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The Use of Low-EPA Fish Oil for Long-Chain Polyunsaturated Fatty Acid Supplementation of Preterm Infants

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ABSTRACT

Because docosahexaenoic acid (DHA) may be an essential nutrient for the visual and early cognitive development of preterm infants, DHA enrichment of preterm formulas has been recommended. This randomized trial was designed to study the n-6 and n-3 fatty acid status of healthy preterm infants fed a formula enriched with a low eicosapentaenoic-fish oil until 4 mo corrected age compared with that of infants fed a standard formula. A reference group of breast-fed infants was studied concurrently. The fatty acid content of red blood cell (RBC) phospholipid was assessed at enrollment, hospital discharge, expected term, and 3 and 6 mo postterm. The DHA content of RBC phospholipid was higher in infants fed the enriched *versus* the standard formula at hospital discharge, expected term, and 3 and 6 mo postterm. However, compared with infants fed the standard formula, infants fed the enriched formula had also higher RBC phospholipid eicosapentaenoic content (0.69 ± 0.15% *versus* 0.25 ± 0.12%, $p < 0.001$), and lower RBC phospholipid arachidonic acid content (15.1 ± 0.93% *versus* 18.8 ± 0.89%; $p < 0.001$). We conclude that supplementing preterm infants with low-eicosapentaenoic fish oil is effective in improving DHA status, but results in worsening of n-6 fatty acid status. We speculate that preterm infants may require a dietary supply of arachidonic acid as well as DHA if the same fatty acid status as that of breast-fed infants is to be achieved. **(*Pediatr Res* 48: 835–841, 2000)**

Abbreviations

AA, arachidonic acid or 20:4n−6
DHA, docosahexaenoic acid or 22:6n−3
EPA, eicosapentaenoic acid or 20:5n−3
LA, linoleic acid or 18:2n−6
LNA, linolenic acid or 18:3n−3
LC-PUFA, long-chain polyunsaturated fatty acid, 20-22 carbon chain length
RBC, red blood cells

The availability of LC-PUFA, such as AA and DHA, is important for early human growth and development (1). Recent studies using stable isotopes have demonstrated that humans, including preterm infants, are capable of synthesizing AA and DHA from linoleic and linolenic acids, respectively (2, 3). However, endogenous synthesis of n-3 fatty acids apparently does not meet the needs of preterm infants for these fatty acids, as suggested by the fatty acid composition of plasma and RBC lipids (4–6). Because preterm infants fed unsupplemented formulas resulting in lower levels of n-3 LC-PUFA in plasma and tissue lipids may be at risk for impaired neural or visual system development (7–12), LC-PUFA enrichment of preterm formulas has been recommended (13–16).

An easy way to enrich formula with n-3 LC-PUFA is to add fish oil, which contains n-3 LC-PUFA. In earlier trials, preterm infants were fed formulas supplemented with fish oils containing DHA (0.2–0.35%) and high levels of EPA (0.30–0.65%) from approximately 3 wk of age until 2 mo (4) or 9 mo past expected term (5). The DHA status of supplemented infants was greatly improved, accompanied by better visual acuity at 4 mo postterm (7, 11, 12, 17). However, in one trial, the supplemented group exhibited poor overall growth throughout the first year of life (18), which was significantly correlated with lower plasma phosphatidylcholine (PC) AA; this was thought to result from the high EPA content of the supplemented formula (19).

A more recent study evaluated the use of low-EPA fish oil as a supplement for preterm formula (9). Infants were fed an enriched formula (0.2% DHA; 0.06% EPA) until 2 mo past expected term. By this time, infants fed the enriched formula

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Table 1. *Fatty acid profiles (percentage of total fatty acids by weight) of the standard and LC-PUFA enriched formulas fed in the study.* Fatty acid profile of breast milk is also reported†*

		Standard formula		Enriched formulas	
Fatty acids	Human milk	PTF	TF	PTF	TF
C6:0	0.17 ± 0.03	0.35	0.41	0.34	0.41
C8:0	0.36 ± 0.10	14.32	2.69	16.27	2.97
C10:0	2.12 ± 0.57	8.13	1.87	8.74	2.09
C12:0	8.13 ± 2.48	4.43	13.52	4.02	15.28
C14:0	9.36 ± 2.26	2.13	5.33	2.08	6.20
C16:0	23.35 ± 1.99	15.23	18.99	14.81	17.86
C18:0	7.29 ± 0.75	3.32	4.31	2.88	3.54
C20:0	0.21 ± 0.03	0.22	0.26	0.14	0.25
C18:1 n-9/n-7	31.69 ± 3.83	31.69	32.17	30.64	31.66
C18:2 n-6	10.98 ± 3.71	17.95	18.23	17.78	17.17
C20:4 n-6	0.56 ± 0.08	—	—	0.02	0.03
C18:3 n-3	0.74 ± 0.15	1.60	1.57	1.10	1.07
C20:5 n-3	0.24 ± 0.21	—	—	0.05	0.09
C22:6 n-3	0.42 ± 0.11	—	—	0.37	0.45
Total n-6 FA %	12.56 ± 4.17	17.95	18.23	17.88	17.31
Total n-3 FA %	1.49 ± 0.16	1.60	1.57	1.54	1.64
FA (mg/100 mL)	3.10 ± 0.3	3.74	3.49	3.60	3.51

* Preterm standard and enriched formulas (PTF) were fed from inclusion until expected term; thereafter, term standard and enriched formulas (TF) were fed until 4 mo postterm.

† Mean +/− SD values from samples collected from 5 of the 10 breast-fed infants.

had the same RBC phospholipid content of DHA as at birth but it decreased as soon as the supplemented formula was discontinued. No significant variation of RBC phospholipid AA content was observed in infants fed the enriched formula. This study suggests that DHA supplementation for more than 2 mo postterm is necessary for optimal accumulation of DHA, and also that supplementation with low-EPA fish oil may prevent the adverse effects of fish oil on n-6 LC-PUFA status and growth.

To evaluate this possibility, we have undertaken a randomized study to assess the effect of feeding a formula enriched with a high DHA low-EPA fish oil until 4 mo postterm on the LC-PUFA status of preterm infants. A nonrandomized group of breast-fed infants was also studied concurrently as a reference group.

METHODS

Subjects. This longitudinal, prospective randomized study of healthy preterm infants was conducted at the Department of Neonatology of the Edouard Herriot Hospital, Lyon, France. The preterm infants enrolled were appropriate for gestational age according to the curves described by Usher and McLean (20). Birth weight was between 700 and 1500 g. Gestational age was determined from the date of the mother's last menstrual period and was confirmed by early ultrasound and clinical evaluation at birth (21). Exclusion criteria included major neonatal morbidity (*e.g.* congenital anomalies, respiratory treatment for more than 10 d, congenital infection, necrotizing enterocolitis, and bowel resection), a postnatal age of more than 21 d, requirement for supplemental oxygen or treatments (*e.g.* diuretics and corticosteroids) that could influence growth and development, a failure to achieve full enteral feeding of 150 mL/kg/d by a postnatal age of 21 d, and a maternal history of cocaine/alcohol abuse, diabetes, hyperlipidemia, or abnormal dietary patterns (strict vegetarian diets).

A research nurse monitored the infants enrolled during hospitalization. Infants were weighed daily using a calibrated infant balance. Length was determined weekly to the nearest 0.1 cm using an infant measuring board. Head circumference also was measured weekly at the largest occipitofrontal circumference with a nonstretchable paper tape. Volume of intake and feeding tolerance were recorded daily. The attending neonatologist and resident physicians who were familiar with the study provided clinical care. After discharge, type of milk and volume of formula intake were recorded daily by the parents. Intake of formula was determined by the difference in prefeeding and postfeeding weights of each feeding unit offered. Weight, length, and head circumference were measured by the research nurse at each visit (*i.e.* expected term, 3 and 6 mo postterm). No infants were excluded from the study, either during hospitalization or during the outpatient follow-up phase of the study.

Diets. Enteral feeding of all infants was started during the first week of life with pooled, pasteurized breast milk (Lyon Lactarium, France) as usual in the unit. For the formula-fed infants, permission for study entry was sought only if the mothers had previously decided not to breast-feed their infants. Twenty-three infants were randomly assigned to receive either a control formula ($n = 12$) or a formula with LC-PUFA ($n = 11$). Formula feeding began during the first 3 wk of life. After enrollment, all infants were fed exclusively with their assigned formula. Preterm formulas were fed from enrollment through expected term, at which time term formulas with or without LC-PUFA, according to assignment, were fed. These were fed through 4 mo postterm, when all infants were switched to a standard formula without LC-PUFA.

Table 2. *Clinical data of the preterm infants according to their diet;* they received their own mother's milk (human milk) or were randomly assigned to receive a standard formula without LC-PUFA or an enriched formula with LC-PUFA.†*

	n	Postnatal age (d)	Gestational age (wk)	Weight (g)	Length (cm)	HC (cm)
Birth						
Human milk	10	—	28.8 ± 1.9	1325 ± 138	40.4 ± 2.0	26.9 ± 1.3
Standard formula	12	—	29.7 ± 1.4	1235 ± 161	39.3 ± 2.2	27.5 ± 1.1
Enriched formula	11	—	29.4 ± 1.4	1275 ± 172	39.6 ± 2.4	27.3 ± 1.9
Inclusion						
Human milk	10	16.3 ± 3.3	31.1 ± 2.0	1353 ± 179	41.2 ± 2.1	27.3 ± 1.4
Standard formula	12	17.1 ± 3.0	32.1 ± 1.6	1313 ± 236	39.4 ± 1.6	28.0 ± 1.4
Enriched formula	11	17.4 ± 4.0	31.8 ± 1.2	1286 ± 205	39.6 ± 1.8	27.5 ± 1.9
Discharge						
Human milk	10	41 ± 7.1	34.7 ± 1.4	2001 ± 141	42.8 ± 1.2	30.9 ± 1
Standard formula	12	41.3 ± 7.7	35.6 ± 1.1	2043 ± 247	43.8 ± 2.4	32 ± 1.4
Enriched formula	11	42.5 ± 8.8	35.4 ± 1	2007 ± 107	43.1 ± 1	31.1 ± 1.2
Expected term						
Human milk	10	77.2 ± 12.6	39.8 ± 1	3312 ± 564	49.2 ± 1.4	34.8 ± 0.7
Standard formula	12	77.0 ± 11.6	40.7 ± 0.8	3284 ± 463	49.0 ± 2.1	35.5 ± 1.8
Enriched formula	11	71.9 ± 10.7	39.8 ± 1.6	2966 ± 440	47.7 ± 1.4	34.3 ± 2.2
3 mo postterm						
Human milk	10	169.4 ± 11	53.0 ± 1.4	6013 ± 824	59.6 ± 1.9	40.9 ± 1.0
Standard formula	12	163.3 ± 11.5	53.0 ± 1.8	5266 ± 722	58.1 ± 2.3	39.4 ± 2.9
Enriched formula	11	163.5 ± 11.5	52.7 ± 1.1	5426 ± 765	58.2 ± 1.6	39.7 ± 1.9
6 mo postterm						
Human milk	10	262.9 ± 17.6	66.4 ± 1.5	7775 ± 1002	66.4 ± 4.3	44.2 ± 1.3
Standard formula	12	257.7 ± 9.1	66.5 ± 1.1	6708 ± 845	65.0 ± 2.5	43.7 ± 2.1
Enriched formula	11	262.4 ± 18.5	66.8 ± 1.7	7109 ± 1109	65.7 ± 2.4	42.5 ± 2.0

HC, head circumference.

* Results are expressed as mean +/− SD.

† No significant differences have been found between groups by using the Kruskal-Wallis method and Dunn's post hoc analysis.

The formulas were specially prepared for the study. All formulas were ready-to-feed, and met or exceeded the levels of energy, protein, and micronutrients as recommended for preterm and term formulas by the European Society for Pediatric Gastroenterology and Nutrition (13, 22). The standard and enriched formulas differed only in the amounts and sources of dietary fatty acids. The oil blend consisted of vegetable oils. The control formulas contained no added LC-PUFA, whereas the enriched formulas provided DHA from a high-DHA low-EPA fish oil (ROPUFA "30" n-3 INF oil, Roche, Basel, Switzerland), which contains at least 30% of LC-PUFA in the form of glycerides such as docosapentaenoic acid, EPA, and DHA (ratio of DHA to EPA = 4.8/1). The DHA content of the supplemented formulas was within the ranges reported for human milk in the French and European populations (6, 23). Both formulas provided the same amount of total fatty acids as well as the same total amount of n-3 and n-6 fatty acids. Full details of the fatty acid composition of the formulas are given in Table 1.

A nonrandomized group of 10 breast-fed infants was also included in this study. From enrollment until discharge, these infants received 100% of their enteral intake as prefrozen milk from their mother supplemented with a standardized human milk fortifier (Eoprotin®, Milupa, Friedrichsdorf, Germany) to assure adequate macro- and micronutrient intake (24). After discharge, the mothers were encouraged to continue breast-feeding as long as possible. If mothers were unable to provide sufficient human milk for full feeding, the standard formula without LC-PUFA was used to supplement the feeding until the end of the study.

All parents were advised about feeding in a uniform manner. The goal was to ensure that each infant be allowed to consume his or her desired intake. Formulas were provided to parents without restriction. Dietary histories were obtained at each visit to estimate the volume of formula intake and to obtain information about use of other nutrient sources. Parents were advised against use of solid food before 4 mo corrected age and advised that formula should remain the major food source for their infant until 6 mo corrected age.

Blood sampling and fatty acid analysis. The composition of fatty acids in total RBC phospholipids was measured to assess the effect of dietary fatty acids to ensure compliance. A 1-mL blood sample was obtained from each infant by venipuncture at enrollment and discharge, as well as expected term and at 3 mo and 6 mo postterm. No blood sample was taken within 10 d after a blood transfusion, except for one breast-fed infant who received a blood transfusion 7 d before enrollment. However, removing the data from this sample changes neither the mean value nor the statistical analysis. After removal of plasma by centrifugation, RBC were washed and immediately resuspended in methanol containing BHT as an antioxidant. Samples were stored under these conditions at −70°C until analysis. Lipid extraction was performed within 2 wk of sample collection.

During the hospital stay, samples of human milk were obtained from five of the breast-feeding mothers and stored at −70°C until analysis for total fat and fatty acid composition. The fatty acid composition observed was similar to that previously published in a French population (6).

Table 3. *Red blood cell phospholipid contents of n-6 fatty acids (percentage of total fatty acids by weight) of preterm infants fed human milk, standard formula (control), and an n-3 LC-PUFA-supplemented formula (low EPA-fish oil) until 4 mo postterm**

	Human milk (n = 10)	Standard formula (n = 12)	Enriched formula (n = 11)	p†
C18:2 n-6				
Enrollment	7.88 ± 2.03	8.11 ± 1.37	8.26 ± 1.27	0.65
Discharge	9.00 ± 1.05 a	11.5 ± 1.38 b	10.7 ± 1.10	0.001
Term	11.3 ± 0.81	11.7 ± 0.75	11.6 ± 1.35	0.54
3 mo postterm	12.8 ± 1.33	13.3 ± 0.97	12.5 ± 0.92	0.18
6 mo postterm	12.6 ± 1.48	13.3 ± 0.97	12.1 ± 1.10	0.08
C20:3 n-6				
Enrollment	2.16 ± 0.34 a	2.65 ± 0.33 b	2.50 ± 0.45	0.01
Discharge	2.30 ± 0.28	2.51 ± 0.51	2.57 ± 0.46	0.35
Term	2.20 ± 0.38 a	2.72 ± 0.54 b	2.47 ± 0.44	0.03
3 mo postterm	2.11 ± 0.37	2.44 ± 0.39	2.17 ± 0.49	0.13
6 mo postterm	1.99 ± 0.25 a	2.40 ± 0.31 b	2.43 ± 0.61	0.02
C20:4 n-6				
Enrollment	19.9 ± 1.42	19.5 ± 1.40	19.8 ± 0.80	0.86
Discharge	19.5 ± 1.01 a	18.7 ± 1.43	18.3 ± 0.68 b	0.02
Term	18.3 ± 1.41 a	18.0 ± 1.43	17.1 ± 0.62 b	0.04
3 mo postterm	18.4 ± 1.22 a	18.8 ± 0.89 a	15.1 ± 0.93 b	<0.001
6 mo postterm	19.0 ± 1.49 a	19.2 ± 1.14 a	17.2 ± 1.37 b	0.01
C22:4 n-6				
Enrollment	3.39 ± 0.35	3.52 ± 0.51	3.38 ± 0.42	0.90
Discharge	3.50 ± 0.25	3.73 ± 0.32	3.48 ± 0.26	0.10
Term	4.32 ± 0.62 a	4.40 ± 0.78 a	3.61 ± 0.28 b	0.004
3 mo postterm	4.61 ± 0.60 a	4.65 ± 0.74 a	3.03 ± 0.41 b	<0.0001
6 mo postterm	4.34 ± 0.41 a	4.21 ± 0.73 a	3.43 ± 0.53 b	0.003
C22:5 n-6				
Enrollment	1.13 ± 0.31	1.18 ± 0.21	1.17 ± 0.37	0.71
Discharge	1.00 ± 0.16	1.09 ± 0.23	1.02 ± 0.24	0.60
Term	0.98 ± 0.15	1.20 ± 0.28 a	0.92 ± 0.12 b	0.005
3 mo postterm	1.12 ± 0.27 a	1.18 ± 0.18 a	0.82 ± 0.10 b	0.002
6 mo postterm	1.03 ± 0.17	1.03 ± 0.18	0.89 ± 0.14	0.10
Total n-6				
Enrollment	34.7 ± 1.9	35.2 ± 1.6	35.4 ± 1.4	0.70
Discharge	35.6 ± 1.0	37.9 ± 1.5 a	36.5 ± 0.8 b	0.002
Term	37.6 ± 1.6	38.6 ± 2.0 a	36.2 ± 1.4 b	0.006
3 mo postterm	39.5 ± 1.7 a	40.9 ± 0.6 a	34.2 ± 0.8 b	<0.0001
6 mo postterm	39.4 ± 1.2 a	40.5 ± 1.5 a	36.4 ± 1.1 b	<0.0001

Values with different letters are significantly different ($p < 0.05$). HM, human milk; SF, standard formula; EF, enriched formula.

* Results expressed as mean +/− SD.

† Kruskal-Wallis method and Dunn's post hoc analysis for between-group comparison.

Fatty acid analysis of blood and milk samples was performed as previously described (6). Lipids from RBC and milks were extracted with methanol/chloroform (1/2). Lipid classes were separated by thin-layer chromatography. The silica gel bands containing RBC phospholipids were immediately scraped and methylated with boron trifluoride in methanol. The fatty acid methyl esters were separated and quantitated by capillary gas liquid chromatography. Identification of individual fatty acids was accomplished by comparison with standard mixtures purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Individual fatty acids were quantified by automatic integration and expressed as percentages of the total fatty acids with 14 or more carbons.

Ethics. The project was approved by the local ethics committee. All parents were informed of the details of the study, and written consent was obtained in all cases.

Statistical methods. Statistical analysis was performed using SigmaStat (SPSS Inc., Chicago, IL, U.S.A.). Sample sizes were estimated to allow detection of a 1.2 SD difference (6) in RBC phospholipid DHA contents between formula-fed groups (power = 80%; $p < 0.05$). The final sample size required is seven infants. Anticipating a 30% loss to follow-up, we planned a recruitment of 9 infants for each of the three diet groups, and achieved enrollment of 10–12 per groups. Descriptive statistics were computed for all dependent variables. The Kruskal-Wallis analysis of variance on ranks was used to compare outcome variables of the three experimental groups. When p values were significant, multiple comparisons were performed; because the sample sizes of the diet groups differed, the Dunn's test with pairwise multiple comparison procedures was used. Correlations between fatty acid contents were calculated by Spearman rank correlations. Results were considered significant if the p value was less than or equal to 0.05.

RESULTS

Inclusion characteristics and growth. The clinical characteristics at birth and at enrollment did not differ significantly

among the feeding groups (Table 2). All groups were fed identically during the pre-enrollment period and there were no statistically significant differences between groups in the total amount of milk fed, the proportion of breast milk fed, or the amount of enteral intake at enrollment. Growth of the three groups did not differ during the study (Table 2). Mean values for weight, length, and head circumference at the various ages were comparable with those usually observed in studies of preterm infants.

RBC phospholipid fatty acid concentrations. The fatty acid profiles of the RBC phospholipids of the three groups were similar at enrollment. No significant differences in the content of saturated and monounsaturated fatty acids were observed between groups during the course of the study (data not shown).

The difference in dietary lipid composition, in contrast, had a strong influence on the n-6 and n-3 fatty acid pattern of RBC phospholipids of formula-fed infants (Tables 3 and 4). Because the standard formula contained no LC-PUFA, infants fed this formula had, as expected, a continuous decrease in RBC phospholipid DHA content from enrollment through the end of the study, when it was 50% lower than observed at enrollment (Table 4). During the same period, RBC phospholipid AA content remained stable in this group (Table 3).

In contrast, the RBC phospholipid DHA content of infants fed the enriched formula was significantly higher than that of the standard formula-fed group during the entire study, peaking at 9% of total fatty acids at 3 mo postterm. Despite the low content of EPA in the formula, the RBC phospholipid content of EPA increased in the supplemented formula group from enrollment until 3 mo postterm. A dramatic decrease in RBC phospholipid content of AA also was observed in this group. RBC phospholipid AA content at 3 mo and 6 mo postterm were significantly lower than those observed in the standard formula group. However, at 6 mo postterm (*e.g.* 2 mo after the supplementation was stopped) the RBC phospholipid AA content of this group was 14% higher than at 3 mo.

No statistically significant variation in the total n-6 and n-3 fatty acid contents of RBC phospholipid was observed in the breast-fed group between enrollment and discharge. After discharge, mothers progressively stopped breast-feeding their infants and commenced feeding standard formula. As a consequence, the total amount of breast milk fed dropped from 100% at discharge to 71% at term and only 3 of the 10 mothers continued to breast-feed their infant beyond expected term. Consequently, the RBC phospholipid fatty acid pattern changed dramatically after discharge, and tended to resemble that of the standard formula group.

Table 4. *Red blood cell phospholipid content of n-3 fatty acids (percentage of total fatty acids by weight) of preterm infants fed human milk, standard formula (control), and an n-3 LC-PUFA-supplemented formula (low EPA-fish oil) until 4 mo postterm**

	Human milk (n = 10)	Standard formula (n = 12)	Enriched formula (n = 11)	p†
C18:3 n-3				
Enrollment	0.07 ± 0.09	0.08 ± 0.07	0.12 ± 0.12	0.64
Discharge	0.15 ± 0.17	0.14 ± 0.10	0.08 ± 0.09	0.54
Term	0.13 ± 0.07[a]	0.11 ± 0.07[a]	0.03 ± 0.04[b]b	0.003
3 mo postterm	0.15 ± 0.05[a]	0.17 ± 0.09[a]	0.09 ± 0.04[b]	0.02
6 mo postterm	0.16 ± 0.04	0.17 ± 0.04	0.14 ± 0.03	0.07
C20:5 n-3				
Enrollment	0.40 ± 0.14	0.35 ± 0.10	0.39 ± 0.17	0.84
Discharge	0.56 ± 0.28	0.35 ± 0.13	0.42 ± 0.10	0.15
Term	0.47 ± 0.17[a]	0.28 ± 0.12[b]	0.45 ± 0.14	0.01
3 mo postterm	0.28 ± 0.13[a]	0.25 ± 0.12[a]	0.69 ± 0.15[b]	<0.0001
6 mo postterm	0.53 ± 0.16	0.45 ± 0.19	0.55 ± 0.21	0.19
C22:5 n-3				
Enrollment	1.02 ± 0.45	0.98 ± 0.28	1.06 ± 0.52	0.98
Discharge	1.52 ± 0.48	1.43 ± 0.44	1.29 ± 0.35	0.64
Term	1.82 ± 0.30[a]	1.70 ± 0.21[a]	1.38 ± 0.26[b]	0.004
3 mo postterm	2.04 ± 0.37[a]	2.02 ± 0.28[a]	1.50 ± 0.21[b]	0.0002
6 mo postterm	2.32 ± 0.48	2.36 ± 0.29[a]	2.05 ± 0.25[b]	0.04
C22:6 n-3				
Enrollment	6.12 ± 0.83	5.89 ± 0.87	6.02 ± 1.27	0.80
Discharge	6.39 ± 0.67[a]	4.86 ± 0.47[b]	6.57 ± 0.59[a]	<0.0001
Term	5.52 ± 1.09[a]	3.79 ± 0.65[b]	6.88 ± 0.76[a]	<0.0001
3 mo postterm	3.91 ± 1.32[a]	2.95 ± 0.69[a]	9.22 ± 0.92[b]	<0.0001
6 mo postterm	3.51 ± 0.82[a]	2.78 ± 0.61[a]	6.61 ± 1.03[b]	<0.0001
Total n-3				
Enrollment	7.6 ± 0.7	7.3 ± 0.9	7.6 ± 1.5	0.59
Discharge	8.6 ± 1.0[a]	6.8 ± 0.8[b]	8.4 ± 0.7[a]	0.003
Term	7.9 ± 1.4[a]	5.9 ± 0.7[b]	8.7 ± 0.7[a]	<0.0001
3 mo postterm	6.4 ± 1.6[a]	5.4 ± 0.8[a]	11.5 ± 1.1[b]	<0.0001
6 mo postterm	6.5 ± 1.0[a]	5.8 ± 0.9[a]	9.3 ± 1.3[b]	<0.0001

Values with different letters are significantly different ($p < 0.05$). HM, human milk; SF, standard formula; EF, enriched formula.

* Results expressed as mean +/− SD.

† Kruskal-Wallis method and Dunn's post hoc analysis for between-group comparison.

Across all infants, there was a statistically significant positive correlation between RBC phospholipid DHA content and RBC phospholipid EPA content at term as well as at 3 mo and 6 mo postterm ($r = 0.46$, $p = 0.008$; $r = 0.90$, $p = 0.0001$; and $r = 0.39$, $p = 0.02$, respectively). These correlations also were statistically significant at 3 mo postterm when human milk and enriched formula-fed groups were examined separately ($r = 0.80$, $p = 0.005$; $r = 0.74$, $p = 0.01$, respectively).

DISCUSSION

This randomized study was designed to assess the effects of feeding a preterm formula supplemented with low-EPA fish oil *versus* a standard formula until 4 mo postterm on the LC-PUFA status of healthy preterm infants. One goal of our study was to determine whether deterioration of DHA status before 6 mo postterm could be prevented. Indeed, a short period of DHA supplementation, until term (6) or until 2 mo (9), is not sufficient to prevent a drop in DHA status during the ensuing months. By supplementing formula for 4 mo after term, normal RBC phospholipid DHA content was maintained until 6 mo postterm.

Another objective of the study was to determine whether the decrease of n-6 PUFA status associated with the use of a high-EPA fish oil could be prevented with the use of a low-EPA modified fish oil. In a previous trial, preterm infants fed such a source of LC-PUFA until 2 mo postterm had no significant decrease in RBC phospholipid AA content compared with the unsupplemented formula group (9). However, a decrease in the AA content was suspected because of a lower ratio of RBC PE AA to RBC PE DHA in the supplemented formula fed group throughout the study. In contrast to this earlier study, the LC-PUFA supplementation in our study had a strong influence on the RBC phospholipid content of AA, which decreased significantly over time in the LC-PUFA enriched group.

The observed decrease in RBC phospholipid AA status might be related to the higher RBC phospholipid content of EPA and/or DHA, which, in turn, might interfere with incorporation of AA into RBC phospholipids (1). The overall effect of these variations resulted, however, in no significant differences in total LC-PUFA content. This observed decrease in RBC phospholipid AA content might also be related to an inhibitory effect of EPA on the desaturation of linoleic acid to AA (1). By using a low-EPA fish oil, the 79% increase in RBC phospholipid EPA content between enrollment and 3 mo postterm in the supplemented group was not expected, but most likely resulted from the small amount of EPA present in the LC-PUFA supplement. However, the strong positive correlation between RBC phospholipid DHA and RBC phospholipid EPA suggests that retroconversion of DHA to EPA might also have occurred (25).

The RBC phospholipid DHA content of the breast milk-fed group remained stable until the mothers started to lessen and finally stop breast-feeding their infants. At 3 mo postterm, the DHA content of RBC phospholipid was slightly higher in the breast milk-fed group than the nonsupplemented group. At the end of the study, however, there was no statistically significant difference between the breast milk-fed group and the standard formula-fed group. This suggests that breast-feeding preterm infants only during the hospital stay is not sufficient to maintain levels of DHA in RBC phospholipids during the first several months of life. This finding is particularly important in countries where the average duration of breast-feeding is only a few weeks.

We conclude from this study that supplementing formulas with a low-EPA fish oil for 4 mo postterm is an effective way to maintain a normal DHA status during the period of supplementation and for 2 mo afterward. However, this supplementation resulted in an increase in RBC phospholipid n-3 fatty acids at the expense of n-6 fatty acids. Further studies are needed to explore the possible clinical effects of subnormal AA status in preterm infants, but it seems likely that formula-fed preterm infants require a dietary supply of preformed AA as well as DHA if the biochemical status of breast-fed infants is to be achieved.

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