Fatty acid composition in the breast milk of Sri Lankan mothers during exclusive breastfeeding

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Abstract
Introduction: Published data on the fatty acid (FA) composition of breast milk (BM) of Sri-Lankan mothers are limited.

Objectives: To report the FA composition in BM within the first 6 months of lactation and to study whether the fat mass of lactating mothers would influence FA composition of their BM.

***Method**:* During the present cross-sectional study, mid-stream milk samples from 48 exclusively breastfeeding mothers were collected and analysed by gas chromatography. Deuterium dilution technique was used to measure maternal body composition.

Results: Most abundant FA was lauric acid (22.3±5.2%). Oleic acid, palmitic acid and myristic acid were also found in high amounts (21.9±4.4%, 19.2±2.6% and 15.7±2.7%, respectively). C18, C14:1 and C20:3n6 percentages were significantly different in the three phases of lactation (0-2, 2-4 and 4-6 months). Percentage of docosapentaenoic acid showed a significant positive correlation with the age of the mother. Amounts of C14:1, C16, C18, C18:1c, C20:4n6, C22:5n3 and C22:n3 were positively correlated significantly to mother's body weight. Further, C16:1 showed a positive significant correlation to the percentage FM of mother.

Conclusions: FA composition of BM from Sri-Lankan mothers showed wide variations in C8, C18:1t, C18:3n3, C20, C20:3n6, C20:4n6, C22:5n3 and C22:6n3. Amounts of arachidonic acid and DHA were high but linoleic and linolenic acid percentages were low.

(Key Words: Human milk, Fatty acids, Essential fatty acids, DHA, Arachidonic acid)

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Introduction

Breast milk (BM) is recommended for infants during the first half of infancy. Milk fat contains the necessary nutrients responsible for infant growth[1]. Body stores of fatty acids (FAs) in infants are limited[2]. However, their rapidly growing tissues require higher amounts of FA deposition[2,3]. It is well established that gestational age, phase of lactation, parity, ethnicity and geographic region affect the composition of FAs in BM[4-7]. Studies have been conducted to measure FA content[5,6] but data on Sri-Lankan mothers are scarce.

Objectives

To report the composition of FAs in human milk during the first 6 months of lactation and to assess the effect of maternal fat mass on composition in BM.

Method

Forty-eight healthy exclusively breastfeeding mothers were recruited to this cross-sectional study to represent three phases of exclusive breastfeeding (16 in each group) from birth to 6 months (two months intervals) from well-baby clinics in the region, using purposive random sampling. Age range of mothers was 18–40 years and their parity was less than 4. All infants of selected mothers were full term and healthy. Mothers with multiple gestation, hypertension, heart disease, hypothyroidism and pregnancy complications were excluded from study. Mothers whose infants had chronic diseases, congenital abnormalities, or diseases that could affect breastfeeding or growth were also excluded. Demographic and anthropometric data of mothers and infants were collected using a questionnaire.

Collection of breast milk: A single mid-stream milk sample (25 mL) was collected from each mother between 9.00-11.00 hours. Samples were collected into sterile polypropylene bottles. Mothers were requested to collect breast milk samples by hand expression from one breast. They were provided with labelled sterile sample collecting bottles and gloves and were instructed to clean the breast and hands thoroughly before sample collection and sit on a chair or floor comfortably. To collect mid-stream milk, mothers were requested to put baby to breast and after 5 minutes collect the milk and then continue feeding[8]. Sample bottles were sealed tightly with parafilm and transported to India on dry ice, where analyses were done at the St. John's Research Institute, Bangalore.

Sample analysis: FAs in BM (from 500µl of milk) were analysed by gas chromatography using a flame ionization detector (Varian 3800; Varian, Palo Alto, CA, USA). Procedure included extraction of total lipids and trans-methylation of all FAs with BF3-methanol[9]. After that FA methyl esters were separated. This was done based on chain length and the degree of saturation by injecting onto a capillary column (50 m, 0.2 mm, FAME, Varian) with nitrogen as the carrier gas. Individual FAs were identified against reference FAs. The odd-chain FA named heptadecanoic acid (C17:0) was used as an internal standard in each sample. Based on the internal standard, total FA contents of each sample were calculated. It was expressed as a percentage of total content of identified FAs. Body composition of mothers was measured by using deuterium-dose-to-mother technique[10].

Ethical issues: Approval for the study was obtained from the Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka (dated 09/03/2012). Participants were recruited entirely on a voluntary basis. Written informed consent was obtained from all participants after explaining procedures and outcomes of the study.

Statistical analysis: SPSS, version 20 (SPSS Inc., Chicago) was employed for the data analysis. Differences of FA composition between groups were analysed using independent sample test (Kruskal-Wallis Test). Correlation between composition of FAs and maternal characteristics, mother's body composition and demographic data was assessed by Spearman's rho correlation using SPSS and the significance was set at $p < 0.05$.

Results

Mothers' average age was 27.8 ± 6.2 years. Mean parity was 2±1. Mean % FM of mothers was 23.5 ± 7.9. Though 71% mothers had passed G.C.E ordinary level or higher levels of education, only about 8% were employed. Mean income of the families was 22,865 LKR per month (approximately 115 USD). Demographic characteristics, anthropometry and body composition of the lactating mothers are shown in Table 1.

Table 2 presents the composition of individual FAs according to the infants' age groups. It gives the amounts of individual FA measured (as a percentage to total FAs) in human milk. Capric (C10), lauric (C12), myristic (C14), palmitic (C16) and stearic (C18) acids were among the main saturated fatty acids (SFA). Oleic acid (C18:1) and palmitoleic acid (C16:1) were the key monounsaturated fatty acids (MUFA) and linoleic acid (C18:2 n6) was the predominant polyunsaturated fatty acid (PUFA). The other detectable PUFAs were a-linolenic acid (C18:3 n3), dihomo-g-linolenic acid (C20:3 n6), arachidonic acid (20:4 n6) and docosahexaenoic acid (C22:6 n3, DHA). No significant differences were found between the three age groups with respect to percentages except for myristoleic acid: C14:1 (p=0.040), ocatadecanoic acid: C18 (p=0.028) and dihomo-γ-linolenic acid: C20:3n6 (p=0.023). Myristoleic acid and octadecanoic acid percentages were higher in the group of mothers whose infants were 2-4 months age. The % dihomo-γ-linolenic acid (DGLA) was found to decline across three age categories.

Table 1: ***Demographic characteristics, anthropometry and body composition of lactating mothers (n=48)***

Information	Age group*			
	Group 1	Group 2	Group 3	All mothers
Age (years) - Mean ± SD	25.5 ± 4.5	31.8 ± 6.0	25.9 ± 6.1	27.8 (6.2)
Parity - Mean ± SD	2 ± 1	2 ± 1	2 ± 1	2 ± 1
Monthly income (Sri Lanka rupees)	20,000.00	24,375.00	24,250.00	22,856.00
Body weight (kg) - Mean ± SD	51.1 ± 8.5	54.7 ± 10.5	47.5 ± 13.0	51.3 ± 11.0
Height (m) - Mean ± SD	1.52 ± 0.05	1.54 ± 0.03	1.51 ± 0.06	1.52 ± 0.05
BMI (kg/m^2) - Mean ± SD	22.2 ± 3.3	23.0 ± 4.0	2.6 ± 4.9	22.0 ± 4.1
Fat mass (kg) - Mean ± SD	39.1 ± 5.9	40.5 ± 5.1	36.1 ± 6.8	12.6 ± 6.2
Fat Free Mass (kg) - Mean ± SD	12.1 ± 5.2	14.3 ± 6.6	11.4 ± 6.7	38.7 ± 6.1
% FM - Mean ± SD	23.0 ± 7.2	24.9 ± 8.5	22.3 ± 8.3	23.5 ± 7.9

**Group 1: Mothers whose infants are <2 months old, Group 2: Mothers whose infants are 2-4 months old, Group 3: Mothers whose infants are 4-6 months old*

Table 2: ***Fatty acid composition (as % to the total fatty acid content) of human milk****

Fatty Acid	Infant's age groups			All infants
	< 2months	2-4 months	4-6months	
Octanoic acid (C8)	0.26 ± 0.19	0.26 ± 0.21	0.12 ± 0.13	0.21 ± 0.19
Decanoic acid (C10)	3.09 ± 0.88	2.44 ± 0.70	2.66 ± 0.56	2.73 ± 0.76
Lauric acid (C12)	22.64 ± 5.81	20.94 ± 6.20	23.23 ± 3.31	22.27 ± 5.24
Myristic acid (C14)	15.49 ± 3.11	15.13 ± 2.82	16.39 ± 2.23	15.66 ± 2.74
Myristoleic acid (C14:1)	0.69 ± 0.34[a]	0.86 ± 0.19[a]	0.69 ± 0.22[a]	0.75 ± 0.26
Palmitic acid (C16)	19.11 ± 2.65	20.13 ± 3.07	18.26 ± 1.59	19.17 ± 2.58
Palmitoleic acid (C16:1)	4.56 ± 1.88	4.95 ± 1.41	4.31 ± 1.52	4.6 ± 1.61
Octadecanoic acid (C18)	3.12 ± 0.46[c]	3.46 ± 0.43[c]	3.08 ± 0.53[c]	3.22 ± 0.50
Eladic acid (C18:1t)	0.05 ± 0.14	0.18 ± 0.38	0.09 ± 0.30	0.10 ± 0.29
Oleic acid (C18:1c)	21.73 ± 4.76	22.63 ± 5.06	21.28 ± 3.26	21.88 ± 4.38
Linoleic acid (C18:2n6)	7.24 ± 1.61	6.9 ± 1.31	7.92 ± 1.66	7.36 ± 1.56
γ - Linolenic acid (C18:3n3)	0.26 ± 0.15	0.46 ± 0.70	0.34 ± 0.16	0.35 ± 0.42
Arachidic acid (C20)	0.04 ± 0.11	0.04 ± 0.08	0.02 ± 0.05	0.03 ± 0.08
Dihomo-γ- linolenic acid (C20:3n6)	0.84 ± 1.38[b]	0.79 ± 1.09[b]	0.33 ± 0.15[b]	0.65 ± 1.03
Arachidonic acid (C20:4n6)	0.56 ± 0.14	1.11 ± 1.34	0.56 ± 0.15	0.75 ± 0.81
Docosapentaenoic acid (C22:5n3)	0.06 ± 0.09	0.06 ± 0.09	0.03 ± 0.06	0.05 ± 0.08
Docosahexaenoic acid (C22:6n3)	0.60 ± 0.37	1.12 ± 1.22	0.63 ± 0.28	0.78 ± 0.78

**There were 16 breast milk samples in each age group; results are presented as mean ± SD*
[a,b,c] superscripts given in a raw indicated significant difference ($p<0.05$)

Association of each %FA with maternal characteristics such as age, body weight, BMI, parity and %FM was assessed using Spearman's rho correlation (Table 3).

Table 3: ***Spearman's correlation (r) of the % FA with maternal characteristics [a]***

Fatty acid	Mother's age	Mother's bodyweight	Parity	Mother's BMI	%FM of mother
C8	0.086 (0.561)	0.264 (0.070)	0.082 (0.580)	0.293 (0.044) *	0.177 (0.240)
C10	-0.193 (0.188)	-0.207 (0.158)	0.012 (0.933)	-0.093 (0.529)	-0.054 (0.722)
C12	-0.135 (0.362)	-0.362 (0.011) *	-0.029 (0.843)	-0.284 (0.051)	-0.183 (0.223)
C14	-0.202 (0.169)	-0.470 (0.001) **	-0.122 (0.407)	-0.368 (0.010) *	-0.272 (0.067)
C14:1	0.228 (0.119)	0.320 (0.026) *	0.147 (0.319)	0.315 (0.029) *	0.404 (0.005) **
C16	0.088 (0.553)	0.376 (0.009) **	0.034 (0.817)	0.354 (0.014) *	0.178 (0.237)
C16:1	0.039 (0.795)	0.264 (0.070)	-0.013 (0.930)	0.270 (0.063)	0.336 (0.022) *
C18	0.150 (0.310)	0.289 (0.046) *	0.100 (0.499)	0.264 (0.070)	0.089 (0.555)
C18:1t	0.223 (0.128)	0.046 (0.756)	-0.002 (0.988)	0.178 (0.225)	0.011 (0.940)
C18:1c	0.129 (0.381)	0.311 (0.031) *	0.003 (0.982)	0.174 (0.238)	0.137 (0.364)
C18:2n6	0.073 (0.624)	0.097 (0.513)	0.035 (0.814)	0.067 (0.652)	0.051 (0.736)
C18:3n3	0.181 (0.219)	0.001 (0.996)	0.073 (0.620)	-0.061 (0.680)	-0.013 (0.930)
C20	0.035 (0.811)	-0.099 (0.502)	-0.008 (0.959)	-0.084 (0.568)	0.169 (0.262)
C20:3n6	-0.009 (0.950)	0.116 (0.432)	0.007 (0.960)	0.024 (0.874)	-0.088 (0.559)
C20:4n6	0.206 (0.160)	0.366 (0.011)**	0.149 (0.312)	0.287 (0.048) *	0.093 (0.540)
C22:5n3	0.290 (0.045)*	0.326 (0.024)*	0.175 (0.234)	0.294 (0.042) *	0.140 (0.354)
C22:6n3	0.258 (0.076)	0.448 (0.001) **	0.082 (0.578)	0.405 (0.004) **	0.171 (0.257)

[a] Results are presented as correlation coefficient (p value)
**Correlation is significant at the 0.05 level*

Age of mothers showed a significant positive correlation for the level of docosapentaenoic acid (DPA) (r=0.290, p=0.045). However, DPA content of BM of Sri-Lankan mothers was low. Mother's body weight had a negative correlation with % of lauric acid (r=-0.362) and myristic acid (r=-0.470) which reached a statistically significant level (p =0.01). On the other hand, mother's body weight was positively correlated with myristoleic acid (r=0.320, p=0.026), palmitic acid (r=0.376, p=0.009), octadecanoic acid (r=0.289, p=0.046), oleic acid (r=0.311, p=0.031), arachidonic acid (r=0.366, p=0.011), docosapentaenoic acid (r=0.326, p=0.024) and docosahexaenoic acid (r=0.448, p=0.001). Maternal parity did not show any significant association with any of maternal parameters assessed. % FM of mothers showed significant positive correlations with myristoleic (r=0.404, p=0.005) and palmitoleic (r=0.336, p=0.022) acids. However, no significant associations were found between the percentages of any of the FAs with infant parameters studied (i.e., birth weight, weight gain or BMI

Discussion

This study investigated FA composition (with regards to 17 FAs) of human milk during exclusive breast feeding. Lauric acid (C12; 22.3% ± 5.2) was found to be the main FA component followed by oleic acid (C18:1c; 21.9%), palmitic acid (C16; 19.2 %) and myristic acid (C14; 15.7%). Fat is among the most important nutrients in human milk[11]. It contributes to 45-55% of energy content in human milk[12]. Milk fat provides indispensable PUFAs (omega-6 and omega-3) which affect the health, growth and development of infant[13]. Studies have shown a considerable variation in FA composition in BM[4-7]. According to literature, content of fat in human milk is more variable than content of other macronutrients. Factors affecting the variability of BM FA composition include ethnicity, population, extent of maternal fat stores in body, maternal nutritional status, parity etc.[4,5,6]. Gestational age, duration of breastfeeding and time of day are also reported to affect the composition of FAs in BM[1]. Content of PUFA in BM is a function of the mothers' dietary intake.

Finley DA, *et al.,* (1985) determined FA composition of 172 human milk samples of 57 Californian mothers (range 1-9 months)[14]. Results showed that C18:1c and C16 as the most abundant fatty acids (31.7% and 23.3% respectively). However, lauric acid (C12) and myristic acid (C14) percentages were low (5.6% and 8.0%) in Californian mother compared to Sri-Lankan mothers[14]. Presence of high level of lauric acid in BM of Sri-Lankan mothers could be due to coconut oil consumption which is rich in lauric acid. All Sri Lankans have a habit of using coconut products (mainly in the form of oil and milk) in cooking. Kuipers RS, *et al*[15] studied BM composition of mothers from Chole, Tanzania. Their study reported high levels of C12 (20.17%), C14 (21.19%), arachidonic acid (0.50 %) and docosahexaenoic acid (0.73 %), but low levels of linoleic acid (4.23 %).

Results of our study have indicated differences in amounts of saturated, MUFA and PUFA levels during the progression of lactation. Percentage octanoic acid (C8) was found to be reduced in the 4-6-month age group compared to the <2 month and 2-4 month age group (0.12% vs. 0.26%). However, difference was not significant. The percentage of octadecanoic acid (C18) was significantly reduced in the 4-6 months old group compared to the <2 month and 2-4-month-old group. Further, percentages of both arachidonic acid (C20) and docosapentaenoic acid (C22:5n3) were reduced in the 4-6-month-old group compared to <2 months and 2-4-month-old groups (0.02% vs. 0.04% and 0.03% vs. 0.06%). However, the differences were not significant. Percentages of dihomo-γ-linolenic acid (C20:3n6) were reduced across the age groups significantly. Yuhas R, *et al*[16] analysed the BM FAs from lactating mothers in Canada, Australia, Japan, Chile, Philippines, China, Mexico, United States and United Kingdom. They found that SFA content was relatively constant in all above mentioned countries, except Philippines**.** In Philippines, lauric and myristic acid levels were significantly high compared to most other countries. MUFAs have shown little variation other than low levels of oleic acid in Philippines and high levels of erucic acid in China. Levels of arachidonic acid (C20:4n-6) was similar in all countries ranging from 0.36 - 0.49%.

DHA and other long chain polyunsaturated fatty acids (LCPUFA) are essential both in prenatal and postnatal periods, since LCPUFA synthesis from their precursors, α-linolenic acid and linoleic acid cannot meet infants' high demand. Postnatal DHA status has been reported to be vital for visual acuity, neurodevelopment, and behaviour[17]. Though BM contains a sufficient amount of LCPUFA (DHA and arachidonic acid) to meet the above needs, LCPUFA composition of human milk mainly depends on the maternal diet[17]. Mean arachidonic acid percentage in this study was higher (0.75±0.8) than reported elsewhere. Average DHA levels were between 0.17 to 0.99%. Highest levels have been reported from Japan and the lowest levels from Canada and U.S. in study by Yuhas R, *et al*[16]. Percentage DHA of Sri Lankan mothers of the three phases of lactation; namely <2month, 2-4 months and 4-6 months were 0.6% ± 0.4, 1.12%±1.2 and 0.63%±0.28 respectively.

Brenna JT, *et al*[18] reported 106 human BM studies that used modern analytical techniques enabling highly accurate results. This study reported the average levels of 0.32 ± 0.22% (range: 0.06-1.4%) DHA and 0.47 ± 0.13% (range: 0.24-1.0%) arachidonic acid in BM respectively. However, we are reporting mean DHA (0.78% ± 0.78) and arachidonic acid (0.75 ± 0.81) contents higher than that of the Brenna *et.al* report[18]. DHA content of BM is dependent on maternal diet. Quinn EA, *et al*[19] investigated the above association in marginally nourished Filipino women (n=117) nursing infants less than 2 years of age. According to their results BM DHA has shown a positive, dose-response relationship to the fish consumption of the mothers. This study has suggested that increasing fish consumption of the mothers might be a cost-effective method for maximizing the delivery of DHA to the infants in populations with marginal energy intakes during the period of lactation.

Lee PS, *et al*[20] estimated DHA contents in BM of 136 Sri Lankan mothers in three areas in Sri Lanka (Colombo, Kandy and Matara,), with a different levels of sea fish access. Study participants were 6-12-month-old mother-baby pairs. This study further studied the effect of dietary fish on BM FA levels by assessing food intake using a food frequency questionnaire. Study reported that DHA levels of BM among mothers who lived in all above three locations were high (0.79%, 0.53% and 0.37% respectively)[20]. It further indicated that the DHA levels of BM correlated with consumption of fish. Mean DHA levels reported from our study are comparable with values reported from mothers who participated from Matara, Sri Lanka, in their study. Present study was conducted in Galle, Sri Lanka, which is in close proximity to Matara and both areas are from the coastal belt of Sri Lanka where sea-fish consumption among the population is high. Both studies have been conducted on Sri-Lankan mothers who are Sinhalese; thus, they possess a similar genotype. Based on the findings of Lee PS, *et al*[20], we suggest that the higher DHA levels reported in this study may also be due to high sea-fish consumption.

Essential FA (Linoleic and linolenic) content in BM possesses a special interest due to their physiological significance. Linoleic and linolenic acids and their derivatives arachidonic acid and docosahexaenoic acid are valuable for proper development and growth of infants, especially for the nervous system. Studies conducted in Germany have reported about 10.8 % of linoleic acid and 0.3-2.4% of linolenic acid in human milk[1]; however, we are reporting 7.4% ± 1.6 and 0.35 % ± 0.4 levels in Sri Lanka. Finley DA, *et al* have also reported higher linoleic and linolenic acid percentages in human milk compared to values reported by this study[14].

Present study did not show a significant association between the % of fatty acids and parity. However, Lassek WD *et al*[21] have studied the effect of parity on body fat distribution in American women. They reported that when parity increases, body fat estimated by skinfold decreases. When waist circumference increases, a relative decrease has been observed in lower body fat. They have postulated that fat mobilization in the lower body at the time of pregnancy and lactation might be helpful to satisfy the needs of the developing brain for essential fatty acids and energy of infants during the period of peak growth[21].

Maternal age did not show a significant correlation with any of FAs except for the percentage of docosapentaenoic acid (DPA) in the present study. DPA level in BM of Sri-Lankan mothers was low. Argov-Argaman N, *et al*[22] studied the effect of maternal age on FA composition by analysing colostrum and transition milk from mothers aged above and below 37 years, respectively. It reported a low-fat content and about 10-times higher levels of omega 6 fatty acids, eicosadecanoic, and arachidonic acids in transition milk of younger group mothers. Further study indicated that the gestational age is affecting the total fat and omega 3 and omega 6 fatty acids in colostrum compositions of the elder group. It was concluded that age would affect the FA composition[22].

Present study did not evaluate the diet of lactating mothers as the main determinant of fat composition in BM. Only healthy mothers were recruited for this study. Their BMI and body composition were in the desirable range. Therefore, none of them were malnourished. According to the monthly income, participants belonged to middle-socio-economic stratum of Sri Lanka. Therefore, we assumed that mothers would consume a normal healthy Sri Lankan diet with more coconut oil and coconut milk compared to other countries. Their sea-fish consumption should also be higher as they are living in the coastal belt in Sri Lanka where accessibility and affordability for sea-fish is higher.

Conclusions

Lauric, palmitic, myristic and oleic acids were the most abundant fatty acids in Sri-Lankan mothers' breast milk during the exclusive breastfeeding period. Breast milk of Sri-Lankan mothers contains fairly high amounts of arachidonic acid and DHA. However, percentages of linoleic and linolenic acids that are considered essential are low compared to values reported elsewhere.

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