Acıbadem Univ. Sağlık Bilim. Derg. 2025; 16 (1)
https://doi.org/10.31067/acusaglik.1411541

The Longitudinal Association Between Human Milk Composition and Nutritional Status of Exclusively Breastfeeding Mothers

Şule Aktaç[1], Simay Kundakçi[1], Güleren Sabuncular[1], Zehra Margot Çelik[1], Ayşe Hümeyra İslamoğlu[1], Perran Boran[2], Fatma Esra Güneş[3]

[1] Marmara University, Faculty of Health Sciences, Department of Nutrition and Dietetic, İstanbul, Turkey
[2] Marmara University School of Medicine, Department of Pediatrics, İstanbul, Turkey
[3] Istanbul Medeniyet University, Faculty of Health Sciences, Department of Nutrition and Dietetic, İstanbul, Turkey

Şule AKTAÇ
0000-0002-2158-5015
Simay KUNDAKÇI
0000-0002-7962-2624
Güleren SABUNCULAR
0000-0001-5922-295X
Zehra Margot ÇELİK
0000-0002-4622-9252
Ayşe Hümeyra İSLAMOĞLU
0000-0002-2138-5996
Perran BORAN
0000-0002-9885-7656
Fatma Esra GÜNEŞ
0000-0003-1693-6375

Correspondence: Şule Aktaç
Marmara University, Faculty of Health Sciences, Department of Nutrition and Dietetic, İstanbul, Turkey
Phone: +90 505 484 06 41
E-mail: suleaktac@hotmail.com

Received: 28.12.2023
Accepted: 03.09.2024

ABSTRACT

Purpose: The presented study investigated the human milk composition changes associated with the nutritional status of exclusively breastfeeding mothers.

Methods: A total of 59 volunteer mothers whose infants were followed at a well-child outpatient clinic were included in the study. During a six-month study period, anthropometric measurements and body composition of the mothers were evaluated at monthly visits, energy and macronutrients of breast milk were analyzed, and 24-hour food consumption records were taken every 15 days. The study conducted between June 2017 and September 2018 was completed by 15 mothers.

Results: A statistically significant downward trend for total protein (tau-b = -0.208; $p < 0.001$) and true protein (tau-b = -0.230; $p < 0.001$), and upward trend for total lactose (tau-b = 0.119; $p = 0.032$) was determined in human milk of the duration of first 6 months. A weak positive correlation was found between maternal body fat percentage and human milk lactose content, and between fat-free mass and true protein content in the first month of lactation. It was stated that there was a weak negative correlation between the percentage of protein and carbohydrate intake of the mothers in the 1st and 6th months and the energy, protein, and fat composition of human milk.

Conclusions: It was concluded that the macronutrient components of human milk had a trend in the first 6 months of lactation and the composition was affected by the mothers' fat mass and protein intake, especially in the first month.

Keywords: Body Composition, Body Mass Index, Human Milk, Macronutrient, Nutrient Intakes, Energy.

ÖZET

Amaç: Bu çalışmada, emziren annelerin beslenme durumu ile ilişkili anne sütü bileşimindeki değişiklikler araştırılmıştır.

Yöntemler: Bebekleri çocuk sağlığı izlem polikliniğinde takip edilen 59 gönüllü anne çalışmaya dahil edilmiştir. Altı aylık çalışma sürecinde, aylık ziyaretlerle annelerin antropometrik ölçümleri ve vücut kompozisyonları değerlendirilmiş, anne sütünün enerji ve makro besin öğeleri analiz edilmiş, her 15 günde bir 24 saatlik besin tüketim kaydı alınmıştır. Haziran 2017 - Eylül 2018 tarihleri arasında yürütülen çalışmayı 15 anne tamamlamıştır.

Bulgular: İlk altı ay boyunca anne sütünün toplam protein (tau-b = -0,208; $p < 0,001$) ve true protein (tau-b = -0,230; $p < 0,001$) için istatistiksel olarak anlamlı bir azalma eğilimi ve toplam laktoz için artış eğilimi (tau-b = 0,119); $p = 0,032$) saptanmıştır. Emzirmenin birinci ayında, annenin vücut yağ yüzdesi ile anne sütü laktoz bileşimi ve yağsız doku kütlesi ile true protein içeriği arasında pozitif yönlü zayıf bir ilişki saptanmıştır. Annelerin 1. ve 6. aylardaki protein ve karbonhidrat alım yüzdesi ile anne sütünün enerji, protein ve yağ bileşimi arasında negatif yönlü zayıf bir ilişki olduğu tespit edilmiştir.

Sonuçlar: Anne sütünün makro besin ögesi bileşemi emzirmenin ilk 6 ayında bir değişme eğilimi gösterdiği ve bileşimin özellikle ilk ayda annelerin yağ kütlesi ve protein alımından etkilendiği belirlenmiştir.

Anahtar Kelimeler: Vücut kompozisyonu, beden kütle indeksi, anne sütü, makrobesin, besin alımı, enerji.

Human milk (HM) is the uniquely balanced nutrition that contains macronutrients, micronutrients, hormones, antibodies, and bioactive molecules that are necessary for infants (1). The World Health Organization (WHO) and United Nations Children's Fund (UNICEF) recommend that every newborn should exclusively breastfeed for the first 6 months of life, followed by HM with complementary foods for at least 2 years (2).

The composition of HM changes rapidly from the time of birth. Initially, it changes from colostrum to transitional milk and from transitional milk to mature milk (3). It is difficult to determine HM consumption because it is dynamic and changes for several reasons. Changes occur during lactation due to time of day or night or within a single feed due to duration of sucking the breast or due to the time of the pumping session. It also changes between populations. The nutritional status of the lactating mother may also affect energy density and nutrient content of the HM (4-5). Vitamins and trace elements vary in HM depending on the diet and body storages (6).

Obesity, which is increasing in Turkey as in the whole world, is especially common in the female population and affects both maternal and infant health (7-8). The literature emphasizes that higher maternal BMI is associated with higher protein concentrations in human milk (9-10). Also, higher human milk fat content has been reported in overweight and obese women compared to normal weight women (11). Additionally, early changes in milk composition have been detected in the milk of obese mothers, with increases in potentially obesity-promoting fatty acids and decreases in protective fatty acids (12). Bzikowska et al. (2018), found that carbohydrate composition in human milk is also not associated with maternal BMI or the body composition of breastfeeding women (11).

The effect of the mother's nutritional status on HM is not clear in the literature, especially studies with exclusive breastfeeding are limited. The aim of this study is to investigate the longitudinal association between body composition and BMI of the mother, on macronutrient composition of the HM.

Materials and Methods

Study Design and Participants

This study was conducted as a cross-sectional descriptive design between June 2017 and September 2018 in the well-child outpatient clinic of the university hospital, in Istanbul. Ethics committee approval was received from Marmara University School of Medicine, Clinical Research Ethics Committee in May 2017 (Ethics Committee Number: 09.2017.340).

Those who were included in the study were volunteer mothers who started to feed on postpartum day one and who were found to have no contraindication in body composition analysis when they came to the outpatient clinic for follow-up during the first 30 days after birth. Exclusion criteria were gestational age less than 37 weeks and more than 43 weeks, infants weighing less than 2.5 kg and more than 4.5 kg, mothers who smoked and/or consumed alcohol, mothers who experienced problems that delayed the start of breastfeeding, mothers with acute or chronic diseases at the time of data collection and mothers who did not breastfeed exclusively. Mothers who could not complete the study were excluded from the study.

The plan of the research prepared according to the Consolidated Standards for Reporting Studies (CONSORT) 2010 flow chart (13) is shown in Figure 1. At the baseline of the study, 70 mothers were interviewed, and 65 mothers were found eligible for the study. After excluding mothers who did not meet the criteria's, 59 volunteer mothers were enrolled. During the study period of 6 months, 44 mothers were lost at follow-up for various reasons, such as going to different clinics for follow-up, the absence of HM, initiation of formula and/or complementary feeding (Figure 1).

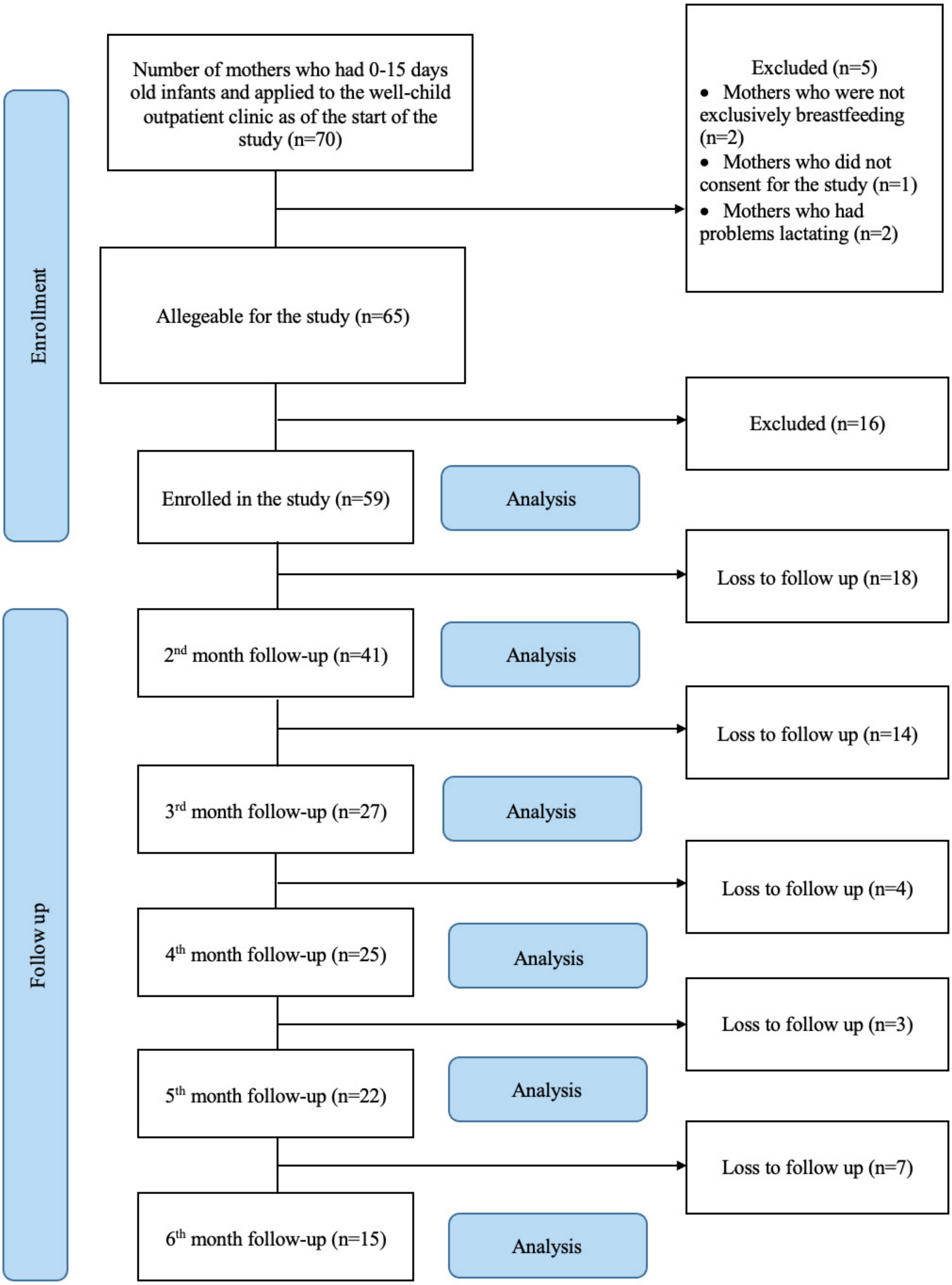

Figure 1: CONSORT 2010 Diagram

Data Collection

At the baseline, a questionnaire form that was constructed by the researchers was filled out in face-to-face interviews. The questionnaire consisted of questions about demographic characteristics, delivery method, weight gain during pregnancy and breastfeeding characteristics.

Collection and analysis of the human milk

Four mL of mature HM was obtained from mothers at the well-child outpatient clinic and the analysis was repeated 2 times for the reliability of the results and the mean value was used. Samples were collected monthly for 6 months, between 9 am and 12 am to minimize possible circadian influences on the milk composition. After the mother fed the infants from the right breast, the milk obtained by hand expression from the right breast was placed in Eppendorf tubes. The samples were analyzed with Miris® HMA TM (Human Milk Analyzer) within 2 hours after expression. Total protein, true protein, fat, lactose, and energy values were determined (14). The HMA provided a calculation of 'total protein' which refers to the protein content based on the total amount of nitrogen in a sample and 'true protein' regarding the correction for non-protein nitrogen compounds, and reflects only the content of actual protein, thus the "true" denotation. Miris HMA uses the factor 6.38 to convert N content to protein content. Additionally, HMA enabled the evaluation of fat and lactose concentrations in HM samples. The calculation of energy was based on the following conversion factors: 4.0, 9.25, 4.4 kcal per 100 mL for lactose, fat and protein, respectively (15).

Anthropometry and body composition parameters

The height (accuracy ± 0.1 cm) and body weight (accuracy ± 0.1 kg) of the mothers were measured according to the standards (16). Body mass index (BMI) was calculated according to WHO classification. Mothers were divided into three groups; normal weight if BMI was between 18.50-24.99 kg/m^2, overweight if BMI was between 25.00-29.99 kg/m^2 and obese if BMI was ≥ 30.00 kg/m^2 (17). Body composition analysis was performed by the bioelectrical impedance method using Inbody 270 device. Total fat percentage, fat mass and lean body mass (LBM) were determined. During the measurement, the mothers' feet were bare and dry, it was confirmed that the mother did not eat or drink at least 3 hours before measurement. This was repeated once a month for 6 months.

Food Consumption

Twenty-four-hour dietary recalls were obtained from the mothers who participated in the study every 15 days. Every month, when HM was collected the food consumptions were taken in face-to-face interviews, and at other times they were asked on the phone throughout the study period. Monthly two 24-hour dietary recalls were taken from each participant and monthly averages were calculated. The information obtained from the dietary recalls was recorded and evaluated by using BeBiS (Nutrition Information Systems) 8.2 nutrition program (18).

Physical activity level

The level of physical activity (PA) was assessed using the short version of the International Physical Activity Questionnaire - Short Form (IPAQ-SF) and it was applied to the mothers monthly for 6 months (19). The validity and reliability of IPAQ for Turkish society was conducted by Sağlam in 2010 (20). The Short Version of the IPAQ (7 questions) assesses physical activity in 4 areas, including leisure, home, work, and transport-related physical activity. Physical activity level is classified as inactive, minimally active, and highly active according to general scores. MET-scoring method, vigorous PA score is equal to vigorous weekly PA expenditure multiplied by 8 METs. Moderate weekly PA expenditure is multiplied by 4 METs and walking PA by 3.3 METs to calculate moderate and walking PA score, respectively. Total PA score is the sum of vigorous, moderate, and walking PA scores. Based on their total and/or vigorous PA score, the subjects were classified into three PA categories (PA class): low, moderate, and high (19).

Statistical Analysis

All statistical analyses were performed using the SPSS 20.0 package program. The Kolmogorov–Smirnov test was used to assess the normality of the distributions. Wilcoxon Signed Rank test was used to test for differences between mothers' characteristics and HM composition according to month of lactation. Kendall's tau-b correlation was applied to analyze the measure of the strength and direction of association that exists between HM composition and month of lactation. The correlation of mothers' characteristics and HM composition were performed using the Spearman's correlation test. The results were evaluated at a 95% confidence interval and a significance level of $p < 0.05$.

Results

A total of 59 mothers participated in the study, 36 mothers completed the evaluations in the third month and 15 mothers completed the evaluations in the sixth month (Figure 1). At the first evaluation, the median age of the mothers was 27.0 years. The percentage of mothers aged between 25-29 was 37.3%. It was found that 35.6% of the mothers were secondary school graduates and the frequency of mothers who graduated from primary and high school was equal (20.3%). It was determined that the majority of mothers (93.2%) were not working. It was stated that 27.1% of the mothers participating in the study had their first birth and 35.6% had their second birth.

Lactation month and HM composition correlation analysis showed that month of lactation was moderately negatively associated with total protein (Kendall's tau-b = -0.208) and true protein (Kendall's tau-b = -0.208) ($p < 0.001$), and positively weak associated with lactose (Kendall's tau-b = 0.119) ($p = 0.032$). It was determined that there was no significant association between energy and fat content of HM and lactation months (Figure 2).

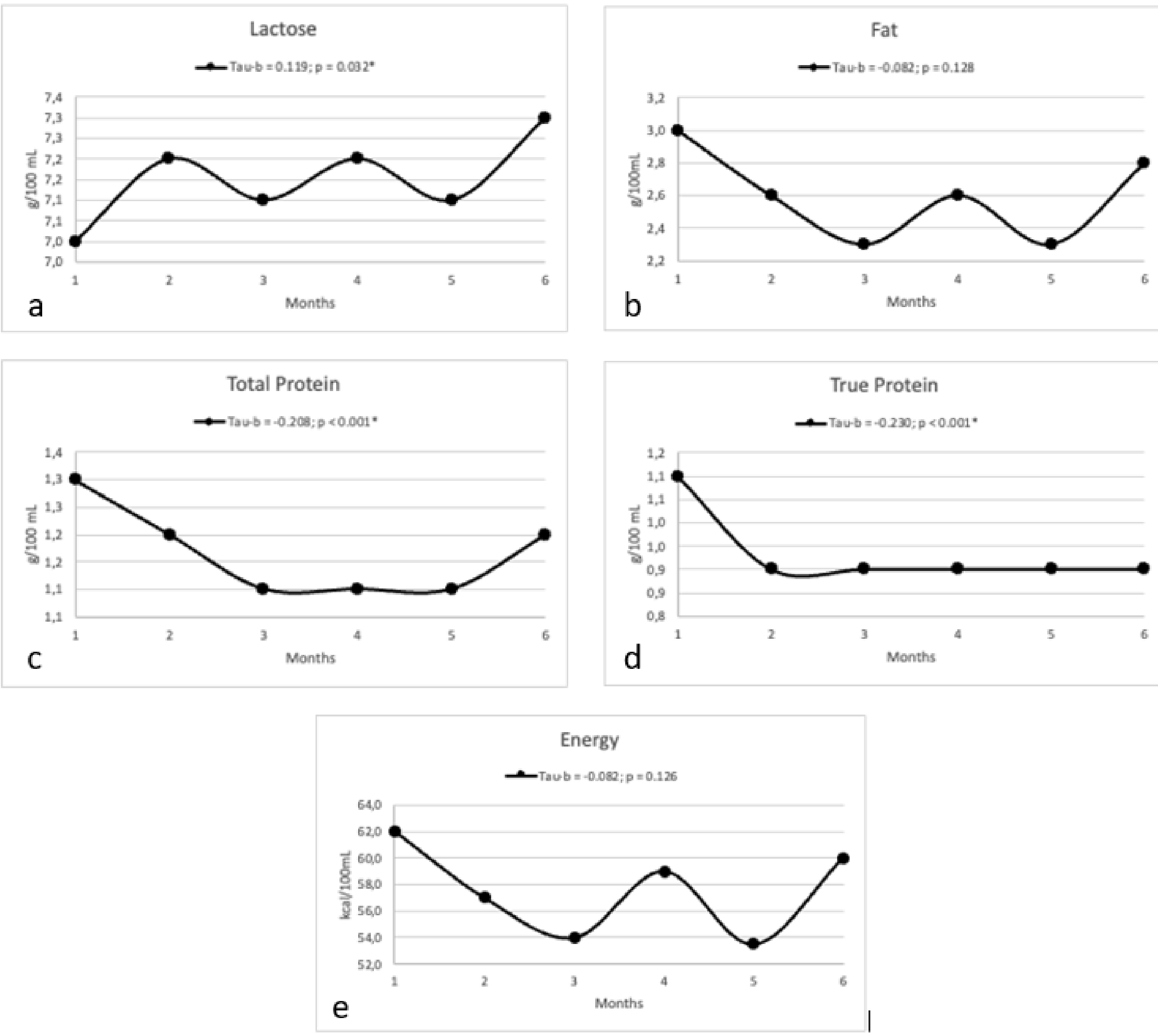

Figure 2: Composition of human milk trend in 0-6 months of lactation

Anthropometric measurements, energy and nutrient intakes, MET-scores, and the human milk composition during the six-month lactation period are presented in Table 1. Body fat percentage increased at the 3rd month compared to the 1st month ($p = 0.004$). Body weight, BMI, energy and macronutrient intakes and physical activity levels of the mothers did not change significantly during the first 6 months of lactation ($p > 0.05$).

The correlation between HM composition and mothers' anthropometric measurements, energy and nutrient intakes, and MET-scores are shown in Table 2. There was a weak positive correlation between body fat mass percentage and HM lactose content in addition to fat free mass and true protein content at the 1st month. There was also a weak negative correlation between maternal protein intake and the percentage of energy from protein and the energy and fat content of HM at the 1st month. A moderately negative correlation was found between the percentage of energy from lactose and true protein content of HM at the 6th month.

Table 1: Comparison of anthropometric measurements, energy and nutrient intakes and MET-scores of mothers according to lactation month

Characteristic	Month of lactation **Median (IQR 25th-75th quartiles)**						p1	p2
	1. month (n=59)	2. month (n=36)	3. month (n=26)	4. month (n=24)	5. month (n=21)	6. month (n=15)		
Anthropometric data and body composition measures								
Weight (kg)	74.0 (62.2 – 80.5)	74.2 (63.7 – 84.9)	75.5 (67.4 – 87.3)	77.6 (68.3 – 85.9)	78.3 (67.9 – 88.8)	84.7 (69.0 – 92.4)	0.751	0.950
Body mass index (kg/ m2)	27.1 (24.4 – 32.5)	28.3 (24.3 – 32.9)	30.1 (26.6 – 33.7)	29.2 (26.3 – 33.8)	32.4 (26.1 – 34.8)	32.4 (26.1 – 36.0)	0.628	0.975
Body fat mass (%)	35.3 (29.1 – 39.6)	36.9 (29.7 – 42.6)	39.9 (33.2 – 44.2)	38.9 (29.6 – 43.2)	40.1 (34.2 – 45.6)	40.3 (35.8 – 46.5)	**0.004**	0.272
Fat free mass (%)	25.9 (23.6 – 28.0)	24.8 (23.3 – 28.5)	24.8 (23.8 – 28.4)	24.9 (24.0 – 28.3)	27.6 (23.5 – 28.8)	27.8 (23.3 – 29.1)	**0.010**	0.443
Energy and macronutrient intake								
Energy (kcal)	1687.4 (1350.5 – 1920.0)	1526.8 (1292.8 – 1803.6)	1454.0 (1325.9 – 1835.7)	1436.2 (1156.2 – 1724.0)	1367.1 (1227.5 – 1725.0)	1389.3 (1162.7 – 1623.4)	0.585	0.233
Protein (g)	52.3 (45.1 – 69.6)	50.6 (42.1 – 61.4)	54.5 (47.1 – 65.9)	49.1 (41.5 – 61.7)	54.6 (37.1 – 63.1)	53.2 (41.1 – 62.1)	0.509	0.820
Protein (% kcal)	13.9 (11.3 – 15.4)	13.7 (11.6 – 15.5)	14.5 (12.1 – 16.7)	14.0 (12.1 – 16.1)	14.3 (11.5 – 15.7)	16.4 (13.9 – 17.3)	0.210	0.100
Fat (g)	65.5 (51.9 – 78.6)	61.3 (52.4 – 79.9)	58.6 (52.1 – 79.7)	65.5 (53.3 – 75.0)	59.9 (49.8 – 69.9)	55.2 (50.3 – 72.3)	0.741	0.609
Fat (% kcal)	36.2 (31.3 – 40.8)	37.6 (33.4 – 41.5)	38.6 (32.8 – 42.5)	39.6 (34.4 – 45.5)	39.5 (33.8 – 45.4)	39.4 (34.5 – 46.2)	0.539	0.427
Carbohydrates (g)	208.5 (163.0 – 256.2)	183.3 (141.9 – 218.6)	183.7 (129.8 – 204.7)	150.5 (120.2 – 201.8)	159.4 (134.7 – 227.7)	152.6 (125.8 – 188.0)	0.439	0.233
Carbohydrates (% kcal)	49.1 (43.8 – 53.3)	47.8 (42.8 – 52.8)	46.3 (39.4 – 52.5)	43.6 (40.2 – 51.4)	46.8 (42.6 – 50.3)	44.8 (36.6 – 49.9)	0.820	0.733
MET-score	0.0 (0.0 – 346.5)	0.0 (0.0 – 396.0)	198.0 (0.0 – 540.0)	0.0 (0.0 – 297.0)	0.0 (0.0 – 132.0)	0.0 (0.0 – 198.5)	0.324	0.183

1Difference between First and Third Month of Lactation
2 Difference between First and Sixth Month of Lactation
Wilcoxon signed rank test for paired samples.

Table 2:. Correlations between human milk composition and mothers' characteristics

	Month of lactation	Composition of Human Milk				
		Energy[1]	Total Protein[2]	True Protein[2]	Fat[2]	Lactose[2]
Anthropometric data and body composition measures		r*				
Weight (kg)	1	0.064	0.018	0.055	0.017	0.167
	3	-0.027	-0.226	-0.324	0.085	-0.186
	6	0.391	-0.039	-0.132	0.370	-0.354
Body mass index (kg/ m²)	1	0.013	-0.151	-0.091	-0.012	0.198
	3	0.009	-0.225	-0.259	-0.065	0.052
	6	0.454	-0.027	-0.118	0.407	-0.395
Body fat mass (%)	1	0.055	-0.219	-0.196	0.040	**0.281***
	3	0.019	-0.168	-0.193	-0.041	0.059
	6	0.497	0.094	-0.006	0.423	-0.153
Fat free mass (%)	1	0.033	0.254	**0.276***	-0.024	-0.066
	3	-0.062	-0.212	-0.329	0.172	-0.372
	6	-0.057	-0.207	-0.173	0.022	-0.373
Energy and macronutrient intake						
Energy (kcal)	1	0.055	-0.058	-0.025	0.020	0.040
	3	-0.139	-0.034	0.125	-0.252	0.213
	6	-0.215	0.022	-0.014	-0.210	0.070
Protein (g)	1	**-0.310***	0.058	0.092	**-0.325***	-0.050
	3	-0.010	-0.270	-0.271	0.122	-0.234
	6	-0.474	-0.196	-0.235	-0.470	0.227
Protein (% kcal)	1	**-0.274***	0.097	0.106	**-0.276***	-0.057
	3	-0.079	-0.107	-0.293	0.054	-0.201
	6	-0.314	-0.095	-0.067	-0.343	-0.084
Fat (g)	1	-0.030	-0.193	-0.169	-0.033	0.028
	3	-0.159	-0.199	-0.081	-0.138	0.034
	6	-0.176	0.299	0.233	-0.210	0.118
Fat (% kcal)	1	0.048	-0.180	-0.205	0.078	-0.045
	3	-0.136	-0.162	-0.231	0.032	-0.168
	6	-0.116	0.506	0.487	-0.160	-0.034
Carbohydrates (g)	1	0.018	0.027	0.057	-0.021	0.090
	3	0.030	0.048	0.186	-0.136	0.330
	6	-0.083	-0.184	-0.179	-0.061	0.045
Carbohydrates (% kcal)	1	0.068	0.179	0.190	0.029	0.040
	3	0.050	0.111	0.191	-0.134	0.360
	6	0.129	-0.427	**-0.554***	0.177	0.104
MET-score	1	-0.214	0.019	0.080	-0.221	0.004
	3	0.028	0.049	0.213	-0.054	-0.043
	6	-0.424	-0.322	-0.276	-0.421	0.545

1 Energy is presented as kilocalories (kcal) per 100 mL.
2 Macronutrients and dry matter are presented as grams per 100 mL.

Discussion

The present study indicated that there was a decrease in the protein content and an increase in the lactose content of the HM in the 6-month period. It was found that especially in the first month, body fat mass and protein intake affect the composition of human milk. However, no correlation was found between other macronutrient intakes of mothers and HM composition in any other months.

While many studies of HM composition have been conducted, components of HM are still being investigated. According to the studies evaluating the composition of HM; the average fat content does not differ greatly; it has been found to range from 3.2 to 3.6 g/dl between populations. Lactose and protein content of HM is less variable; mature milk contains between 6.3-8.1 g/dl lactose and 0.9-1.2 g/dl protein (4,21). In line with previous studies, HM macronutrient content did not change significantly according to the months. In this study, it was determined that the HM fat content was 2.7-3.3 g/dl, the lactose content was 6.9-7.2 g/dl, the protein content was 1.0-1.2 g.

Previous studies have shown that the protein content of HM decreases rapidly during the first month and then gradually towards the sixth month of lactation (22-23). Bzikowska-Jura et al. found a statistically significant downward trend for total protein (tau-b = –0.31) and true protein (tau-b = –0.30) concentration in HM in the duration of 6 months (18). In line with Bzikowska-Jura et al. this study stated that lactation month was moderately negatively associated with total protein and true protein, in addition a positive weak association with lactose was found (11). Although the literature and our study show that there are changes in HM over a 6-month period, there is not enough data to claim that this situation is the same for all populations.

When the literature is examined, it is seen that HM fat increases as BMI increases (24-28). Daniel et al. showed that for every 1 kg/m^2 increase in BMI, the HM fat content increases by 0.56 g/L (24). In another study, fat content in HM was higher at 6 months ($p = 0.002$) and protein content at 5 and 6 months of postpartum ($p < 0.03$) in overweight mothers compared to mothers with normal BMI (28). In the literature, it has been stated that there is a correlation between mothers' BMI and HM composition (24-29) and there are differences in HM composition according to BMI classification (28); in this study, unlike other studies, it was determined that there was no correlation between BMI and HM fat content.

Maternal BMI was positively associated with a higher protein level in HM (26-30). Daniel et al. found no significant association between maternal BMI and HM total protein (24), protein content in HM did not appear to differ between overweight and/or obese and normal weighted women (27). Similar to the studies of Daniel et al. (24) and Leghi et al. (27), there was no correlation found between BMI and the protein content of HM in this study.

Research shows that association of maternal BMI and HM lactose content is inconsistent. While Lenghi et al., found that higher BMI was associated with higher lactose concentrations at different stages of lactation (27), in other studies it was determined that BMI was negatively correlated with the lactose content of HM (26-31). On the contrary Daniel et al., did not find any relationship between BMI and HM lactose content (24). In a study conducted with 2632 mothers in Korea, it was found that the mothers' BMIs were negatively correlated with the lactose content of HM (26). In a meta-analysis, a total of 31 studies (5078 lactating women) were included in the qualitative synthesis and nine studies (872 lactating women) in the quantitative synthesis, BMI was associated with different lactose concentrations at different stages of lactation (27). In another study, women with high BMI had significantly lower HM lactose content at 4 and 6 months postpartum than those in the normal BMI range (28). In this study, similar to the study of Daniel et al. (24), it was determined that there was no correlation between BMI and the lactose content of HM.

In the literature, the results of the relationship between the body composition of the mother and the energy and macronutrient content of HM are contradictory. As for the fat content of HM, there are studies that demonstrate higher body fat mass is related with higher HM fat content (27,32) whereas, other studies found no correlation (1,31). Some studies found a correlation between body composition and lactose content of HM (27,31), other studies found no correlation (1,33). For the protein content of the HM; a study shows higher body fat percentage is related to higher HM protein content, it also demonstrates that this correlation weakens as the lactation month progresses (33), contrarily there are studies that did not find any correlation between body composition and HM protein content (1,31). In a meta-analysis, maternal obesity was associated with higher HM fat and lactose concentrations at different stages of lactation (27). In the presented study, there was no correlation found between the fat content of the HM and maternal body composition at any month. However, a weak positive correlation between body fat mass percentage and HM lactose content was found in addition to fat free mass and true protein content at 1st

month. In the early period of lactation, maternal body fat stores are used during pregnancy to support milk production. Therefore, the physiological effects of rapid fat mobilization make it difficult to establish a correlation between mothers' ideal weight and HM fat concentration in the first 3-4 months of lactation (29).

Maternal diet has been reported to affect the HM composition, with conflicting results (5, 11, 34-35). Yang et al. (2014) found that dietary intake had a less impact on HM fat content than maternal BMI (25). In a study, HM fat content seemed to have a weak positive correlation with maternal fat intake, although it was not statistically significant (1). Quinn et al. showed that the contents of fat and energy of HM increase with the duration of breastfeeding, however no relationship was found with maternal diet (31). The studies conducted by Iranpour et al. (2013) and Daud et al. (2013) demonstrated that maternal food intake during lactation did not modify the composition of HM (3,29). In another study, significant positive associations were found between protein, fat, carbohydrate and energy intake, and levels of macronutrients in HM, especially in protein content (30). Unlike other studies present study shows that maternal protein intake and the percentage of energy from protein has a weak negative correlation with the energy and fat content of HM at 1st month. A moderately negative correlation was found between the percentage of energy from lactose and true protein content of HM at 6th months.

This study has multiple strengths. First of all, the samples were obtained as hindmilk during the day, immediately after breastfeeding, with the same technique (by hand expressing) and always from the same breast (right), only mothers who exclusively breastfed were included. Secondly, advanced techniques and protocols were used to assess the milk collection, which allowed possible errors in HM composition to be minimized. Thirdly, it was a longitudinal study in which milk samples from 0-6 months were collected. Also, the participants from different socioeconomic levels were presented in the study and the body composition of the mothers was measured by researchers with advanced techniques.

Limitations of the present study include food consumption records were obtained with a recall method based on mothers' declaration and some were done with telephone interviews. This method does not reflect daily changes in diet and all dietary habits. Also, HM was collected and analyzed only once a month.

Conclusions

Knowing and alternating the body composition and maternal diet will affect HM content and this will be guiding in terms of planning education and counseling services to lactating women. The information obtained from this study will contribute to the development of strategies for mothers in the period of breastfeeding and improve the HM composition to ensure optimal feeding of infants.

Due to the small number of studies in this field and the inconsistency of the data, it is thought that the effect of the mothers' body mass index on the macronutrient content of HM should be evaluated in larger sample groups and randomized controlled studies.

Declarations

Funding

This study was supported by Marmara University Scientific Research Projects Commission (BAPKO) with SAG-A-120917-0496 number.

Conflicts of Interest

The authors declare no competing interests.

Ethics Approval

Ethics committee approval was received from Marmara University School of Medicine, Clinical Research Ethics Committee in May 2017 (Ethics Committee Number: 09.2017.340).

Availability of Data and Material

Data may be available from the principal investigator, Dr. Şule Aktaç, upon reasonable request.

Authors' Contributions

All authors critically reviewed the draft manuscript and approved the final version of the manuscript for publication.

Conceived and designed the analysis – ŞA

Collected the data – ŞA, SK, ZMÇ, AHİ and GS

Contributed data or analysis tools – ŞA, SK, ZMÇ, AHİ, GS, PB and FEG

Performed the analysis – ŞA, SK, ZMÇ, AHİ, GS

Wrote the paper - ŞA, SK, ZMÇ, AHİ, GS, PB and FEG

Acknowledgments

We would like to thank the participants and Marmara University Pendik Training and Research Hospital Well-child Polyclinic nurses for their dedication and hard work in collecting the samples. This study was supported by Marmara University Scientific Research Projects Commission (BAPKO) with SAG-A-120917-0496 number.

References

1. Kurniati AM, Sunardi D, Sungkar A, et al. Associations of maternal body composition and nutritional intake with fat content of Indonesian mothers' breast milk. Paediatr Indones. 2016;56:297-303. DOI: 10.14238/pi56.5.2016.297-303
2. World Health Organization. Breastfeeding. Available from: https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding. [Last accessed: 01/30/2023].
3. Daud AZ, Mohd-Esa N, Azlan A, Chan YM. The trans fatty acid content in human milk and its association with maternal diet among lactating mothers in Malaysia. Asia Pac J Clin Nutr. 2013;22:431–42. DOI: 10.6133/apjcn.2013.22.3.09
4. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatr Clin. N Am. 2013;60:49-74. DOI: 10.1016/j.pcl.2012.10.002
5. Keikha M, Bahreynian M, Saleki M, et al. Macro- and micronutrients of human milk composition: Are they related to maternal diet? A comprehensive systematic review. Breastfeed Med. 2017;12:517–27. DOI: 10.1089/bfm.2017.0048
6. Chung MY. Factors affecting human milk composition. Pediatr Neonatol. 2014;55:421-22. DOI: 10.1016/j.pedneo.2014.06.003
7. Hacettepe University Institute of Population Studies. 2018 Turkey Demographic and Health Survey. Hacettepe University Institute of Population Studies, T.R. Presidency of Turkey Directorate of Strategy and Budget and TUBITAK, Ankara, Turkey, 2019.
8. Duvekot JJ. Pregnancy and obesity: practical implications. Eur Clinics Obstet Gynaecol. 2005;1:74-88. DOI: 10.1007/s11296-005-0019-0
9. Hahn WH, Jeong T, Park S, et al. Content fat and calorie of human milk is affected by interactions between maternal age and body mass index. J Matern Fetal Neonatal Med. 2018;31(10):1385–8. DOI: 10.1080/14767058.2017.1315660
10. Young BE, Patinkin Z, Palmer C, et al. Human milk insulin is related to maternal plasma insulin and BMI: but other components of human milk do not differ by BMI. Eur J Clin Nutr. 2017;71(9):1094–100. DOI: 10.1038/ejcn.2017.75
11. Bzikowska-Jura A, Czerwonogrodzka-Senczyna A, Oledzka G, et al. Maternal nutrition and body composition during breastfeeding: association with human milk composition. Nutrients. 2018;10(10):1379. DOI: 10.3390/nu10101379
12. Ellsworth L, Perng W, Harman E, et al. Impact of maternal overweight and obesity on milk composition and infant growth. Matern Child Nutr. 2020;16(3):e12979. DOI: 10.1111/mcn.12979
13. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. J Pharmacol Pharmacother. 2010;1:100-7. DOI: 10.1186/1741-7015-8-18
14. Groh-Wargo S, Valentic J, Khaira S, et al. Human milk analysis using mid-infrared spectroscopy. Nutr Clin Pract. 2016;31:266–72. DOI: 10.1177/0884533615596508
15. Kulesza-Brończyk B, Bień A, Sobieraj P, et al. Factors affecting total protein and lactoferrin in human milk. Scientific Reports. 2023;13(1):22434. DOI: 10.1038/s41598-023-50124-1
16. Casadei K, Kiel J. Anthropometric Measurement. In: StatPearls. StatPearls Publishing, Treasure Island (FL); 2023. PMID: 30726000.
17. World Health Organization. Body Mass Index (BMI) classifications. Available from: http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi. [Last access: 01/30/2023].
18. Ebispro for Windows, Stuttgart, Germany; Turkish Version: BeBiS 8.2.
19. PAQ committee. Guidelines for data processing and analysis of the International Physical Activity Questionnaire (IPAQ). Available from: https://ugc.futurelearn.com/uploads/files/bc/c5/bcc53b14-ec1e-4d90-88e3-1568682f32ae/IPAQ_PDF.pdf. [Last access: 01/30/2023].
20. Saglam M, Arikan H, Savci S, et al. International physical activity questionnaire: reliability and validity of the Turkish version. Perceptual and Motor Skills. 2010;111:278-84. DOI: 10.2466/06.08.PMS.111.4.278-284
21. Miller EM, Aiello MO, Fujita M, et al. Field and laboratory methods in human milk research. Am J Hum Biol. 2013;25:1–11. DOI: 10.1002/ajhb.22334
22. Butte NF, Garza C, Johanson CA, et al. Longitudinal changes in milk composition of mothers delivering preterm and term infants. Early Hum Dev. 1984;9:153–62. DOI: 10.1016/0378-3782(84)90096-3
23. Saarela T, Kokkonen J, Koivisto M. Macronutrient and energy contents of human milk fractions during the first six months of lactation. Acta paediatrica. 2005;94(9):1176-81. DOI: 10.1111/j.1651-2227.2005.tb02070.x
24. Daniel AI, Shama S, Ismail S, et al. Maternal BMI is positively associated with human milk fat: a systematic review and meta-regression analysis. Am J Clin Nutr. 2021;113:1009-22. DOI: 10.1093/ajcn/nqaa410
25. Yang T, Zhang Y, Ning Y, et al. Breast milk macronutrient composition and the associated factors in urban chinese mothers. Chin Med J. 2014;127:1721–25. DOI: 10.3760/cma.j.issn.0366-6999.20133260
26. Chang N, Jung JA, Kim H, et al. Macronutrient composition of human milk from Korean mothers of full term infants born at 37-42 gestational weeks. Nutr Res Prac. 2015;9:433-38. DOI: 10.4162/nrp.2015.9.4.433
27. Leghi GE, Netting MJ, Middleton PF, et al. The impact of maternal obesity on human milk macronutrient composition: A systematic review and meta-analysis. Nutrients. 2020;*12*:934. DOI: 10.3390/nu12040934
28. Sims CR, Lipsmeyer ME, Turner DE, et al. Human milk composition differs by maternal BMI in the first 9 months postpartum. Am J Clin Nutr. 2020;112:548-57. DOI: 10.1093/ajcn/nqaa098
29. Iranpour R, Kelishadi R, Babaie S, et al, Comparison of long chain polyunsaturated fatty acid content in human milk in preterm and term deliveries and its correlation with mothers' diet. J Res Med Sci. 2013;18:1–5.
30. Binder C, Baumgartner-Parzer S, Gard LI, et al. Maternal Diet Influences Human Milk Protein Concentration and Adipose Tissue Marker. Nutrients. 2023;15:433. DOI: 10.3390/nu15020433
31. Quinn EA, Largado FE, Power M, et al. Predictors of breast milk macronutrient composition in Filipino mothers. Am J Hum Biol. 2012;24:533-40. DOI: 10.1002/ajhb.22266
32. Butte NF, Villalpando S, Wong WW, et al. Human milk intake and growth faltering of rural Mesoamerindian infants. Am J Clin Nutr 1992;55:1109-16. DOI: 10.1093/ajcn/55.6.1109
33. Kugananthan S, Gridneva Z, Lai C, et al. Associations between maternal body composition and appetite hormones and macronutrients in human milk. Nutrients. 2017;9:252. DOI: 10.3390/nu9030252
34. Bzikowska A, Czerwonogrodzka-Senczyna A, Weker H, et al. Correlation between human milk composition and maternal nutritional status. Rocz Państwowego Zakładu Hig 2018;69:363–67. DOI:10.32394/rpzh.2018.0041
35. Bravi F, Di Maso M, Eussen S, et al. Dietary Patterns of Breastfeeding Mothers and Human Milk Composition: Data from the Italian MEDIDIET Study. Nutrients. 2021;13:1722. DOI: 10.3390/nu13051722