Fatty Acid Profiles of Infants Fed Formulas Supplemented with Long-chain Polyunsaturated Fatty Acids

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The quality of the dietary fatty acid supply during early life is of increasing interest, as lipids are structural components of all tissues and may play an important role in the neurodevelopment of infants. Supplementation of infant formulas with long-chain polyunsaturated fatty acids (LCPUFAs) has been tested in several studies, but no consensus has been reached on the recommended concentrations and sources for LCPUFA supplementation. Our aim in this chapter is to review recent published clinical trials dealing with LCPUFA supplementation of infant formulas and to compare these results with those of two clinical trials we performed in European study centers. We tested whether LCPUFA supplementation close to the lower levels found in breast milk might achieve red blood cell (RBC) phospholipid fatty acid concentrations close to those found in breast-fed infants. The selection of RBC phospholipids as the main markers is based on the fact that in nutritional deficiency, these are correlated with tissue composition, especially in neural tissues such as the retina or the cerebral cortex. An additional aim of the study was to compare the results of two LCPUFA-supplemented study formulas that differed in the LCPUFAs source used for supplementation.

SYNTHESIS AND METABOLISM OF LONG-CHAIN POLYUNSATURATED FATTY ACIDS

The nutritional importance of specific lipids was first revealed through the work of Burr and Burr in 1929 (1). These investigators introduced the concept that specific components of fat may be indispensable for proper growth and development of
animals and humans. Two fatty acids, linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3), were described as essential nutritional factors.

According to the number of carbon atoms between the terminal double bond and the n (ω) carbon (located at the methyl end of the molecule), one can distinguish n-9, n-6, and n-3 fatty acids (Fig. 1). Mammals can introduce additional double bonds in the n-9 position; hence n-9 fatty acids can be synthesized from saturated fats. In contrast, humans can neither insert double bonds in the n-6 or n-3 positions, nor transform n-6 to n-3 or vice versa. These two independent families of fatty acids, the n-6 and n-3 fatty acids, are known today to be essential for physiologic human cell function. Fatty acids containing 20 or more carbons and with two or more double bonds are synthesized from essential 18-carbon fatty acid by sequential alternating desaturation and elongation; they are named LCPUFAs and are synthesized from the major dietary essential fatty acids, linoleic acid and α-linolenic acid. These bind to a microsomal enzyme system for further desaturation and elongation to C20 and C22 LCPUFAs, such as arachidonic acid (AA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3; Fig. 1) (2). The conversion of dietary linoleic acid and α-linolenic acid is under active regulation. The controlling step of the pathway is the competition of the respective substrates (α-linolenic acid for the n-3 fatty acid and linoleic acid for the n-6) for δ-6-desaturase, which is necessary for the synthesis of both n-3 and n-6 LCPUFAs. The affinity for substrate increases with the number of double bonds; thus α-linolenic acid (C18:3n-3) is the preferred substrate. If there is a lack of n-3 fatty acids in the diet, desaturation of the n-6 compounds causes a significant increase in docosapentaenoic acid (C22:5n-6); however, if both n-3 and n-6 fatty acids are deficient, n-9 fatty acids, such as eicosatrienoic acid (C20:3n-9), accumulate (Fig. 1) (3). This can lead to an inhibition of the elongation of the dietary essential fatty acid and thus reduce the necessary LCPUFA supply.

In contrast, an excess of dietary linoleic acid associated with the intake of some vegetable and corn oils may decrease the formation of DHA from α-linolenic acid because the δ-6-desaturase is inhibited by excess substrate. This can lead to an inhibition of the elongation of the dietary essential fatty acid and thus reduce the necessary LCPUFA supply. In addition, AA neosynthesis is reduced when excess linoleic acid or α-linolenic acid is provided (3–6). In this instance, the conversion of C18:2n-6 and C18:3n-3 to AA and DHA is no longer sufficient; thus not only linoleic acid and α-linolenic acid but also AA and DHA can be considered essential nutrients under such circumstances (7).

**FIG. 1.** Essential fatty acid and long-chain polyunsaturated fatty acid (LCPUFA) metabolism. Desaturation and elongation of dietary omega-3 (n-3), n-6, and n-9 fatty acids; n-9 fatty acids derive from de novo synthesis or from dietary sources; parent n-3 (C18:3n-3) and n-6 (C18:2n-6) fatty acids cannot be synthesized and depend on dietary intake. δ-Desaturases introduce double bonds at 9, 6, and 5 carbons from the carboxylic end of the fatty acid chain, and elongation occurs two carbons at a time.
n-3  n-6  n-9

C18:3n-3  C18:2n-6  C18:1n-9
(α-linolenic acid, ALA) (linoleic acid, LA) (oleic acid)

↓  ↓  ↓

δ-6 - Desaturase

↓  ↓  ↓

C18:4n-3  C18:3n-6  C18:2n-9
(γ-linolenic acid, GLA)

↓  ↓  ↓

Elongation

↓  ↓  ↓

C20:4n-3  C20:3n-6  C20:2n-9
(dihomo-γ-linolenic acid)

↓  ↓  ↓

δ-5 - Desaturase

↓  ↓  ↓

C20:5n-3  C20:4n-6  C20:3n-9
(eicosapentaenoic acid, EPA) (arachidonic acid, AA) (eicosatrienoic acid, ETA)

↓  ↓  ↓

Elongation

↓  ↓  ↓

C22:5n-3  C22:4n-6  C22:3n-9

↓  ↓  ↓

δ-4 - Desaturase

↓  ↓  ↓

C22:6n-3  C22:5n-6  C22:4n-9
(docosahexaenoic acid, docosahexaenoic acid) (docosapentaenoic acid, DPA)
FUNCTIONS OF LONG-CHAIN POLYUNSATURATED FATTY ACIDS

Essential LCPUFAs have an important structural function as components of phospholipids in biologic membranes. These participate in the regulation of membrane-associated cell functions, such as enzyme activity, cell fusion, endocytosis, permeability, and transmembrane transport (2,8-11). Furthermore, they serve as precursors for the synthesis of prostaglandins, thromboxanes, and leukotrienes.

The n-3 and n-6 Fatty Acids

The n-3 and n-6 fatty acids play an important role during fetal and postnatal development of the retina and brain. The retina has a high content of DHA (35-60% of total fatty acid) (12) in the outer segment of the photoreceptor cells, which effect the transduction of light absorption into electrical signals. Accumulation of DHA in the retina is associated with enhanced retinal response to light (13) and visual acuity (14). Recently published data indicate that preterm infants who are either breast-fed or fed a LCPUFA-supplemented formula might have better visual development than infants fed NS formulas (15).

In term infants, supplementation of a standard term infant formula with DHA or with DHA and AA during the first months of life has led to significantly improved visual acuity compared with that in infants fed NS formulas (16-18). However, in one trial, the higher-rated visual acuity of infants fed supplemented formulas was only transient, and the red cell phospholipid DHA concentrations of supplemented infants did not differ from those of NS infants at the end of the trial (19). The interpretation of the visual studies is still debated (20) because there also are published reports that did not show any effect of LCPUFA supplementation on visual development (21). Another factor that might explain the discrepancies among the results from these studies is the different methods used to measure visual acuity.

Whereas the effects of LCPUFA supplementation on visual development are still controversial, there is consensus about the importance of n-3 and n-6 LCPUFAs for brain development. Fatty acid accretion in the fetal brain has been studied in vitro by analyzing brain tissue from dead infants of different gestational ages. In two studies (22,23) in which the n-6 and n-3 LCPUFA accretion in the brain was measured, n-6 LCPUFA incorporation into neuronal tissue was found to be 2 to 3 times higher than n-3 incorporation. Several clinical studies on preterm and term infants have shown better performance in neurodevelopmental tests when they were fed breast milk or n-3 and n-6 LCPUFA-enriched formulas than when they were fed conventional NS formulas (24-28). Agostoni et al. (29) reported a scoring advantage in development tests for term 4-month-old infants fed an AA- and DHA-supplemented formula compared with infants fed a normal, NS formula. Willats et al. (28) found similar results when administering a problem-solving test to 10-month-old infants. Although there is evidence for a neurodevelopmental advantage of breast-fed infants or infants fed n-3 and n-6 LCPUFA–supplemented formulas in the first months of life, there is still a need to evaluate diet-induced changes in relation to long-term developmental outcome.
FATTY ACID PROFILES OF INFANTS WITH LCPUFAS

CLINICAL TRIALS AND LONG-CHAIN POLYUNSATURATED FATTY ACIDS SUPPLEMENTATION

Long-Chain Polyunsaturated Fatty Acids Concentrations Used in Clinical Trials

During intrauterine development, the fetus does not seem to depend on active endogenous LCPUFA synthesis. Comparisons of maternal and cord blood fatty acid composition at birth have led to the conclusion that LCPUFAs are transferred across the placenta (30). After birth, breast-fed infants have higher plasma concentrations of LCPUFAs than do formula-fed infants (31,32) because, in contrast to conventional formulas, breast milk contains a full complement of n-6 and n-3 fatty acids, including DHA and AA, which formula-fed infants must synthesize from precursors. Human breast milk usually contains ~ 7–18% linoleic acid, 0.4–1.5% α-linolenic acid, and variable amounts of n-6 and n-3 LCPUFAs. Infant formulas provide similar or higher levels of linoleic acid and α-linolenic acid, but only small amounts of or no LCPUFAs. Recent studies have shown that even formulas supplemented with n-3 and n-6 recommended for preterm and term infants may not have optimal amounts of n-6 and n-3 fatty acids for the period of rapid development of the central nervous system (33–36).

Human milk does not have a uniform lipid composition, because the type of fatty acid composition depends on the mother’s diet during pregnancy and lactation. Thus the question of how much LCPUFAs to give in feeds cannot be answered by taking “the human milk” as a reference. Usually the concentration of AA, the main n-6 LCPUFA in human milk, is 1.5 to 2 times higher than that of DHA, the most important of the n-3 series. The ratio of total n-6 to total n-3 ranges from 5:1 up to 15:1 if oils high in linoleic acid are consumed by the mother. In healthy Australian women (n = 23) Makrides et al. (37) found breast-milk linoleic acid concentrations of 13.6% of total (wt/wt) at week 6 of lactation, which remained stable up to week 30. α-Linolenic acid concentrations (0.89% of total, wt/wt) also remained stable during this lactation period, with a small peak at week 16 (0.94%), whereas AA and DHA concentrations both showed a tendency to decline (AA, 0.45–0.39%; DHA, 0.26–0.19%). Fatty acid concentrations in breast milk in 15 German breast-feeding women between weeks 12 and 16 of lactation showed slightly lower concentrations of α-linolenic acid (0.81%; wt/wt), AA (0.36%), and DHA (0.22%), and a definitely lower linoleic acid concentration (10.7%; wt/wt), compared with those of the Australian group (38). As the same methods were used in both trials, the differences probably reflect cultural factors in food preferences in the two populations.

In previous clinical trials, the supplemented fatty acid and LCPUFA concentrations have shown major differences depending on the source of LCPUFAs used (linoleic acid, 11–33% of total fatty acids; α-linolenic acid, 0.3–4.9%; AA, 0.01–0.9%; DHA, 0.1–0.5%) (7). In 1991, the Committee on Nutrition of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) recommended linoleic acid concentrations of 4.5–10.8% of total energy and a linoleic acid to α-linolenic acid ratio of 5:1 to 15:1 in formulas given to
term and low-birthweight infants. In accordance with the Scientific Committee for Food of the European Union, ESPGHAN decided to set an upper limit for linoleic acid supplementation (10% of energy), but no recommendation was made for \( \alpha \)-linolenic acid supplementation (39). The committee thought that LCPUFA supplementation of infant formulas was likely to be advantageous, but before making any definite recommendations, they requested more data on clinical trials with LCPUFA-enriched formulas for term infants.

**Long-Chain Polyunsaturated Fatty Acids Sources Used for Supplementation**

Vegetable oils from maize, safflower, and sunflower contain linoleic acid predominantly and only a small amount of or even no \( \alpha \)-linolenic acid, whereas oils derived from soybean and low-erusic rapeseed oils contain ample \( \alpha \)-linolenic acid. An important LCPUFA source is fish, which incorporate n-3 fatty acids from marine zooplankton and phytoplankton (40,41) and further elongate them to eicosapentaenoic acid (EPA; C20:5n-3) and DHA (Fig. 1). The higher the fat content of the fish, the higher its content of n-3 fatty acid, because fish concentrate EPA and DHA as triglycerides, mainly in the adipose tissue (42,43). Egg phospholipids are another important source of LCPUFAs for supplementing infant formulas. More recently, single-cell oils with a high concentration of DHA or AA also have been produced by biofermentation and extraction. They offer a promising new source of LCPUFAs. The industrial production of AA, EPA, and DHA from fungal or bacterial strains has been successful, and the use of these fatty acids will become more common depending on their purity, bioavailability, toxicologic testing, and cost. Therefore, regulatory hurdles to add LCPUFAs to formulas for term infants still exist in some countries.

**Analytic Methods**

As well as the concentrations and sources of the LCPUFAs used for supplementation of infant formulas, the methods for quantification of the LCPUFAs play an important role in assessing the published data. Dietary fatty acid composition affects the fatty acid concentrations in the plasma, but usually these reflect only the recent dietary intake and may provide information about the bioavailability of the supplemented fatty acid. More-detailed information about long-term effects of LCPUFA supplementation can be achieved by analyzing the LCPUFA concentrations of the RBC membranes. The LCPUFA content of the phospholipid fraction of the RBC membranes can readily be measured and has been found to correlate best with tissue fatty acid composition.

Expression of LCPUFA concentrations in RBCs in quantitative terms would facilitate comparison of the results of the different trials; however, there is no consensus about what to relate the concentrations to (sample volume, number of cells, hemoglobin, and so on). Thus most of the results are expressed as a percentage of the total fatty acid concentrations found in the sample. However, the variable number of identified fatty acids used as “total fatty acids” and the differences in calculations based on weight (wt/wt%) or concentration (mol%) renders the comparison of results of different trials difficult.
RED BLOOD CELL FATTY ACID PROFILES OF INFANTS FED LONG-CHAIN POLYUNSATURATED FATTY ACIDS–SUPPLEMENTED FORMULAS

During two prospective clinical trials with whey-adapted starter formulas ("NAN" trial), and partially hydrolyzed starter formulas ("hypoallergenic formulas"; BEBA HA Start trial) carried out in European study centers (Italy and Germany), we compared RBC fatty acids of healthy infants who were fed breast milk or formulas with and without LCPUFA supplementation. The concentrations of the supplemented LCPUFAs (AA and DHA) in the test formulas of both studies were comparable (AA, 0.21–0.24% of total fatty acids; DHA, 0.13–0.14% of total fatty acids; Table 1) and

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Name</th>
<th>Formula 1 (NAN; NS)</th>
<th>Formula 2 (N1.8; NS)</th>
<th>Formula 3 (N1.8/LCPUFA) + BEBA HA Start</th>
<th>LCPUFA</th>
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<tr>
<td>Saturated</td>
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<tr>
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<td>0.11</td>
<td>0.10</td>
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</tr>
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<td>LCPUFA</td>
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<tr>
<td>C18:2n-6</td>
<td>Linoleic acid (LA)</td>
<td>14.96</td>
<td>15.7</td>
<td>15.05</td>
<td>15.69</td>
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<td>C18:3n-3</td>
<td>α-Linolenic acid (ALA)</td>
<td>1.78</td>
<td>1.9</td>
<td>1.69</td>
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<tr>
<td>C18:3n-6</td>
<td>γ-Linolenic acid (GLA)</td>
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<tr>
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<td>Eicosadienoic acid</td>
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<td>Arachidonic acid (AA)</td>
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<td>0.24</td>
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<td>C20:5n-3</td>
<td>Eicosapentaenoic acid (EPA)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C22:6n-3</td>
<td>Docosahexaenoic acid (DHA)</td>
<td>0.13</td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
</tbody>
</table>

Values expressed as percentage of total fatty acids (wt/wt).

*a Formula 1 and formula 2 of the NAN trial are combined as the non–LCPUFA-supplemented group (NS). Formula 3 of the NAN trial corresponds to the NAN LCPUFA supplemented group (N1.8/LCPUFA) and BEBA HA Start, to the LCPUFA-supplemented formula of the BEBA HA Start trial.
corresponded to the lower level of LCPUFA concentrations found in breast milk. LCPUFAs derived from fish oil/single-cell oil were added to the Italian study formulas, and those from egg phospholipids, to the German study formulas. In most of the recent published trials, higher LCPUFA concentrations were used for formula supplementation (Table 1).

Study Protocols and Methods

NAN Trial

In this trial healthy newborn infants were either breast-fed or fed one of three formulas from day 5 of life onward. In addition to anthropometric data, dietary records were obtained at 30 and 122 days of life. At these visits, venous blood was obtained (if parental consent was available) in heparinized tubes and centrifuged. The plasma was immediately frozen and kept frozen for further analytic procedures. RBCs were washed 3 times with a 0.9% sodium chloride solution, with sodium-ethylenediaminetetraacetic acid (EDTA) as antioxidant, and then rapidly frozen at −80°C. The time of the last feed was recorded.

After thawing, the RBC lipids were extracted (44), and the phospholipid fraction separated by using solid-phase aminopropyl columns (45). The extracts were methylated with boron trifluoride methanol for 1 h at 100°C, and 1 µl of the supernatant was injected into the column by using a Hewlett Packard 5890 gas chromatograph equipped with a precolumn (2.2 m) and a DB23 capillary column (60 m). The concentrations of the injected sample (in µmol/l) were calculated in relation to an internal standard (1,2-dinonadecanoyl-sn-glycero-3-phosphatidylcholine) added before the initial lipid extraction, and expressed as percentage of total fatty acids (C12:0 to C24:1). The analytic interseries (day-to-day) precision was determined at physiologic LCPUFA concentrations with an RBC pool. The coefficient of variation (n = 11) was linoleic acid, 5.0%; α-linolenic acid, 17.7%; AA, 5.2%; and DHA, 6.3%.

The three isocaloric formulas fed to the infants differed in protein and fatty acid composition (Tables 1 and 2). Formula 1 was a whey-adapted conventional starter

<table>
<thead>
<tr>
<th>Casein/whey ratio</th>
<th>Formula 1 (NAN; NS)</th>
<th>Formula 2 (N1.8; NS)</th>
<th>Formula 3 (N 1.8/LCPUFA)</th>
<th>BEBA HA Start + LCPUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g/100 kcal)</td>
<td>2.24</td>
<td>1.83</td>
<td>1.83</td>
<td>2.25</td>
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<tr>
<td>Carbohydrates (g/100 kcal)</td>
<td>10.8</td>
<td>11.2</td>
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<tr>
<td>Fat (g/100 kcal)</td>
<td>5.3</td>
<td>5.3</td>
<td>5.3</td>
<td>5.1</td>
</tr>
</tbody>
</table>

a See Table 1.
formula (NAN); formula 2 was a whey-adapted starter formula containing acid whey, with a protein content of 1.83 g/100 kcal and a casein/whey ratio of 30:70 (N1.8); and formula 3 (N1.8/LCPUFA) had the same protein content as formula 2, but was prepared with modified sweet whey (casein/whey ratio, 30:70). LCPUFA composition (Table 1) of the three formulas differed markedly, because formula N1.8/LCPUFA was enriched with AA and DHA. The AA source used for supplementation was a single-cell oil (ROPUFA; Hoffmann La Roche, Switzerland), and low-EPA fish oil was the source for the supplemented DHA (Hoffmann La Roche). As formulas 1 and 2 did not differ in fatty acid and LCPUFA composition, the two groups were combined for statistical analysis (nonsupplemented group, NS). A breast-fed group served as the control group (Table 2).

**BEBA HA Start Trial**

In this study healthy newborn infants were either breast-fed or fed a commercially available partially hydrolyzed infant formula (BEBA HA START) during their first 4 months of life. The formula composition was comparable to that of formula 1 in the NAN trial (Table 2), except for the protein and LCPUFA sources. The total amount of supplemented DHA and AA in BEBA HA Start was similar to that of NAN (N1.8/LCPUFA, Table 1). Egg phospholipids were used as the LCPUFA source for BEBA HA Start. Blood-sampling procedures and methods of LCPUFA analysis were the same as those in the NAN trial.

The breast-fed groups from both studies were combined because differences in RBC phospholipid fatty acid concentrations of the infants in Italy and Germany at ages 30 and 122 days were small and statistically not significant: total saturated fatty acids (C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0), total monounsaturated fatty acids (C14:ln-5, C16:ln-7t, C16:ln-7c, C18:ln-9t, C18:ln-9c, C20:ln-9, C22:ln-9, C24:ln9; t, trans; c, cis stereoisomer), AA, DHA, total n-6, and total n-3 fatty acid concentrations (Kruskal–Wallis test). The breast-fed group served as the reference group. The numbers of infants at ages 30 and 122 days from whom blood could be collected is indicated in Table 3.

The data were checked for normal distribution but showed a large deviation; therefore we present the results as quartiles and not as mean and standard deviations. Statistical comparisons between groups were made by using a nonparametric test (the Mann–Whitney test).

| TABLE 3. Numbers of infants in each age group in which blood could be obtained* |  |
|---|---|---|---|---|
| Age | NS | N1.8/LCPUFA | BEBA HA Start | Breast-fed |
| 30 ± 2 d | 33 | 16 | 6 | 36 |
| 122 ± 4 d | 23 | 14 | 16 | 30 |

*See Table 1.
Comparison of Fatty Acid and Long-chain Polyunsaturated Fatty Acids Concentrations in Red Cell Phospholipids of Infants Fed NAN, with or without Long-chain Polyunsaturated Fatty Acids Supplementation, and Breast Milk

There were no differences in saturated fatty acids (C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0) between the three groups at ages 30 and 120 days. In contrast, the monounsaturated fatty acids (C14:1n-5, C16:1n-7t, C16:1n-7c, C18:1n-9t, C18:1n-9c, C20:1n-9, C22:1n-9, C24:1n-9) were lower in the breast-fed group than in either of the formula groups at age 30 days \((p < 0.005)\) and remained lower at age 120 days \((p = 0.026)\) compared with those of the NS group.

**n-6 Fatty Acids**

The total n-6 fatty acid concentrations (C18:2n-6tt, C18:2n-6c, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:2n-6, C22:4n-6; tt, trans-trans stereoisomer) did not differ at age 30 days between the groups, whereas at the end of the study, the breast-fed infants had significantly lower total n-6 concentrations in RBC phospholipids than did infants fed the NS or LCPUFA-supplemented formulas \((p < 0.05; \text{Fig. 2})\). At age 30

FIG. 2. Total omega-6 (n-6) fatty acid concentrations of red blood cell phospholipids [percentage of total fatty acids (FAs)] of infants at age 120 days. Box plots of total n-6 FA concentrations (C18:2n-6tt, C18:2n-6c, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:2n-6, C22:4n-6; tt, trans-trans; c, cis stereoisomer; results in mol% of total FAs) of breast-fed infants (BF) and infants fed non-long-chain polyunsaturated fatty acid (LCPUFA)-supplemented formulas (NS), or two different LCPUFA-supplemented formulas (N1.8/LCPUFA is enriched by 0.21% of total fatty acids with single-cell oil arachidonic acid and by 0.13% of total with fish oil docosahexaenoic acid (DHA); BEBAHA Start is enriched by 0.24% of total FAs with AA and by 0.14% of total with DHA, both from egg phospholipids). Boxes, lower and upper quartiles; crosses, medians; notches, errors of means; whiskers, nonoutlier minimal and maximal values; dots, extreme values (>1.5 x interquartile range).
FATTY ACID PROFILES OF INFANTS WITH LCPUFAS

221

FIG. 3. Arachidonic acid (AA) concentrations of red blood cell phospholipids (percentage of total fatty acids) in infants at age 120 days. Box plots of AA concentrations (C20:4n-6) of breast-fed infants (BF) and infants fed non-long-chain polyunsaturated fatty acid (LCPUFA)-supplemented formulas (NS), or two different LCPUFA-supplemented formulas (N1.8/LCPUFA is enriched by 0.21% of total fatty acids with a single-cell oil AA and by 0.13% of total with fish oil docosahexaenoic acid; BEBA HA Start is enriched by 0.24% of total fatty acids with AA and by 0.14% of total with docosahexaenoic acid, both from egg phospholipids). Boxes, lower and upper quartiles; crosses, medians; notches, errors of means; whiskers, nonoutlier minimal and maximal values; dots, extreme values (>1.5 \times interquartile range).

days, AA concentrations were significantly higher in the breast-fed group than in either formula group (p < 0.005) and were even higher at age 120 days compared with those of the NS group (p = 0.032). In contrast, AA concentrations in the LCPUFA-supplemented group (N1.8/LCPUFA) were no longer significantly lower than in the breast-fed group at age 120 days (Fig. 3).

n-3 Fatty Acids

Total n-3 fatty acids (C18:3n-3, C18:4n-3, C20:3n-3, C20:5n-3, C22:3n-3, C22:5n-3, C22:6n-3) were present in slightly higher concentrations in the breast-fed group at age 30 days (p < 0.05) than in the formula groups. At age 120 days, supplementation of formula with DHA led to a clear increase in total n-3 fatty acid concentrations in the LCPUFA-supplemented group, and differences between the breast-fed group and the LCPUFA-supplemented formula group were small (N1.8/LCPUFA) for DHA and total n-3 fatty acid concentrations (Figs. 4 and 5). In contrast, infants fed the NS formulas still showed significantly lower total n-3 fatty acid and DHA concentrations compared with the breast-fed group and the LCPUFA-supplemented group (Figs. 4 and 5).
FIG. 4. Total n-3 fatty acid (FA) concentrations of red blood cell phospholipids (percentage of total fatty acids) in infants at age 120 days. Box plots of total n-3 FA concentrations (C18:3n-3, C18:4n-3, C20:3n-3, C20:5n-3, C22:3n-3, C22:5n-3, C22:6n-3; results in mol% of total fatty acids) in breast-fed infants (BF) and infants fed non-long-chain polyunsaturated fatty acid (LCP-UFA)-supplemented formulas (NS), or two different LCP-UFA-supplemented formulas (N1.8/LCPUFA is enriched by 0.21% of total fatty acids with a single-cell oil arachidonic acid (AA) and by 0.13% of total FAs with DHA from fish oil; BEBA HA Start is enriched by 0.24% of total FAs with AA and by 0.14% of total with DHA, both from egg phospholipids). Boxes, lower and upper quartiles; crosses, medians; notches, errors of means; whiskers, nonoutlier minimal and maximal values; dots, extreme values (>1.5 x interquartile range).

FIG. 5. Docosahexaenoic acid (DHA) concentrations of red blood cell phospholipids (percentage of total fatty acids) in infants at age 120 days. Box plots of DHA concentrations (C22:6n-3) in breast-fed infants (BF) and infants fed non-long-chain polyunsaturated fatty acid (LCP-UFA)-supplemented formulas (NS), or two different LCP-UFA-supplemented formulas (N1.8/LCPUFA is enriched by 0.21% of total fatty acids with arachidonic acid (AA) from single-cell oil and by 0.13% of total fatty acids with DHA from fish oil; BEBA HA Start is enriched by 0.24% of total fatty acids with AA and by 0.14% of total with DHA, both from egg phospholipids). Boxes, lower and upper quartiles; crosses, medians; notches, errors of means; whiskers, nonoutlier minimal and maximal values; dots, extreme values (>1.5 x interquartile range).
Is There Any Influence of the Different Long-chain Polyunsaturated Fatty Acid Sources Used for Supplementation of Infant Formulas?

To determine whether the source of the supplemented LCPUFA plays a role, we compared the results of the RBC phospholipid LCPUFA concentrations in the NAN trial with those in the BEBA HA Start trial.

RBC phospholipid LCPUFAs in the infants fed the BEBA HA Start formula showed the same pattern of saturated and monounsaturated fatty acids at ages 30 and 120 days as did the supplemented NAN formula group (N1.8/LCPUFA). No difference was found for total n-6 between any of the formula groups and the breast-fed group at age 1 month. In contrast to the NS and supplemented formula group in the NAN trial, total n-6 concentrations of RBC phospholipids in the BEBA HA Start group did not differ from those found in breast-fed infants at age 120 days (Fig. 2). AA concentrations were higher in the BEBA HA group than those in the NS NAN group at age 30 days, but were comparable to the NAN-supplemented formula group and the breast-fed group at the end of the trial (Fig. 3). Total n-3 fatty acid and DHA levels in RBC phospholipids in the NAN-supplemented and the BEBA HA groups showed the same results at ages 30 and 120 days (Figs. 4 and 5). These results indicate that the different sources used for AA supplementation—a single-cell oil for the NAN formula and egg phospholipids for the BEBA HA Start formula—appear not to influence the AA concentrations in RBC phospholipids after 4 months of feeding the formulas. In addition, AA concentrations of the two supplemented formula groups were comparable to those found in the breast-fed group at age 120 days. However, the much higher variance of the AA results in the BEBA HA Start group (Fig. 3), the cause of which remains unknown, leads to loss of discriminatory power. Our results furthermore showed no significant influence in the different DHA sources used for supplementation in the NAN and BEBA HA Start trials.

CONCLUSIONS

Feeding infants with formulas of differing specific fatty acid compositions usually results in measurable differences in plasma lipid composition. The selection of RBC phospholipids as the main markers is based on the fact that in nutritional deficiency, these are correlated with tissue composition, especially in neural tissues such as the retina or the cerebral cortex. The fatty acid composition of human milk differs from that of the NS infant formulas, particularly in their LCPUFA concentrations. Recent studies have shown that infants fed DHA- and AA-supplemented formulas have LCPUFA concentrations in the RBC phospholipids that are comparable to those of breast-fed infants throughout the first year of life (16,21,24,46).

In the studies presented here, we supplemented formulas with DHA and AA to achieve concentrations close to the lower levels found in human milk and lower than those in most former clinical trials. Our results from two clinical trials (NAN and BEBA HA) confirm that these low concentrations of DHA and AA in formulas given during the first 4 months result in RBC phospholipid AA and DHA concentrations similar to those in breast-fed infants. The AA concentrations showed substantial
variance in the BEBA HA formula group and so did not differ statistically from the NS group at age 4 months; however, there was a clearly significant benefit of supplementing infant formulas with DHA. These low-level supplements appear to provide sufficient quantities of both LCPUFAs at age 4 months.

The recent development of single-cell oils enabled us to use a single-cell AA for formula enrichment (N1.8/LCPUFA) and to compare it with a formula supplemented with AA from egg phospholipid (BEBA HA Start). We found no significant differences between the two supplemented formulas at ages 30 and 120 days, but the small number of infants at age 30 days (n = 6) in the BEBA HA Start group and the higher variance of the AA results in this group suggest that more infants should be studied before jumping to conclusions. Comparison of the different sources of DHA supplementation did not reveal any differences; both originated from natural sources (fish oil or egg phospholipids).

If we assume that the breast-milk group used as reference provides an optimal supply of LCPUFAs, our data indicate that formulas can be supplemented with lower concentrations of LCPUFAs than have been used so far.

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