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Fatty acid composition of human milk during the 1st month after term and preterm delivery

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Abstract The fatty acid composition of human breast milk was determined longitudinally after term and preterm delivery by high resolution gas liquid chromatography. Milk samples were obtained at days 5, 10, 20 and 30 after term (n = 38) or preterm (n = 19) delivery. The saturated fatty acids C10:0 and C12:0 and the polyunsaturates linoleic acid (C18:2ω-6) and α-linolenic acid (C18:3ω-3) increased significantly from day 5 to day 10, whereas arachidonic acid (C20:4ω-6), total ω-6 long-chain polyunsaturates (LCP), docosahexaenoic acid (C22:6ω3) and total ω-3 LCP decreased significantly. Term and preterm milk did not differ in percentage content of linoleic acid, α-linolenic acid and LCP at any time point. Preterm milk contained significantly more medium and intermediate chain fatty acids (C10:0, C12:0 and C14:0) than term milk on days 5 (12.28 vs 9.78%; $P > 0.05$), 10 (16.25 vs 12.62%; $P > 0.05$) and 20 (17.29 vs 13.47%; $P > 0.005$).

Conclusion The milk of mothers of preterm infants is not better suited to meet the high LCP requirements of their infants during the first weeks after birth. The slightly higher proportion of medium and intermediate chain fatty acids in preterm milk during the 1st month after birth might be advantageous for the fat and calcium absorption of preterm infants.

Key words Lipids · Infant nutrition · Medium chain fatty acids · Long-chain polyunsaturated fatty acids · *Trans* fatty acids

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Abbreviations *DHA* docosahexaenoic acid (C22:6ω3) · *IQR* inter quartile range · *LCP* long-chain polyunsaturated fatty acids

Introduction

The supply of essential polyunsaturated fatty acids with human milk lipids is of major importance for growth and development of the nursing infant [10]. Newborn and particularly low birth weight infants have only very limited body stores of polyunsaturated fatty acids, but high requirements for deposition in their rapidly growing tissues. In addition to the provision of the classical essential fatty acids, the perinatal supply and metabolism of long-chain polyunsaturated fatty acids (LCP), such as arachidonic (C20:4ω6) and docosahexaenoic (C22:6ω3, DHA) acids, are receiving increased attention. The extent of LCP incorporation into the structural lipids of the growing brain and other membrane rich tissues was shown to affect neural function [10], but the activity of endogenous LCP synthesis is relatively low in the postnatal period [13]. Therefore, in infants LCP levels in plasma and tissues are largely determined by the dietary intake of preformed LCP [12, 15, 21, 25]. Observational studies indicate a possible relationship between availability of arachidonic acid and infant growth [7, 19, 22], and results of randomized studies document an advantage of a postnatal LCP supply for early visual and cognitive development in both preterm [6, 8, 29] and term infants [1, 26].

Compared to healthy fullterm infants, premature infants are considered to have greater LCP requirements because of their lower body stores and higher growth rate [14]. Luukkainen et al. [24] reported a higher LCP content in the milk of a small number of mothers after premature versus term delivery, whereas Bitman et al. [4] found no difference. Since this question is of potential relevance for the choice of feeding in preterm infants and for designing formulae for low birth weight infants, we studied the compositional changes of fatty acids in the

Table 1 Clinical data of the mothers

	Term $n = 38$	Preterm $n = 19$	
Age (years)	32.12 ± 4.55	29.9 ± 2.9	P < 0.05
Gravida	2.6 ± 1.08	2.9 ± 1.66	
Parturition	1.75 ± 0.76	1.9 ± 0.74	
Gestational age (weeks)	39.91 ± 0.64	28.63 ± 3.72	P < 0.01
Weight prior to pregnancy (kg)	62.42 ± 8.99	65.89 ± 14.06	
Weight at delivery (kg)	74.97 ± 9.37	73.53 ± 13.4	
Height (m)	1.67 ± 0.06	1.68 ± 0.08	
Body mass index prior to delivery	22.45 ± 2.78	23.40 ± 4.10	
Body mass index at delivery	27.00 ± 2.99	26.13 ± 3.80	
Cesarean Sectio	6	11	P < 0.01

breast milk of sizeable groups of mothers giving birth to infants at term and preterm during the 1st month of lactation.

Subjects and methods

Subjects and sample collection

Human milk samples were donated by apparently healthy women, who were asked to participate in the study during their stay at the obstetric department. The study protocol had been reviewed by the ethical committee of the Medical Faculty, University of Munich, and informed consent was obtained. The participating women had given birth to single infants born at term ($n = 38$) or preterm ($n = 19$) (Table 1). They collected one sample each of fore- and hindmilk by manual expression in the mornings on days 5, 10, 20 and 30 post partum. If the infant did not nurse due to prematurity, an aliquot of milk was collected in the morning with an electric or manual breastpump. The samples were immediately frozen at −20°C until analysis. All mothers consumed a unrestricted omnivorous diet, and unusual dietary habits and food fads were excluded by interviews.

Analytical methods

Frozen samples were thawed only once. Fat was extracted with methanol-chloroform and fatty acids transesterified with methanolic HCL as previously described [20]. Fatty acid methylesters were determined by high-resolution capillary gas liquid chromatography (HP Series II 5980 A, Hewlett Packard, Böblingen, Germany) with on column injection and flame ionisation detection [11]. Peak identification was assured by comparison with authentic standards (NuChekPrep, Elysian, MN, USA) and by mass spectrometry (HP Series 5971 MSD).

Statistical analysis

Minitab for Windows, Vers. 9.2 (Minitab, State College, PA, USA) was used for statistical analysis. Differences over time within groups were analysed with Kruskal Wallis nonparametric one way analysis of variance, which allows evaluation of data sets with varying sample size. Differences between the term and preterm milk at single time points were evaluated with the Mann-Whitney test. The level of significance was set at $P < 0.05$.

Results

Clinical data

The clinical data of the mothers delivering at term and preterm (Table 1) show no difference in mean age, parity, height, weight and body mass index prior to pregnancy and weight and body mass index at delivery. According to the study design, gestational age and mode of delivery differed significantly.

Presentation of fatty acid data

Since a skewed data distribution was observed for some low concentrated fatty acids, results are presented as median values and interquartile ranges (IQR).

Term milk

There was no difference in the percentage composition of fatty acids in fore- and hindmilk samples of the first and second breast nursed (data not shown), therefore, the results of fore- and hindmilk samples of both breasts of each subject and sampling time were pooled for further evaluation (Table 2).

Saturated fatty acids

The saturated fatty acids C10:0 and C12:0 increased significantly ($P < 0.0001$) from day 5 to day 10, whereas C16:0 and C24:0 declined during this time period ($P < 0.005$) (Table 2). There was no further change after day 10.

ω-6 Polyunsaturated fatty acids

Linoleic acid (C18:2ω6) increased significantly ($P < 0.05$) from day 5 to day 10 but showed no further change by day 20 and 30. In contrast, its immediate metabolite γ-linolenic acid (C18:3ω-6) remained constant throughout the entire study period. Total ω-6 LCP with 20 and 22 carbon atoms as well as arachidonic acid decreased significantly until day 20, but dihomo-γ-linolenic acid (C20:3ω-6) did not decline further after the 10th day.

ω-3 Polyunsaturated fatty acids

α-Linolenic acid (C18:3ω3) increased significantly from day 5 to day 10 ($P < 0.01$), but total ω-3 LCP including docosahexaenoic acid (C22:6ω3, DHA) decreased from day 5 to day 10 ($P < 0.01$), and DHA levels continued to decline significantly until day 20 ($P < 0.001$).

Table 2 Fatty acid composition of total milk lipids (% of total fatty acids) as median and (IQR) for term and preterm milk from day 5 to day 30

	5 days term = 32	preterm = 19	10 days term = 38	preterm = 19	20 days term = 38	preterm = 17	30 days term = 38	preterm = 13
C10:0	0.50	0.46	0.95[*1]	1.06[*1]	1.05	1.15	1.01	1.08
	(0.10)	(0.09)	(0.22)	(0.13)	(0.26)	(0.22)	(0.30)	(0.24)
C12:0	3.42	4.17	5.18[*1]	6.19[*1,*5]	5.39	7.17[*4]	5.21	6.07
	(0.91)	(0.81)	(1.31)	(1.88)	(1.19)	(1.17)	(1.50)	(1.64)
C14:0	5.86	7.65	6.49	9.00[*5]	7.03	8.97[*5]	6.90	7.93
	(1.25)	(1.50)	(1.47)	(1.67)	(1.21)	(1.70)	(2.05)	(2.02)
C16:0	24.97	24.79	22.56[*2]	22.62	23.18	22.58	22.47	22.84
	(2.36)	(1.24)	(2.00)	(2.11)	(2.09)	(1.10)	(2.31)	(1.26)
C18:0	7.07	6.34	7.42	7.54[*5]	7.74	7.85	7.40	7.65
	(0.73)	(1.59)	(0.90)	(0.94)	(0.75)	(0.90)	(0.82)	(0.72)
Total Sat	43.65	44.16	43.05	47.41[*3,*5]	46.28	48.09[*5]	44.30	46.78
	(3.55)	(3.20)	(3.67)	(3.60)	(3.83)	(3.50)	(5.00)	(2.46)
C18:1n–9	32.10	33.83	32.10	30.81[*3]	31.97	29.81	31.50	31.38
	(1.87)	(2.30)	(2.61)	(2.36)	(1.73)	(2.98)	(2.49)	(3.95)
Total trans	0.96	1.01	1.07	1.07	1.49	1.13	1.13	1.26
	(0.35)	(0.40)	(0.41)	(0.43)	(0.56)	(0.31)	(0.46)	(0.27)
C18:2n–6	8.86	9.88	10.30[*3]	9.56	10.47	10.17	11.33	10.49
	(1.30)	(1.86)	(2.15)	(1.60)	(2.10)	(1.57)	(2.17)	(1.95)
C18:3n–6	0.14	0.11	0.15	0.11	0.18	0.16	0.18	0.17
	(0.31)	(0.09)	(0.08)	(0.09)	(0.09)	(0.07)	(0.10)	(0.03)
C20:2n–6	0.57	0.54	0.42[*1]	0.39[*2]	0.31[*1]	0.32[*3]	0.30	0.32
	(0.12)	(0.08)	(0.06)	(0.06)	(0.06)	(0.06)	(0.07)	(0.06)
C20:3n–6	0.53	0.50	0.43[*2]	0.45	0.38	0.41	0.38	0.41
	(0.13)	(0.15)	(0.11)	(0.05)	(0.06)	(0.04)	(0.08)	(0.08)
C20:4n–6	0.72	0.74	0.59[*1]	0.64[*2]	0.50[*1]	0.51[*1]	0.45	0.48
	(0.07)	(0.12)	(0.09)	(0.08)	(0.06)	(0.04)	(0.07)	(0.03)
C22:4n–6	0.23	0.24	0.15[*1]	0.14[*1]	0.10[*1]	0.11[*1]	0.08	0.11
	(6.00)	(0.08)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)
Total n–6 LCP	2.15	2.13	1.59[*2]	1.69[*2]	1.35[*1]	1.35[*1]	1.28	1.31
	(0.34)	(0.32)	(0.20)	(0.19)	(0.17)	(0.13)	(0.19)	(0.19)
Total n–6	11.57	12.20	12.25	11.11	12.09	11.64	13.06	12.02
	(1.94)	(2.34)	(2.17)	(1.75)	(2.32)	(1.70)	(2.50)	(1.50)
C18:3n–3	0.65	0.67	0.81[*2]	0.67	0.78	0.78	0.90	0.73
	(0.07)	(0.12)	(0.17)	(0.09)	(0.11)	(0.15)	(0.20)	(0.14)
C20:3n–3	0.09	0.09	0.05[*2]	0.00	0.05	0.05	0.05	0.00
	(0.03)	(0.05)	(0.03)	(0.04)	(0.04)	(0.04)	(0.04)	(0.00)
C20:5n–3	0.04	0.00	0.00[*3]	0.00	0.04	0.05	0.05	0.00
	(0.08)	(0.04)	(0.03)	(0.05)	(0.05)	(0.05)	(0.07)	(0.04)
C22:5n–3	0.22	0.22	0.18[*1]	0.15[*1]	0.15	0.15	0.15	0.15
	(0.05)	(0.05)	(0.04)	(0.04)	(0.04)	(0.01)	(0.03)	(0.01)
C22:6n–3	0.46	0.43	0.39[*3]	0.35[*3]	0.27[*1]	0.24[*3]	0.23	0.24
	(0.08)	(0.08)	(0.06)	(0.06)	(0.07)	(0.05)	(0.06)	(0.05)
Total n–3 LCP	0.80	0.73	0.66[*1]	0.60[*1]	0.53	0.42	0.48	0.42
	(0.21)	(0.17)	(0.11)	(0.13)	(0.15)	(0.07)	(0.16)	(0.07)
Total n–3	1.51	1.38	1.51	1.30	1.43	1.13	1.52	1.13
	(0.32)	(0.20)	(0.26)	(0.16)	(0.32)	(0.07)	(0.45)	(0.07)
Total LCP	3.08	2.85	2.34[*1]	2.30[*2]	1.90[*1]	1.66[*2]	1.80	1.66
	(0.46)	(0.55)	(0.28)	(0.30)	(0.33)	(0.16)	(0.30)	(0.16)
n6/n3 LCP	2.60	2.71	2.69	2.71	2.56	2.88	2.58	2.88
	(0.55)	(0.42)	(0.63)	(0.68)	(0.63)	(0.72)	(0.61)	(0.72)

[*1] $P < 0.001$, [*2] $P < 0.01$, [*3] $P < 0.05$ versus prior study day, [*4] $P < 0.01$, [*5] $P < 0.05$ versus term milk

Preterm milk

Saturated fatty acids

Similar to term milk, the saturated fatty acids C10:0 and C12:0 increased significantly ($P < 0.0001$) from day 5 to day 10. In contrast to the term milk samples, the decreasing trend of C16:0 values between days 5 and 10 did not reach statistical significance, whereas the increase of C18:0 and of total saturated fatty acids during the same time period was statistically significant ($P < 0.05$).

ω-*6 Polyunsaturated fatty acids*

Linoleic acid and dihomo-γ-linolenic acid did not change significantly in preterm milk over time, but arachidonic acid, C20:2ω-6 and C22:4ω-6 fell from day 5

to day 10 ($P < 0.005$) and further to day 20 ($P < 0.01$). Total ω-6 LCP also declined significantly until day 20, while total ω-6 polyunsaturated fatty acids did not change over time.

ω-3 Polyunsaturated fatty acids

Values of C22:5ω3, DHA and total ω-3 LCP declined significantly from day 5 to 10 ($P < 0.0001$, $P < 0.05$ and $P < 0.01$, respectively). DHA levels and total ω-3 polyunsaturated fatty acids continued to decline significantly until day 20 ($P < 0.05$ and $P < 0.01$, respectively).

Term versus preterm milk

To facilitate comparison of our results with those of other investigators, means and standard errors for physiologically important fatty acids are presented in Table 3. Preterm milk contained significantly more medium and intermediate chain fatty acids (C10:0, C12:0 and C14:0) than term milk on days 5, 10 (both $P \leq 0.05$) and 20 ($P \leq 0.005$) (Table 3). There were no statistically significant differences of arachidonic and docosahexaenoic acids and of total LCP content between mature and preterm human milk at any time point (Fig. 1).

Discussion

The concept of supplying preformed LCP in the nutrition of preterm infants has only recently been developed, and LCP supplemented preterm infant formulas presently available in Europe tend to approach the fatty acid pattern of human milk [14]. There has been a controversy as to whether or not the composition of milk of mothers of preterm infants may be more suitable for the specific needs of preterm infants [2–4, 18] and, therefore, may also be a better model for designing preterm infant formula. An increased nitrogen content in preterm milk was reported in the early 1980s, and the hypothesis was raised that this may serve to meet the high amino acid requirements of low birth weight infants, but this concept has later been dismissed [3]. More recently, increased contents of medium-chain fatty acids [4] and of LCP [24] in preterm milk have been reported and interpreted as a possible benefit for the feeding of preterm babies.

In this study, we found no LCP enrichment of preterm versus term milk, with a similar decline of contents over time both in term and preterm milk. Milk fatty acid values showed a large interindividual variation at all time points. In accordance with our results, other studies reported a changing milk fatty acid composition with the duration of lactation, with a marked rise in linoleic acid and a decline in arachidonic acid and DHA during the first weeks after birth [5, 16, 17]. Bitman et al. [4] compared the milk composition of 46 mothers delivering prematurely with a small group of 6 mothers after a term delivery over 80 days after birth and found no significant difference between preterm and term milk, but the same decline of arachidonic acid and DHA with duration of lactation in both groups [4]. Luukkainen et al. [24] found a similar decline of arachidonic acid and DHA levels over the 1st month of lactation. Thereafter, no further decrease was observed in preterm milk but LCP continued to decline in a small group of mothers ($n = 5$–10) delivering at term. In contrast to our study, these authors were unable to obtain milk samples from more than 40% – 70% of their mothers at any given time point, which somewhat limits the conclusions that can be drawn from their study. Nonetheless it is conceivable that mothers of term infants develop a greater depletion of arachidonic acid and DHA body stores after longer lactation. In comparison to mothers of premature infants, those delivering at term provide a markedly greater placental LCP transfer during the longer duration of pregnancy, and also greater milk volumes to their larger infants. Since LCP transferred to the young through the placenta and the breast may be derived from maternal stores, mothers giving birth at term might be less able to maintain high milk LCP levels than mothers after preterm delivery.

Relatively high arachidonic acid and DHA contents of colostrum and transitional milk were found in the present and in other studies, which may be partly caused by the higher proportion of LCP-rich phospholipids to total milk lipids during early stages of lactation [16, 17]. The high proportion of LCP in milk lipids immediately after birth may benefit the newborn infant in the 1st week of life, when the total amount of breast milk consumed is still low but the infants' LCP requirements are high [14]. However, an extension of this hypothesis to premature infants is not convincing, since their apparently higher LCP requirements should extend over many weeks beyond the maturational pattern of breast milk fatty acids observed in this study, which is just as rapid in preterm as in term milk (Table 2). It appears more likely that the observed changes in fatty acid composition of milk lipid composition after birth are related to maturational changes of the secretory process, with conversion of mammary glandular cells from presecretory to actively milk-synthesizing cells [3, 4].

Incomplete maturation of milk secretion processes prior to term birth may also explain the higher contents of medium-and intermediate-chain fatty acids (C10:0, C12:0) in preterm than in term milk in our and in Bitman's study [4]. Medium-and intermediate-chain fatty acids can by synthesized *de novo* in the mammary gland, an immature uptake of long-chain lipids from the circulation and their conversion into long-chain milk lipids after preterm delivery may lead to a compensatory stimulation of *de novo* fat synthesis [27]. Levels of C10:0 in C12:0 in milk are also enhanced by maternal carbohydrate consumption [17, 20], but in our study there is no

Table 3 Fatty acid composition of major fatty acids (% of total fatty acids) as mean and SE

	5 days		10 days		20 days		30 days	
	term = 32	preterm = 19	term = 38	preterm = 19	term = 38	preterm = 17	term = 38	preterm = 13
C10:0+C12:0+C14:0	11.04	12.81	13.79**	16.64	14.02*	17.88	13.55*	16.7
	4.33	3.38	4.54	4.38	3.81	5.19	4.68	5.68
C16:0	24.56	23.84	22.29	22.74	23.16	22.42	22.76	22.74
	(0.76)	(0.73)	(0.61)	(0.69)	(0.56)	(0.46)	(0.46)	(0.45)
C18:0	7.23	6.66	7.60	7.61	7.69	8.03	7.47	7.72
	(0.19)	(0.21)	(0.19)	(0.34)	(0.20)	(0.36)	(0.21)	(0.37)
Total Sat	43.67	44.14	44.73*	48.22	45.70**	49.22	44.69	47.66
	(1.18)	(0.98)	(0.96)	(1.14)	(0.79)	(1.37)	(0.94)	(1.17)
C18:1n–9	32.16	33.49	31.59	29.73	32.00	30.98	31.09	31.62
	(0.70)	(1.53)	(0.60)	(2.20)	(0.47)	(0.96)	(0.81)	(1.19)
Total trans	0.96	1.18	1.31	1.18	1.49	1.21	1.23	1.27
	(0.14)	(0.15)	(0.38)	(0.19)	(0.13)	(0.14)	(0.15)	(0.21)
C18:2n–6	9.44	9.86	10.99	10.34	10.47	9.75	11.81	10.46
	(0.58)	(0.50)	(0.55)	(0.64)	(0.68)	(0.53)	(0.58)	(0.66)
C18:3n–6	0.31	0.10	0.18	0.11	0.22	0.13	0.29	0.16
	(0.11)	(0.02)	(0.11)	(0.02)	(0.06)	(0.03)	(0.07)	(0.02)
C20:3n–6	0.59	0.55	0.45	0.46	0.39	0.40	0.41	0.40
	(0.04)	(0.05)	(0.03)	(0.03)	(0.02)	(0.03)	(0.03)	(0.03)
C20:4n–6	0.72	0.76	0.60	0.62	0.50	0.50	0.46	0.48
	(0.02)	(0.03)	(0.02)	(0.03)	(0.02)	(0.01)	(0.03)	(0.02)
Total n–6 LCP	2.23	2.16	1.66	1.67	1.34	1.33	1.31	1.26
	(0.09)	(0.13)	(0.05)	(0.06)	(0.04)	(0.06)	(0.09)	(0.06)
Total n–6	12.09	12.22	13.01	12.07	12.37	11.19	13.62	11.85
	(0.64)	(0.55)	(0.57)	(0.68)	(0.71)	(0.57)	(0.60)	(0.70)
C18:3n–3	0.66	0.73	0.84	0.74	0.82	0.78	0.96	0.74
	(0.03)	(0.11)	(0.05)	(0.06)	(0.04)	(0.05)	(0.06)	(0.05)
C20:3n–3	0.08	0.06	0.05	0.03	0.05	0.04	0.04	0.01
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)
C22:6n–3	0.45	0.45	0.39	0.37	0.3	0.29	0.28	0.25
	(0.03)	(0.03)	(0.02)	(0.03)	(0.02)	(0.08)	(0.04)	(0.01)
Total n–3 LCP	0.87	0.78	0.65	0.61	0.58	0.54	0.60	0.44
	(0.06)	(0.05)	(0.04)	(0.05)	(0.04)	(0.14)	(0.08)	(0.04)
Total n–3	1.62	1.57	1.58	1.38	1.51	1.36	1.70	1.17
	(0.08)	(0.14)	(0.07)	(0.09)	(0.07)	(0.15)	(0.12)	(0.08)
Total LCP	3.12	2.95	2.32	2.28	1.95	1.90	1.96	1.70
	(0.12)	(0.16)	(0.07)	(0.08)	(0.06)	(0.15)	(0.13)	(0.08)
n6/n3 LCP	2.67	2.80	2.68	2.86	2.51	2.69	2.55	2.85
	(0.76)	(0.17)	(0.13)	(0.20)	(0.19)	(0.36)	(0.18)	(0.26)

*$P < 0.01$, **$P < 0.05$ versus preterm milk

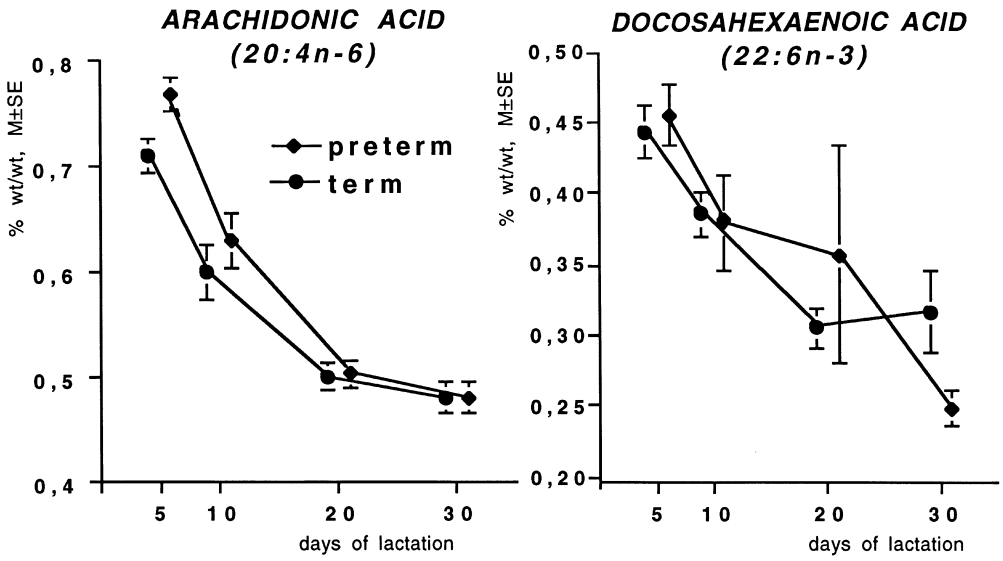

Fig. 1 Arachidonic and docosahexaenoic acid contents in total lipids of human milk from mothers of preterm ($n = 19$) and term ($n = 38$) infants on days 5, 10, 20 and 30 of lactation. There was a significant decrease of LCP from day 5 to 20, but no differences between term and preterm milk

indication for a different dietary intake of mothers delivering preterm. Fatty acids with 10 and 12 carbon atoms are more easily absorbed by preterm infants than long-chain triglycerides, and they may be beneficial for calcium balance [9, 14], which might result in some advantage of feeding preterm infants there own mother's milk over donor term milk. However, there is no indication that this small difference in milk composition represents an evolutionary developmental pattern of the mammary gland to accommodate the immature digestive system of preterm babies. The survival of significant numbers of very premature infants is a relatively recent phenomenon, and it appears unlikely that evolution would have anticipated their specific needs.

We conclude that although human milk feeding has great advantages not only for term but also for preterm infants, the fatty acid composition of human milk from mothers delivering preterm babies is not specifically adapted to meet their infants' LCP needs. There is no scientific basis to simply deduct the essential fatty acid needs of preterm infants from the composition of their own mother's milk. Rather, one should attempt to define the nutrient needs of premature infants by adequate investigation of fatty acid metabolism and functional effects. It is quite conceivable that the results of such studies might even reveal the need for supplementing human milk fed to preterm infants with certain lipids, as it has been previously established for protein and minerals [18, 23, 28].

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