI at 18 months corrected age that did not reach statistical significance ($p = 0.2$), although there were 50% fewer children (5.2 vs. 10.5%, $p = 0.03$) with significant cognitive delay in the high-DHA group [27]. Furthermore, DHA-supplemented girls and infants born weighing <1,250 g had a 5-point improvement in mental development scores compared with control [27]. The efficacy of high DHA in infants born <1,500 g was also recently reported, show-

ing improved problem solving and better recognition memory at 6 months corrected age [29], indicating that higher DHA doses than currently found in infant formulas or the breast milk of women with Westernized diets may be needed for preterm infants. In both trials of high-DHA feeding, no adverse effects were reported [27, 29].

Probiotics, Prebiotics and Synbiotics

Emerging evidence suggests that the microbiota disturbances during early life may have both short- and long-term consequences extending into adulthood. The pathogenesis of diseases such as asthma, allergy, atopy, type 1 diabetes and inflammatory bowel disease has been linked to abnormal intestinal colonization. This has led to an interest in the development of strategies aimed at manipulating bacterial colonization, including

These products available as drugs, medical foods, dietary supplements or in infant formula are currently gaining worldwide popularity and are increasingly being used in the pediatric population.

the administration of probiotics or prebiotics or a combination of both (synbiotics). These products available as drugs, medical foods, dietary supplements or in infant formula are currently gaining worldwide popularity and are increasingly being used in the pediatric population. However, uncertainty exists regarding the efficacy and safety of their use, particularly in preterm infants.

Probiotics

According to the most widely used definition, probiotics are defined as ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’ [30]. The rationale for probiotic supplementation of preterm infants is based on data demonstrating differences in the establishment of the intestinal microbiota in preterm infants. Compared with healthy full-term infants, the intestinal microbiota in preterm infants features a low number of species, and there is significantly delayed colonization with anaerobes, particularly bifidobacteria [31–33]. Additionally, preterm infants are often cared for in intensive care units and receive broad-

Table 3. Risk analysis in preterm neonates in the probiotic group versus controls

	Relative risk	95% CI
NEC (n = 1,488)	0.35	0.23–0.55
All-cause mortality	0.42	0.29–0.62
Sepsis (n = 1,519)	0.98	0.81–1.18

spectrum antibiotics, which further contribute to differences in colonization patterns.

Prevention of NEC

The possible health consequences of abnormal patterns of colonization in preterm infants are not known. However, it has been speculated that they may contribute to increased susceptibility to infections and NEC pathogenesis. The latter is one of the most severe life-threatening gastrointestinal diseases, and it is characterized by various degrees of mucosal or transmural necrosis of the intestine. The incidence of NEC in infants is 5–10% [34]. The highest incidence is reported in infants with birth weights <1,000 g and the incidence decreases with increasing birth weights [35]. The exact cause of NEC remains unclear. However, in addition to prematurity, factors such as formula feeding, intestinal hypoxia-ischemia and colonization with pathogenic microbiota are considered to play a role in the pathogenesis of NEC [36]. While clearly the most effective strategy for preventing NEC is feeding with human milk, it also has been suggested that the enteral administration of probiotics to preterm newborns could prevent infections and NEC and reduce the use of antibiotics [37].

A number of systematic reviews, with or without a meta-analysis, have reviewed data on the effects of the enteral administration of probiotics on the risks of NEC and

Fig. 2. Probiotics compared with control in the prevention of NEC in preterm infants: analysis based on probiotic strain(s). Interpretation: if visually the horizontal line representing the results of an individual trial does *not* cross the line of no effect, there is 95% chance that there is a real difference between the groups. If the horizontal line *does* cross the line of no effect, there is no significant difference between the treatments and/or the sample size was too small to allow one to be confident about where the true result lies.

mortality in preterm infants [38–41]. Among them, the most recent is the updated meta-analysis by Deshpande et al. [41] (search date: March 2009), which identified 11 RCTs, including 4 recent trials, and involved 2,176 preterm infants. Compared with the control group, preterm neonates in the probiotic group had a reduced risk of NEC and all-cause mortality, but there was no difference between groups in the risk of sepsis (table 3). Heterogeneity between trials was low ($I^2 = 0\%$), suggesting that the benefit appears to be a true class effect despite known differences between individual probiotic microorganisms.

From a methodological point of view, this is a high-quality meta-analysis for which the results should be reliable and valid. The major concern with regard to this meta-analysis, as with many other meta-analyses in the area of probiotics, is whether it is appropriate to pool data on different microorganisms. First, there is evidence that the beneficial effects of probiotics, particularly the immunomodulatory effects of individual probiotics observed in the host, differ greatly and are strain specific. Second, probiotics vary by organism. In addition to the most commonly used lactic acid bacteria (e.g. lactobacilli or bifidobacteria), the yeast *Saccharomyces boulardii* is often used. All of these probiotics have different properties and anti-pathogenic mechanisms. Consequently, their efficacy may vary. Third, the dose of probiotics may be important, as has been documented [42].

According to the ESPGHAN Committee on Nutrition [1], presently available data do not permit recommending

the routine use of prebiotics or probiotics as food supplements in preterm infants to prevent NEC. The Committee also recommended that each probiotic strain and potential combinations need to be characterized separately for each product. This position does not mean that the use of probiotics for preventing NEC should be totally discarded. Rather, in settings in which the incidence of NEC is high, one may consider the use of probiotics – alone or in combination. However, care should be given to choose those that are the best studied, with the highest effect size and the best safety profile [43]. Figure 2 depicts a meta-analysis of the effects of probiotics for preventing NEC, with subgroup analyses based on the type of probiotic administered. In addition to data presented in the meta-analysis by Deshpande et al. [41], it also includes the results of one of the most recently published trials [44]. Clearly, not all probiotic microorganisms are equal in preventing NEC.

Efficacy by Probiotic Strain(s)

As stated, the ESPGHAN Committee on Nutrition recommended that each probiotic strain and potential combinations need to be characterized separately for each product. Given this consideration, table 4 summarizes the evidence, from RCTs or their systematic review/meta-analysis, of the effects of probiotics compared with placebo or no intervention in preterm infants. Of note, only a limited number of probiotic strains have been assessed in clinical trials. One of the best studied is *Bifido-*

Table 4. Summary of evidence from RCTs or their systematic reviews/meta-analysis (MA) of the effects of probiotics compared with placebo or no intervention in preterm infants

Probiotic(s)	Outcomes	Study design	Effect
Various probiotics pooled together	prevention of NEC	MA	↓ (see fig. 2)
Various probiotics pooled together	death due to all causes	MA	↓
Various probiotics pooled together	prevention of sepsis	MA	nonsignificant
<i>B. animalis</i> ssp. <i>lactis</i> CNCM I-3446 (also known as <i>B. lactis</i> Bb12)	prevention of NEC ≥2 risk of sepsis use of antibiotics	MA MA MA	nonsignificant nonsignificant nonsignificant
<i>B. longum</i> BB536 and <i>L. rhamnosus</i> GG	tolerance of enteral feeding	RCT	nonsignificant
<i>L. rhamnosus</i> GG	risk of urinary tract infection, sepsis, NEC enteric colonization by <i>Candida</i> species	RCT RCT	nonsignificant ↓
<i>L. rhamnosus</i> ATCC 55730	feeding tolerance	RCT	↓ episodes of regurgitation; ↓ mean daily crying time
	gastrointestinal motility	RCT	more frequent stools

bacterium animalis ssp. *lactis* CNCM I-3446 (also known as *B. lactis* Bb12). A recent systematic review [45] of 4 RCTs [46–49] found that compared with administration of a control product, *B. lactis* supplementation has the potential to increase fecal bifidobacteria counts and to reduce Enterobacteriaceae and *Clostridium* spp counts. It also can reduce stool pH and fecal calprotectin concentrations, increase fecal immunoglobulin A and short-chain fatty acid concentrations [48], and decrease intestinal permeability. Compared with controls, *B. lactis* supplementation had no effect on the risk of NEC stage ≥ 2 ; however, the power of these studies does not allow for a definitive statement regarding a reduced risk of NEC. *B. lactis* supplementation also had no effect on the risk of sepsis or use of antibiotics, but it did have some effects on anthropometric parameters. No adverse effects in any of the 4 RCTs were noted.

Lactobacillus GG has the potential to colonize the gut of preterm infants, although the degree of colonization varies [50–52], and data on the ability of such colonization to reduce the bowel reservoir of pathogenic bacteria are not consistent [51]. In one study, administration of *Lactobacillus* GG compared with placebo had no effect on the risk of urinary tract infections, NEC or sepsis [53]. Another study showed that supplementation with *Lactobacillus* GG has the potential to significantly reduce the incidence and intensity of enteric colonization by *Candida* species in preterm infants [54]. Other probiotics studied include *Bifidobacterium longum* BB536 and *Lactobacillus* GG (no effect on gastrointestinal tolerance of enteral feeding in very-low-birth-weight infants but may improve gastrointestinal tolerance in infants weighing $>1,000$ g) [55] and *Lactobacillus reuteri* ATCC55730 (may stimulate gastric emptying) [56, 57].

Long-Term Effects

Only 1 trial has addressed the long-term effects of probiotic administration to preterm infants to date. Participants in 1 RCT designed to evaluate the efficacy of probiotics in preventing NEC were subsequently enrolled in a follow-up study. At 3 years corrected age, oral administration of *Lactobacillus acidophilus* and *Bifidobacterium infantis* from 1 week after birth until discharge had no effect on growth, neurodevelopmental outcomes and sensory outcomes [58].

Probiotics
Life microorganisms which when administered in adequate amounts confer a health benefit on the host [30].
Prebiotics
Non-digestible food components that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon thereby improving host health [59].
Synbiotics
A combination of both.

Prebiotics

The term ‘prebiotic’ was introduced by Gibson and Roberfroid [59] in 1995, who defined prebiotics as ‘non-digestible food components that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thereby improving host health’. Oligosaccharides that are contained in human breast milk are considered to be the prototype of prebiotics, since they have been shown to facilitate the growth of bifidobacteria and lactobacilli in the colon of breast-fed neonates [60–62].

One systematic review/meta-analysis [63] that examined primarily the efficacy and safety of prebiotic oligosaccharide supplementation of formula in reducing the incidence of NEC and sepsis as well as improving physical

growth in preterm infants was identified. The methodological quality of this systematic review was good. By searching the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE and CINAHL databases and proceedings of relevant conferences, the authors identified 4 RCTs that qualified for inclusion in the review [64–67]. One of these trials [66] was first published as an abstract only, but it was later published as a full paper [68]. Sample sizes in the studies ranged from 20 to 56 infants; only 126 preterm infants were included in the review. The prebiotic oligosaccharides used in these studies were galacto-oligosaccharides/fructo-oligosaccharides or fructo-oligosaccharides alone. The duration of supplementation ranged from 14 to 30–33 days. Authors of 2 RCTs reported that NEC did not occur in

The major concern with regard to this meta-analysis, as with many other meta-analyses in the area of probiotics, is whether it is appropriate to pool data on different microorganisms.

any of their infants. Authors of the other 2 RCTs did not report data related to NEC or sepsis. A meta-analysis of the data from 3 RCTs ($n = 106$) showed no significant difference in weight gain between the prebiotic and control groups (WMD -1.6 g/day, 95% CI -3.76 to 0.57). A meta-analysis of the data from the 2 trials that evaluated stool flora showed a statistically significant increase in bifidobacterial counts in the prebiotic-supplemented group compared with the control group (WMD 0.53 , 95% CI 0.33 – 0.73). The authors of the review concluded that prebiotic-supplemented formula increased stool colony counts of bifidobacteria and lactobacilli in preterm neonates without adversely affecting weight gain. They also stated that available evidence is insufficient to derive any clear conclusions and does not support the routine supplementation of preterm formula with prebiotics.

Synbiotics

The term 'synbiotic' is used 'when a product contains both probiotics and prebiotics' [69]. In a recently published RCT [70], 90 preterm infants received a dietary supplement containing 2 *Lactobacillus* species plus fructo-oligosaccharides, a supplement containing several species of lactobacilli and bifidobacteria plus fructo-oligosaccharides, or placebo (a dilute preparation of Pregestamil formula) twice daily for 28 days or until discharge if earlier. The study found that preterm infants who received the supplement containing several species of lactobacilli and bifidobacteria plus fructo-oligosaccharides were more likely to become colonized with bifidobacteria. There were no significant differences in weight gain or the content of short-chain fatty acids in the stool between groups.

Inconclusive data regarding

- the effectiveness of prebiotics in preterm infants
- a possible relationship between the administration of synbiotics and health outcomes in preterm infants

Safety

In 2004, the ESPGHAN Committee on Nutrition commented on the probiotic bacteria in dietetic products for infants and stated that the probiotics used thus far in clinical trials can be generally considered as safe. However, surveillance regarding the detection of possible side effects, such as cases of infection in high-risk groups for which there is very little systematic evidence available, is needed. The Committee concluded that the available data

are not sufficient to support the safety of probiotic use in healthy newborns and very young infants with immature defense systems, immunocompromised infants, premature infants and infants with congenital heart disease [71]. In the most recent comment on enteral nutrient supply for preterm infants, the Committee stated that efficacy and safety should be established for each probiotic or prebiotic product to be used in preterm infants [43]. While there are no data to suggest that any categories of preterm infants are at higher risk than others, it is noteworthy that data related to infants of very low birth weights ($<1,000$ g) are very limited.

Conclusions

LCPUFA, particularly DHA, supplementation of infant formula for preterm infants has consistently demonstrated better visual development of preterm infants compared with unsupplemented formulas. In addition, there is increasing evidence to suggest that LCPUFA supplementation for preterm infants is also related to improvements in more global measures of development, without any adverse effects. It is, however, important to note that the doses of DHA tested in the infant formula interventions for preterm infants have been rather conservative – amounts were designed to match the concentrations of DHA observed in the milk of women consuming Westernized diets rather than mimicking the levels of DHA supplied in utero. Newer studies comparing dietary DHA concentrations that match in utero accumulation rates with dietary DHA concentrations typical in the milks of women consuming little fish or supplemented infant formulas demonstrate that these higher DHA doses are also related with improvements in domains of cognitive development. Although further work is needed to better understand the optimal DHA requirements of preterm infants, it is clear that a dietary source of DHA is important to support neurodevelopment.

Probiotics and prebiotics have the potential to prevent and treat many disorders in preterm infants. To date, the most promising application is the prevention of NEC by the administration of certain probiotics. Many other benefits of administering probiotics and/or prebiotics to preterm infants are, however, largely unproven. The latest comment by the ESPGHAN Committee on Nutrition is that there is not enough available evidence that the use of probiotics or prebiotics in preterm infants is safe. Efficacy and safety should be established for each probiotic and/or prebiotic product. It was also stated that at the present time, a lack of evidence does not permit recom-

mending the routine use of prebiotics or probiotics as food supplements in preterm infants. However, clearly the concept that probiotics and/or prebiotics could have a role to play in reducing morbidity and/or mortality of preterm infants is worthy of further studies. Further research should specify strain-specific outcomes and determine optimal dosing schedules. Safety and long-term follow-up studies are of particular interest.

Disclosure Statement

Hania Szajewska has participated as a clinical investigator, and/or advisory board member, and/or speaker for Arla, Biocodex, Danone, Nestlé Nutrition Institute, Nutricia, and Mead Johnson. Maria Makrides serves on scientific advisory boards for Nestlé, Nutricia and Fonterra. Associated honoraria are paid to her institution to support the continuing education of early career researchers. The writing of this article was supported by Nestlé Nutrition Institute.

References

- Agostoni C, Buonocore G, Carnielli VP, et al: Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2010;50:85–91.
- Innis SM: Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. *J Pediatr* 2003;143:S1–S8.
- Innis SM: Fatty acids and early human development. *Early Hum Dev* 2007;83:761–766.
- Bazan NG: Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. *Curr Opin Clin Nutr Metab Care* 2007;10:136–141.
- Clandinin MT, Chappell JE, Heim T, Swyer PR, Chance GW: Fatty acid utilization in perinatal de novo synthesis of tissues. *Early Hum Dev* 1981;5:355–366.
- Smithers LG, Gibson RA, McPhee AJ, Makrides M: Effect of two doses of docosahexaenoic acid (DHA) in the diet of preterm infants on infant fatty acid status: results from the DINO trial. *Prostaglandins Leukot Essent Fatty Acids* 2008;79:141–146.
- Birch D, Birch E, Hoffman DR, et al: Retinal development of very low birthweight infants fed diets differing in n-3 fatty acids. *Invest Ophthalmol Vis Sci* 1992;33:2365–2376.
- Carlson SE, Werkman SH, Rhodes PG, et al: Visual-acuity development in healthy preterm infants: effect of marine-oil supplementation. *Am J Clin Nutr* 1993;58:35–42.
- Carlson SE, Cooke RJ, Werkman SH, et al: First year growth of preterm infants fed standard compared to marine oil n-3 supplemented formula. *Lipids* 1992;27:901–907.
- Simmer KN, Schulzke SM, Patole S: Long-chain polyunsaturated fatty acid supplementation in preterm infants. *Cochrane Database Syst Rev* 2008;1:CD000375.
- Gibson RA, Makrides M: LCPUFA and the growth of preterm or term infants. A systematic review and meta-analysis. *Maternal and Infant LCPUFA Workshop*. Kansas City, 2003, pp 1–21.
- Smithers LG, Gibson RA, McPhee A, et al: Effect of long-chain polyunsaturated fatty acid supplementation of preterm infants on disease risk and neurodevelopment: a systematic review of randomized controlled trials. *Am J Clin Nutr* 2008;87:912–920.
- Carlson SE, Werkman SH, Tolley EA: Effect of long-chain n-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia. *Am J Clin Nutr* 1996;63:687–697.
- O'Connor DL, Hall R, Adamkin D, et al: Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective randomized controlled trial. *Pediatrics* 2001;108:359–371.
- Clandinin MT, Van Aerde JE, Merkel KL, et al: Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. *J Pediatr* 2005;146:461–468.
- Carlson SE: Lipid requirements of very-low-birth-weight infants for optimal growth and development; in Dobbing J (ed): *Lipids, Learning, and the Brain: Fats in Infant Formulas*. Columbus, Ross Laboratories, 1993, pp 188–207.
- Foreman-van Drongelen MMHP, van Houwelingen AC, Kester AD, Blanco CE, Hasaart TH, Horntsa G: Influence of feeding artificial-formula milks containing docosahexaenoic and arachidonic acids on the postnatal long-chain polyunsaturated fatty acid status of healthy preterm infants. *Br J Nutr* 1996;76:649–667.
- Fewtrell MS, Morley R, Abbott RA, et al: Double-blind, randomized trial of long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. *Pediatrics* 2002;110:73–82.
- Fewtrell MS, Abbott RA, Kennedy K, et al: Randomized, double-blind trial of long-chain polyunsaturated fatty acid supplementation with fish oil and borage oil in preterm infants. *J Pediatr* 2004;144:471–479.
- Fang PC, Kuo HK, Huang CB, Ko TY, Chen CC, Chung MY: The effect of supplementation of docosahexaenoic acid and arachidonic acid on visual acuity and neurodevelopment in larger preterm infants. *Chang Gung Med J* 2005;28:708–715.
- van Wezel-Meijler G, van der Knaap MS, Huisman J, Jonkman EJ, Valk J, Lafeber HN: Dietary supplementation of long-chain polyunsaturated fatty acids in preterm infants: effects on cerebral maturation. *Acta Paediatr* 2002;91:942–950.
- Vanderhoof J, Gross S, Hegyi T, et al: Evaluation of a long-chain polyunsaturated fatty acid supplemented formula on growth, tolerance, and plasma lipids in preterm infants up to 48 weeks postconceptual age. *J Pediatr Gastroenterol Nutr* 1999;29:318–326.
- Carlson SE, Montalto MB, Ponder DL, Werkman SH, Korones SB: Lower incidence of necrotizing enterocolitis in infants fed a preterm formula with egg phospholipids. *Pediatr Res* 1998;44:491–498.
- Yip E, Lee J, Sheehy Y: Breast-feeding in neonatal intensive care. *J Paediatr Child Health* 1996;32:296–298.
- Furman L, Minich N, Hack M: Breastfeeding of very low birth weight infants. *J Hum Lact* 1998;14:29–34.
- Espy KA, Senn TE: Incidence and correlates of breast milk feeding in hospitalized preterm infants. *Soc Sci Med* 2003;57:1421–1428.
- Makrides M, Gibson RA, McPhee A, et al: Neurodevelopmental outcome of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial. *JAMA* 2009;301:175–182.
- Smithers LG, Gibson RA, McPhee AJ, Makrides M: Higher dose of docosahexaenoic acid in the neonatal period improves visual acuity of preterm infants: results of a randomized controlled trial. *Am J Clin Nutr* 2008;88:1049–1056.
- Henriksen C, Haugholt K, Lindgren M, et al: Improved cognitive development among preterm infants attributable to early supplementation of human milk with docosahexaenoic acid and arachidonic acid. *Pediatrics* 2008;121:1137–1145.
- Joint Food and Agriculture Organization of the United Nations and World Health Organization Working Group: *Report on Drafting Guidelines for the Evaluation of Probiotics in Food*. London, 2002.

- 31 Gewolb IH, Schwalbe RS, Taciak VL, et al: Stool microflora in extremely low birth-weight infants. *Arch Dis Child Fetal Neonatal* Ed 1999;80:F167–F173.
- 32 Magne F, Suau A, Pochart P, et al: Fecal microbial community in preterm infants. *J Pediatr Gastroenterol Nutr* 2005;41:386–392.
- 33 Blakey JL, Lubitz L, Barnes GL, et al: Development of gut colonisation in pre-term neonates. *J Med Microbiol* 1982;15:519–529.
- 34 Lin PW, Nasr TR, Stoll BJ: Necrotizing enterocolitis: recent scientific advances in pathophysiology and prevention. *Semin Perinatol* 2008;32:70–82.
- 35 Llanos AR, Moss ME, Pinzón MC, et al: Epidemiology of neonatal necrotizing enterocolitis: a population-based study. *Paediatr Perinat Epidemiol* 2002;16:342–349.
- 36 Hsueh W, Caplan MS, Qu XW, et al: Neonatal necrotizing enterocolitis: clinical considerations and pathogenetic concepts. *Pediatr Dev Pathol* 2003;6:6–23.
- 37 Caplan MS, Jilling T: Neonatal necrotizing enterocolitis: possible role of probiotic supplementation. *J Pediatr Gastroenterol Nutr* 2000;30:S18–S22.
- 38 Deshpande G, Rao S, Patole S: Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials. *Lancet* 2007;369:1614–1620.
- 39 Alfaleh K, Bassler D: Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev* 2008;1:CD005496.
- 40 Barclay AR, Stenson B, Simpson JH, et al: Probiotics for necrotizing enterocolitis: a systematic review. *J Pediatr Gastroenterol Nutr* 2007;45:569–576.
- 41 Deshpande G, Rao S, Patole S, et al: Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. *Pediatrics* 2010;125:921–930.
- 42 Szajewska H: Advances and limitations of evidence-based medicine: impact for probiotics. *Ann Nutr Metab* 2010;57(suppl):6–9.
- 43 Tarnow-Mordi W, Wilkinson D, Trivedi A, Sinn J, Dutta S, Parikh T, Lin HC: Re: ESPGHAN commentary and education that probiotics substantially reduce all-cause mortality and necrotizing enterocolitis in preterm infants. *J Pediatr Gastroenterol Nutr* 2010;50:694, author reply 694–695.
- 44 Mihatsch WA, Vossbeck S, Eikmanns B, Hoegel J, Pohlandt F: Effect of *Bifidobacterium lactis* on the incidence of nosocomial infections in very-low-birth-weight infants: a randomized controlled trial. *Neonatology* 2010;98:156–163.
- 45 Szajewska H, Guandalini S, Morelli L, Van Goudoever JB, Walker A: Effect of *Bifidobacterium animalis* subsp *lactis* supplementation in preterm infants: a systematic review of randomized controlled trials. *J Pediatr Gastroenterol Nutr* 2010;51:203–209.
- 46 Mohan R, Koebnick C, Schildt J, Schmidt S, Mueller M, Possner M, Radke M, Blaut M: Effects of *Bifidobacterium lactis* Bb12 supplementation on intestinal microbiota of preterm neonates: a double placebo controlled, randomised study. *J Clin Microbiol* 2006;44:4025–4031.
- 47 Stratiki Z, Costalos C, Sevastiadou S, et al: The effect of a bifidobacter supplemented bovine milk on intestinal permeability of preterm infants. *Early Hum Dev* 2007;83:575–579.
- 48 Mohan R, Koebnick C, Schildt J, et al: Effects of *Bifidobacterium lactis* supplementation on body weight, fecal pH, acetate, lactate, calprotectin and IgA in preterm infants. *Pediatr Res* 2008;64:418–422.
- 49 Mihatsch W, Vossbeck S, Franz AR, et al: Effect of *Bifidobacterium lactis* on the incidence of nosocomial infections in preterm infants. *Pediatr Res* 2004;56:495.
- 50 Stansbridge EM, Walker V, Hall MA, Smith SL, Millar MR, Bacon C, Chen S: Effects of feeding premature infants with *Lactobacillus* GG on gut fermentation. *Arch Dis Child* 1993;69:488–492.
- 51 Millar MR, Bacon C, Smith SL, Walker V, Hall MA: Enteral feeding of premature infants with *Lactobacillus* GG. *Arch Dis Child* 1993;69:483–487.
- 52 Reuman PD, Duckworth DH, Smith KL, Kagan R, Bucciarelli RL, Ayoub EM: Lack of effect of *Lactobacillus* on gastrointestinal bacterial colonization in premature infants. *Pediatr Infect Dis* 1986;5:663–668.
- 53 Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF: Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm neonates: a prospective double-blind study. *Biol Neonate* 2002;82:103–108.
- 54 Manzoni P, Mostert M, Leonessa ML, Priolo C, Farina D, Monetti C, Latino MA, Gomirato G: Oral supplementation with *Lactobacillus casei* subspecies *rhannosus* prevents enteric colonization by *Candida* species in preterm neonates: a randomized study. *Clin Infect Dis* 2006;42:1735–1742.
- 55 Rougé C, Piloquet H, Butel MJ, Berger B, Rochat F, Ferraris L, et al: Oral supplementation with probiotics in very-low-birth-weight preterm infants: a randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr* 2009;89:1828–1835.
- 56 Indrio F, Riezzo G, Raimondi F, Bisceglia M, Cavallo L, Francavilla R: The effects of probiotics on feeding tolerance, bowel habits, and gastrointestinal motility in preterm newborns. *J Pediatr* 2008;152:801–806.
- 57 Indrio F, Riezzo G, Raimondi F, Bisceglia M, Cavallo L, Francavilla R: Effects of probiotic and prebiotic on gastrointestinal motility in newborns. *J Physiol Pharmacol* 2009;60(suppl 6):27–31.
- 58 Chou IC, Kuo HT, Chang JS, et al: Lack of effects of oral probiotics on growth and neurodevelopmental outcomes in preterm very low birth weight infants. *J Pediatr* 2010;156:393–396.
- 59 Gibson GR, Roberfroid MB: Dietary modulation of the human colonic microbiota. Introducing the concept of prebiotics. *J Nutr* 1995;125:1401–1412.
- 60 Eshach Adiv O, Berant M, Shamir R: New supplements to infant formulas. *Pediatr Endocrinol Rev* 2004;2:216–224.
- 61 Dai D, Walker WA: Protective nutrients and bacterial colonization in the immature human gut. *Adv Pediatr* 1999;46:353–382.
- 62 Quigley EMM, Quera R: Small intestinal bacterial overgrowth: roles of antibiotics, prebiotics, and probiotics. *Gastroenterology* 2006;130:S78–S90.
- 63 Srinivasjois R, Rao S, Patole S: Prebiotic supplementation of formula in preterm neonates: a systematic review and meta-analysis of randomised controlled trials. *Clin Nutr* 2009;28:237–242.
- 64 Boehm G, Lidestri M, Casetta P, Jelinek J, Negretti F, Stahl B, et al: Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of faecal bifidobacteria in preterm infants. *Arch Dis Child Fetal Neonatal* Ed 2002;86:F178–F181.
- 65 Mihatsch WA, Hoegel J, Pohlandt F: Prebiotic oligosaccharides reduce stool viscosity and accelerate gastrointestinal transport in preterm infants. *Acta Paediatr* 2006;95:843–848.
- 66 Indrio F, Riezzo G, Montagna O, Valenzano E, Mautone A, Boehm G: Effect of a prebiotic mixture of short chain galacto-oligosaccharides and long chain fructo-oligosaccharides on gastric motility in preterm infants. *J Pediatr Gastroenterol Nutr* 2007:e122.
- 67 Kapiki A, Costalos C, Oikonomidou C, Triantafyllidou A, Loukatou E, Petrohilou V: The effect of a fructo-oligosaccharide supplemented formula on gut flora of preterm infants. *Early Hum Dev* 2007;83:335–339.
- 68 Indrio F, Riezzo G, Raimondi F, Francavilla R, Montagna O, Valenzano ML, et al: Prebiotics improve gastric motility and gastric electrical activity in preterm newborns. *J Pediatr Gastroenterol Nutr* 2009;49:258–261.
- 69 Schrezenmeier J, de Vrese M: Probiotics, prebiotics, and synbiotics: approaching a definition. *Am J Clin Nutr* 2001;73:361S–364S.
- 70 Underwood MA, Salzman NH, Bennett SH, Barman M, Mills DA, Marcobal A, Tancredi DJ, Bevins CL, Sherman MP: A randomized placebo-controlled comparison of 2 prebiotic/probiotic combinations in preterm infants: impact on weight gain, intestinal microbiota, and fecal short-chain fatty acids. *J Pediatr Gastroenterol Nutr* 2009;48:216–225.
- 71 ESPGHAN Committee on Nutrition: Probiotic bacteria in dietetic products for infants: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2004;38:365–374.