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Comparing the fatty acid levels of preterm and term breast milk in Turkish women

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Aim: Lipids are the main source of calories and considered very important in infant growth. We aimed to compare fatty acid composition of term and preterm breast milk. This is the first study that compares the fatty acid levels of preterm and term breast milk in Turkish women.

Materials and methods: Breast milk samples were obtained from mothers of term (n = 15) and preterm (n = 15) infants on postnatal days 3, 7, and 28. Fatty acid composition of human breast milk was determined longitudinally by gas-chromatography/mass spectrometry.

Results: There were 31 fatty acids measured in the milk samples. In the first month, 17 fatty acid levels had significant differences. In group comparison, some fatty acids (C14:0, C16:0, C18:1 and C20:5) had significantly increased in the preterm group (P = 0.041, P = 0.046, P = 0.027, P = 0.033, respectively), whereas myristoleic acid (C14:1) and eicosanoic acid (C20:0) had significantly increased in the term group (P = 0.015, P = 0.048, respectively).

Conclusion: Term and preterm milk have different compositions of fatty acids. Breast milk composition changes over time. As a general conclusion, breast milk provides the lipid requirements of infants.

Key words: Breast milk, fatty acid, preterm, term

1. Introduction

Breast milk has been recommended as the sole source of food for healthy infant growth during the first 6 month of life (1,2). Milk fat is the major source of energy, essential fatty acids, and fat-soluble vitamins for breast-fed infants (3). Newborn and particularly preterm infants have only very limited body stores of fatty acids (FAs), but they have high requirements for deposition in their rapidly growing tissues (4–6). On the other hand, the FA composition of breast milk shows considerable variation with regards to factors such as the duration of pregnancy, stage of lactation, maternal parity, and geographic region (7–15). Many studies have been performed to determine the content of FA in breast milk collected from mothers with preterm and term infants, but the information on the composition of milk from Turkish women has remained incomplete (16,17). In this study, we reported on the compositional changes of FA in breast milk during the first month of lactation and compared FA composition of breast milk donated by mothers giving birth with full-term and preterm infants.

2. Materials and methods

2.1. Subjects

In this longitudinal study, 30 healthy volunteer mothers were included (preterm n = 15, term n = 15) from the postnatal wards on the day after delivery. Gestational age was determined according to the last menstrual period of the mothers and early ultrasound findings of pregnancy. All infants were Ballard scored. Infants' first and fifth minute APGAR scores and the delivery method were found by scanning the records of the hospital. Infants' head circumference, crown rump length, weight changes, maternal age, gravidity, and parity information were recorded. For comparison, 5 mL of breast milk was collected from mothers of preterm (n = 15) and term

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neonates (n = 15) on days 3, 7, and 28 in the postpartum period by an electrical pump between 08:00 and 11:00 hours before nourishing the baby and from only 1 breast. All milk samples were stored at –80 °C until analyzed. The study was explained to the mothers and their written consent was obtained prior to enrollment. The institutional medical ethics committee's approval of the project was obtained.

2.2. Analysis of fatty acids in breast milk

FA methyl esters were prepared with sodium methylate and methanolic boron trifluoride and extracted into hexane (Sigma, USA) by following the method developed by Molto-Puigmarti et al. for chromatographic analysis (18). Subsequently, they were separated and quantified by fast-gas chromatography with flame ionization detection according to the same method. Each sample was analyzed in duplicate. The methyl esters were separated with a Shimadzu QP2010 Plus GC/MS by a 0.25 × 0.25 mm × 30 m fused silica column. FAs were identified by comparing the retention times to those of authentic standards (Sigma). FA content has been expressed as percent (wt/wt) of total FA, because percent values may reflect essential FA status better than absolute plasma concentrations (19).

Operating conditions were as follows: injector port temperature 250 °C; helium as carrier gas at a linear velocity of 24.69 cm/s at 110 °C; inlet pressure 255 kPa; split ratio 1:50 and injection volume 1 μL; detector temperature 270 °C; H_2 flow 40.0 mL/min; air flow 450 mL/min; N_2 make up gas 40.0 mL/min; sampling rate 50 Hz. A programmed temperature run was used to separate fatty acids. The initial oven temperature was 110 °C, isothermal for 1 min. It was increased at a rate of 55 °C/min to 160 °C and held for 7.5 min. Next, the temperature was increased at a rate of 20 °C/min to 170 °C, held for 2 min, then increased at 4.5 °C/min to 190 °C with no hold, and finally increased at 9 °C/min to 230 °C and held for 5 min. Total analysis time was 26 min.

2.3. Statistics

The compliance of FA levels in milk with the normal distribution was evaluated by Shapiro–Wilk test. Values were presented as mean ± SD for normal distribution and [median (IQR)] for the non-fit normal distribution. Group comparisons were made by using the Mann–Whitney U test or Student's t-test. Depending on the distribution, repeated measure analysis of variance (ANOVA) or Friedman nonparametric analysis of variance were performed in order to investigate changes of FA levels by time (days 3, 7, and 28). SPSS 15.0 for Windows (SPSS Inc., USA) was used for the statistical analyses. Differences were considered significant at $P < 0.05$.

3. Results

When the age, gravidity, and parity data of those who were included the study were evaluated, there were no statistically significant differences among groups. There are significant differences in other clinical parameters ($P < 0.05$). Clinical data of the participants are shown in Table 1.

Table 1. Clinical data of participants.

	Preterm (n = 15) Mean ± SD (Median)	Term (n = 15) Mean ± SD (Median)
Primiparity (yes/no)	8/7	8/7
Maternal age at delivery	29.33 ± 5.6 (29)	28.2 ± 3.0 (28)
*Mode of delivery (C-section/vaginal)	10 / 5	1 / 14
Baby's sex (M/F)	7/8	6/9
*Duration of pregnancy (weeks)	34.1 ± 2.7 (35)	40.1 ± 0.8 (40)
*APGAR score (1st min)	8.6 ± 0.7 (9)	9.1 ± 0.5 (9)
*APGAR score (5th min)	9.8 ± 0.4 (10)	10 ± 0.0 (10)
*Birth weight (g)	2211 ± 614 (2340)	3396 ± 304 (3400)
*Birth length (cm)	44.5 ± 3.8 (45.5)	49.9 ± 1.4 (50)
*Birth head circumference (cm)	31.7 ± 2.4 (32.3)	35.2 ± 1.1 (35.5)

*: Statistically significant difference between preterm and term groups ($P < 0.05$).

The saturated fatty acids (SFAs) accounted for the majority of fatty acids in the breast milk. C14:0, C16:0, and C18:0 were the main saturated fatty acids found in the milk. The predominant monounsaturated fatty acid (MUFA) was C18:1. The most abundant n-6 polyunsaturated fatty acid (PUFA) was C18:2 in milk. n-3 FAs were the smallest PUFA component of breast milk. Preterm total SFA percentage, MUFA percentage, and n-3 PUFA percentage levels were higher than term on days 3, 7, and 28. Term milk n-6 PUFA percentage levels were higher than preterm on days 3, 7, and 28 (see Table 2). Total SFA percentage, MUFA percentage and PUFA percentage levels were in a

Table 2. FA composition (weight percentage of total FA) of breast milk at first month of lactation.

Fatty acids (FA)	Day 3		Day 7		Day 28	
	Preterm	Term	Preterm	Term	Preterm	Term
[a]C 10:0	0.03 (0.15)	0.26 (0.36)	0.06 (0.85)	0.04 (0.18)	0.23 (0.41)	0.13 (0.39)
[a]C 11:0	trace	0.02 (0.02)	0.02 (0.02)	trace	trace	trace
[a]C 12:0	3.5 (4.38)	1.58 (5.31)	4.93 (3.95)	4.97 (3.81)	5.37 (6.06)	6.91 (2.59)
[b]C 13:0	0.04 ± 0.02	0.06 ± 0.08	0.06 ± 0.03	0.06 ± 0.04	0.04 ± 0.02	0.05 ± 0.03
[a]C 14:0	Y 8.83 ± 2.49	7.78 ± 5.9	10.44 ± 3.14	9.05 ± 1.95	9.06 ± 2.9	9.6 ± 1.93
[b]C 15:0	0.6 ± 0.26	0.64 ± 0.61	0.66 ± 0.31	0.63 ± 0.32	0.5 ± 0.16	0.64 ± 0.28
[a]C 16:0	Y19.75 ± 1.66	19.66 ± 9.33	25 ± 10.29	19.7 ± 6.29	23.1 ± 7.08	23.05 ± 8.91
[b]C 17:0	0.71 ± 0.28	0.65 ± 0.62	0.81 ± 0.33	0.69 ± 0.37	0.65 ± 0.2	0.69 ± 0.4
[b]C 18:0	8.74 ± 3.05	7.95 ± 6.34	8.39 ± 6.9	9.33 ± 5.08	9.96 ± 4.69	8.45 ± 4.06
[a]C 20:0	0.51 ± 0.17	§0.65 ± 0.58	0.58 ± 0.23	0.5 ± 0.29	0.54 ± 0.18	0.53 ± 0.25
[b]C 21:0	1.09 ± 0.52	0.94 ± 0.68	1.24 ± 0.48	0.89 ± 0.37	0.94 ± 0.42	0.81 ± 0.43
[a]C 22:0	0.23 ± 0.1	0.35 ± 0.38	0.27 ± 0.11	0.24 ± 0.12	0.25 ± 0.07	0.26 ± 0.11
[b]C 23:0	0.13 ± 0.09	0.18 ± 0.21	0.21 ± 0.12	0.13 ± 0.08	0.11 ± 0.07	0.16 ± 0.11
[b]C 24:0	0.32 ± 0.11	0.47 ± 0.42	0.36 ± 0.18	0.33 ± 0.21	0.26 ± 0.11	0.27 ± 0.14
% SFA	44.48	41.53	53.02	46.57	51.02	51.56
[a]C 14:1	0.23 ± 0.13	§0.27 ± 0.31	0.33 ± 0.17	0.32 ± 0.21	0.25 ± 0.12	0.38 ± 0.18
[a]C 15:1	trace	0.01 (0.01)	trace	trace	0.01 ± 0.01	0.02 (0.01)
[a]C 16:1	2.5 (2.84)	2.14 (2.78)	1.7 (2.36)	3.22 (2.49)	2.28 (2.3)	1.64 (5.29)
[b]C 17:1	0.47 ± 0.26	0.34 ± 0.42	0.51 ± 0.28	0.37 ± 0.31	0.44 ± 0.16	0.43 ± 0.37
[b]C 18:1	Y 20.33 (10.2)	19.09 (15.59)	15.25 (4.22)	14 (6.79)	14.33 (8.58)	13.31 (8.15)
[b]C 20:1	1.39 ± 0.41	1.21 ± 0.92	1.23 ± 0.55	1.05 ± 0.58	1 ± 0.41	0.86 ± 0.35
[b]C 22:1n9	0.34 ± 0.1	0.33 ± 0.25	0.3 ± 0.12	0.25 ± 0.15	0.2 ± 0.12	0.17 ± 0.08
[b]C 24:1	0.72 ± 0.26	0.78 ± 0.51	0.77 ± 0.39	0.74 ± 0.53	0.64 ± 0.41	0.37 ± 0.13
% MUFA	25.99	24.17	20.1	20	19.15	17.17
[a]C 18:2n6c	0.27 (8.4)	6.8 (13.59)	0.48 (3.12)	6.98 (12.43)	0.56 (13.76)	9.28 (10.14)
[a]C 18:2n6t	22.57 ± 5.8	19.35 ± 13.1	19.1 ± 12.77	19.75 ± 6.97	23.47 ± 7.31	16.3 ± 6.94
[a]C 18:3n6	0.33 ± 0.21	2.15 ± 7.23	0.46 ± 0.2	0.29 ± 0.14	0.47 ± 0.16	0.63 ± 0.36
[b]C 20:2	2.22 ± 0.79	2.11 ± 1.64	2.12 ± 1.05	1.8 ± 0.87	1.49 ± 0.68	1.41 ± 0.51
[b]C 20:4n6	1.73 ± 0.66	1.82 ± 1.41	1.9 ± 0.79	2.66 ± 3.68	1.36 ± 0.39	1.81 ± 0.91
% n-6 PUFA	27.12	32.23	24.08	31.48	27.35	29.43
[a]C 18:3n3	1.12 ± 0.53	0.84 ± 0.73	1.59 ± 0.87	1.16 ± 0.57	1.59 ± 0.78	1.2 ± 0.67
[a]C 20:3n3	0.11 (0.08)	0.12 (0.13)	0.17 (0.17)	0.09 (0.15)	0.08 (0.15)	0.05 (0.02)
[a]C 20:5n3	0.08 (0.07)	0.06 (0.14)	0.12 (0.11)	0.09 (0.09)	Y 0.17 (0.15)	0.08 (0.06)
[a]C 22:6n3	1.09 (0.9)	1.08 (0.9)	0.93 (0.31)	0.68 (0.91)	0.65 (0.97)	0.52 (0.52)
% n-3 PUFA	2.4	2.1	2.81	2.02	2.49	1.85

§: Term > preterm ($P \leq 0.05$), Y: preterm > term ($P \leq 0.05$). SFA: saturated FA, MUFA: monounsaturated FA, PUFA: polyunsaturated FA. Data are shown as mean ± SD (for normal distribution) or median (IQR) (if they do not fit normal distribution). [a]: Change of FA level with the progression of lactation is statistically significant ($P < 0.05$), [b]: change by time is not significant ($P > 0.05$).

comparable range to other published values (20–23). Total FA levels of preterm and term breast milk changes on days 3, 7, and 28 of lactation are shown in the Figure.

In this study we determined the levels of 31 FAs in breast milk. As a result of analysis of variance, FA levels were found to change over time in 17 FAs ($P < 0.05$), but 14 of them did not ($P > 0.05$). Statistically significant differences were found between groups in 9 fatty acids, but 3 of them were ignored since they were present only in trace amounts. In group comparison, some FAs (SFAs C14:0 and C16:0 on day 3, MUFA C18:1 on day 3, and C20:5 on day 28) significantly increased in the preterm group compared to the term group ($P = 0.041$, $P = 0.046$, $P = 0.027$, $P = 0.033$, respectively), whereas myristoleic acid (C14:1) and eicosanoic acid (C20:0) on day 3 significantly increased in the term group ($P = 0.015$, $P = 0.048$, respectively). Preterm and term FA compositions of breast milk are shown in Table 2.

4. Discussion

Although breast milk is universally considered to be the optimal form of nutrition for newborn infants, the fat content and FA composition of breast milk show considerable variability. Populations, the effects of the extent of maternal FA body stores, nutritional status, parity, and various other factors may affect the variability of breast milk FA composition (7–15). One of these factors is the gestational age. In this study we argue that preterm infants may need more FA than term infants. Thus, we investigated whether there are differences in their mothers' milk. Furthermore, we determined FA composition and time-dependent changes of breast milk in Turkey.

There are many studies on breast milk FA composition, but the results are not consistent. Kumbhat et al. (24) reported the difference in fat concentration in preterm and term milk as nonsignificant. Paul et al. (25) reported a significant increase in fat concentration with the progression of lactation, but no significant difference between term and preterm milk. Gross et al. (26) observed a nonsignificant difference in fat concentration in both the progression of lactation and decreased gestation. Bitman et al. (27) did not find any difference when comparing FA composition of breast milk on day 42 of lactation in women giving birth to very preterm (26 to 30 weeks), preterm (31 to 36 weeks), and term (37 to 40 weeks) infants. Similarly, Genczel-Boroviczeny et al. (28) did not find any

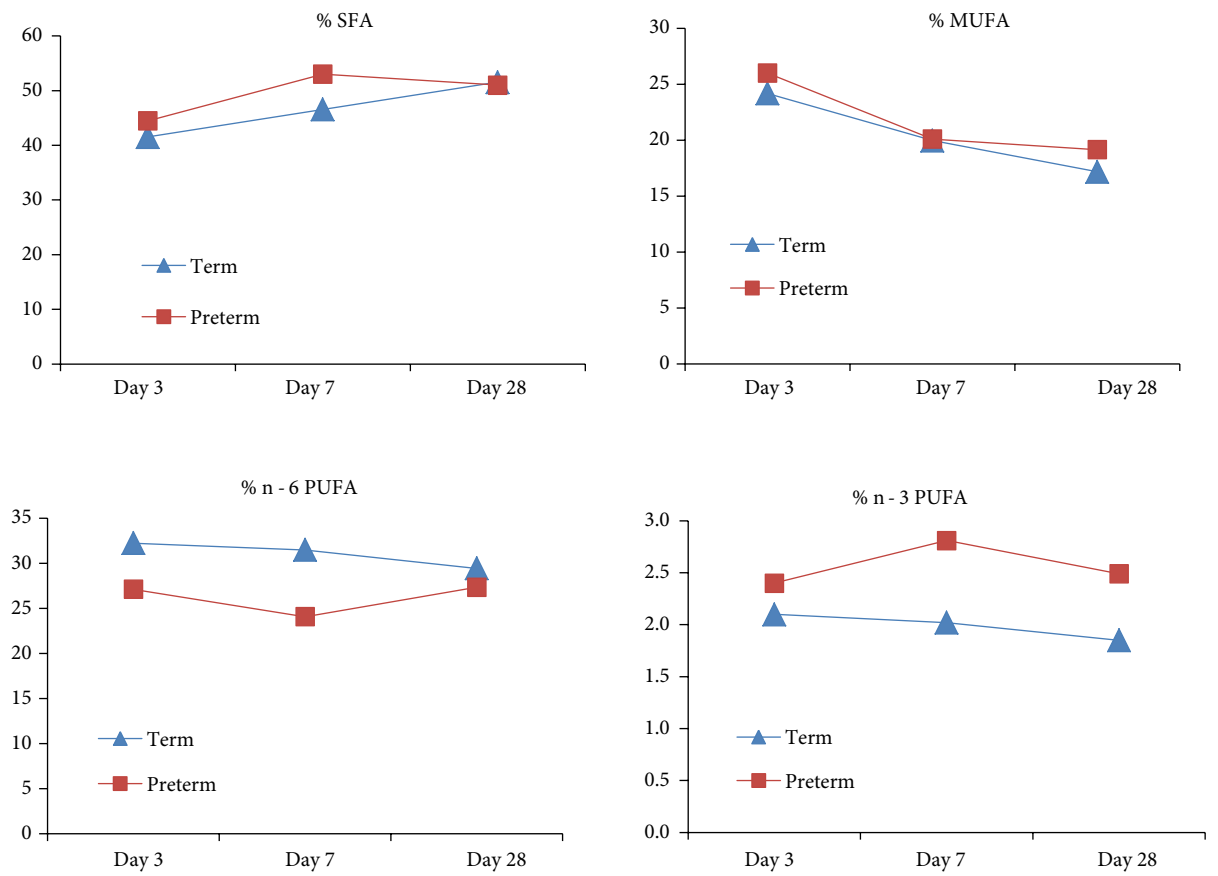

Figure. Changes of total FA levels of preterm and term breast milk at first month of lactation.

differences on days 5, 10, 20, and 30 of lactation in mothers of preterm as compared to term infants. Contrary to all of them, Luukkainen et al. (29) reported significantly higher contributions of C20:4 and C22:6 to the FA composition of breast milk in mothers of preterm rather than term infants. Kovacs et al. (30) reported significant differences in long-chain PUFA (LCPUFA), C20:4, and C22:6 between term and preterm breast milk.

In this study, we found some differences in total SFA percentage, MUFA percentage, and PUFA percentage levels. When all FAs compositions were evaluated, while the contribution of MUFA percentage and n-6 PUFA percentage decreased, SFA percentage levels increased with the progression of lactation. Although term and preterm FA composition changed together in the SFA percentage, MUFA percentage, and n-6 PUFA percentage FA groups, the term and preterm groups showed different types of changes in n-3 PUFA percentage. On the other hand, significant changes during the progression of lactation were found in 17 fatty acid levels. Contrary to Kovacs et al. and Luukkainen et al. (29,30), we did not find any significant difference in C20:4, C22:6, and LCPUFAs, but we found statistically significant differences in some FA levels (C14:0, C16:0, C18:1, C20:5, C14:1, and C20:0) between the preterm and term groups.

Newborn, and especially preterm, infants have only very limited body stores of FAs. It is shown that essential FAs are very important for the development of visual acuity, the nervous system, and later growth in fetal and neonatal stages (31–34). Thus, it is extremely important to provide adequate nutrients and especially essential FAs in early infancy. As is well known, arachidonic acid (C20:4), docosahexaenoic acid (DHA; C22:6) and eicosapentaenoic acid (EPA; C20:5) can be synthesized by chain elongation and desaturation of essential FA, but linoleic acid (LA; C18:2n-6) and alpha-linolenic acid (ALA; C18:3n-3) cannot be. Moreover, endogenous synthesis of these FAs from their precursors LA and ALA is limited in preterm infants.

In our study, we found increased ALA levels in the preterm group. Additionally, we measured cis and trans isoforms of LA. While cis-LA (C18:2n-6c) levels were higher (day 7) in the term group (did not fit normal distribution), trans-LA (C18:2n-6t) levels were higher in the preterm group but not did not differ significantly ($P > 0.05$). We think that further studies with greater numbers of samples can give more information about LA levels and their physiological role in breast milk. In our study, the majority of the differences were on day 3 of lactation. These data suggest that breast milk, and especially early infancy period colostrum, may have an important role for preterm nutrition. When previously reported studies are evaluated, similar results to our study can be observed (30,35). Nevertheless, according to some studies there are no differences in these FA levels between preterm and term groups (26–28).

The control of growth in neonates is very complex. FAs are considered important in neonates' development. In fact, FAs may have a central or key factor role on stimulating the development of newborns. Breast milk is universally considered to be the optimal nutrition for infants, and breast milk contains a considerable amount of LCPUFAs (36–38). However, the content (39–42) and FA composition of breast milk may show considerable variety between populations (9,10). Furthermore, the stage of lactation also has an important role in determining the FA composition of breast milk. This study determined changing levels of FAs by the progression of lactation in breast milk from mothers of preterm neonates and compared them to those from mothers of term infants. The results of this study and other similar studies can help to improve infant formula, but more detailed studies are needed.

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