Essential fatty acids in full term infants fed breast milk or formula

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Abstract

To determine the biochemical effects of the fatty acid composition of plasma lipids, two groups of 10 healthy full term infants who were either exclusively breast fed or received a formula with similar contents of linoleic and α linolenic acids, but without long chain polyunsaturated (LCP) fatty acids, were studied prospectively. Plasma phospholipid, triglyceride, and sterol ester fatty acids were determined at the age of 2, 4, and 8 weeks by high resolution capillary gas chromatography.

Breast fed infants maintained stable LCP fatty acid concentrations throughout the study. Formula fed infants had significantly lower median values of arachidonic acid (AA) at the ages of 2 (6.9 v 9.5% wt/wt) and 4 weeks (5.9 v 7.9%) and docosahexaenoic acid (DHA) at the ages of 4 (1.1 v 1.7%) and 8 weeks (1.0 v 1.7%) in plasma phospholipids. Median AA values in triglycerides were also significantly lower in the infants receiving formula at

the ages of 2 (0.4 v 0.6%) and 4 weeks (0.3 v 0.6%).

It is concluded that formula fed full term infants are unable to match the ω -3 and ω -6 LCP status of breast fed full term infants until at least two months after birth.

(Arch Dis Child 1995; 72: F23-F28)

Keywords: essential fatty acids, term infants, breast milk, formula.

Long chain polyunsaturated (LCP) fatty acids are essential for early human growth and development.¹⁻³ LCP fatty acids of the ω -6 and ω -3 families are synthesised from linoleic (C18:2n-6, LA) and α linolenic (C18:3n-3, ALA) acids, respectively (figure). (Short formulae for fatty acids state the number of carbon atoms in the acyl chain, the number of double bonds and, in the case of an unsaturated fatty acid, the distance of the terminal double bond from the ω carbon atom.) Large amounts of ω -6 and ω -3 LCP, predominantly arachidonic (C20:4n-6, AA) and docosahexaenoic (C22:6n-3, DHA) acids, are deposited in the developing brain and retina during prenatal and early postnatal growth.⁴⁻⁶ In preterm infants the availability of DHA is related to development of visual function,⁷⁻¹⁰ and that of AA to body growth.^{11 12} The incorporation of LCP into formulas for low birthweight infants has been recommended in Europe,¹³ because it was shown to improve biochemical and some functional variables.

It is less clear whether formula fed, healthy infants born at term would also benefit from preformed dietary LCP. Term infants have larger endogenous LCP pools at birth, have less neonatal pathology, and their rate of tissue accretion is much slower than that of preterm infants. However, it is not known whether the activity of endogenous LCP synthesis is fully active in term infants after birth. Breast milk contains small, but nutritionally important, amounts of preformed ω -6 and ω -3 LCP fatty acids,¹⁴ but most commercially available infant formulas do not.¹⁵ Therefore, we studied prospectively the biochemical effects of feeding human milk or a vegetable oil based formula on the fatty acid composition of plasma lipid classes in healthy infants during the first two months of life.

Methods

Twenty apparently healthy, full term, and appropriate for gestational age infants were enrolled in the study at the Department of Paediatrics, University Medical School of Pécs,

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Accepted 20 July 1994

Metabolic pathways for the synthesis of the major LCP fatty arachidonic (C20:4n-6) and docosahexaenoic (C22:6n-3) (DHA) acids. Recent evidence²² suggests that the synthesis of DHA (C22:6n-3) from eicosapentaenoic acid (C20:5n-3) is an indirect conversion, including chain elongation to C24:5n-3, δ -6-desaturation, and peroxisomal chain shortening.

Table 1 Major fatty acids in total lipids of human milk in Hungary¹⁶ and formula¹⁵ used in the study (% wt/wt of total fatty acids)

	Human milk*	Formula†
Total saturated fatty acids	40.9	48.4
Total cis-monounsaturated fatty acids	44.8	34.4
Omega-6 polyunsaturated fatty acids:		
Linoleic acid (C18:2n-6)	11.0	13.2
Arachidonic acid (C20:4n-6)	0.5	ND
Omega-3 polyunsaturated fatty acids:		
Alpha-linolenic acid (C18:3n-3)	1.2	1.0
Docosahexaenoic acid (C22:6n-3)	0.1	ND
Total LCP	1.5	0.1

*Mean of 13 breast milk samples; †median of four determinations. ND=not detectable; LCP=long chain polyunsaturated fatty acid.

after the study protocol had been approved by the local ethics committee and the informed consent of the parents had been obtained. Breast feeding was encouraged, but the method of feeding was determined by the parents. The infants were either breast fed (n=10) or received a commercially available formula (n=10) based on cow's milk protein and vegetable fat (Pre-Aptamil; Milupa, Puch, Austria). Infants were regarded as formula fed if the amount of human milk did not exceed 20% of the daily intake. However, by the age of 2 weeks all infants in the formula fed group had been fully weaned. The fatty acid compo-

sition of breast milk in Hungary¹⁶ and the formula used in this study¹⁵ are shown in table 1. All infants were fed as required.

Anthropometric measurements (body weight, length, head and chest circumferences) were taken and venous blood samples for fatty acid analysis obtained at the ages of 2, 4, and 8 weeks. We aimed at blood sampling in the late morning and at least two hours after the last meal, but this time interval to the last feeding was not always achieved, in particular in breast fed infants who suckled frequently. Blood for fatty acid analysis was collected into tubes containing sodium ethylene-diamine-tetra-acetate (1 mg/ml), centrifuged immediately, and blood plasma was stored at -20°C until analysis.

Fatty acid analysis was carried out at the Kinderpoliklinik at the University of Munich. Plasma lipids were extracted from 0.25 ml plasma with chloroform-methanol¹⁷ after the addition of internal standards. Lipid classes were separated by thin layer chromatography.¹⁸ Fatty acids in plasma phospholipids, triglycerides, and sterol esters were transesterified with methanol and hydrochloric acid¹⁹ and analysed by high resolution capillary gas liquid chromatography using a Hewlett-Packard Series II 5890 gas chromatograph with on-column injection and flame ionisation detection. The technical parameters of chromatography have been described in detail

Table 2 Fatty acid content (mg/l) and composition (% wt/wt) of plasma phospholipids in infants fed breast milk (BM) or formula (F) (median (Q₁, Q₃))

		Age (weeks)		
		2	4	8
Sum (mg/l)				
	BM	1222 (1090, 1437)	1055 (933, 1238)	890 (715, 986)*
	F	1214 (1020, 1290)	927 (855, 1478)	1059 (950, 1207)*
Saturated fatty acids				
Total	BM	48.47 (47.50, 49.38)	47.77 (46.38, 48.07)	46.89 (46.23, 47.71)
	F	47.87 (45.90, 49.41)	47.71 (46.92, 48.17)	47.90 (46.28, 48.56)
Cis-monounsaturated fatty acids				
Total	BM	16.10 (15.19, 17.43)	16.32 (15.07, 16.79)	15.99 (15.23, 17.41)
	F	17.56 (16.54, 18.20)	16.24 (15.34, 16.96)	15.13 (14.12, 15.63)
Trans-fatty acids				
Total	BM	0.51 (0.24, 0.69)	0.29 (0.18, 0.64)	0.43 (0.26, 0.80)
	F	0.48 (0.24, 0.60)	0.58 (0.20, 0.73)	0.30 (0.18, 0.61)
N-6 PUFA				
C18:2n-6	BM	20.50 (15.70, 22.70)	16.47 (11.58, 20.73)	16.54 (12.57, 20.23)
	F	21.37 (19.10, 22.42)	22.50 (21.98, 23.81)	24.27 (22.87, 24.98)
C18:3n-6	BM	0.10 (0.02, 0.11)	0.11 (0.06, 0.14)	0.14 (0.10, 0.18)
	F	0.14 (0.05, 0.18)	0.11 (0.08, 0.15)	0.10 (0.08, 0.12)
C20:2n-6	BM	0.28 (0.25, 0.31)	0.31 (0.24, 0.37)	0.38 (0.27, 0.42)
	F	0.33 (0.23, 0.40)	0.29 (0.26, 0.38)	0.31 (0.28, 0.38)
C20:3n-6	BM	2.42 (2.19, 2.67)****	2.89 (2.46, 3.26)	3.57 (3.00, 3.80)****
	F	2.63 (2.43, 2.98)	2.90 (2.58, 3.71)	2.98 (2.24, 3.30)
C20:4n-6	BM	9.46 (7.96, 10.94)*	7.90 (6.71, 11.53)*	7.90 (5.42, 12.18)
	F	6.89 (6.36, 7.68)*	5.88 (5.36, 7.17)*	5.99 (4.78, 6.57)
C22: 4n-6	BM	0.38 (0.34, 0.49)	0.44 (0.34, 0.83)	0.48 (0.40, 1.09)
	F	0.42 (0.36, 0.53)	0.51 (0.36, 1.20)	0.44 (0.32, 0.71)
n-6 LCP	BM	12.94 (10.05, 15.47)	13.56 (11.99, 15.88)**	12.29 (9.56, 15.98)
	F	11.32 (9.20, 11.98)	10.06 (8.68, 12.59)**	10.02 (8.76, 11.11)
n-6 PUFA	BM	33.37 (31.77, 34.68)	33.69 (30.03, 34.83)	34.07 (33.18, 35.31)
	F	32.99 (29.61, 34.17)	34.33 (32.07, 34.63)	34.38 (33.25, 35.80)
N-3 PUFA				
C18:3n-3	BM	0.28 (0.14, 0.37)	0.17 (ND, 0.33)	0.18 (0.02, 0.32)
	F	0.34 (0.27, 0.40)	0.34 (0.25, 0.38)	0.29 (0.26, 0.36)
C20:5n-3	BM	0.13 (0.11, 0.41)	0.13 (0.07, 0.48)	0.10 (0.02, 0.17)
	F	0.11 (0.09, 0.16)	0.13 (0.06, 1.05)	0.12 (0.10, 0.16)
C22:5n-3	BM	0.22 (0.17, 0.30)	0.22 (0.09, 0.29)	0.15 (0.07, 0.22)
	F	0.18 (0.16, 0.26)	0.15 (0.06, 0.23)	0.22 (0.10, 0.32)
C22:6n-3	BM	2.05 (1.17, 2.22)	1.74 (1.49, 2.57)*	1.73 (1.19, 2.96)*
	F	1.52 (1.31, 1.92)***	1.09 (0.83, 1.51)*	0.96 (0.89, 1.18)***
n-3 LCP	BM	2.21 (1.54, 2.80)	2.04 (1.70, 3.01)	1.90 (1.41, 3.17)
	F	1.99 (1.77, 2.30)****	1.75 (1.32, 2.33)	1.43 (1.09, 1.58)****
n-3 PUFA	BM	1.93 (1.70, 2.32)	2.25 (1.90, 2.89)	2.05 (1.62, 3.21)
	F	2.26 (1.94, 2.76)****	2.04 (1.67, 2.58)	1.77 (1.51, 2.13)****

PUFA=polyunsaturated fatty acid; n-5 LCP=C20:2n-6+C20:3n-6+C20:4n-6+C22:4n-6; n-6 PUFA=n-6 LCP+C18:2n-6+C18:3n-6; n-3 LCP=C20:5n-3+C22:5n-3+C22:6n-3; n-3 PUFA=n-3 LCP+C18:3n-3.

*p<0.05, **p<0.01 between groups; ***p<0.01; ****p<0.001 between 2 and 8 weeks.

Table 3 Fatty acid content (mg/l) and composition (% wt/wt) of plasma triglycerides in infants fed breast milk (BM) or formula (F) (median (Q_1 , Q_3))

		Age (weeks)		
		2	4	8
Sum (mg/l)				
	BM	1278 (824, 1821)	778 (604, 1014)	786 (662, 1223)
	F	1184 (907, 1529)	990 (612, 1497)	1026 (876, 1404)
Saturated fatty acids				
Total	BM	40.01 (39.09, 40.66)	40.64 (37.81, 46.54)	42.34 (38.65, 46.19)*
	F	35.49 (32.79, 36.80)	39.32 (36.49, 40.24)	37.36 (34.99, 39.81)*
Cis-monounsaturated fatty acids				
Total	BM	41.97 (40.46, 46.19)	48.02 (42.67, 49.86)	45.60 (40.93, 48.53)
	F	47.21 (46.57, 50.22)	45.43 (43.99, 45.98)	45.58 (44.21, 48.85)
Trans-fatty acids				
Total	BM	0.80 (0.46, 1.23)	0.45 (0.22, 1.10)	0.80 (0.34, 1.60)
	F	0.29 (0.20, 0.72)	0.51 (0.43, 0.99)	0.31 (0.14, 0.56)
N-6 PUFA				
C18:2n-6	BM	15.32 (11.08, 16.81)	9.97 (6.07, 12.24)*	10.42 (5.35, 14.74)
	F	14.23 (12.18, 14.69)	13.60 (12.16, 14.41)*	14.00 (13.58, 15.16)
C18:3n-6	BM	0.09 (0.04, 0.13)	0.08 (ND, 0.10)	0.08 (0.03, 0.11)
	F	0.10 (0.01, 0.16)	0.04 (ND, 0.12)	0.10 (0.07, 0.19)
C20:2n-6	BM	0.27 (0.17, 0.29)*	0.24 (0.09, 0.35)	0.20 (0.04, 0.38)
	F	0.10 (0.07, 0.17)*	0.08 (ND, 0.19)	0.12 (0.07, 0.14)
C20:3n-6	BM	0.09 (ND, 0.26)	0.10 (0.02, 0.34)	0.11 (0.09, 0.25)
	F	0.16 (0.09, 0.23)	0.22 (0.13, 0.35)	0.15 (0.12, 0.22)
C20:4n-6	BM	0.61 (0.56, 0.66)*	0.61 (0.40, 0.89)*	0.52 (0.22, 0.88)
	F	0.40 (0.32, 0.51)*	0.32 (0.25, 0.38)*	0.31 (0.23, 0.59)
n-6 LCP	BM	1.09 (0.93, 1.32)	1.18 (0.66, 1.65)	1.05 (0.51, 1.58)
	F	0.81 (0.56, 1.13)	0.72 (0.42, 1.10)	0.70 (0.45, 0.94)
n-6 PUFA	BM	16.59 (12.09, 18.20)	11.89 (7.37, 13.36)	11.88 (6.30, 15.43)
	F	15.41 (13.26, 15.58)	14.32 (12.91, 15.71)	14.56 (14.37, 16.19)
N-3 PUFA				
C18:3n-3	BM	0.30 (0.16, 0.55)	0.21 (0.05, 0.39)	0.24 (0.10, 0.35)
	F	0.06 (ND, 0.26)	0.10 (ND, 0.30)	0.10 (ND, 0.28)
C22:6n-3	BM	0.11 (0.05, 0.17)	0.13 (0.04, 0.18)*	0.09 (ND, 0.23)
	F	0.07 (0.03, 0.20)	0.08 (0.05, 0.14)*	0.09 (0.04, 0.40)
n-3 LCP	BM	0.16 (0.06, 0.24)	0.13 (0.05, 0.22)	0.16 (0.06, 0.31)
	F	0.12 (0.05, 0.26)	0.08 (ND, 0.21)	0.13 (0.04, 0.41)
n-3 PUFA	BM	0.57 (0.32, 0.87)	0.46 (0.22, 0.64)	0.50 (0.37, 1.03)
	F	0.49 (0.30, 0.69)	0.50 (0.31, 0.66)	0.37 (0.22, 0.61)

PUFA=polyunsaturated fatty acid; n-6 LCP=C20:2n-6+C20:3n-6+C20:4n-6+C22:4n-6; n-6 PUFA=n-6 LCP+C18:2n-6+C18:3n-6; n-3 LCP=C20:5n-3+C22:5n-3+C22:6n-3; n-3 PUFA=n-3 LCP+C18:3n-3.

* $p<0.05$.

elsewhere.²⁰ Identification of fatty acids was secured by comparison with authentic standards and mass spectrometry (Hewlett-Packard Series 5971 MSD).

Fatty acid results were expressed as percentage (wt/wt) of all fatty acids detected with a chain length between 12 and 22 carbon atoms, because percentage values may better reflect the essential fatty acid status than absolute plasma concentrations.¹⁵ The fatty acid data are presented as median and quartile values, because skewed distributions were found, especially in the lower concentrated fatty acids. Results were evaluated using Minitab, version 8 extended.²¹

Clinical data were compared by Student's unpaired t test; Mann-Whitney's two sided rank test was used for intergroup comparison of fatty acid profiles and Wilcoxon's one sample rank test for the analysis of age related changes. Differences of $p<0.05$ were regarded as significant.

Results

The breast and formula fed groups had similar birth weights (3389 SD (298) v 3504 (390) g) (not significant) and gestational ages (38.0 (1.6) v 39.6 (0.8) weeks; $p<0.05$). There was no difference between the groups in weight gain or head and chest circumferences. Longitudinal growth was significantly greater in formula fed infants (1.01 (0.22) v 0.74 (0.24) cm/week; $p<0.05$).

Breast fed infants maintained relatively stable values for precursor and product essential

fatty acids in all lipid classes throughout the study period (tables 2–4). In plasma phospholipids there was no significant difference between the two groups for the precursor fatty acids linoleic acid (C18:2n-6) and α linolenic acid (C18:3n-3), although the values tended to be higher in formula fed infants (table 2). Contributions of AA at the ages of 2 and 4 weeks and DHA at the ages of 4 and 8 weeks were significantly higher in breast fed than in formula fed infants. Dihomo- γ -linoleic acid (C20:3n-6) increased significantly in breast fed infants between the ages of 2 and 8 weeks. DHA, total ω -3 LCP and total ω -3 polyunsaturated fatty acids (PUFA) remained stable in breast fed infants; they decreased significantly in the infants receiving formula between the ages of 2 and 8 weeks. No difference was seen between the two groups in total LCP and total PUFA values. At the end of the study period, the sum of plasma phospholipid fatty acids was slightly higher in infants fed formula. However, the observed differences in fatty acid values between the two groups remained significant when data were calculated as absolute fatty acid concentrations (mg/l).

AA in triglycerides were significantly higher in breast fed than in formula fed infants at the ages of 2 and 4 weeks, even though the precursor linoleic acid was significantly higher in the formula fed group (table 3). DHA was significantly higher in breast fed infants than in those receiving formula at 4 weeks (table 3). In sterol esters linoleic acid and total ω -6 PUFA concentrations increased significantly between the ages of 2 and 8 weeks in the formula fed

Table 4 Fatty acid content (mg/l) and composition (% wt/wt) of plasma sterol esters in infants fed breast milk (BM) or formula (F) (median (Q_1 , Q_3))

		Age (weeks)		
		2	4	8
Sum (mg/l)				
	BM	743 (547, 783)	597 (427, 738)	598 (441, 714)
	F	600 (521, 731)	566 (481, 630)	611 (586, 712)
Saturated fatty acids				
Total	BM	25.35 (24.32, 25.78)	25.87 (27.02)	24.76 (23.48, 28.48)
	F	27.05 (22.21, 34.70)	22.99 (20.93, 25.71)	23.17 (20.67, 26.29)
Cis-monounsaturated fatty acids				
Total	BM	37.02 (30.22, 38.96)	35.56 (32.92, 43.50)	36.91 (29.18, 45.40)
	F	34.49 (31.58, 36.40)	33.60 (33.08, 34.47)	33.26 (30.46, 35.78)
Trans-fatty acids				
Total	BM	0.20 (0.10, 0.25)****	0.39 (0.16, 0.60)	0.52 (0.37, 0.66)****
	F	0.39 (ND, 1.00)	0.49 (0.16, 0.99)	0.29 (0.50, 0.78)*
N-6 PUFA				
C18:2n-6	BM	31.38 (29.77, 37.69)	30.86 (24.15, 36.87)	33.28 (21.49, 38.56)
	F	30.87 (27.75, 35.73)****	38.33 (34.08, 40.65)	39.40 (35.49, 41.56)****
C18:3n-6	BM	0.46 (0.19, 0.65)	0.39 (0.24, 0.64)	0.34 (0.26, 0.80)
	F	0.64 (0.46, 0.89)	0.85 (0.60, 0.99)	0.63 (0.10, 0.65)
C20:3n-6	BM	0.57 (0.45, 0.75)	0.60 (0.54, 0.71)	0.59 (0.33, 0.61)
	F	0.57 (0.44, 0.70)	0.55 (ND, 0.75)	0.50 (0.10, 0.65)
C20:4n-6	BM	3.60 (3.30, 5.11)	4.42 (3.48, 5.59)	3.08 (2.27, 0.17)
	F	2.90 (2.63, 3.74)	2.50 (2.04, 3.37)	2.72 (2.14, 3.10)
n-6 LCP	BM	4.29 (4.00, 5.36)	5.10 (4.15, 6.16)	3.63 (2.81, 6.47)
	F	4.20 (3.28, 5.02)	3.14 (2.30, 5.30)	3.27 (2.37, 3.86)
n-6 PUFA	BM	36.92 (34.25, 43.56)	36.97 (30.73, 42.23)	37.53 (25.33, 46.15)
	F	35.89 (31.16, 41.09)***	42.53 (38.95, 44.71)	42.91 (39.75, 46.29)***
N-3 PUFA				
C18:3n-3	BM	0.13 (0.10, 0.29)***	0.10 (ND, 0.14)	0.21 (0.14, 0.26)***
	F	0.15 (ND, 0.24)	0.08 (ND, 0.18)	0.18 (ND, 0.24)
C22:6n-3	BM	0.22 (0.03, 0.29)	0.24 (0.13, 0.26)	0.15 (0.11, 0.34)
	F	0.14 (ND, 0.65)	0.07 (ND, 0.33)	0.15 (ND, 0.40)
n-3 LCP	BM	0.27 (0.03, 0.37)	0.24 (0.13, 0.34)	0.35 (0.12, 0.44)
	F	0.25 (ND, 0.50)	0.11 (ND, 0.57)	0.18 (ND, 0.45)
n-3 PUFA	BM	0.35 (0.07, 0.50)	0.35 (0.17, 0.52)	0.55 (0.26, 0.72)
	F	0.41 (ND, 0.84)	0.28 (0.04, 0.77)	0.31 (ND, 0.86)

PUFA=polyunsaturated fatty acid; ND=not detectable; n-6 LCP=C20:2n-6+C20:3n-6+C20:4n-6+C22:4n-6; n-6 PUFA=n-6 LCP+C18:2n-6+C18:3n-6; n-3 LCP=C20:5n-3+C22:5n-3+C22:6n-3; n-3 PUFA=n-3 LCP+C18:3n-3.

* $p < 0.05$ between groups; *** $p < 0.005$; **** $p < 0.01$ between 2 and 8 weeks.

group; they remained unchanged in breast fed infants (table 4). The concentrations of trans-isomeric fatty acids increased significantly in the breast fed infants between 2 and 8 weeks and reached significantly higher values than in infants receiving formula at the age of 2 months (table 4).

Discussion

The percentage contributions to plasma fatty acids of the two major LCP fatty acids (arachidonic and docosahexaenoic acids) and of total ω -6 or ω -3 LCP were significantly higher in breast fed infants than in those receiving formula. In plasma phospholipids nearly twofold higher DHA values were measured in breast fed infants even at the age of 2 months. The relatively more pronounced depletion of DHA than of AA may reflect the more complex synthesis of DHA. Arachidonic acid synthesis from linoleic acid involves three enzyme steps; recent biochemical data suggest that the synthesis of DHA from α linolenic acid comprises four additional steps (figure).²²

In accordance with previous observations in preterm infants,²³ we also found significantly higher trans-fatty acid values in term infants fed human milk. The difference between breast fed and formula fed infants can be explained by the fact that human milk contains appreciable amounts of trans-fatty acids, while most infant formulas do not.¹⁵

Phospholipids account for about half of the total plasma lipids during early life, therefore most LCP fatty acids are transported in the

blood stream by phospholipids. In this study the contribution of plasma phospholipid AA to total plasma AA (83 to 106 mg/l, calculated as the sum of AA in phospholipids, triglycerides, and sterol esters) was between 66% and 79%, whereas plasma phospholipid DHA represented 85–92% of total plasma DHA (17 to 28 mg/l, calculated as the sum of DHA in phospholipids, triglycerides, and sterol esters). Plasma phospholipid concentrations can alter membrane phospholipid concentrations, as has been shown in erythrocyte membranes during parenteral nutrition²⁴; thus differences between formula fed infants and those receiving breast milk may be clinically important.

A few other studies have addressed the question of the effect of various diets on the early postnatal essential fatty acid status of term infants.^{25–30} In most of these investigations both AA and DHA concentrations tended to be equal or higher in breast fed infants than in those receiving formula. The variability of the lipid fractions investigated (erythrocyte phosphatidylethanolamine, phosphatidylcholine, serine, or plasma lipid fractions), the different dietary interventions applied, and the wide variety in the time of blood sample collection make it difficult, however, to draw firm conclusions.³¹ Moreover, in all investigations except one³⁰ chromatography with packed columns was used. This offers lower resolution and precision of fatty acid determination, particularly for LCP, than current analytical methodology with capillary columns.²³ In no previous study has peak identification been verified by mass spectrometry.

Preterm infants fed with formula containing precursor essential fatty acids but no LCP show a rapid depletion of DHA, AA, and other LCP in plasma and tissue lipids. This may interfere with the maturation of membrane rich tissues such as the brain and the retina.^{7-10 32 33} In preterm infants DHA deficiency has been associated with impaired visual function determined by Teller's forced choice preferential looking test^{8 10} and by electroretinogram.^{7 9} The initial results of an ongoing study by Carlson and coworkers also suggested that reduced ω -3 LCP concentrations are associated with altered performance on the Bayley mental developmental index.³⁴ Low ω -6 LCP fatty acids, on the other hand, were related to reduced body weight.^{11 12} Furthermore, Leaf and coworkers reported a positive correlation between both ω -6 and ω -3 fatty acids and measurements of fetal growth and maturation in preterm infants.³⁵ Therefore, the Committee on Nutrition of the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) commented that, 'enrichment of metabolites of both linoleic and alpha-linolenic acid approximating levels typical for human milk lipids (n-6 LCP 1%, n-3 LCP 0.5% of total fatty acids) is desirable for formulas for low birth weight infants'.¹³

In spite of the substantial differences between the nutritional status of preterm and term infants, the possibility that dietary deficiency of LCP fatty acids may lead to functional disturbances in full term infants also cannot be excluded. It has been suggested that the activity of the LCP synthesising enzymes is lower immediately after birth than at later ages.^{36 37} Farquharson and coworkers showed that early LCP intake also affects tissue fatty acid compositions. In subcutaneous tissue triglycerides in a group of predominantly term infants who died of cot death both AA and DHA values were significantly higher in breast fed than in formula fed infants.³⁸ Moreover, the mean percentage contribution of DHA to cerebral cortex grey matter was significantly higher in infants previously fed breast milk than in those who had received formula.³⁹

Recent observational studies in term infants have suggested possible functional deficiencies related to biochemical deficiency of ω -3 LCP. Poorer performance in visual evoked potential tests was associated with lower ω -3 LCP concentrations in a group of breast fed and formula fed infants investigated at the age of 5 months.⁴⁰ In another observational study the random dot stereo acuity and letter matching ability of 36 month old, full term, breast fed infants were better than those of formula fed infants, and the differences were correlated with a dietary ω -3 sufficiency index obtained at the age of 4 months.⁴¹ Nevertheless, at present there is no clear evidence of functional differences related to different LCP status in term infants in prospective randomised studies.⁴²

LCP enrichment of infant formulas is technically feasible, but it may increase the costs of production considerably. LCP fatty acids may also be more prone to oxidation than their pre-

cursor essential fatty acids, which might affect the antioxidant status of the infant and the shelf-life of LCP enriched formulas. There is an urgent need to determine in prospective clinical trials whether the addition of LCP fatty acids to infant formulas reduces biochemical differences in the LCP fatty acid status between breast fed and formula fed infants and whether this affects function.

We gratefully acknowledge the financial support of Deutsche Forschungsgemeinschaft, Bonn, Germany (Ko 912/4-2).

TD was the recipient of a scholarship from Milupa, Austria.

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