Influence of Formulas with Borage Oil or Borage Oil Plus Fish Oil on the Arachidonic Acid Status in Premature Infants

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ABSTRACT: Several studies have reported that feeding γ-linolenic acid (GLA) has resulted in no increase in arachidonic acid (AA) in newborns. This result was ascribed to the eicosapentaenoic acid (EPA)-rich fish oil used in these formulas. Docosahexaenoic acid (DHA) sources with only minor amounts of EPA are now available, thus the addition of GLA to infant formulas might be considered an alternative to AA supplementation. Sixty-six premature infants were randomized to feeding one of four formulas [ST: no GLA, no long-chain polyunsaturated fatty acids; BO: 0.6% GLA (borage oil); BO + FOLOW: 0.6% GLA, 0.3% DHA, 0.06% EPA; BO + FOHIGH: 0.6% GLA, 0.3% DHA, 0.2% EPA] or human milk (HM, nonrandomized) for 4 wk. Anthropometric measures and blood samples were obtained at study entry and after 14 and 28 d. There were no significant differences between groups in anthropometric measures, tocopherol, and retinol status at any of the studied time points. The AA content of plasma phospholipids was similar between groups at study start and decreased significantly until day 28 in all formula-fed groups, but not in the breast-fed infants [ST: 6.6 ± 0.2%, BO: 6.9 ± 0.3%, BO + FOLOW: 6.9 ± 0.4%, BO + FOHIGH: 6.7 ± 0.2%, HM: 8.6 ± 0.5%, where values are reported as mean ± standard error; all formulas significantly different ($P \leq 0.05$) from HM]. There was no significant influence of GLA or fish oil addition to the diet. GLA had only a very limited effect on AA status which was too small to obtain satisfactory concentrations (concentrations similar to breast-fed babies) under the circumstances tested. The effect of GLA on AA is independent of the EPA and DHA content in the diet within the dose ranges studied.

Paper no. L8539 in *Lipids 36,* 555–566 (June 2001).

The addition of suitable amounts of long-chain polyunsaturated fatty acids (LC-PUFA) to infant formulas, especially arachidonic (AA) and docosahexaenoic (DHA) acids, establishes concentrations of these fatty acids in infant plasma phospholipids equivalent to those found in breast-fed infants (1,2). Evidence is accumulating that the availability of appreciable LC-PUFA concentrations is associated with the neurological development of preterm (3) and term babies (4,5). This is ascribed to the importance of these fatty acids for membrane properties, as there are high relative concentrations of DHA in retina and brain (6). AA and other 20-carbon atom fatty acids [dihomo-γ-linolenic (DGLA) and eicosapentaenoic (EPA)] are important precursors for eicosanoids (7). Although functional effects of pure AA supplementation have not been investigated in humans, a stable supply of AA, as achieved by breast feeding (8), seems desirable for formula-fed babies. In rats negative effects of low AA plasma concentrations, induced by fish oil feeding, on postnatal development have been shown (9). Furthermore, AA status has been associated with infant growth (10,11).

An exogenous supply of LC-PUFA is required to achieve plasma concentrations equivalent to those found in breast-fed babies, although newborns already synthesize LC-PUFA endogenously from the essential precursors linoleic (LA) and α-linolenic acid during the first days after birth (12–14). Although the pathways of conversion have been elucidated, the question whether the low plasma and tissue concentrations found without dietary supplement are due to the high metabolic demand of LC-PUFA or to low conversion has not been answered (15). The pathways of n-3 and n-6 essential fatty acid conversion to AA and DHA share the enzymes for Δ-6, Δ-5 desaturation and chain elongation, but it is not yet known whether there are different variants of these enzymes (16). The initial step is a Δ-6 desaturation, which converts LA to γ-linolenic acid (GLA). There are strong indications that this is the rate-limiting step for the endogenous synthesis of AA (17). Based on this concept, an exogenous supply of GLA might increase the plasma concentration of n-6 LC-PUFA in infants, as was reported to occur in adults (18).

Many manufacturers produce infant formulas containing from 0.4 to 1.0% of fatty acids as LC-PUFA (19). Although fish oils are readily available as a source for n-3 LC-PUFA, the introduction of AA is a greater challenge. Egg phospholipids and single-cell oils are the primary AA sources used in infant formulas, but they are comparatively expensive or have not been approved for formula use in all countries. Alternatively, readily available sources of GLA-containing oils, e.g., borage, black currant seed or evening primrose oil, could be

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Abbreviations: AA, arachidonic acid (20:4n-6); BO, borage oil; Chol, cholesterol; DGLA, dihomo-γ-linolenic acid (20:3n-6); DHA, docosahexaenoic acid (22:6n-3); EPA, eicosapentaenoic acid (20:5n-3); GLA, γ-linolenic acid (18:3n-6); HM, human milk; LA, linoleic acid (18:2n-6); LC-PUFA, long-chain polyunsaturated fatty acids (≥ 20 C atoms, ≥ 2 double bonds); MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SAT, saturated fatty acid; ST, standard formula; TG, triglycerides.

added to the formulas (20). These oils have been considered safe in older children even in pharmacological doses (21).

Some studies investigated the influence of a GLA and n-3 LC-PUFA supply to term infants and found no positive effects on AA percentage in red blood cell phospholipids (22–24). Since formulas tested in these studies also contained fish oils rich in EPA, it appeared possible that the lack of a positive effect of the GLA supplement might have been due to an inhibiting effect of high EPA and DHA supply on AA synthesis (8,20). This is based on the observation that feeding n-3 LC-PUFA-rich marine oil caused increased concentrations of n-3 LC-PUFA and decreased AA concentrations in infants and adults (25,26). However, in term infants a formula containing 0.5% GLA only marginally increased the AA level, although EPA contributed only 0.07% to dietary fatty acids (22). DHA sources that contain only minor amounts of EPA are now readily available, thus we investigated the inclusion of a slightly higher dose of GLA into a preterm formula as an alternative to AA supplementation.

We compared four different formulas for preterm infants containing: (i) no GLA and no LC-PUFA, (ii) 0.6% GLA and no LC-PUFA, (iii) 0.6% GLA and 0.3% DHA combined with 0.06% EPA, and (iv) 0.2% EPA and 0.3% DHA plus 0.6% GLA. A nonrandomized reference group of breast-fed infants was also followed. While the majority of the previous infant studies analyzed red blood cells, which might have an attenuated and delayed response to dietary changes, we chose to analyze plasma phospholipids.

Since LC-PUFA supplementation of the diet decreased the tocopherol/lipid ratio in the erythrocytes of infants and the plasma vitamin E concentrations in adults (27,28) and since in newborns serious health risks have been associated with low vitamin E levels (29), we also analyzed plasma concentrations of lipid-soluble vitamins to detect potential influences of the various fatty acid supplies on the infants' antioxidative status (30).

SUBJECTS AND METHODS

Premature infants were recruited for the study at the Children's Hospital Gyor (Hungary) from December 1994 to May 1997. Inclusion criteria were a birth weight below 1800 g and full enteral feeding with more than 120 mL milk/kg/d. Excluded from the study were any infants with serious metabolic or congenital anomalies; infants on artificial ventilation; infants with septic infections, gastrointestinal problems or metabolic diseases; and infants receiving any parenteral lipids during the study period. Systemic corticosteroid therapy during the study was only allowed for up to 3 d, otherwise subjects were excluded. According to the standard hospital protocol for parenteral nutrition, all participating infants were fed a 20% lipid emulsion based on soybean oil. Up to 10% of total energy intake from sources other than the corresponding study diet were permitted.

The study protocol was approved by the Ethical Committee of the Hungarian Medical Association. After explanation of the study protocol, written parental consent for participation was obtained for all infants before enrollment. If mothers provided HM, the infants were fed with breast milk. If mothers chose not to provide breast milk, the infants were randomized double blind to one of the four formula groups without further stratification. Randomization numbers were computer-generated (Roche, Basel, Switzerland) and allocated sequentially in the order of enrollment to the infants. Double blinding was achieved, as the identity of the dispensed formula was provided in a sealed envelope for each infant. Only in case of an immediately reportable adverse event was this envelope to be opened.

The study formulas were produced by Nutricia (Zoetermeer, The Netherlands) based on the low birth weight infant formula "Nenatal." The formulas delivered 80 kcal per 100 mL, and contained 11% (of energy) protein (whey/casein = 3:2), 40% carbohydrates (mainly lactose and polysaccharides), and 49% fat. The dietary fat (4.4 g/100 mL) was based on a blend of milk fat, coconut oil, soy oil, sunflower oil, and canola oil. These were the only fat components of the standard formula (ST), whereas the GLA content was increased by the addition of borage oil (BO). The two other formulas (BO + FOLOW, BO + FOHIGH) additionally contained fish oils differing in their EPA/DHA ratio (Hoffmann-La Roche Ltd., Basel, Switzerland). Both were composed to deliver 0.3% DHA but contained either 0.06% (BO + FOLOW) or 0.2% EPA (BO + FOHIGH). Table 1 shows the detailed fatty acid composition of the different formulas and of the breast milk fed (mean and standard error of individual samples), as analyzed in our laboratory. In the case of breast milk feeding, a 5-mL sample of breast milk was collected on days 0 and 14. All formulas contained 0.1 mg vitamin A and 1.3 mg vitamin E per 100 mL.

On the day of enrollment (study day 0), 2 wk later (study day 14), and 4 wk later (study day 28) anthropometric measures were recorded and blood samples were taken. At all time points a deviation of maximally 1 d from the date determined by the protocol was tolerated. Weight, length, and head circumference were measured according to standard hospital procedures. Blood samples (0.5 mL) were taken by venipuncture and immediately transferred into EDTA-containing tubes. Blood cells and plasma were separated by centrifugation at $1000 \times g$ for 5 min. A plasma aliquot of at least 200 μL was frozen immediately at −80°C for later analysis. Red blood cells were washed three times in 0.9% aqueous NaCl solution, hemolyzed, and stored at −80°C until analysis (data not shown). The remaining portion of the sample was used for the determination of routine laboratory parameters: alanine-amino-transferase, γ-glutamyltranspeptidase, total bilirubin, creatinine, total protein, glucose, carbamide-N, sodium, potassium, and calcium. For further assessment of possible adverse effects a series of hematological measures were performed, e.g., red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, hemoglobin E, white blood cell count, platelet count, and hemogram. Pulse rate and blood pressure were also recorded.

As differences of 20% or greater in the primary study parameter, AA concentration in plasma phospholipids, were considered clinically important, the sample size was planned on the data of Decsi and Koletzko (31) who reported a rela-

TABLE 1
Fatty Acid Composition (%w/w) of the Formulas Used in the Study (results of our own analyses) and from the Individual Human Milk Samples on Day 0 and Day 14[a]

					HM	
Fatty acid	ST (n = 2)	BO (n = 4) (mean ± SE)	BO + FOLOW (n = 2)	BO + FOHIGH (n = 2)	Day 0 (n = 12) (mean ± SE)	Day 14 (n = 13) (mean ± SE)
∑SAT	41.10/41.34	41.53 ± 0.07	40.87/40.96	40.44/40.64	46.32 ± 1.76	47.47 ± 1.17
∑MUFA	44.05/43.88	43.75 ± 0.11	44.02/44.09	44.07/43.88	34.68 ± 1.12	34.19 ± 1.09
∑TRANS	0.05/0.05	0.03 ± 0.01	0.05/0.06	0.07/0.07	0.72 ± 0.14	0.85 ± 0.14
20:3n-9	ND	ND	ND	ND	0.01 ± 0.01	0.01 ± 0.01
18:2n-6	13.47/13.40	12.88 ± 0.03	12.73/12.74	12.97/12.97	15.65 ± 1.25	15.15 ± 1.14
18:3n-6	0.01/0.01	0.65 ± 0.02	0.60/0.60	0.64/0.64	0.15 ± 0.01	0.17 ± 0.01
20:2n-6	ND	0.01 ± 0.00	0.01/0.01	0.02/ND	0.40 ± 0.04	0.32 ± 0.03
20:3n-6	0.01/0.01	0.01 ± 0.00	0.01/0.01	0.01/0.01	0.43 ± 0.03	0.41 ± 0.03
20:4n-6	0.01/0.01	0.01 ± 0.00	0.03/0.03	0.02/0.02	0.51 ± 0.03	0.47 ± 0.03
22:2n-6	0.06/0.06	0.06 ± 0.00	0.06/0.06	0.05/0.05	0.05 ± 0.01	0.04 ± 0.01
22:4n-6	ND	ND	ND	ND	0.14 ± 0.01	0.12 ± 0.01
18:3n-3	1.21/1.20	1.06 ± 0.09	1.20/1.20	1.26/1.26	0.51 ± 0.05	0.43 ± 0.04
18:4n-3	0.02/0.02	0.02 ± 0.01	0.02/0.02	0.04/0.04	0.03 ± 0.01	0.03 ± 0.01
20:3n-3	ND	ND	ND	ND	0.02 ± 0.01	0.01 ± 0.01
20:5n-3	0.01/0.01	ND	0.06/0.06	0.15/0.15	0.03 ± 0.01	0.02 ± 0.01
22:5n-3	0.01/0.01	0.01 ± 0.00	0.02/0.02	0.02/0.02	0.11 ± 0.01	0.10 ± 0.01
22:6n-3	ND	ND	0.24/0.25	0.25/0.25	0.22 ± 0.03	0.20 ± 0.03
LC-PUFA	0.10/0.10	0.10 ± 0.00	0.42/0.42	0.52/0.51	1.95 ± 0.12	1.73 ± 0.12
18:2n-6/18:3n-3	11.10/11.12	12.39 ± 1.06	10.61/10.63	10.30/10.33	33.92 ± 3.87	38.64 ± 4.56

[a]ST, standard formula; BO, borage oil; BO + FOLOW, borage oil + 0.3% DHA and 0.06% EPA; BO + FOHIGH, borage oil + 0.3% DHA and 0.2% EPA; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SAT, saturated fatty acids; MUFA, monounsaturated fatty acids; TRANS, *trans* fatty acids; ND, not detected; LC-PUFA, long-chain polyunsaturated fatty acids.

tive standard deviation of 24% for AA. With 13 infants per group, the considered difference between groups was to be detected with 90% probability and a 5% risk of type I error.

Analysis of plasma phospholipids. After the addition of dipentadecanoyl-phosphatidylcholine as an internal standard, total lipids from 100 µL plasma were extracted into 2 mL hexane/isopropanol (4:1) and subsequently twice into 2 mL of hexane (32). Extracts were combined and dried under a stream of N_2. For application on silica gel plates (Merck KG, Darmstadt, Germany), the residue was taken up in chloroform/methanol (1:1). Phospholipids were isolated by development of the plates in *n*-heptane/diisopropylether/glacial acetic acid (60:40:3, by vol) (33). After visualization with 2,7-dichlorofluorescein, phospholipid-containing bands were scraped off and transferred into reaction vials. Synthesis of fatty acid methyl esters was performed by heating the phospholipids to 85°C for 45 min after dissolution in 1.5 mL 3M methanolic hydrochloric acid. After neutralization of the reaction mixture with carbonate buffer, methyl esters were extracted twice into 1 mL hexane and the combined extracts taken to dryness under nitrogen. For storage (–80°C) until gas chromatographic analysis, the samples were dissolved in 40 µL hexane (containing 2 g/L butylhydroxytoluene) and transferred into microvials.

Gas chromatography was performed on an HP5890 series II gas chromatograph (Hewlett-Packard, Waldbronn, Germany), equipped with a split/splitless injector (250°C, split ratio = 1:20, column head pressure 1.20 bar) and a flame-ionization detector (300°C). Separation of individual fatty acid methyl esters was achieved with a BPX70 column (SGE, Weiterstadt, Germany) with 60 m length and 0.32 mm inner diameter. The temperature program started at 130°C, increased at a rate of 3°C/min up to 200°C, and continued without delay up to 210°C at a rate of 1°C/min. For identification and determination of response factors, commercially available standards were used [Nu-Chek-Prep, Elysian, MN; Sigma, Deisenhofen, Germany; docosapentaenoic (n-6) acid methyl ester was a gift from Omega Tech, Boulder, CO]. Chromatographic data were recorded and evaluated using EZChrom Elite Ver. 2.1 software (Scientific Software Inc., Pleasanton, CA). Because of the small plasma volumes, samples were weighed in addition to volumetric dosage, and absolute plasma concentrations are given as mg fatty acid methyl ester per kg plasma.

Analysis of milk lipids. Milk samples were analyzed as previously described (34). Briefly, after the addition of 100 µL potassium oxalate (3.5%) solution, lipids from 1 mL milk were extracted into 3 mL of a mixture of ethanol/*tert*-butylmethylether/petrolether (1:1:1). To obtain quantitative recovery, a second extraction with 2 mL *tert*-butyl-methylether/petrolether (1:1) was performed. Extracts were combined and transferred into a preweighed vial; solvents were evaporated under nitrogen, and the remainder was dried in a desiccator overnight. Total lipid content could now be determined gravimetrically. For transesterification at 90°C for 60 min, the lipids were dissolved in 2 mL 1.5 M methanolic hydrochloric acid

and 1 mL hexane. After the addition of 2 mL water, the methyl esters were extracted into 2 mL of hexane and analyzed by gas chromatography as described for plasma samples.

Analyses of vitamins A and E. These analyses were performed as previously described from 100 µL plasma samples by high-performance liquid chromatography (35). After the addition of 100 µL ethanol to the samples, vitamins were extracted three times into 1 mL hexane and the combined extracts taken to dryness. For chromatography the extract was reconstituted in 100 µL mobile phase [acetonitrile/tetrahydrofuran/methanol/ammonium acetate (1%) = 684:220:68:28] containing tocol as standard for quantification. A high-performance liquid chromatography system (L6200 pump, AS 2000 autosampler, L4250 ultraviolet-visible detector, F-1050 fluorescence detector; Merck), equipped with a reversed-phase column (LiChrospher 100, RP-18, 25 cm long, Merck) was used for chromatography. With a flow rate of 0.65 mL/min, retinol, α-tocopherol and δ-tocopherol could be separated, while β- and γ-tocopherol coeluted. For retinol detection the photometer was set to 325 nm, and tocopherols were detected by their fluorescence at 330 nm after excitation at 298 nm. Data were recorded and evaluated with EZChrom Elite Ver. 2.1 software (Scientific Software Inc., Pleasanton, CA).

Total triglycerides (TG) and total cholesterol (Chol) were determined using a Vitros 250 autoanalyzer (Johnson & Johnson, Neckargmünd, Germany).

Statistical analysis. Data characterizing subjects are given as means ± standard deviations. One-way analysis of variance was used to detect statistically significant differences ($P \leq 0.05$) between groups. Analytical results for fatty acids and lipid-soluble vitamins are presented as means ± standard errors of the mean. Statistical evaluation of the results at the end of the study was performed by analysis of variance, including the corresponding values at study start as covariates. Differences between group means were taken as statistically significant if the 95% confidence interval (Scheffé method for correction of multiple testing) of differences between groups did not include 0. Coefficients of correlation were calculated according to Pearson and were taken as significant if P was equal to or less than 0.05. Individual changes with time were investigated by paired t-tests (significance level 0.05). Calculations were performed using S Plus, Ver. 4.5 (Mathsoft Inc., Seattle, WA).

RESULTS

Sixty-six infants with gestational ages between 25 and 37 wk were enrolled into the study. One infant from group ST was excluded from the study because of infectious disease. Thus the results from 65 infants who completed the study were evaluated. There were no statistically significant differences between the five dietary groups in clinical characteristics, body weight, length, head circumference, and postnatal age (Table 2). Although infants in groups BO and BO + FOHIGH tended to be older, there were no significant differences at study start and study end.

Infants presented very similar fatty acid compositions of plasma phospholipids across all groups at study start (Table 3). Saturated fatty acids (SAT) contributed almost one-half and *cis*-monounsaturated about 20% of the total. The only major difference at study start was a lower GLA content in the plasma phospholipids of the breast-fed group, although 0.15% GLA was detected in HM fat.

Tendencies indicating the statistically analyzed values at study end could already be observed for all investigated parameters at study day 14; thus there seemed to be a continuous trend during the whole study period.

TABLE 2
Clinical and Anthropometric Description of the Infants (mean ± SD) Studied at Birth, Day 0 (study start), Day 14, and Day 28 (study end)

	ST	BO	BO + FOLOW	BO + FOHIGH	HM
Number of infants	13	13	13	14	13
Birth weight (g)	1494 ± 269	1302 ± 229.3	1500 ± 199	1362 ± 284	1404 ± 290
Age at study start (d)	21.2 ± 10.4	32.4 ± 18.2	22.6 ± 10.9	33.9 ± 15.1	29.5 ± 13.3
			Body weight[a] (g)		
Day 0	1522 ± 222	1422 ± 188	1535 ± 198	1516 ± 223	1532 ± 250
Day 14	1840 ± 275	1790 ± 249	1888 ± 223	1865 ± 319	1822 ± 294
Day 28	2315 ± 325*	2239 ± 306*	2388 ± 250*	2391 ± 377*	2239 ± 380*
			Length[a] (cm)		
Day 0	43.2 ± 2.9	42.2 ± 2.1	42.9 ± 3.4	42.8 ± 1.7	42.7 ± 2.5
Day 14	44.0 ± 2.4	44.2 ±1.9	44.1 ± 2.7	44.6 ± 1.9	44.0 ± 2.2
Day 28	46.6 ± 2.9*	46.0 ± 2.3*	46.9 ± 1.7*	46.2 ± 1.9*	46.3 ± 2.5*
			Head circumference[a] (cm)		
Day 0	30.0 ± 2.0	28.2 ± 2.8	29.2 ± 1.7	29.9 ± 1.6	28.9 ± 2.0
Day 14	31.3 ± 1.8	30.7 ± 1.7	31.5 ± 1.7	31.5 ± 1.7	30.6 ± 1.1
Day 28	32.9 ± 1.5*	32.2 ± 1.5*	33.0 ± 1.1*	32.86 ± 1.6*	32.2 ± 0.9*

[a]Asterisk indicates a significant change ($P \leq 0.05$) from day 0 to day 28. For abbreviations for formulas used see Table 1.

TABLE 3
Fatty Acid Composition (%w/w, mean ± SE) of Infant Plasma Phospholipids at Study Start (day 0), After 2 wk on Study Diet (day 14), and at Study End After 4 wk (day 28)

	ST	BO	BO + FOLOW	BO + FOHIGH	HM
			Day 0		
∑SAT	48.01 ± 0.87	47.53 ± 0.53	47.72 ± 0.71	48.21 ± 0.32	48.28 ± 0.46
∑MUFA	20.69 ± 0.78	21.64 ± 1.13	20.11 ± 0.78	19.61 ± 0.86	19.44 ± 0.45
∑TRANS	0.86 ± 0.07	0.89 ± 0.06	0.83 ± 0.07	0.87 ± 0.04	0.94 ± 0.07
20:3n-9	0.78 ± 0.22	0.96 ± 0.31	0.48 ± 0.09	0.81 ± 0.21	0.47 ± 0.15
18:2n-6	13.94 ± 0.94	13.73 ± 1.20	14.49 ± 0.90	14.57 ± 1.08	14.72 ± 0.44
18:3n-6	0.11 ± 0.03	0.12 ± 0.02	0.10 ± 0.03	0.12 ± 0.03	0.01 ± 0.01
20:2n-6	0.39 ± 0.04	0.47 ± 0.03	0.44 ± 0.02	0.46 ± 0.02	0.46 ± 0.02
20:3n-6	2.71 ± 0.17	2.68 ± 0.12	2.69 ± 0.11	2.83 ± 0.17	2.83 ± 0.17
20:4n-6	9.37 ± 0.52	8.55 ± 0.41	9.80 ± 0.36	9.18 ± 0.42	9.47 ± 0.35
22:2n-6	0.55 ± 0.04	0.50 ± 0.04	0.49 ± 0.04	0.56 ± 0.05	0.59 ± 0.04
22:4n-6	0.50 ± 0.05	0.55 ± 0.03	0.53 ± 0.03	0.55 ± 0.03	0.51 ± 0.02
20:3/20:4	0.30 ± 0.02	0.32 ± 0.02	0.28 ± 0.02	0.32 ± 0.03	0.30 ± 0.02
∑n-6 LC-PUFA	13.51 ± 0.62	12.74 ± 0.50	13.95 ± 0.35	13.57 ± 0.49	13.85 ± 0.42
18:3n-3	ND	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	ND
20:5n-3	0.06 ± 0.03	0.15 ± 0.02	0.14 ± 0.05	0.09 ± 0.04	0.04 ± 0.02
22:5n-3	0.19 ± 0.04	0.27 ± 0.05	0.18 ± 0.04	0.25 ± 0.04	0.24 ± 0.03
22:6n-3	1.85 ± 0.09	1.93 ± 0.14	1.97 ± 0.08	1.87 ± 0.10	2.01 ± 0.14
∑n-3 LC-PUFA	2.10 ± 0.13	2.35 ± 0.18	2.30 ± 0.12	2.22 ± 0.13	2.29 ± 0.16
∑PUFA	30.43 ± 1.02	29.94 ± 1 .40	31.33 ± 0.80	31.30 ± 1.06	31.33 ± 0.71
∑PUFA/∑SAT	0.64 ± 0.03	0.63 ± 0.03	0.66 ± 0.02	0.65 ± 0.02	0.65 ± 0.02
∑LC-PUFA	16.39 ± 0.72	16.05 ± 0.46	16.72 ± 0.40	16.60 ± 0.51	16.61 ± 0.54
			Day 14		
∑SAT	43.86 ± 0.44	43.91 ± 0.46	44.36 ± 0.68	44.56 ± 0.46	47.98 ± 0.52
∑MUFA	23.77 ± 0.37	23.60 ± 0.38	23.33 ± 0.56	22.99 ± 0.54	18.94 ± 0.43
∑TRANS	0.55 ± 0.03	0.54 ± 0.03	0.52 ± 0.02	0.53 ± 0.05	0.97 ± 0.05
20:3n-9	0.19 ± 0.04	0.20 ± 0.03	0.17 ± 0.03	0.13 ± 0.04	0.39 ± 0.11
18:2n-6	18.38 ± 0.60	17.81 ± 0.53	16.59 ± 0.38	16.93 ± 0.38	16.31 ± 0.60
18:3n-6	0.06 ± 0.02	0.15 ± 0.02	0.16 ± 0.02	0.13 ± 0.02	0.08 ± 0.03
20:2n-6	0.42 ± 0.02	0.43 ± 0.02	0.40 ± 0.02	0.41 ± 0.02	0.49 ± 0.02
20:3n-6	2.51 ± 0.17	3.17 ± 0.11	3.25 ± 0.18	3.06 ± 0.11	2.88 ± 0.14
20:4n-6	7.37 ± 0.38	7.33 ± 0.22	7.57 ± 0.24	7.43 ± 0.28	8.57 ± 0.33
22:2n-6	0.52 ± 0.05	0.52 ± 0.05	0.51 ± 0.06	0.63 ± 0.04	0.57 ± 0.03
22:4n-6	0.36 ± 0.03	0.38 ± 0.02	0.32 ± 0.03	0.32 ± 0.03	0.54 ± 0.03
20:3/20:4	0.35 ± 0.03	0.44 ± 0.02	0.43 ± 0.03	0.42 ± 0.03	0.34 ± 0.02
∑n-6 LC-PUFA	11.18 ± 0.42	11.83 ± 0.22	12.05 ± 0.26	11.85 ± 0.24	13.05 ± 0.37
18:3n-3	0.05 ± 0.02	0.09 ± 0.02	0.03 ± 0.01	0.05 ± 0.02	0.01 ± 0.01
20:5n-3	0.14 ± 0.03	0.13 ± 0.04	0.36 ± 0.02	0.42 ± 0.04	0.08 ± 0.03
22:5n-3	0.16 ± 0.03	0.20 ± 0.03	0.16 ± 0.04	0.22 ± 0.02	0.32 ± 0.02
22:6n-3	1.66 ± 0.13	1.55 ± 0.12	2.28 ± 0.12	2.17 ± 0.09	1.89 ± 0.14
∑n-3 LC-PUFA	1.95 ± 0.15	1.87 ± 0.15	2.80 ± 0.14	2.82 ± 0.11	2.29 ± 0.15
∑PUFA	31.82 ± 0.26	31.96 ± 0.48	31.79 ± 0.44	31.91 ± 0.28	32.11 ± 0.68
∑PUFA/SAT	0.73 ± 0.01	0.73 ± 0.02	0.72 ± 0.02	0.72 ± 0.01	0.67 ± 0.02
∑LC-PUFA	13.33 ± 0.52	13.91 ± 0.28	15.01 ± 0.40	14.80 ± 0.26	15.72 ± 0.44
			Day 28		
∑SAT	44.08 ± 0.45^{a,*}	43.95 ± 0.47^{b,*}	40.95 ± 3.45^{c,*}	44.52 ± 0.40^{d,*}	47.54 ± 0.63^{a,b,c,d}
∑MUFA	24.00± 0.42^{a,*}	23.63 ± 0.31^{b}	21.35 ± 1.87^{c,*}	23.05 ± 0.43^{d,*}	19.30 ± 0.63^{a,b,c,d}
∑TRANS	0.49 ± 0.06^{a,*}	0.54 ± 0.03^{b,*}	0.54 ± 0.07^{c,*}	0.58 ± 0.04^{d,*}	0.90 ± 0.06^{a,b,c,d}
20:3n-9	0.29 ± 0.03*	0.16 ± 0.02^{a,*}	0.19 ± 0.03*	0.14 ± 0.03^{b,*}	0.50 ± 0.15^{a,b}
18:2n-6	18.66 ± 0.34^{a,*}	18.41 ± 0.34*	17.31 ± 0.51*	17.64 ± 0.27*	16.51 ± 0.84^{a,*}
18:3n-6	0.09 ± 0.02	0.15 ± 0.01	0.15 ± 0.03	0.14 ± 0.02	0.09 ± 0.03*
20:2n-6	0.45 ± 0.02	0.42 ± 0.02	0.44 ± 0.02	0.39 ± 0.02*	0.46 ± 0.02
20:3n-6	2.69 ± 0.13	3.11 ± 0.17	3.29 ± 0.18*	3.00 ± 0.09	2.86 ± 0.19
20:4n-6	6.64 ± 0.21^{a,*}	6.94 ± 0.27^{b,*}	6.87 ± 0.38^{c,*}	6.71 ± 0.20^{d,*}	8.57 ± 0.47^{a,b,c,d}
22:2n-6	0.59 ± 0.05	0.58 ± 0.05	0.48 ± 0.04	0.62 ± 0.05	0.63 ± 0.04
22:4n-6	0.31 ± 0.01^{a,*}	0.35 ± 0.03^{b,*}	0.32 ± 0.03^{c,*}	0.25 ± 0.02^{d,*}	0.52 ± 0.04^{a,b,c,d}
20:3/20:4	0.41 ± 0.02*	0.46 ± 0.03*	0.50 ± 0.04^{a,*}	0.45 ± 0.02*	0.34 ± 0.02^{a}
∑n-6 LC-PUFA	10.69 ± 0.27^{a,*}	11.40 ± 0.32*	10.52 ± 0.95^{b,*}	10.99 ± 0.23^{c,*}	13.05 ± 0.55^{a,b,c}
18:3n-3	0.05 ± 0.02*	0.10 ± 0.02^{a,*}	0.06 ± 0.02*	0.06 ± 0.02*	0.01 ± 0.01^{a}
20:5n-3	0.12 ± 0.03^{a}	0.13 ± 0.04^{b,c}	0.35 ± 0.06^{b,d,*}	0.41 ± 0.05^{a,c,e,*}	0.06 ± 0.03^{d,e}
22:5n-3	0.15 ± 0.03^{a,b}	0.20 ± 0.03^{c}	0.26 ± 0.02^{a,*}	0.20 ± 0.03^{d}	0.32 ± 0.02^{b,c,d,*}
22:6n-3	1.37 ± 0.07^{a,b,*}	1.33 ± 0.13^{c,d,*}	2.20 ± 0.12^{a,c,e}	2.27 ± 0.09^{b,d,f,*}	1.72 ± 0.14^{e,f,*}
∑n-3 LC-PUFA	1.64 ± 0.09^{a,b,*}	1.66 ± 0.16^{c,d,*}	2.60 ± 0.27^{a,c,e,*}	2.88 ± 0.13^{b,d,f,*}	2.10 ± 0.16^{e,f}
∑PUFA	31.42 ± 0.28	31.87 ± 0.45	29.48 ± 2.50	31.85 ± 0.22	32.26 ± 0.48
∑PUFA/∑SAT	0.71 ± 0.01*	0.73 ± 0.02*	0.72 ± 0.02*	0.72 ± 0.01*	0.68 ± 0.02
∑LC-PUFA	12.62 ± 0.35^{a,*}	13.22 ± 0.42*	13.30 ± 1.20*	14.01 ± 0.27*	15.65 ± 0.68^{a}

[a]Common roman letter superscripts within a row indicate a significant difference ($P \leq 0.05$) between groups. For abbreviations see Table 1.

After 4 wk on the study diet, the percentage of SAT fatty acids decreased, while *cis*-monounsaturated fatty acids (MUFA) increased in all formula groups (Tables 3,4). In contrast, there were no changes in the breast-fed group, reflecting a higher percentage of SAT and a lower percentage of *cis*-MUFA in breast milk compared to the formula diets (Tables 3,4). The *trans* fatty acid content in the plasma lipids of breast-fed babies was almost twice as high as in all formula-fed groups, which corresponds to about 0.8% *trans* fatty acids in breast milk and no detectable *trans*-MUFA in formulas based on plant oil. Mead acid (20:3n-9) percentage decreased significantly over time in all formula groups, but not in HM-fed infants. In the diet, the percentage of the precursor oleic acid was higher in the formulas than in breast milk (43 vs. 31%), but traces of Mead acid were detectable only in breast milk.

At day 28, on the diets with 0.6% GLA there was no marked difference in phospholipid GLA content (0.1% in group ST vs. 0.15% in the other formula groups, not significant); only the breast-fed group showed a significant increase with time. No obvious difference was caused by additional dietary supply of n-3 LC-PUFA in the diet. The same trend was observed for the absolute concentrations, with all mean values below 2.5 mg/kg plasma. There were no significant differences in the content (absolute and relative) of DGLA, which is obtained by chain elongation of GLA. LA content increased significantly with time in all groups, but only the differences between groups ST and HM evolved to significance at day 28. The concentration of the elongation product of LA, 20:2n-6, like LA, showed no significant group differences, although only HM contained 20:2n-6. The content of the major n-6 LC-PUFA AA, which was very similar in all groups on day 0, decreased significantly until day 28 in all formula groups but not in the breast milk (0.5% AA) group, indicating that supplementation with GLA did not increase the AA content (Table 3). After 4 wk on the study diets, the AA percentage was significantly higher in the HM group, whereas no other group differences approached statistical significance. Absolute concentrations were only different between ST and HM (Table 4). The chain elongation product of AA (22:4n-6) was significantly lower in the formula-fed groups than in HM, but this cannot be ascribed to elongase activity since only breast milk contained 22:4n-6 (0.14%).

Addition of fish oil that was either high or low in EPA content did not further decrease n-6 LC-PUFA but markedly increased the DHA content in phospholipids (Tables 3,4). Mean EPA percentage was about twice as high in these groups as in all other groups, although EPA contributed only 0.06 and 0.15% to dietary fatty acids. Absolute concentrations of EPA were more than sixfold higher in the fish oil groups than in the HM group and twofold higher than in the other formula groups, respectively (Table 4). In contrast to EPA and DHA, the levels of the intermediate 22:5n-3 tended to be higher in HM infants, who received an exogenous supply (0.1%) of 22:5n-3. There was no significant difference between groups BO + FOLOW and BO + FOHIGH in any fatty acid concentration.

There was a marked influence of fish oil supply on the ratio of n-6 LC-PUFA to n-3 LC-PUFA. The ratios at day 28 were similar in breast-fed infants and in infants from groups ST and BO, whereas the n-6/n-3 LC-PUFA ratio dropped significantly ($P \leq 0.05$) from 6.27 ± 0.33 (M ± SE) to 4.23 ± 0.30 in BO + FOLOW and from 6.36 ± 0.36 to 3.97 ± 0.27 in BO + FOHIGH, respectively, and in BO it increased significantly from 5.72 ± 0.39 to 7.36 ± 0.48.

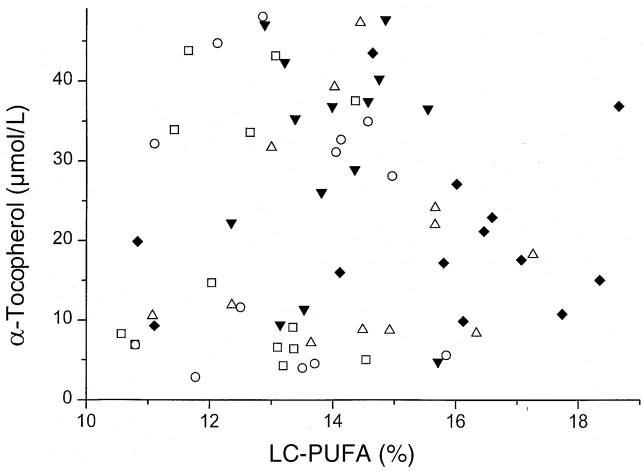

FIG. 1. Individual α-tocopherol concentrations (µmol/L) in relation to the percentage of LC-PUFA in plasma phospholipids (□ ST, ○ BO, △ BO + FOLOW, ▼ BO + FOHIGH, ◆ HM) showing neither a significant difference between groups nor a significant correlation for any of the groups. Abbreviations: LC-PUFA, long-chain polyunsaturated fatty acids; ST, standard formula; BO, borage oil; BO + FOLOW, borage oil + 0.3% docosahexaenoic acid (DHA) and 0.06% eicosapentaenoic acid (EPA); BO + FOHIGH, BO + 0.3% DHA + 0.2% EPA; HM, human milk.

The amount of antioxidative vitamins in the formulas (0.1 mg retinol and 1.3 mg tocopherol per 100 mL) resulted in plasma concentrations of retinol and α-tocopherol that were not different from breast-fed infants (Table 5). There was no detectable influence of LC-PUFA addition to the formulas on this parameter. In addition, there was no significant correlation between LC-PUFA percentage and α-tocopherol concentration (Fig. 1). Only for δ-tocopherol, which showed low concentrations in all infants and which is an isomer with low biological activity (36), could significant differences be observed between BO and HM as well as BO + FOHIGH and HM. The adjustment of α-tocopherol and retinol concentrations for Chol and TG by multiple regression according to Jordan *et al.* (37) did not reveal significant differences between groups (Table 5). In all groups there was a trend for α-tocopherol to increase with time, but it reached significance at day 28 only in groups BO and BO + FOHIGH.

DISCUSSION

This study was designed to evaluate the influence of added GLA in preterm infant formula on the composition of n-6 LC-PUFA in infant plasma phospholipids. The added GLA resulted in dietary intakes of at least 32–34 mg/kg/d in groups BO, BO + FOLOW and BO + FOHIGH, which is close to the sum of GLA, DGLA, and AA typically ingested with breast

TABLE 4
Absolute Concentrations of Fatty Acids in Plasma Phospholipids (mg/kg plasma, mean ± SE) for the Three Time Points Investigated[a]

	ST	BO	BO + FOLOW	BO + FOHIGH	HM
			Day 0		
Total fatty acids	1042.7 ± 59.7	1171.5 ± 51.8	1113.5 ± 41.2	1029.9 ± 43.4	963.2 ± 46.7
∑SAT	500.1 ± 28.6	555.7 ± 22.9	530.9 ± 20.2	496.3 ± 20.4	465.0 ± 23.3
∑MUFA	217.6 ± 17.7	253.2 ± 16.1	222.3 ± 8.7	201.0 ± 10.8	186.4 ± 9.0
∑TRANS	9.0 ± 0.9	10.3 ± 0.7	9.1 ± 0.8	9.0 ± 0.7	9.0 ± 0.6
20:3n-9	9.2 ± 2.9	11.2 ± 3.7	5.3 ± 1.0	8.3 ± 2.3	4.9 ± 1.8
18:2n-6	141.0 ± 7.7	161.9 ± 17.1	164.0 ± 15.3	150.9 ± 14.0	141.2 ± 6.8
18:3n-6	1.2 ± 0.4	1.4 ± 0.3	1.1 ± 0.3	1.2 ± 0.3	0.1 ± 0.1
20:2n-6	4.1 ± 0.4	5.5 ± 0.5	4.9 ± 0.4	4.7 ± 0.2	4.4 ± 0.3
20:3n-6	28.5 ± 2.5	31.3 ± 2.0	29.9 ± 1.6	29.2 ± 2.3	27.4 ± 2.3
20:4n-6	98.9 ± 9.3	100.7 ± 7.6	108.9 ± 5.4	94.8 ± 6.3	92.1 ± 6.4
22:2n-6	5.7 ± 0.5	5.7 ± 0.4	5.4 ± 0.4	5.7 ± 0.6	5.6 ± 0.4
22:4n-6	5.4 ± 0.7	6.4 ± 0.5	5.9 ± 0.4	5.6 ± 0.6	4.9 ± 0.3
∑n-6 LC-PUFA	142.6 ± 12.4	149.7 ± 10.0	155.1 ± 6.6	140.1 ± 8.3	134.3 ± 8.6
18:3n-3	ND	0.4 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	ND
20:5n-3	0.7 ± 0.3	1.8 ± 0.3	1.5 ± 0.5	0.9 ± 0.36	0.4 ± 0.2
22:5n-3	1.9 ± 0.4	3.2 ± 0.6	2.03 ± 0.5	2.6 ± 0.4	2.4 ± 0.3
22:6n-3	19.5 ± 1.7	22.8 ± 2.2	22.0 ± 1.2	19.5 ± 1.6	19.5 ± 1.8
∑n-3 LC-PUFA	22.0 ± 2.0	27.8 ± 2.7	25.5 ± 1.6	23.1 ± 2.0	22.3 ± 2.0
∑PUFA	316.0 ± 19.5	352.3 ± 25.8	351.0 ± 19.7	323.7 ± 19.9	302.8 ± 16.7
∑LC-PUFA	173.8 ± 15.4	188.7 ± 11.3	185.8 ± 7.6	171.4 ± 9.8	161.5 ± 11.0
			Day 14		
Total fatty acids	1142.7 ± 68.9	1210.2 ± 65.8	1136.4 ± 54.4	1124.3 ± 43.4	1063.7 ± 66.6
∑SAT	501.2 ± 30.6	530.2 ± 27.6	503.5 ± 24.7	501.9 ± 22.3	509.9 ± 32.6
∑MUFA	271.7 ± 17.0	286.9 ± 18.2	264.5 ± 12.8	257.1 ± 8.7	201.8 ± 14.4
∑TRANS	6.4 ± 0.7	6.5 ± 0.4	5.9 ± 0.4	6.126 ± 0.7	10.4 ± 0.9
20:3n-9	2.4 ± 0.5	2.5 ± 0.4	2.0 ± 0.4	1.5 ± 0.5	4.5 ± 1.8
18:2n-6	207.0 ± 9.8	216.1 ± 13.9	188.8 ± 11.8	190.8 ± 9.4	171.8 ± 10.1
18:3n-6	0.7 ± 0.2	1.8 ± 0.3	1.7 ± 0.3	1.5 ± 0.3	0.9 ± 0.4
20:2n-6	4.8 ± 0.4	5.2 ± 0.4	4.6 ± 0.3	4.6 ± 0.3	5.2 ± 0.4
20:3n-6	28.8 ± 2.6	38.5 ± 2.7	36.7 ± 2.4	34.3 ± 1.6	30.6 ± 2.6
20:4n-6	86.4 ± 9.2	88.1 ± 4.5	87.0 ± 6.1	83.7 ± 4.8	92.0 ± 7.2
22:2n-6	5.83 ± 0.6	6.1 ± 0.5	5.6 ± 0.5	7.2 ± 0.6	6.2 ± 0.6
22:4n-6	4.3 ± 0.7	4.5 ± 0.3	3.8 ± 0.4	3.6 ± 0.3	5.7 ± 0.5
∑n-6 LC-PUFA	130.2 ± 12.0	142.3 ± 7.0	137.6 ± 7.9	133.3 ± 6.0	139.7 ± 10.5
18:3n-3	0.6 ± 0.2	1.1 ± 0.2	0.4 ± 0.2	0.6 ± 0.2	0.1 ± 0.1
20:5n-3	1.6 ± 0.4	1.7 ± 0.6	4.0 ± 0.3	4.7 ± 0.5	0.9 ± 0.4
22:5n-3	1.8 ± 0.3	2.6 ± 0.4	1.8 ± 0.4	2.5 ± 0.2	3.4 ± 0.2
22:6n-3	19.2 ± 1.9	18.7 ± 1.9	26.3 ± 2.2	24.2 ± 1.0	20.4 ± 2.2
∑n-3 LC-PUFA	22.6 ± 2.3	22.9 ± 2.4	32.1 ± 2.6	31.4 ± 1.3	24.7 ± 2.5
∑PUFA	363.4 ± 21.8	386.69 ± 21.6	362.6 ± 20.0	359.1 ± 14.7	341.7 ± 21.6
∑LC-PUFA	155.2 ± 1.0	167.74 ± 8.9	171.6 ± 10.5	166.2 ± 6.8	168.9 ± 13.4
			Day 28		
Total fatty acids	1139.0 ± 44.1	1195.9 ± 65.0	1202.2 ± 47.2	1154.0 ± 57.0	1077.1 ± 67.7
∑SAT	501.4 ± 18.3	523.7 ± 26.3	532.5 ± 20.1*	514.3 ± 26.7*	510.2 ± 29.7*
∑MUFA	274.2 ± 13.2*	282.8 ± 15.6	279.3 ± 15.2*	265.1 ± 12.3*	209.7 ± 18.0
∑TRANS	5.7 ± 0.8^{a,*}	6.4 ± 0.4^{b,*}	6.9 ± 0.6*	6.8 ± 0.7^{c,*}	9.6 ± 0.7^{a,b,c}
20:3n-9	3.4 ± 0.4	2.0 ± 0.4^{a,*}	2.4 ± 0.5*	1.6 ± 0.3^{b,*}	5.7 ± 1.9^{a,b}
18:2n-6	212.3 ± 8.9*	221.4 ± 14.6*	208.0 ± 9.5*	203.2 ± 9.6*	176.3 ± 12.3*
18:3n-6	1.1 ± 0.3	1.8 ± 0.2	1.9 ± 0.4	1.7 ± 0.2	1.0 ± 0.3*
20:2n-6	5.1 ± 0.3*	5.1 ± 0.4	5.3 ± 0.3	4.5 ± 0.2	4.9 ± 0.3
20:3n-6	30.5 ± 1.7	37.8 ± 3.5	39.7 ± 3.1*	34.8 ± 2.2*	30.4 ± 2.5
20:4n-6	75.6 ± 3.8^{a,*}	82.5 ± 4.7	82.1 ± 4.7*	77.7 ± 5.0*	93.8 ± 10.2^{a}
22:2n-6	6.7 ± 0.6	6.8 ± 0.6	5.7 ± 0.5	7.4 ± 0.8*	6.8 ± 0.6*
22:4n-6	3.6 ± 0.2^{a,*}	4.2 ± 0.4^{b,*}	3.9 ± 0.3^{c,*}	3.0 ± 0.4^{d,*}	5.6 ± 0.6^{a,b,c,d}
∑n-6 LC-PUFA	121.5 ± 5.3	136.5 ± 8.3	136.7 ± 6.7*	127.4 ± 7.9	141.6 ± 12.5
18:3n-3	0.6 ± 0.2*	1.2 ± 0.2*	0.8 ± 0.2*	0.8 ± 0.2*	0.1 ± 0.1
20:5n-3	1.4 ± 0.4^{a,b}	1.7 ± 0.5^{c,d}	4.2 ± 0.8^{a,c,e,*}	4.6 ± 0.5^{b,d,f,*}	0.7 ± 0.4^{e,f}
22:5n-3	1.8 ± 0.4^{a,b}	2.5 ± 0.4	3.2 ± 0.2^{a,*}	2.3 ± 0.3	3.4 ± 0.3^{b,*}
22:6n-3	15.7 ± 1.1^{a,b}	16.1 ± 1.8^{c,d,*}	26.5 ± 1.9^{a,c,*}	26.3 ± 1.8^{b,d,e,*}	18.7 ± 2.1^{e}
∑n-3 LC-PUFA	18.9 ± 1.4^{a,b}	20.2 ± 2.4^{c,d,*}	33.9 ± 2.6^{a,c,e,*}	33.3 ± 2.2^{b,d,f,*}	22.8 ± 2.7^{e,f}
∑PUFA	357.7 ± 14.0	383.0 ± 24.1	383.6 ± 15.6	367.9 ± 18.9	347.5 ± 22.8*
∑LC-PUFA	143.7 ± 6.9	158.7 ± 10.4	172.9 ± 9.1	162.3 ± 9.8	170.1 ± 15.4

[a]Asterisk indicates a significant change ($P \leq 0.05$) from Day 0 to Day 28; common roman letter superscripts within a row indicate a significant difference ($P \leq 0.05$) between groups. For abbreviations see Table 1.

TABLE 5
Concentration (μmol/L, mean ± SE) of Retinol and Tocopherol Isomers in Infant Plasma at Study Start (day 0), After 2 wk on Study Diets (day 14), and After 4 wk on Study Diets (day 28)[a]

	ST	BO	BO + FOLOW	BO + FOHIGH	HM
			Day 0		
Retinol	0.49 ± 0.08	0.38 ± 0.03	0.39 ± 0.05	0.36 ± 0.04	0.43 ± 0.05
α-Tocopherol	13.03 ± 1.52	12.08 ± 1.86	12.18 ± 1.66	14.48 ± 1.92	13.80 ± 1.90
δ-Tocopherol	0.11 ± 0.03	0.09 ± 0.02	0.1 ± 0.02	0.11 ± 0.03	0.08 ± 0.01
β,γ-Tocopherol	0.61 ± 0.18	0.59 ± 0.14	0.48 ± 0.11	0.67 ± 0.21	0.49 ± 0.12
α-Tocopherol adj.	13.78 ± 1.33	11.13 ± 1.41	12.88 ± 1.63	15.20 ± 1.94	14.18 ± 1.87
Retinol adj.	0.51 ± 0.06	0.37 ± 0.03	0.41 ± 0.05	0.41 ± 0.04	0.43 ± 0.05
Chol (mg/dL)	101.3 ± 9.3	112.5 ± 8.6	101.9 ± 5.2	89.5 ± 6.6	104.7 ± 11.2
TG (mg/dL)	81.3 ± 8.9	116.6 ± 17.4	82.0 ± 8.6	78.6 ± 7.2	86.8 ± 7.4
			Day 14		
Retinol	0.34 ± 0.03	0.35 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.35 ± 0.03
α-Tocopherol	18.15 ± 3.7	19.26 ± 4.02	14.53 ± 2.95	26.24 ± 4.15	16.66 ± 1.84
δ-Tocopherol	0.27 ± 0.06	0.37 ± 0.09	0.22 ± 0.04	0.39 ± 0.06	0.08 ± 0.01
β,γ-Tocopherol	1.52 ± 0.44	1.5 ± 0.38	0.89 ± 0.17	1.68 ± 0.24	0.51 ± 0.06
α-Tocopherol adj.	17.78 ± 3.97	18.17 ± 3.60	14.81 ± 3.09	26.95 ± 3.86	17.26 ± 2.01
Retinol adj.	0.34 ± 0.03	0.33 ± 0.03	0.37 ± 0.03	0.39 ± 0.03	0.34 ± 0.03
Chol (mg/dL)	106.8 ± 8.6	117.5 ± 10.4	108.3 ± 5.4	95.0 ± 4.5	113.0 ± 11.5
TG (mg/dL)	99.4 ± 11.8	109.2 ± 12.6	87.7 ± 8.1	83.5 ± 11.1	81.2 ± 7.7
			Day 28		
Retinol	0.44 ± 0.04	0.39 ± 0.04	0.41 ± 0.03	0.46 ± 0.04	0.42 ± 0.05
α-Tocopherol	19.47 ± 4.44	22.1 ± 4.6*	19.84 ± 3.88	30.44 ± 3.72*	20.54 ± 2.83*
δ-Tocopherol	0.25 ± 0.05	0.37 ± 0.08[a,*]	0.28 ± 0.05*	0.43 ± 0.05[b,*]	0.12 ± 0.03[a,b]
β,γ-Tocopherol	1.36 ± 0.31	1.43 ± 0.31*	1.19 ± 0.2*	1.69 ± 0.18[a,*]	0.62 ± 0.13[a]
α-Tocopherol adj.	18.75 ± 4.54	20.58 ± 4.69*	20.16 ± 3.81	30.14 ± 3.70*	20.73 ± 2.99
Retinol adj.	0.43 ± 0.04	0.37 ± 0.04	0.39 ± 0.02	0.48 ± 0.03	0.40 ± 0.05
Chol (mg/dL)	110.5 ± 6.3	115.8 ± 9.5	116.7 ± 5.5*	100.0 ± 5.4	116.6 ± 11.0
TG (mg/dL)	104.4 ± 13.1	108.2 ± 8.2	85.1 ± 11.7	99.7 ± 16.0	87.4 ± 10.2

[a]Common roman letter superscripts within a row indicate a significant difference between groups; asterisk indicates a significant change ($P \leq 0.05$) from day 0 to day 28. Chol, cholesterol; TG, triglycerides; for other abbreviations see Table 1.

milk (38) and agrees reasonably well with the observed intake of about 43 mg/kg/d in the studied breast-fed infants.

The formulas showed a LA/α-linolenic acid ratio of about 11, which was markedly different from the mean ratio of 36 in human milk lipids, caused by the low α-linolenic acid content of human milk. The slightly higher LA content of 15.4% in breast milk compared to 13% in the formulas was not reflected by higher LA concentrations in the plasma phospholipids and is not assumed to cause an increase in AA, as the addition of LA to formulas showed only small effects on circulating AA (39).

There were no significant differences between groups with respect to plasma TG and total Chol concentrations, even though breast milk contains considerably more Chol than formulas for infants based on plant oil (40). This lack of a difference might be due to the fact that the infants were studied in the period when enteral feeding had just started. However, the concentrations were similar to values observed previously in newborn infants (41,42).

Total fatty acids in plasma phospholipids did not differ between groups or change with time and were well within the range of previously observed values in preterm infants of this age (36). AA levels were similar in all groups at study start, while there were clear differences between the formula groups and the breast milk group at the later time points, reaching significance at day 28. The decrease with time in infants not supplemented with AA is in agreement with earlier reports (36,43,44). The addition of GLA either alone or in combination with fish oil did not significantly improve the AA status as compared to the unsupplemented group. This confirms previous observations in studies where infant formulas had been supplemented with 0.5 or 0.3% GLA in addition to EPA and DHA (0.4 and 0.3%) or (0.6 and 0.4%), respectively, and either an unchanged or a decreased AA content in red blood cell lipids was observed (23,24). Thus, even in the absence of dietary n-3 LC-PUFA, an intake of up to 0.6% GLA failed to produce a significant increase of AA status. On the other hand, GLA supplementation in children and adults showed differing effects on AA status (18,45). Johnson *et al*. (18) reported a significant increase of total plasma AA percentage after 3 wk of GLA (1.5–6 g/d) intake, which corresponded to intakes ≥20 mg/kg/d assuming a body weight of 70 kg. Animal studies clearly showed that GLA is converted to AA (16), but whether there is an increase of AA concentrations in humans seems to depend on further factors. Stable isotope studies in term infants identified Δ-6 desaturation as the limiting step in the endogenous conversion of dietary LA to AA (13). The principal activity of the conversion of LA *via* GLA and DGLA to AA in neonates has been demonstrated *in vitro* (46) and *in vivo* (12–14), but the addition of the intermediate GLA to the diet does not seem to im-

prove the yield of AA. As it can be assumed that GLA has been efficiently absorbed (47), there must be some other physiological reason.

Although DGLA was delivered only in trace amounts with all tested formulas, DGLA showed plasma phospholipid concentrations in the GLA-containing formula groups that were higher than in the HM group, but lower values were present in the ST group, although differences were not significant (Table 3). It appears that 0.6% of GLA in the diet may well replace about 0.4% of DGLA, as provided with breast milk lipids (22), thus pointing toward rapid elongation. This is in accordance with other findings in neonates, healthy adults, and patients with atopic dermatitis (18,24,48). A further indication for an active fatty acid elongase in the studied preterm neonates is the finding that 20:2n-6, the elongation product of LA, was found in similar concentrations in all feeding groups, although only breast milk contained appreciable amounts of this fatty acid.

Phospholipid GLA concentrations did not respond to dietary GLA supply, which may be explained by the low affinity of GLA toward incorporation into phospholipids (49). Moreover, lipoprotein lipase preferentially hydrolyzes fatty acids with 16 or 18 carbon atoms from chylomicrons as compared to polyenoic fatty acids with 20 carbon atoms (50). There appears to be more uptake of the shorter fatty acids by tissues for oxidation, while a lower proportion is available for conversion to LC-PUFA or direct incorporation into phospholipids in the liver. This is in agreement with the observation of Leyton *et al*. (51) who reported that 27% of orally administered GLA in rats was oxidized to CO_2 within 24 h, while only 14% each of DGLA or AA were recovered in breath. Brouwer *et al.* (52) and Woltil *et al.* (53) reported that dietary GLA is preferentially incorporated into Chol esters in adults and that a significantly higher GLA content in plasma Chol esters of low birth weight infants was found when they were fed a formula with 0.3% GLA than when they were fed HM.

Obviously, dietary GLA was efficiently elongated to DGLA, possibly already in the intestine (54). Elongation products and remaining precursors reached the circulation after incorporation into chylomicrons, and it is possible that from these lipoproteins GLA was preferentially eliminated and oxidized. Thus only a negligible portion of GLA might have been available in the liver for conversion into AA and incorporation into phospholipids. Owing to the active elongation, the ingested GLA appears in the liver converted to DGLA, and might be handled similarly to dietary DGLA. After feeding deuterated DGLA to adults and measuring the concentration of deuterated AA in plasma, it was estimated that only a very low percentage of exogenous DGLA is converted to AA (55,56). The study by Emken *et al*. (56) offers a possible explanation for an inconsistent effect of GLA feeding on AA concentrations, as they noted that in subjects with an AA intake of 0.2 g/d, only about 2% of DGLA was desaturated, while this percentage increased to 18% with an AA intake of 1.7 g/d. In a larger group of subjects it was demonstrated that the high AA intakes were followed by elevated AA concentrations in all plasma lipid fractions and blood cells (57). Hence, the effect of GLA supplementation might depend on the current AA status. As there was no AA intake in the formula groups studied and AA concentrations were lower than those observed by Emken *et al.*, in combination with a possible immaturity of the desaturating enzyme system, these findings could explain the absence of an increase of AA after GLA supplementation.

This study also investigated the influence of fish oil supplementation on infant plasma phospholipid composition. The addition of 0.3% DHA established phospholipid DHA values similar to those found in the HM-fed group, and even exceeded them in a population with rather low HM contents of DHA. These findings are in accordance with published studies in term infants; e.g., Horby-Jorgensen *et al*. (24) reported 1.9% DHA in red blood cell phosphatidylcholine after feeding a formula with 0.3% DHA for 1 mon compared to 1.25% in an unsupplemented group. In preterm infants 0.2% DHA compared to 0% DHA in dietary fatty acids caused a concentration difference of about 5 mg/L in plasma total phospholipid DHA after 4 wk (25). In previous studies, dietary fish oil without simultaneous addition of preformed AA into the formula had led to AA concentrations below those of groups with LC-PUFA unsupplemented (25), which was explained by the competition of EPA and DHA for the incorporation into phospholipids (58) and an inhibitory effect of n-3 fatty acids on desaturase activities (59,60). It has been postulated that DHA and not EPA is the potent desaturase inhibitor in humans and that it mainly affects Δ-6 desaturation (61). The absence of a further AA decrease with DHA intake may be explained by the GLA supply in both fish oil-supplemented groups, thereby circumventing the Δ-6 desaturation step. Effects similar to those seen here were reported by Ward *et al*. (62) in brain phospholipids of artificially reared rat pups on diets varying in their GLA content from 0.5 to 3% and with either 0.5 or 2.5% DHA (62). They demonstrated that even 3% GLA in dietary lipids did not significantly increase AA content compared to 0.5% GLA. In contrast to our results, Makrides *et al*. (23) found a lower AA value of 10.2 ± 1.0% (mean ± SD) of total fatty acids from erythrocytes in term infants fed for 16 wk a formula with 0.58% EPA, 0.36% DHA, and 0.27% GLA compared to an unsupplemented control group (12.9 ± 1.3%), but they did not study a group fed the same dose of n-3 LC-PUFA but no GLA.

Tocopherol and retinol did not differ between groups on day 28 in either the absolute concentrations or in α-tocopherol and retinol concentrations after adjustment by multiple regression. At study day 0, 50% of the infants showed α-tocopherol concentrations below a level of 12.4 μmol/L, considered as the threshold for vitamin E sufficiency (63). This number decreased to 38% at day 28 with mean values for all groups from day 14 on above the threshold of sufficiency. Although there are suggestions to aim at much higher vitamin E concentrations, the intake in the formula-fed groups was 1.6 mg vitamin E/100 kcal (1.9 mg vitamin E/g PUFA), which is well above recommendations of the European Society for Pediatric Gastroenterology Hepatology and Nutrition (64).

In conclusion, our results are in line with previous findings (22,24,53,65) and show that addition of 0.6% GLA to formula diets does not increase AA content in plasma phospholipids of preterm infants and does not achieve values similar to comparable HM-fed infants. This effect does not seem to depend on the n-3 LC-PUFA content of the diet with the dose ranges studied. Although AA in the diet cannot be substituted by equimolar amounts of GLA, its addition may have other beneficial effects, e.g., increased high density lipoproteins (66). For the achievement of a LC-PUFA status similar to breast-fed infants, the supplementation of AA and n-3 LC-PUFA seems necessary.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Oross Istváné, Tóth Lászlóné, Dr. Kóbor Lászlóné, and Töröcsik Lászlóné for expert technical assistance. This study was supported in part by Deutsche Forschungsgemeinschaft, Bonn, Germany (Ko 912/5-2), and Hoffmann-La Roche Ltd., Basel, Switzerland. The experimental study formulas were kindly provided by Nutricia, Zoetermeer, The Netherlands.

REFERENCES

1. Clandinin, M.T., Van Aerde, J.E., Parrott, A., Field, C.J., Euler, A.R., and Lien, E.L. (1997) Assessment of the Efficacious Dose of Arachidonic and Docosahexaenoic Acids in Preterm Infant Formulas: Fatty Acid Composition of Erythrocyte Membrane Lipids, *Pediatr. Res. 42,* 819–825.
2. Koletzko, B., Demmelmair, H., and Socha, P. (1998) Nutritional Support of Infants and Children: Supply and Metabolism of Lipids, in *Bailliere's Clinical Gastroenterology* (Goulet, O., ed.), pp. 671–696, Bailliere Tindall, London.
3. Carlson, S.E., Werkman, S.H., Rhodes, P.G., and Tolley, E.A. (1993) Visual-Acuity Development in Healthy Preterm Infants: Effect of Marine-Oil Supplementation, *Am. J. Clin. Nutr. 58,* 35–42.
4. Willats, P., Forsyth, J.S., DiModugno, M.K., Varma, S., and Colvin, M. (1998) Effect of Long-Chain Polyunsaturated Fatty Acids in Infant Formula on Problem Solving at 10 Months of Age, *Lancet 352,* 688–691.
5. Birch, E.E., Garfield, S., Hoffman, D.R., Uauy, R., and Birch, D.G. (2000) A Randomized Controlled Trial of Early Dietary Supply of Longchain Polyunsaturated Fatty Acids and Mental Development in Term Infants, *Dev. Med. Child Neurol. 42,* 174–181.
6. Koletzko, B., Diener, U., Fink, M., Demmelmair, H., von Schönaich, P., and Bernsau, U. (1999) Supply and Biological Effects of Long-Chain Polyunsaturated Fatty Acids (LCPUFA) in Premature Infants, in *Nutrition of the Extremely Low Birthweight Infant* (Ziegler, E., Lucas, A., and Moro, G., eds.), pp. 33–48, Lippincott-Raven, Philadelphia.
7. Smith, W.L., Borgeat, P., and Fitzpatrick, F.A. (1991) The Eicosanoids: Cyclooxygenase, Lipoxygenase, and Epoxygenase Pathways, in *Biochemistry of Lipids, Lipoproteins and Membranes* (Vance, J., ed.), pp. 297–325, Elsevier Science Publishers, Amsterdam.
8. Hamosh, M., and Salem, N., Jr. (1998) Long-Chain Polyunsaturated Fatty Acids, *Biol. Neonate 74,* 106–120.
9. Amusquivar, E., Rupérez, F.J., Barbas, C., and Herrera, E. (2000) Low Arachidonic Acid Rather Than α-Tocopherol Is Reponsible for the Delayed Postnatal Development in Offspring of Rats Fed Fish Oil Instead of Olive Oil During Pregnancy and Lactation, *J. Nutr 130,* 2855–2865.
10. Koletzko, B., and Braun, M. (1991) Arachidonic Acid and Early Human Growth: Is There a Relation? *Ann. Nutr. Metab. 35,* 128–131.
11. Carlson, S.E., Cooke, R.J., Werkman, S.H., and Tolley, E.A. (1992) First Year Growth of Preterm Infants Fed Standard Compared to Marine Oil n-3 Supplemented Formula, *Lipids 27,* 901–907.
12. Carnielli, V.P., Wattimea, D.J.L., Luijendijk, I.H.T., Boerlage, A., Degenhart, H.J., and Sauer, P.J.J. (1996) The Very Low Weight Premature Infant Is Capable of Synthesizing Arachidonic and Docosahexaenoic Acids from Linoleic and Linolenic Acids, *Pediatr. Res. 40,* 169–174.
13. Szitanyi, P., Koletzko, B., Mydlilova, A., and Demmelmair, H. (1999) Metabolism of ^{13}C-Labelled Linoleic Acid in Newborn Infants During the First Week of Life, *Pediatr. Res. 45,* 669–673.
14. Sauerwald, T.U., Hachey, D.L., Jensen, C.L., Chen, H., Anderson, R.E., and Heird, W.C. (1997) Intermediates in Endogenous Synthesis of C22:6ω-3 and C20:4ω-6 by Term and Preterm Infants, *Pediatr. Res. 41,* 183–187.
15. Gibson, R.A., and Makrides, M. (1998) The Role of Long Chain Polyunsaturated Fatty Acids (LCPUFA) in Neonatal Nutrition, *Acta Paediatr. 87,* 1017–1022.
16. Luthria, D.L., and Sprecher, H. (1995) Metabolism of Deuterium-Labeled Linoleic, 6,9,12-Octadecatrienoic, 8,11,14-Eicosatrienoic, and Arachidonic Acids in the Rat, *J. Lipid Res. 36,* 1897–1904.
17. Sprecher, H., Voss, A., Careaga, M., and Hadjiagapiou, C. (1987) Interrelationships Between Polyunsaturated Fatty Acid and Membrane Lipid Synthesis, in *Polyunsaturated Fatty Acids and Eicosanoids* (Lands, W.E.M., ed.), pp. 154–168, American Oil Chemists' Society, Champaign.
18. Johnson, M.M., Swan, D.D., Surette, M.E., Stegner, J., Chilton, T., Fonteh, A.N., and Chilton, F.H. (1997) Dietary Supplementation with Gamma-Linolenic Acid Alters Fatty Acid Content and Eicosanoid Production in Healthy Humans, *J. Nutr. 127,* 1435–1444.
19. Koletzko, B., and Sinclair, A. (1999) Long-Chain Polyunsaturated Fatty Acids in Diets for Infants: Choices for Recommending and Regulating Bodies and for Manufacturers of Dietary Products, *Lipids 34,* 215–220.
20. Horrobin, D.F. (1993) Fatty Acid Metabolism in Health and Disease: the Role of Δ-6-Desaturase, *Am. J. Clin. Nutr. 57,* 732S–737S.
21. Morse, P.F., Horrobin, D.F., Manku, M.S., and Stewart, J.C.M. (1989) Meta-Analysis of Placebo-Controlled Studies of the Efficacy of Gamma-Linolenic Acid in the Treatment of Atopic Eczema, *Br. J. Dermatol. 121,* 75–90.
22. Gibson, R.A., Neumann, M.A., and Makrides, M. (1998) The Effects of Diets Rich in Docosahexaenoic Acid and/or γ-Linolenic Acid on Plasma Fatty Acid Profiles in Term Infants, in *Lipids in Infant Nutrition* (Huang, Y.S., and Sinclair, A.J., eds.), pp. 19–28, AOCS Press, Champaign.
23. Makrides, M., Neumann, M., Simmer, K., Pater, J., and Gibson, R. (1995) Are Long-Chain Polyunsaturated Fatty Acids Essential Nutrients in Infancy? *Lancet 345,* 1463–1468.
24. Horby Jorgensen, M., Holmer, G., Lund, P., Hernell, O., and Fleischer Michaelsen, K. (1998) Effect of Formula Supplemented with Docosahexaenoic Acid and γ-Linolenic Acid on Fatty Acid Status and Visual Acuity in Term Infants, *JPGN 26,* 412–421.
25. Carlson, S.E., Cooke, R.J., Rhodes, P.G., Peeples, J.M., Werkman, S.H., and Tolley, E.A. (1991) Long-Term Feeding of Formulas High in Linolenic Acid and Marine Oil to Very Low Birth Weight Infants: Phospholipid Fatty Acids, *Pediatr. Res. 30,* 404–412.
26. Blonk, M.C., Bilo, H.J.G., Nauta, J.J.P., Popp-Snijders, C., Mul-

der, C., and Donker, J.M. (1990) Dose-Response Effects of Fish-Oil Supplementation in Healthy Volunteers, *Am. J. Clin. Nutr. 52,* 120–127.
27. Koletzko, B., Decsi, T., and Sawatzki, G. (1994) Vitamin E Status of Low Birthweight Infants Fed Formula Enriched with Long-Chain Polyunsaturated Fatty Acids, *Int. J. Vit. Nutr. Res. 65,* 101–104.
28. Meydani, N., Natiello, F., Goldin, B., Free, N., Woods, M., Schaefer, E., Blumberg, J., and Gorbach, S. (1991) Effect of Long-Term Fish Oil Supplementation on Vitamin E Status and Lipid Peroxidation in Women, *J. Nutr. 121,* 484–491.
29. Frank, L. (1992) Antioxidants, Nutrition and Bronchopulmonary Disease, *Clin. Perinatol. 6,* 221–225.
30. Muggli, R. (1989) Dietary Fish Oil Increases the Requirement for Vitamin E in Humans, in *Health Effects of Fish and Fish Oils* (Chandra, R.K., ed.), pp. 201–210, ARTS Biomedical Publishers & Distributors, St. John's, Newfoundland.
31. Decsi, T., and Koletzko, B. (1995) Growth, Fatty Acid Composition of Plasma Lipid Classes, and Plasma Retinol and Alpha-Tocopherol Concentrations in Full-Term Infants Fed Formula Enriched with ω-6 and ω-3 Long-Chain Polyunsaturated Fatty Acids, *Acta Paediatr. 84,* 725–732.
32. Kolarovic, L., and Fournier, N.C. (1986) A Comparison of Extraction Methods for the Isolation of Phospholipids from Biological Sources, *Anal. Biochem. 156,* 244–250.
33. Carnielli, V.P., Pederzini, F., Vittorangeli, R., Luijendijk, I.H.T., Boomaars, W.E.M., Pedrotti, D., and Sauer, P.J.J. (1996) Plasma and Red Blood Cell Fatty Acid of Very Low Birth Weight Infants Fed Exclusively with Expressed Preterm Human Milk, *Pediatr. Res. 39,* 671–679.
34. Fidler, N., Sauerwald, T.U., Koletzko, B., and Demmelmair, H. (1998) Effects of Human Milk Pasteurization and Sterilization on Available Fat Content and Fatty Acid Composition, *J. Pediatr. Gastroenterol. Nutr. 27,* 317–322.
35. Göbel, Y., Schaffer, C., and Koletzko, B. (1997) Simultaneous Determination of Low Plasma Concentrations of Retinol and Tocopherols in Preterm Infants by a High-Performance Liquid Chromatographic Micromethod, *J. Chromatogr. B 688,* 57–62.
36. Koletzko, B., Edenhofer, S., Lipowsky, G., and Reinhardt, D. (1995) Effects of a Low Birthweight Infant Formula Containing Human Milk Levels of Docosahexaenoic and Arachidonic Acids, *J. Pediatr. Gastroenterol. Nutr. 21,* 200–208.
37. Jordan, P., Brubacher, D., Moser, U., Stahelin, H.B., and Gey, K.F. (1995) Vitamin E and Vitamin A Concentrations in Plasma Adjusted for Cholesterol and Triglycerides by Multiple Regression, *Clin. Chem. 41,* 924–927.
38. Koletzko, B., and Rodriguez-Palmero, M. (1999) Polyunsaturated Fatty Acids in Human Milk and Their Role in Early Infant Development, *J. Mammary Gland Biol. Neoplasia 4,* 269–284.
39. Clark, K.J., Makrides, M., Neumann, M., and Gibson, R.A. (1992) Determination of the Optimal Ratio of Linoleic Acid to α-Linolenic Acid in Infant Formulas, *J. Pediatr. 120,* S151–S158.
40. Rodriguez-Palmero, M., Koletzko, B., Kunz, C., and Jensen, R. (1999) Nutritional and Biochemical Properties of Human Milk, Part II: Lipids, Micronutrients, and Bioactive Factors, *Clin. Perinatol. 26/2,* 335–359.
41. Decsi, T., Fekete, M., and Koletzko, B. (1997) Plasma Lipid and Apolipoprotein Concentrations in Full Term Infants Fed Formula Supplemented with Long-Chain Polyunsaturated Fatty Acids and Cholesterol, *Eur. J. Pediatr. 156,* 397–400.
42. Genzel-Boroviczeny, O., D'Harlingue, A.E., Kao, L.C., Scott, C., and Forte, T.M. (1988) High-Density Lipoprotein Subclass Distribution in Premature Newborns Before and After the Onset of Enteral Feeding, *Pediatr. Res. 23,* 543–547.
43. Clandinin, T.M., Parrott, A., Van Aerde, J.E., Hervada, A.R., and Lien, E. (1992) Feeding Preterm Infants a Formula Containing C20 and C22 Fatty Acids Simulates Plasma Phospholipid Fatty Acid Composition of Infants Fed Human Milk, *Early Human Devel. 31,* 41–51.
44. Uauy, R.D., Birch, D.G., Birch, E.E., Tyson, J.E., and Hooman, D.R. (1990) Effect of Dietary Omega-3 Fatty Acids on Retinal Function of Very-Low-Birth-Weight Neonates, *Pediatr. Res. 28,* 485–492.
45. Horrobin, D.F., Ells, K.M., Morse-Fisher, N., and Manku, M.S. (1991) The Effect of Evening Primrose Oil, Safflower Oil and Paraffin on Plasma Fatty Acid Levels in Humans: Choice of an Appropriate Placebo for Clinical Studies on Primrose Oil, *Prostaglandins Leukotrienes Essent. Fatty Acids 42,* 245–249.
46. Poisson, J.-P., Dupuy, R.-P., Sarda, P., Descomps, B., Narce, M., Rieu, D., and De Paulet, A.C. (1993) Evidence That Liver Microsomes of Human Neonates Desaturate Essential Fatty Acids, *Biochim. Biophys. Acta 1167,* 109–113.
47. Morgan, C., Stammers, J., Colley, J., Spencer, S.A., and Hull, D. (1998) Fatty Acid Balance Studies in Preterm Infants Fed Formula Milk Containing Long-Chain Polyunsaturated Fatty Acids (LCP) II, *Acta Paediatr. 87,* 318–324.
48. Marra, F., Riccardi, D., Melani, L., Spadoni, S., Galli, C., Fabrizio, P., Tosti-Guerra, C., Carloni, V., Gentilini, P., and Laffi, G. (1998) Effects of Supplementation with Unsaturated Fatty Acids on Plasma and Membrane Lipid Composition and Platelet Function in Patients with Cirrhosis and Defective Aggregation, *J. Hepatol. 28,* 654–661.
49. Leyton, J., Drury, P.J., and Crawford, M.A. (1987) *In Vivo* Incorporation of Labeled Fatty Acids in Rat Liver Lipids After Oral Administration, *Lipids 22,* 553–558.
50. Ekström, B., Nilsson, A., and Akesson, B. (1989) Lipolysis of Polyenoic Fatty Acid Esters of Human Chylomicrons by Lipoprotein Lipase, *Eur. J. Clin. Invest. 19,* 259–264.
51. Leyton, J., Drury, P.J., and Crawford, M.A. (1987) Differential Oxidation of Saturated and Unsaturated Fatty Acids *in Vivo* in Rats, *Br. J. Nutr. 57,* 383–393.
52. Brouwer, D.A.J., Hettema, Y., van Doormaal, J.J., and Muskiet, F.A.J. (1998) γ-Linolenic Acid Does Not Augment Long-Chain Polyunsaturated Fatty Acid ω-3 Status, *Prostaglandins Leukotrienes Essent. Fatty Acids 59,* 329–334.
53. Woltil, H.A., van Beusekom, C.M., Schaafsma, A., Okken, A., and Muskiet, F.A.J. (1999) Does Supplementation of Formula with Evening Primrose and Fish Oils Augment Long Chain Polyunsaturated Fatty Acid Status of Low Birthweight Infants to That of Breast-Fed Counterparts? *Prostaglandins Leukotrienes Essent. Fatty Acids 60,* 199–208.
54. Christiansen, E.N., Rostveit, T., Norum, K.R., and Thomassen, M.S. (1986) Fatty Acid Chain Elongation in the Rat Small Intestine, *Biochem. J. 273,* 293–295.
55. Boustani, S.E., Causse, J.E., Descomps, B., Monnier, L., Mendy, F., and De Paulet, A.C. (1989) Direct *in Vivo* Characterization of Delta 5 Desaturase Activity in Humans by Deuterium Labeling: Effect of Insulin, *Metabolism 38,* 315–321.
56. Emken, E.A., Adlof, R.O., Duval, S.M., and Nelson, G.J. (1997) Influence of Dietary Arachidonic Acid on Metabolism *in Vivo* of 8*cis*,11*cis*, 14-Eicosatrienoic Acid in Humans, *Lipids 32*, 441–448.
57. Nelson, G.J., Schmidt, P.C., Bartolini, G., Kelley, D.S., Phinney, S.D., Kyle, D., Silbermann, S., and Schaefer, E.J. (1997) The Effect of Dietary Arachidonic Acid on Plasma Lipoprotein Distributions, Apoproteins, Blood Lipid Levels, and Tissue Fatty Acid Composition in Humans, *Lipids 32,* 427–433.
58. Cleland, L.G., James, M.J., Neumann, M.A., D'Angelo, M., and Gibson, R.A. (1992) Linoleate Inhibits EPA Incorporation from Dietary Fish-Oil Supplements in Human Subjects, *Am. J. Clin. Nutr. 55,* 395–399.
59. Garg, M.L., Sebovka, E., Thomson, A.B.R., and Clandinin, M.T. (1988) Delta 6-Desaturase Activity in Liver Microsomes

of Rats Fed Diets Enriched with Cholesterol and/or Omega 3 Fatty Acids, *Biochem. J. 249,* 351–356.
60. Garg, M.L., Thomson, A.B.R., and Clandinin, M.T. (1988) Effect of Dietary Cholesterol and/or ω-3 Fatty Acids on Lipid Composition and Delta 5-Desaturase Activity of Rat Liver Microsomes, *J. Nutr. 118,* 661–668.
61. Grimsgaard, S., Bonaa, K.H., Hansen, J.B., and Nordoy, A. (1997) Highly Purified Eicosapentaenoic Acid and Docosahexaenoic Acid in Humans Have Similar Triacylglycerol-Lowering Effects but Divergent Effects on Serum Fatty Acids, *Am. J. Clin. Nutr. 66,* 649–659.
62. Ward, G.R., Huang, Y.S., Xing, H.C., Bobik, E., Wauben, I., Auestad, N., Montalto, M., and Wainwright, P.E. (1999) Effects of γ-Linolenic Acid and Docosahexaenoic Acid in Formulae on Brain Fatty Acid Composition in Artificially Reared Rats, *Lipids 34,* 1057–1063.
63. Kaempf, D.E., and Linderkamp, O. (1998) Do Healthy Premature Infants Fed Breast Milk Need Vitamin E Supplementation: α- and γ-Tocopherol Levels in Blood Components and Buccal Mucosal Cells, *Pediatr. Res. 44,* 54–59.
64. Aggett, P., Bresson, J.-L., Hernell, O., Koletzko, B., Lafeber, H., Fleischer Michaelsen, K., Micheli, J.-L., Ormisson, A., Salazar de Sousa, J., and Weaver, L. (1998) Comment on the Vitamin E Content in Infant Formulas, Follow-on Formulas, and Formulas for Low Birth Weight Infants, *JPGN 26,* 351–352.
65. Ghebremeskel, K., Leighfield, M., Leaf, A., Costeloe, K., and Crawford, M. (1995) Fatty Acid Composition of Plasma and Red Cell Phospholipids of Preterm Babies Fed on Breast Milk and Formulae, *Eur. J. Pediatr. 154,* 46–52.
66. Van Biervliet, J.P., Vinaimont, R., Vercaemst, R., and Rosseneu, M. (1992) Serum Cholesterol, Cholesteryl Ester, and High-Density Lipoprotein Development in Newborn Infants: Response to Formulas Supplemented with Cholesterol and γ-Linolenic Acid, *J. Pediatr. 120,* S101–S108.

[Received May 24, 2000, and in revised form April 27, 2001; revision accepted May 22, 2001]