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Title: No Evidence for Sex-specificity in Vitamin C, E and Fatty Acid Content of Human Milk from Healthy Polish Mothers

Short title: Fatty Acids and Vitamin Content in Human Milk

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Abstract

Objectives: Human milk (HM) is a complex fluid that meets the nutritional needs of infants. Its composition is associated with environmental, maternal, and foetal variables. It provides nutrients and bioactive substances, including cytokines, immunoglobulins, and constituents with antioxidative properties. Boys are reportedly more susceptible to oxidative stress. This study aimed to determine the relationship between infant sex and the antioxidants vitamins C and E, and the fatty acid (FA) profile of HM. Results of this investigation may infer sex differences for the composition of infant formulas.

Methods: Thirty days after delivery, a sample of HM was collected from 152 healthy, non-smoking mothers of full-term new-borns (77 males) born in good clinical condition. After FAs were extracted

from the fat component, they were converted into methyl esters and separated using high-performance gas chromatography. Tocopherol content was determined using a method described in a previous study. Vitamin C content was determined using reversed-phase high-performance liquid chromatography with ultraviolet detection, as described in the same study.

Results: The study groups (male versus female offspring) did not differ in terms of vitamin and FA content in HM. The only difference found was in gondoic acid 20:1 (n-9), with a higher concentration in the HM of mothers with female offspring (mean 0.63±0.18 versus 0.59±0.15 g/100g FA; $p<0.047$).

Conclusions: Despite the acknowledged differences in the composition of HM associated with infant sex and the increased oxidative stress in males, antioxidant content did not appear to differ according to infant sex. These results suggest that there is no need for the antioxidant content of infant formulas to be sex specific.

Keywords: Fatty acids, ascorbic acid, tocopherols, HM, oxidative stress

What is known

- The composition of human milk (HM) is associated with many variables.
- Male and female new-borns differ in their adaptation to environmental factors such as the level of oxidative stress and infections.
- It is known that the content of some components of HM (lipids, proteins, electrolytes, energy) is influenced by the sex of the offspring in some populations.

What is new

- There was no difference in vitamin C and E concentrations in the HM of mothers of boys versus girls.
- Differences in gondoic acid concentrations in the HM of healthy Polish mothers of males and females were observed; however, its meaning requires further investigation.

Introduction

Human milk (HM) is an ideal nutrient for healthy, full-term infants, not only for its nutritional properties, but also for the presence of bioactive components [1]. HM contains macro- and micronutrients, hormones, immune components, and other metabolites that are important for growth and development. Due to the unique composition and physiological characteristics of HM, current recommendations advise exclusive breastfeeding during the first six months of life [2]. It is well known that the composition of HM varies among mothers and different time points of lactation, and its content can be modulated by environmental, maternal, and foetal factors [3–5].

It has been reported that female and male infants may differ, not only in terms of the pattern and pace of both development and growth but also in susceptibility and response to different factors [6–8].

To date, there have been limited data regarding the impact of infant sex on the composition of HM. Studies have primarily focused on macronutrient profile and energy content of HM, and have reported conflicting and wide ranging results, including advantages for females [9] to no appreciable differences [10] to advantages for males [11]. However, little is known about the relationship between fatty acid (FA) and vitamin concentrations in HM and infant sex. There have been few studies investigating sex differences in plasma vitamin C and FA concentrations and consecutive events. For example, vitamin C intake is negatively associated with abdominal obesity in women [12]. The concentration of some FAs (e.g., linoleic acid [LA] and gamma LA) may also differ between women and men [13]. Fat storage in the subcutaneous adipose tissue appears to be higher in women, whereas it is higher in the visceral adipose tissue in men. Visceral obesity is a well-known risk factor for cardiovascular disease [14]. Because diet is believed to be the major factor influencing FA composition and subsequent development of adipose tissue, it appears reasonable to evaluate FA content in HM, the very first form of human nourishment. Moreover, it has been reported that male infants exhibit higher levels of oxidative stress than girls [7,8]. This raises the question as to whether this is reflected in the composition of HM and, if so, could this be relevant to the composition of infant formulas?

The aim of this study was to evaluate differences in antioxidants (vitamins E, C) and FA in HM, and whether they can be attributed to the sex of the infant. To our knowledge, this is the first study to investigate the antioxidative properties and constituents of HM and their relationship with infant sex.

Materials and Methods

Participants

This cross-sectional study included women who delivered in an obstetric ward of the Copernicus Hospital (a tertiary hospital located in Gdańsk, Poland) and met the following inclusion criteria: age >18 years; Polish ethnicity; no history of chronic conditions or pregnancy complications; normal full-term vaginal delivery of a single neonate (38–40 gestational weeks); good general condition of the neonate (Apgar score, 8–10); and normal birth weight (2500–4000 g). Women who were active smokers, those experiencing acute infection within the previous 3 months, and those with chronic conditions (such as gestational diabetes and/or atopy) or undergoing pharmacotherapy were excluded. On the day of sample collection, the participating mothers were administered a survey addressing the health status of the mothers and children, and the presence of any exclusion criteria. Based on the sex of the infant, participants were divided into two groups: mothers of girls (group I [n=75]) and mothers of boys (group II [n=77]). The study groups were homogenous in terms of age, ethnicity, and socioeconomic status and, as such, likely consumed a similar diet.

HM sampling

HM samples were collected 30 days after delivery. The mammary gland was completely evacuated with the aid of an electric lactator 2 h after the first morning feeding and collected in sterile glass containers. After gentle stirring, 5 mL samples were aliquoted into sterile containers and immediately frozen at -80°C. The remaining milk was fed to the infants.

Analytical protocol

Total fat extraction: Fat in the HM was extracted using the traditional Roese-Gottlieb method [15], which was modified for the purpose of this analysis; the step for fat drying at 102°C was replaced by evaporation of the solvent at 40°C under reduced pressure. Changes in the final stage of extraction of lipids from the HM enabled the quantitative determination of milk fat without altering its quality (e.g., without oxidation of polyunsaturated fatty acids).

FA content: FAs extracted from the HM fat were converted into FA methyl esters (FAMEs) in accordance with a standard ISO procedure [16]. The FAMEs were separated using high-performance gas chromatography (HP-GC) based on the length of the hydrocarbon chain and the degree of FAME unsaturation using a gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a split/splitless injector, flame ionisation detector, and Rtx 2330 column (100 m×0.25 mm; Restek, Bellefonte, PA, USA). Quantitative and qualitative analyses of the FAs were performed against standard solutions of FAMEs (Supelco Bellefonte, PA, USA; Larodan Fine Chemicals, Malmö, Sweden) using the external standard method with correction factors [17]. Results are expressed as weight percentages of all identified FAs with 6- to 24-carbon chain lengths, containing up to six double-bond FAs in total FAs.

Tocopherol content: Tocopherol content in the HM was determined using the method described by Romeu-Nadal et al. [18]. A sample of HM (0.5 mL) was poured into a tube. One millilitre of n-hexane was added, and the mixture was shaken for 1 min and centrifuged at 7000 rpm for 10 min. The organic phase was then poured into a new tube. Extraction was repeated using n-hexane (0.5 mL). The combined organic phase was evaporated under nitrogen to dryness and re-dissolved in n-hexane to prepare a solution suitable for analysis using liquid chromatography (50 mg per 1 mL of n-hexane). Tocopherol concentrations were determined using NP-HPLC with ultraviolet (UV) detection, as described by the respective Polish Standard (PN-EN 12822:2002) and Chavez-Servín et al. [19,20]. The HPLC system comprised a liquid chromatograph (Thermo-Fisher Dionex, Waltham, MA, USA) and a UV detector (SpectraSYSTEM UV3000HR, Thermo Electron Corporation, San Jose, CA, USA). Chromatographic separation was performed using a Separon™ SGX NH_2 column (150×3.0 mm I.D., 3-µm particle size; Tessek) at 20ºC and 25-µL injection volumes. The isocratic elution technique with a hexane/ethyl acetate 85:15 (v:v) mobile phase was used. The flow rate of the mobile phase was 0.6 mL/min and the chromatograms were monitored at a wavelength of 295 nm.

Vitamin C content: Vitamin C content in the HM was determined using reversed-phase high-performance liquid chromatography with UV detection (RP-HPLC/UV) according to the method described by Romeu-Nadal et al. [21]. Vitamin C exists in two forms: ascorbic acid (AsA) and dehydroascorbic acid (DHsA). To determine the total content of vitamin C, DHsA was reduced to AsA using dithiothreitol (DTT). Degradation of AsA was prevented using a 0.56% solution of metaphosphoric acid (V) as a stabilising agent. Test tubes were filled with 300 µL of HM and 800 µL of 0.1 mol/L DTT. The mixture was shaken for 30 s and incubated at room temperature (21-25 °C) for 15 min in the dark. Three hundred microlitres of 0.56% $H_3O_3P_9$ and 20 µL of TCA were added, and the tubes were repeatedly shaken for 30 s. The mixture was centrifuged at 4500×*g* for 10 min at 10 °C. The AsA content of clear solutions was determined by RP-HPLC/UV in a liquid chromatography system (Perkin Elmer, Waltham, MA, USA) equipped with a C-18 column (Merck, Darmstadt, Germany) (125×4.0 mm I.D., 5 µm particle size) and a UV-VIS detector (λ=245 nm). The mobile phase was a mixture of 0.1% acetic acid and methanol (95:5 v/v). The flow rate of the mobile phase was 0.8 mL/min. The AsA content in HM samples (100 mg/mL) was calculated based on calibration curves illustrating the relationship between peak areas and AsA concentrations in reference solutions.

Statistical analysis

Statistical processing of the data was performed using Statistica version 10 (StatSoft Inc., Tulsa, OK, USA). Descriptive variables are expressed as mean and standard deviation (SD). Continuous variables were compared using an independent *t*-test for parametric variables; differences with $p<0.05$ were considered to be statistically significant.

Ethics

All procedures were approved by the local ethics committee, and subjects provided written informed consent before the start of the procedure.

Results

The present study included 152 participants, the characteristics of whom are summarised in Table, Supplemental Digital Content 1. No difference was found between the mothers of female and male neonates in terms of age, parity, maternal weight, neonatal weight at birth, gestational age, and Apgar score at birth.

Table, Supplemental Digital Content 1.

Vitamin content

The results of analysis of α- and γ-tocopherol and AsA content in HM are presented in Table 2. Although the SDs were quite wide in relation to the arithmetic means in the case of tocopherols, there were no differences in either vitamin C or vitamin E content in HM between groups I and II.

Table 1. Vitamin content in milk in study participants.

Parameter	Female neonate (n=75)	Male neonate (n=77)	p value
α-tocopherol (μg/100 ml milk)	434.44±315.11	402.93±222.66	0.288
α-tocopherol (μg/g fat)	146.90±101.731	163.38±88.062	0.358
γ-tocopherol (μg/100 ml milk)	42±23	42±20	0.481
γ-tocopherol (μg/g fat)	18.39±14.082	19.07±14.285	0.801
Vit E (μg/100 ml milk)	356±136.4	407±101.2	0.113
Vit E (μg/g fat)	70.92±32.361	125.90±99.949	0.103
Ascorbic acid (μg/100 ml milk)	53.17±17.59	50.64±16.31	0.265

Values presented as arithmetic means (±SD).

FA composition

Total fat, saturated FAs (SFAs), monounsaturated FAs (MUFAs), and polyunsaturated FAs (PUFAs) content are presented in Table 3. The concentrations of specific FA types were comparable to those in the milk of European women (Table, Supplemental Digital Content 2). No significant differences in the mean content of the analysed components of HM were found between the studied groups. However, there was a tendency toward lower PUFA concentration in the milk for the male

infant (13.39±2.78% weight percentages per 100 g of FAs versus [vs.] 14.02±2.09% in the milk for the female infant; p=0.087).

Table 2. Total fat, SFA, MUFA, and PUFA breast milk contents (weight percentages) in study participants.

Parameter	Female neonate (n=75)	Male neonate (n=77)	p value
Total fat (per 100 g of milk)	3.02±1.28	2.97±1.76	0.146
Total SFAs (% of total FAs)	42.11±4.08	46.98±6.98	0.261
Total MUFAs (% of total FAs)	42.90±2.91	41.98±6.09	0.314
Total PUFAs (% of total FAs)	14.02±2.09	13.39±2.78	0.087

Values presented as arithmetic means (±SD).

Table, Supplemental Digital Content 2.

SFAs

SFAs account for approximately 42% of all HM FAs in mothers of male and female infants. Palmitic acid (16:0) was the most prevalent SFA in both groups (24.03% of total FAs in the milk for the female infant vs. 24.31% in the milk for the male infant), followed by stearic acid (18:0, approximately 6% in each group). No differences in SFA composition were found between the groups.

A tendency toward lower myristic acid concentration was found in the HM of mothers of female infants (5.69±1.60% of the total FAs in the milk for the male infant vs. 5.30±1.32% in the milk for the female infant; p=0.073) (Table 3).

Table 3. Saturated fatty acid breast milk contents in study participants.

Saturated fatty acid (weight percentages per 100 g of fatty acids)	Female neonate (n=75)	Male neonate (n=77)	p value
Caproic acid 6:0	0.02±0.02	0.02±0.02	0.314
Caprylic acid 8:0	0.14±0.06	0.14±0.07	0.318
Capric acid 10:0	1.11±0.44	1.21±0.45	0.108
Undecylic acid 11:0	0.02±0.03	0.02±0.03	0.457
Lauric acid 12:0	4.33±1.32	4.55±1.54	0.214
Tridecylic acid 13:0	0.08±0.51	0.02±0.03	0.138
Myristic acid 14:0	5.30±1.32	5.69±1.60	0.073
Pentadecylic acid 15:0	0.33±0.12	0.34±0.12	0.214
Palmitic acid 16:0	24.03±2.45	24.31±2.60	0.309
Margaric acid 17:0	0.30±0.08	0.31±0.07	0.323
Stearic acid 18:0	6.14±1.00	6.12±1.08	0.400
Arachidic acid 20:0	0.19±0.05	0.19±0.04	0.419
Behenic acid 22:0	0.06±0.02	0.07±0.02	0.196

Lignoceric acid 24:0	0.06±0.06	0.07±0.10	0.152

Values presented as arithmetic means (±SD).

MUFAs

In both groups, MUFAs accounted for approximately 42–43% of all the FAs in HM, and oleic acid (18:1, n-9) was the most prevalent MUFA. Higher mean concentrations of gondoic acid (20:1, n-9) were observed in the HM of the mothers of the female neonates compared to the HM of the mothers of the male neonates (0.63±0.18 in the milk for the female infant vs. 0.59±0.15 in the milk for the male infant; $p<0.047$).

There was a tendency toward a higher concentration of palmitoleic acid (16:1, n-9) in the milk for the male infant compared with the milk for the female infant (2.56±0.64% vs. 2.36±0.72%; $p=0.08$).

PUFAs

LA (18:2, n-6) accounted for approximately 10%, while alpha-LA (ALA, 18:3, n-3) constituted approximately 1% of all breast milk FAs in both groups. No significant differences were found between the study groups in the PUFA profile in HM.

The results of MUFA and PUFA analysis are summarised in Table 4.

Table 4. Mono- and polyunsaturated fatty acid breast milk contents in study participants.

	Female neonate (n=75)	**Male neonate (n=77)**	**p value**
Monounsaturated fatty acid (weight percentages per of 100 g fatty acids)			
Triadecenoic acid 13:1	0.07±0.09	0.07±0.10	0.424
Myristoleic acid 14:1 (n-5)	0.14±0.08	0.14±0.08	0.295
Sum of 15:1 (*cis* + *trans*)	0.09±0.06	0.08±0.04	0.228
Palmitoleic acid 16:1 (n-7)	2.36±0.72	2.56±0.64	0.080
Other 16:1 isomers	0.31±0.14	0.31±0.16	0.464
Sum 17:1 (*cis* + *trans*)	0.21±0.08	0.22±0.07	0.205
Oleic acid 18:1 (n-9)	34.58±3.05	33.84±3.32	0.100
Vaccenic acid 18:1 11	2.26±0.37	2.32±0.37	0.161
Gondoic acid 20:1 (n-9)	0.63±0.18	0.59±0.15	<0.047*
Erucic acid 22:1 (n-9)	0.13±0.07	0.13±0.07	0.230
Nervonic acid 24:1(n-9)	0.08±0.06	0.09±0.07	0.481
Polyunsaturated fatty acids (weight percentages per 100 g of fatty acids)			
Conjugated linoleic acid, isomers 18:2 (*cis trans*)	0.31±0.10	0.31±0.09	0.398
n-6 fatty acids			
Linoleic acid 18:2 (n-6)	10.24±1.88	9.81±1.97	0.108
Gamma-linolenic acid 18:3 (n-6)	0.08±0.03	0.09±0.04	0.273

Eicosadienoic acid 20:2 (n-6)	0.29±0.10	0.30±0.11	0.492
Dihomo-gamma-linolenic acid 20:3 (n-6)	0.36±0.09	0.36±0.12	0.346
Arachidonic acid 20:4 (n-6)	0.48±0.16	0.47±0.15	0.230
Docosadienoic acid 22:2 (n-6)	0.10±0.08	0.08±0.05	0.293
Adrenic acid 22:4 (n-6)	0.04±0.04	0.04±0.04	0.246
Docosapentaenoic acid 22:5 (n-6)	0.21±0.15	0.23±0.25	0.313
Sum of all n-6 PUFAs	11.83±1.85	11.27±2.43	0.083
n-3 fatty acids			
Alpha-linolenic acid 18:3 (n-3)	1.22±0.49	1.17±0.46	0.230
Eicosatrienoic acid 20:3 (n-3)	0.07±0.07	0.07±0.05	0.293
Eicosapentaenoic acid 20:5 (n-3)	0.08±0.14	0.06±0.05	0.426
Docosapentaenoic acid 22:5 (n-3)	0.19±0.09	0.20±0.15	0.477
Docosahexaenoic acid 22:6 (n-3)	0.28±0.13	0.30±0.13	0.336
Sum of all n-3 PUFAs	1.84±0.63	1.78±0.56	0.404

Values presented as arithmetic means (±SD). * significant difference

Summary of results

No statistically significant differences in the concentrations of FAs, vitamin C, and tocopherols according to the sex of the child (i.e., between groups I and II) were found in mature milk. The only statistically significant difference was the higher concentration of gondoic acid in the HM of mothers nursing girls.

Discussion

To our knowledge, this was the first study to investigate possible sex-related differences in the content of the most important antioxidants (vitamin C and tocopherols) and FAs of HM. Results of this study revealed no differences in the concentrations of tocopherols, FAs, and vitamin C in the HM in association with the sex of the offspring. However, some warrant a comment.

The SD of the tocopherols was relatively high. Women enrolled in the study were asked to submit the entire content of the breast 2 h after feeding. Such a wide range of tocopherol concentrations may have resulted from a failure to comply with these instructions. Fat is secreted most abundantly in the hind milk [22]. As such, if the breast is not completely emptied or the time since previous feeding was too short, different fat content would be expected. To avoid misinterpretation, the concentration of tocopherols was expressed as mass (μg) per volume (100 mL) of milk as well as per total fat mass. In our previous studies, we found that the concentration of vitamin C in HM was correlated with its absorption from food [23]. However, the concentration of vitamin E in HM is not correlated with dietary intake [24], although it is significantly higher in the colostrum than in mature milk [24]. According to some authors, the release of vitamins into HM is partially related to the levels of these

substances in the diet of the mother; however, many other studies have highlighted the modes of active and selective transport of these substances into the mammary gland and subsequently into milk [25–29].

FAs are one of the main and most variable compounds in HM. Not only do they provide energy but also contribute to cell membrane structure, are precursors of inflammatory mediators, influence gene expression, and their composition greatly influences the intensity of oxidative stress. There have been only a few studies investigating FA content in HM in relation to infant sex, and the results are conflicting [30–32]. It has been reported that HM secretion, especially its lipid composition, is a highly dynamic, individual biological process. This plasticity may be controlled by feedback mechanisms operating between the mother and child. It has been hypothesised that inappropriate placental transport of FAs would be compensated for after birth, with higher levels of FAs in HM [33]. There are approximately 170 types of FAs in HM, and their composition depends on many factors, including genetics, length of gestation, and the diet of the woman during both pregnancy and lactation [34, 35]. The socioeconomic status of the mothers is correlated with their income, education, and lifestyle, which may in turn determine their dietary choices [36].

Bobinski et al. reported higher levels of eicosenoic acid in the mature and transitional milk of mothers of appropriate for gestational age (AGA), full-term neonates compared with milk for preterm and full-term small appropriate for gestational age (SGA) infants [37].

Interestingly, Fujita et al. reported differences in HM fat content depending on both the sex of the child and socioeconomic status of the mother [4], which supports the Trivers-Willard hypothesis of unequal parental investment in female and male offspring [38].

According to Quinn et al., there are no sex-related differences in the FA composition of HM [10]. The only significant difference found in our study was the higher concentration of gondoic acid in the HM of mothers who delivered females. Gondoic acid (Z11-eikosanoic acid, C20:1, n-9) is present in large amounts in oils from some plants, particularly jojoba oil, in the seed oil of *Limnanthes alba* (Meadowfoam). It is not produced by humans and comes exclusively from the diet [39]. Gondoic acid is one of the few MUFAs and its concentration decreases during lactation. The role and/or function of gondoic acid in humans remains unclear. However, some clinical conditions, such as autism spectrum disorder or schizophrenia, in which abnormal levels of gondoic acid have been found [40,41], may be implicated. Interestingly, gondoic acid is the only MUFA in HM and is significantly and strongly associated with a lower risk of mother-to-child transmission of HIV [42]. Assuming that in the study population of women, there were no major differences in diet, considering that the concentration of gondoic acid varies during lactation, we speculate that gondoic acid could be actively transported from the mammary gland into HM. The significance of our findings is uncertain; as such, further studies are required to confirm this.

Sex-dependent differences in HM composition may be explained by changes in hormones secreted by the placenta during pregnancy and their impact on developing mammary glands [43].

In contrast, boys may consume 8%–10% more milk on average, and more frequently during the day [44]. In addition, fat content is inversely correlated with breast fullness [45]. Another possible mechanism for sex-associated differences in HM composition is microchimerism. This phenomenon involves the migration of a small population of foetal cells, and mature immunological and hematopoietic stem cells during pregnancy into the mother [46,47].

Other factors influencing the composition of milk intended for different sexes cannot be ignored, such as parity, body mass index of the mother, and the length of gestation [48,49].

A possible weakness of our analysis was the incomplete monitoring of HM sampling (total emptying of the breast), and the absence of diet surveillance during pregnancy and after delivery. It is likely that repeated measurements of vitamins C and E and FAs at specified time intervals would have yielded more data and supported more precise conclusions. Another weakness of this study was the lack of a power calculation and no multiple testing (i.e., FDR correction). Undoubtedly, a comparison of the concentrations of these substances in HM and in the serum of the infant would be valuable. However, this type of analysis does not appear to be ethical, considering the necessity of a blood draw (a relatively large volume, thus inflicting pain). In contrast, blood concentration could be influenced not only by absorption of these substances from HM but also by the length of gestation (placental transport).

Nevertheless, although this investigation is the first to report preliminary results, our findings do not suggest any need for the antioxidant content of infant formula to be sex specific. Further studies based on a larger population, with more compounds subjected to analysis and investigation of their interactions are warranted.

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Supplementary content:

Suppl Table 1. Demographic and obstetrical characteristics of study participants.

Parameter	Female neonate (n=75)	Male neonate (n=77)	p value
Age (years)	28.16±4.01	28.03±3.98	0.361
Parity (n)	2 (1-2.8)	2 (1-2.5)	0.450
Maternal weight at delivery (kg)	72.23(54.50-93.00)	72.88 (54.00-93.00)	0.330
Gestational age (weeks)	40 (39-41)	4 (39-41)	0.140
Birth weight (g)	3 319 (2 510-3 625)	3608 (2510-3900)	0.550
Apgar score at 1st min (points)	10	10	0.890

Values presented as arithmetic means (±SD) or medians and interquartile ranges (in parenthesis)

Content of individual fatty acids groups [% of total fatty acids composition] in milk lipids of women from different countries of the world.

Country	SFA Incl.: Sum of 10:0,12:0,14:0	MUFA	PUFA Incl.: C18:2(n-6) C18:3(n-3)
Germany: Koletzko B., Mrotzek M., Bremer H.J. "Fatty acids composition of mature human milk in Germany." *American Journal of Clinical Nutrition*, Vol. 47, 1988, 954-959;	42,76 14,28	42,38	13,82 10,05 0,78
Caribbean: Smith E.N., Martini J.A., Mulder H., Boersime E.R., Muskiet F.A.J. "Estimated biological variation of the mature human milk fatty acids composition." *Prostaglandins, Leukotrienes and Essential Fatty Acids*. Vol. 66, Issue: 5-6, May 2002, 549-555;	56,62 28,98	28,06	13,93 11,26 0,67
Tanzania : Smith et al.	60,53 36,92	22,37	15,57 12,47 0,82
Pakistan : Smith et al.	57,52 23,30	30,93	9,96 8,73 0,34
Kuwait : Hayat L., Al-Sughayer M.A.M, Afzal M. "Fatty acids composition of human milk in Kuwaiti mothers." *Comparative Biochemistry and Physiology*. Part b, 124, 1999, 261-267;	42,86 13,71	37,25	20,27 17,44 0,42
Nepal Glew R.H., Huang Y-S., VanderJagtD.J., Chuang L.T., Bhatts K., Magnussen M. "Fatty acids composition of the milk lipids of Nepalese women: correlation between fatty acids composition of serum phospholipids and melting	51,80 23,70	32,65	13,35 9,05 1,81

point." *Prostaglandins, Leukotrienes and Essential Fatty Acids*. Vol. 65, Issue:3, September 2001, 147-156;			
Nigeria :Knox E., VanderJagt D.J., Shatime D., Huang Y-S, Chunag L.t., Glew R.H. "Nutritional status and intermediate chain-length fatty acids influence the conservation of essential fatty acids in the milk of northern Nigerian women." *Prostaglandins, Leukotrienes and Essential Fatty Acids*. Vol. 63, Issue: 4, October 2000, 195-202;	55,59 29,19	28,29	17,24 14,10 0,51
Saudi Arabia:Al-Othman A.A., El-Fawaz H.A., Hewdy F.M. "Fatty acids composition of mature breast milk of Saudi lactating mothers." *Food Chemistry*, Vol. 57, Issue: 2, October 1996, 211-215;	56,20 15,10	28,29	17,24 8,70 1,10
China: Qi, C., Sun, J., Xia, Y., Yu, R., Wei, W., Xiang, J., Jin, Q., Xiao, H., & Wang, X. (2018). Fatty Acid Profile and the sn-2 Position Distribution in Triacylglycerols of Breast Milk during Different Lactation Stages. *Journal of Agricultural and Food Chemistry*, *66*(12), 3118–3126.	37.60 ± 2.54	34.62 ± 2.87	25.11 ± 2.95 20.43 ± 2.90 1.30 ± 0.17
Sweden: Nilsson, A. K., Löfqvist, C., Najm, S., Hellgren, G., Sävman, K., Andersson, M. X., Smith, L. E. H., & Hellström, A. (2018). Long-chain polyunsaturated fatty acids decline rapidly in milk from mothers delivering extremely preterm indicating the need for supplementation. *Acta Paediatrica, International Journal of Paediatrics*, *107*(6), 1020–1027.	45.48 (42.77–48.73)	41.26 (37.88–43.37)	12,71 10.11 (8.27-12.07) 1.35 (0.97–2.21)
Netherlands: Heijning, B. J. M. Van De, Stahl, B., Schaart, M. W., Beek, E. M. Van Der, Rings, E. H. H. M., & Mearin, M. L. (2017). Fatty acid and amino acid content and composition of human milk in the course of lactation. *Advances in Pediatric Research*, *3*(1), 1–14.	44.4 ± 4,6	39.1± 4,4	15.7± 1,60
Brazil: Berenhauser, A. C., Pinheiro Do Prado, A. C., Da Silva, R. C., Gioielli, L. A., & Block, J. M. (2012). Fatty acid composition in preterm and term breast milk. *International Journal of Food Sciences and Nutrition*, *63*(3), 318–325.	46.88 ± 4.71	33.72 ± 4.79	19.41 ± 2.46