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LONGITUDINAL AND CIRCADIAN VARIATION IN BREASTMILK MACRONUTRIENT COMPOSITION ACROSS THE FIRST SIX MONTHS OF LACTATION – ASSOCIATIONS WITH MATERNAL DETERMINANTS AND INFANT ANTHROPOMETRIC DEVELOPMENT

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ABSTRACT

Background. The composition of breast milk depends on the lactation period, the phase of single breastfeeding, time of day, and frequency of breastfeeding throughout the day, alongside other maternal and infant variables. This study aimed to assess the macronutrient and energy content of breastmilk during the first six months of lactation and evaluation of maternal determinants and association with infant anthropometric development.
Material and methods. Breast milk samples were collected from 47 mothers from Warsaw in the first, third, and sixth month of lactation. Breast milk macronutrient composition was assessed using the MIRIS Human Milk Analyzer. Maternal diet was assessed using 3-day food records in the third and sixth months of lactation. At each study visit, we assessed maternal and infant anthropometric parameters.
Results. Protein content declined consistently from 1.3 g/100 ml in the first month to 1.0 g/100 ml in the sixth month ($p < 0.001$). However, fat, lactose content, and energy values showed no significant changes throughout lactation. Fat content and milk energy value varied over time of the day in the 1st and 3rd month of lactation. There were no significant differences in the protein and lactose content according to the time of the day. Breastmilk fat adversely correlated with pre-pregnancy BMI, but not current BMI. The energy values of the diet were 2180 ±614 and 2046 ±503 kcal ($p > 0.05$), respectively, in the third and sixth month of lactation. The majority of mothers had lower than recommended intakes of vitamin D, folate, vitamins B_1, B_2, B_3, B_6, B_{12}, C, A, E, magnesium, zinc, and calcium for at least one of the study periods. However, maternal dietary intake and anthropometric parameters showed a limited correlation with breastmilk macronutrient composition. We did not observe any significant correlations between breastmilk macronutrient composition and infants' body mass, length, or BMI to age z-score, nor changes in these parameters between the third and sixth month of life.
Conclusion. Our findings support the assumption that breastmilk offers chrononutrition to infants. It is important to consider diurnal changes in its composition, especially when the infant is fed expressed breastmilk. Furthermore, it is necessary to implement educational programs targeting maternal nutrition, as many women do not maintain adequate dietary intake.

Keywords: anthropometric development, breastmilk, Body Mass Index (BMI), chrononutrion, dietary intake

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INTRODUCTION

Health organization worldwide recommends exclusive breastfeeding for the first six months of life (Królak-Olejnik et al., 2017; Szajewska et al., 2021; Meek et al., 2022). According to the limited data available, in Poland, only 4% of infants are exclusively breastfed at this time (Królak-Olejnik et al., 2017), whereas the World Health Organization (WHO) aims to increase this rate to at least 50% globally by 2025 (WHO, 2012). Breastfeeding is associated with a variety of health benefits, primarily due to unique breastmilk composition (Szajewska et al., 2021; Meek et al., 2022; Brockway et al., 2023). During exclusive breastfeeding, human milk provides infants with all the necessary nutrients and bioactives for optimal growth and development (Szajewska et al., 2021; Brockway et al., 2023). Moreover, breastmilk composition is dynamic and changes under the influence of various factors, including those related to lactation physiology, the mother, and the infant, to best match the current situation (Czosnykowska-Łukacka et al., 2018; Bzikowska-Jura et al., 2018; Italianer et al., 2020; Adhikari et al., 2021; Brockway et al., 2023).

Adequate nutrition during the lactation period is of utmost importance, as there is a significant increase in energy, and demand for selected nutrients (Jarosz et al., 2020; Bzikowska-Jura et al., 2023). While maternal diet generally has a limited effect on breastmilk composition with some exceptions, such as water-soluble vitamins and iodine, nutritional deficiencies during this period could have adverse effects on maternal nutritional status (Adhikari et al., 2021; Di Maso et al., 2021). A recent systematic review has indicated that maternal dietary intake generally aligns with dietary recommendations, although deficiencies in certain nutrients, such as vitamin D and potassium, were observed (Di Maso et al., 2021). However, a previous study conducted in Poland reported insufficient energy value as well as deficiencies in vitamin D and calcium intake in the majority of exclusively breastfeeding women (Bzikowska-Jura et al., 2018). Interestingly, Bzikowska-Jura et al. (2018) also found an association between maternal body mass index (BMI) and breastmilk fat, a finding that was further confirmed in a recent meta-regression by Daniel et al. (2021). Nevertheless, due to the considerable variation in methodologies utilized across studies investigating the associations between maternal diet, nutritional status, and breastmilk composition, further research in this area is warranted.

Therefore, the objective of this study was to evaluate the energy value and macronutrient content in breastmilk during the first six months of lactation and to assess maternal determinants of breastmilk composition and associations with infant anthropometric development.

MATERIAL AND METHODS

This study was approved by the Ethics Committee of the Medical University of Warsaw (Resolution No. AKBE/139/15) and was conducted following the Declaration of Helsinki. The study involved 47 mother-infant pairs, who completed the three study visits in the first, third, and sixth months of lactation. All of the participants resided in Central Poland, were in good health, and exclusively breastfed their infants throughout the study period. Detailed characteristics of the study group are presented in Table 1, and the study design has been described previously (Zielinska et al., 2019; Zielinska-Pukos et al., 2022).

Prior to each study visit, mothers were instructed to collect 4 breastmilk samples (comprising both pre- and post-feed milk) to eliminate intra-feeding variation in breastmilk composition. Mothers expressed the same amount (5–10 mL) of pre- and post-feed breastmilk sample from one selected feeding from each interval (06:00–12:00; 12:00–18:00; 18:00–24:00; 24:00–06:00). Breastmilk samples were stored and transported into the laboratory under cooling conditions and protected from light. In the laboratory, each breastmilk sample was vortexed, and an equal amount was transferred to another container to create one pooled sample representative of the study visit. In total, 15 breastmilk samples were collected for each mother (4 samples reflecting diurnal variations at the first, third, and sixth months of lactation, and pooled samples from the first, third, and sixth months of lactation) over the first six months of lactation.

The energy value and macronutrient composition per 100 mL of breastmilk were assessed using the MIRIS Human Milk Analyzer (Miris, Uppsala, Sweden). Immediately following each study visit, fresh

Table 1. Study group characteristics (n = 47)

Variable	n, %	M ±SD / Me (25–75)	Min÷max
Maternal age, years		31.9 ±3.6	22.7 ÷ 40.0
University education	47 (100)		
Self–assessment of the economic situation: very good	19 (41)		
Average monthly income: >1500 PLN per capita	33 (70)		
Marital status: married	40 (85)		
Parity: primiparous	22 (47)		
Pre-pregnancy BMI (kg/m^2); overweight or obesity	5 (11)	21.8 (20.2–23.2)	18.4 ÷ 38.6
BMI at 1st month (kg/m^2); overweight or obesity	10 (21)	23.8 (22.0–24.5)	18.4 ÷ 39.7
BMI at 3rd month (kg/m^2); overweight or obesity	6 (13)	22.6 (21.2–24.0)	18.1 ÷ 40.8
BMI at 6th month (kg/m^2); overweight or obesity	7 (15)	22.4 (20.2–23.7)	19.0 ÷ 42.6
Gestational weight gain, kg		13.4 ±4.2	0.7 ÷ 22.0
Pregnancy duration, Hbd		39.3 (38.0–40.0)	37.0 ÷ 42.0
Mode of delivery (C section)	21 (45)		
Infant gender (boys)	22 (47)		
Weight-to-age z-score in 3rd month		–0.04 ±1.00	–1.47–2.11
Weight-to-age z-score in 6th month		–0.17 ±0.81	–1.60–1.89
Length-to-age z-score in 3rd month		1.37 ±1.68	–1.71–5.53
Length-to-age z-score in 6th month		1.25 ±1.55	–2.05–5.53
BMI z-score in 3rd month		–1.08 ±1.51	–4.17–3.82
BMI z-score in 6th month		–1.18 ±1.38	–4.17–1.63
Weight z-score Δ 3–6 months		–0.15 ±0.68	–1.92–1.57
Length z-score Δ 3–6 months		–0.11 ±1.42	–3.83–3.22
BMI z-score Δ 3–6 months		–0.15 ±1.07	–2.62–2.02

Δ 3–6 months – changes in z-score between the 6th and 3rd month of life; BMI – Body Mass Index; Hbd – hebdomas; weeks of gestation; M – mean; Me – median; PLN – Polish zloty; SD – standard deviation.

6 mL breastmilk samples from each interval and the pooled sample were warmed to 40°C in the air bath and homogenized (1.5 s/ 1 mL of sample) in a sonicator. Breastmilk composition was analyzed in triplicate, and the average of those measurements was used for further analysis.

A three-day dietary record was used to evaluate the mother's eating habits in the third and sixth months of lactation. Additionally, data regarding the use of dietary supplements or pharmaceuticals containing nutrients were collected, and the average intake of nutrients with dietary supplements was calculated. In 3-day food records, participants reported food portions as measured weight, if they had a kitchen scale or using typical household measures. Reported food portions were verified using the "Album of Photographs of Food Products and Dishes" (Szponar, 2000). Energy value and nutrient intake were calculated using

Dieta 5.0 software (National Food and Nutrition Institute, Warsaw, Poland) with national food databases. The energy value and dietary intake were reported as an average of the three recorded days and as the sum of the intake with diet and dietary supplements. The nutritional values obtained were compared to the national requirements for lactating mothers in the first six months of lactation (Jarosz et al., 2020).

The anthropometric measurements (weight and height/length of the mother and infant) were taken at each study visit by a registered dietitian, following to the international standards for anthropometric assessment guidelines (ISAK, 2001; WHO, 2008) to the nearest 0.1 kg or cm using a professional scale with a stadiometer (Seca 799), neonatal scale (SECA 728, Hamburg, Germany) and anthropometric tape (SECA 203, Hamburg, Germany). Based on these measurements, BMI was calculated and interpreted according to the WHO standards (2000), or BMI, body mass, and length-for-age z-scores were calculated using the WHO Anthro Survey Analyzer Software v3.2.2 (Geneva, Switzerland).

Quantitative data were presented in accordance with the normality of distribution (evaluated by the Shapiro-Wilk test): median and upper-lower quartile (unnormal distribution) or mean and standard deviation (normal distribution). Qualitative data were expressed as numbers and percentages. Differences in breastmilk micronutrient composition across the study period or time of day were evaluated with the ANOVA Friedman test (unnormal distribution) or ANOVA with repeated measurements (normal distribution) with appropriate post-hoc tests. Differences in dietary intake between 3 and 6 months of lactation were evaluated using the Wilcoxon matched pair test (unnormal distribution) or t-student for repeated measurements, and the differences in the compliance with nutritional requirements between study visits were evaluated by means of the Chi^2 McNemary test. The correlation between breastmilk composition and maternal dietary intake and nutritional status was evaluated using Spearman rank or gamma correlations. All statistical analyses were conducted in the Statistica 13.3 software (TIBCO Software Inc., Paolo Alto, CA, USA) with a significance level set at 0.05.

RESULTS

The average energy value of breastmilk across the study period was 67 (62.7–73.0) kcal/100 mL, while fat, total protein, and carbohydrates concentrations were 3.8 (3.3–4.4), 1.1 (1.0–1.2), and 6.8 (6.6–7.0) g/100 mL, respectively. No significant differences were observed in energy value, fat, and carbohydrate concentrations across the study period, whereas total and crude protein, as well as dry mass, decreased significantly from the first to sixth month of lactation (Table 2). In the first and third months of lactation, significant circadian differences were observed in the fat and dry mass concentration, whereas in the first month of lactation energy value was also significantly different, depending on the time of the day (Table 3). In the sixth month of lactation, no significant differences were observed in breastmilk composition in terms of the time of the day.

The median maternal energy values of the diet were 2099.3 (1729.7–2549.3) and 2060.3 (1666.4–2483.2), in the third and sixth month of lactation. In the sixth month of lactation, mothers consumed significantly less monounsaturated fatty acids (MUFA), vitamins D, A, E, B_1, B_2, B_3, B_6, folates, zinc, iodine, and calcium compared to the third month of lactation (Table 4). Half or more mothers did not meet the requirements for vitamins D, A, E, B_1, folates, zinc, iodine, calcium, and potassium for at least one study period.

Limited correlations were observed between maternal dietary intake of macro- and micronutrients and breastmilk composition in the third month of lactation, with more correlations observed in the sixth month (Table 5). In the sixth month of lactation, breastmilk fat was correlated with energy value and carbohydrate intake, dry mass with energy value, fat, carbohydrate intakes. Additionally, breastmilk energy value was correlated with the energy value of the maternal diet and intake of fat and carbohydrates. Pre-pregnancy BMI was correlated with breastmilk fat and dry matter in the first and third months of lactation and energy value in the third month. Current BMI was correlated with fat and dry matter in the first month of lactation and energy value and dry matter in the third month. Moreover, in the first month of lactation, an adverse correlation was observed between maternal age and breastmilk energy value (Table 5). No significant

Table 2. Breastmilk energy and macronutrient composition in the first, third, and sixth month of lactation ($n = 47$)

Breastmilk composition per 100 mL	Lactation month M ±SD / Me (25–75) min ÷ max			*p*-value
	1	3	6	
Energy value, kcal	67.7 (63.3–73.0) 57.0 ÷ 89.7	66.0 (61.0–75.0) 49.3 ÷ 95.5	66.0 (61.0–75.0) 49.3 ÷ 95.5	0.615
Fat, g	3.8 (3.2–4.3) 2.2 ÷ 6.0	3.6 (3.1–4.5) 1.7 ÷ 6.9	3.8 (3.5–4.4) 2.2 ÷ 6.4	0.563
Carbohydrates, g	6.7 (6.5–7.0) 5.6 ÷ 7.6	6.8 (6.5–7.0) 5.3 ÷ 7.5	6.8 (6.6–7.1) 5.8 ÷ 7.4	0.077
Total protein, g	1.3 ±0.2[a] 0.9 ÷ 1.7	1.1 ±0.2[b] 0.8 ÷ 1.4	1.0 ±0.1[c] 0.7 ÷ 1.3	<0.001*
Crude protein, g	1.0 ±0.2[a] 0.7 ÷ 1.3	0.8 ±0.1[b] 0.6 ÷ 1.1	0.7 ±0.1[c] 0.5 ÷ 1.0	<0.001*
Dry mass, g	12.3 (11.7–13.2) 11.1 ÷ 16.1	11.8 (11.2–13.2) 10.0 ÷ 15.3	11.9 (11.4–12.3) 10.0 ÷ 15.3	0.006

M – mean; Me – median; SD – standard deviation; * – results of ANOVA for repeated measurements test, other variables were tested using ANOVA Friedman test. The different lower case letters indicate significantly different values.

Table 3. Diurnal variations in breastmilk energy and macronutrient composition in the first, third and sixth month of lactation ($n = 47$)

Breastmilk composition per 100 mL	Time of a day M ±SD / Me (25–75) Min ÷ max				*p*-value
	06:00–12:00	12:00–18:00	18:00–24:00	24:00–06:00	
1	2	3	4	5	6
First month					
Energy value, kcal	69.3 (64.0–76.0)[ab] 39.6 ÷ 93.7	73.0 (67.5–80.0)[a] 46.5 ÷ 107.0	67.7 (60.3–75.0)[b] 52.0 ÷ 96.3	64.3 (58.3–72.5)[b] 51.0 ÷ 91.7	0.001
Fat, g	3.7 (3.2–4.3)[ab] 1.9 ÷ 6.2	4.2 (3.5–4.9)[a] 1.1 ÷ 8.0	3.4 (2.9–4.5)[ab] 1.5 ÷ 6.5	3.2 (2.8–4.0)[b] 1.6 ÷ 6.1	0.003
Carbohydrates, g	6.7 (6.5–7.0) 6.2 ÷ 7.0	6.8 (6.5–7.0) 5.7 ÷ 7.5	6.8 (6.5–6.9) 5.0 ÷ 7.4	6.7 (6.5–7.0) 5.8 ÷ 7.5	0.191
Total protein, g	1.3 (1.1–1.4) 1.0 ÷ 1.9	1.3 (1.2–1.4) 0.9 ÷ 1.8	1.3 (1.2–1.5) 0.9 ÷ 1.7	1.3 (1.1–1.4) 0.9 ÷ 1.7	0.138
Crude protein, g	1.0 (0.9–1.1) 0.7 ÷ 1.5	1.0 (0.9–1.1) 0.7 ÷ 1.4	1.0 (0.9–1.2) 0.7 ÷ 1.3	1.0 (0.9–1.1) 0.7 ÷ 1.4	0.087
Dry mass, g	12.4 (11.7–13.3)[ab] 10.2 ÷ 15.5	12.9 (12.3–13.6)[a] 10.3 ÷ 16.7	12.4 (11.3–13.3)[ab] 9.4 ÷ 15.6	11.9 (11.2–12.9)[b] 10.0 ÷ 19.4	<0.001

Table 3 – cont.

1	2	3	4	5	6
Third month					
Energy value, kcal	70.3 (61.0–77.3) 43.0 ÷ 101.0	67.5 (62.5–79.0) 40.0 ÷ 93.0	66.8 (59.3–73.7) 48.7 ÷ 112.7	61.7 (54.7–70.7) 39.0 ÷ 112.0	0.062
Fat, g	4.1 (3.2–5.0)[a] 1.2 ÷ 7.2	3.9 (3.2–4.9)[ab] 1.0 ÷ 6.1	3.6 (3.0–4.7)[b] 1.5 ÷ 8.9	3.2 (2.4–3.9)[b] 0.7 ÷ 8.8	0.040
Carbohydrates, g	6.6 (6.5–6.9) 5.4 ÷ 7.7	6.8 (6.5–7.0) 5.3 ÷ 7.4	6.7 (6.5–6.9) 4.6 ÷ 7.5	6.6 (6.5–7.0) 5.4 ÷ 7.3	0.202
Total protein, g	1.0 (0.9–1.2) 0.8 ÷ 1.5	1.1 (0.9–1.2) 0.7 ÷ 1.4	1.1 (0.9–1.2) 0.8 ÷ 1.4	1.0 (0.9–1.1) 0.7 ÷ 1.5	0.497
Crude protein, g	0.8 (0.7–0.9) 0.6 ÷ 1.2	0.8 (0.7–0.9) 0.6 ÷ 1.2	0.8 (0.7–0.9) 0.6 ÷ 1.1	0.8 (0.7–0.9) 0.5 ÷ 1.2	0.070
Dry mass, g	12.3 ±1.5[b] 9.2 ÷ 15.9	12.2 ±1.3[b] 8.9 ÷ 15.2	12.1 ±1.4[a] 9.5 ÷ 16.6	11.6 ±1.7[a] 8.6 ÷ 16.6	0.004*
Sixth month					
Energy value, kcal	67.7 (62.0–73.0) 52.0 ÷ 90.7	70.3 (60.0–80.0) 39.3 ÷ 100.5	67.7 (60.7–75.7) 48.7 ÷ 94.3	65.0 (58.0–72.7) 40.0 ÷ 91.0	0.281
Fat, g	3.8 (3.3–4.5) 1.9 ÷ 8.2	4.1 (3.3–5.2) 0.8 ÷ 7.6	3.9 (3.1–4.8) 1.6 ÷ 6.7	3.7 (2.8–4.5) 0.8 ÷ 6.2	0.273
Carbohydrates, g	6.7 (6.5–7.1) 5.4 ÷ 7.6	6.9 (6.7–7.1) 6.0 ÷ 7.6	6.9 (6.7–7.0) 5.3 ÷ 7.6	6.9 (6.6–7.1) 5.5 ÷ 7.5	0.198
Total protein, g	0.9 (0.8–1.0) 0.7 ÷ 1.4	0.9 (0.9–1.0) 0.5 ÷ 1.3	0.9 (0.9–1.0) 0.7 ÷ 1.4	0.9 (0.9–1.0) 0.7 ÷ 1.2	0.768
Crude protein, g	0.7 (0.6–0.8) 0.5 ÷ 1.1	0.7 (0.7–0.8) 0.4 ÷ 1.1	0.7 (0.7–0.8) 0.5 ÷ 1.1	0.7 (0.7–0.8) 0.4 ÷ 1.0	0.349
Dry mass, g	11.9 (11.2–12.5) 9.8 ÷ 15.6	12.1 (11.1–13.3) 8.9 ÷ 15.5	11.9 (11.2–12.7) 10.0 ÷ 15.0	11.6 (10.8–12.3) 9.0 ÷ 14.8	0.168

M – mean; Me – median; SD – standard deviation; *– results of ANOVA for repeated measurements test, other variables were tested using ANOVA Friedman test. The different lower case letters indicate significantly different.

Table 4. Maternal dietary intake in the third and sixth month of lactation in comparison to nutritional recommendations (*n* = 47)

Intake per day	Month of lactation M ±SD Me (25–75)		*p*-value	Dietary recommendation[a]	Below / above recommendation[b] n of participants		*p*-value[d]
	3	6			3 month	6 month	
1	2	3	4	5	6	7	8
Energy, kcal	2099 (1730–2549)	2060 (1666–2483)	0.083	BMI 18.5–24.9 kg/m^2	7	7	0.480
Protein, g	85.1 ±18.3	80.4 ±18.8	0.085	EAR = 1.17 g/kg	13	14	0.998
Total fat, g	85.4 (63.1–102)	76.4 (56.8–92.4)	0.085	–			

Table 4 – cont.

1	2	3	4	5	6	7	8
Carbohydrates, g	262 (227–349)	261 (226–332)	0.200	–			
Protein, % En	16.1 ±2.9	16.0 ±2.4	0.875[c]	RI = 15–20	22	14	0.677
Fat, % En	33.8 ±5.7	32.7 ±7.0	0.289[c]	RI = 20–35	20	15	0.332
Carbohydrates, % En	50.1 ±5.8	51.1 ±7.2	0.275[c]	RI = 45–65	1	3	0.480
SFA, g	32.4 ±12.4	29.1 ±12.6	0.097[c]	–			
MUFA, g	32.7 (23.8–39.6)	29.0 (20.4–36.8)	0.033	–			
PUFA, g	11.3 (8.9–15.0)	10.5 (7.5–14.3)	0.166	–			
Cholesterol, mg	290 (193–360)	283 (190–349)	0.440	–			
Fiber, g	23.5 (19.1–29.1)	21.8 (18.8–28.2)	0.446	individually determined			
Water, ml	2266 (1556–2900)	2133 (1609–2840)	0.472	AI = 2700	16	13	0.579
Vitamin D, µg	27.1 (4.5–54.4)	7.5 (3.4–28.1)	0.000	EAR = 15	21	31	0.016
Vitamin A, µg RE	1218 (828–1739)	984 (690–1307)	0.009	EAR = 900	15	29	0.628
Vitamin E, mg	14.8 (10.8–31.7)	10.5 (7.8–18.0)	0.000	AI = 11	34	22	0.006
Vitamin C, mg	137 (78.5–189)	120 (101–158)	0.775	EAR = 100	18	11	0.169
Vitamin B_{12}, µg	4.2 (3.0–5.7)	3.7 (2.8–4.4)	0.092	EAR = 2.4	4	5	1.000
Vitamin B_6, mg	2.1 (1.7–3.4)	1.9 (1.6–2.4)	0.011	EAR = 1.7	9	17	0.099
Folate, µg	468 (292–889)	382 (293–563)	0.000	EAR = 450	23	29	0.114
Thiamine, mg	1.5 (1.2–2.1)	1.3 (1.0–1.8)	0.019	EAR = 1.3	14	24	0.024
Riboflavin, mg	1.9 (1.7–2.5)	1.9 (1.4–2.2)	0.031	EAR = 1.3	3	19	0.077
Niacin, mg	19.9 (15.0–25.5)	17.8 (14.3–22.4)	0.034	EAR = 13	6	9	0.546
Iron, mg	18.0 (12.7–35.6)	15.6 (12.1–27.8)	0.472	EAR = 7.0	0	0	–
Zinc, mg	12.4 (11.0–14.0)	10.8 (9.3–14.5)	0.033	EAR = 10.4	9	26	0.016
Iodine, µg	217 (117–327)	163 (122–211)	0.004	EAR = 210	23	35	0.003
Sodium, mg	3111 (2625–3765)	2986 (2501–3878)	0.619	AI = 1500	47	46	–
Magnesium, mg	399 (11.4–542)	374 (311–442)	0.058	EAR = 265	6	5	1.000
Calcium, mg	1043 (759–1198)	729 (611–1048)	0.031	EAR = 800	13	26	0.008
Phosphorus, mg	1492 ±383	1412 (1186–1571)	0.144	EAR = 580	0	0	–
Potassium, mg	3482 (2853–3886)	3130 (2725–3991)	0.619	AI = 4000	10	11	1.000

% En – the percentage of total energy; AI – Adequate Intake; BMI – Body Mass Index; EAR – Estimated Average Requirements; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; SFA – saturated fatty acids; RE – retinol equivalents; RI – Reference Intake range; a – nutritional recommendations for the Polish population (Jarosz et al., 2020); b – we reported the number of people with intake ‘above or equal AI norm’, ‘below EAR norm’ and ‘below and above RI norm’; c– results of the t-student test for repeated measurements test, other variables were tested using Wilcoxon matched pair test; d – results of Chi^2 McNemary test.

Table 5. Correlations between maternal factors, infant growth, and breastmilk composition at the first, third and sixth month of lactation ($n = 47$)

Variable	Lactation month	Breastmilk composition per 100 mL					
		energy value	fat	carbohydrates	total protein	crude protein	dry mass
1	2	3	4	5	6	7	8
Maternal age[a]	1	–0.369*	–0.249	0.218	0.116	0.149	–0.200
	3	–0.098	–0.103	0.201	–0.040	–0.024	–0.113
	6	–0.105	–0.133	0.024	–0.017	–0.023	–0.075
Parity[b]	1	0.092	0.124	0.056	0.039	0.059	0.184
	3	–0.027	0.000	–0.182	–0.163	–0.144	–0.085
	6	0.207	0.277*	0.054	–0.55	–0.032	0.109
Pregnancy duration[a]	1	0.215	0.296*	–0.007	–0.084	–0.090	0.208
	3	0.044	0.164	0.058	0.044	0.050	0.239
	6	0.061	0.119	0.059	0.096	0.068	0.116
Pre-pregnancy BMI[a]	1	–0.273	–0.374**	0.053	–0.106	–0.083	–0.305*
	3	–0.335*	–0.318*	0.040	–0.152	–0.172	–0.301*
	6	0.073	–0.001	0.041	–0.119	–0.160	0.030
Current BMI[a]	1	–0.273	–0.333*	–0.005	–0.113	–0.082	–0.299*
	3	–0.341*	–0.259	0.019	–0.142	–0.142	–0.368*
	6	0.070	0.127	0.110	–0.193	–0.258	0.045
Breastfeeding per day[a]	1	–0.067	–0.024	–0.117	0.118	0.095	0.154
	3	–0.169	–0.196	–0.296	–0.075	–0.087	–0.132
	6	–0.067	–0.032	–0.140	0.169	0.167	–0.064
Energy value of maternal diet[a]	3	–0.055	–0.140	0.055	0.134	0.163	0.011
	6	0.361*	0.290*	0.055	0.091	0.127	0.400**
Protein intake[a]	3	–0.061	–0.107	0.027	0.261	0.217	–0.006
	6	0.241	0.200	0.086	0.120	0.129	0.256
Total fat intake[a]	3	–0.128	–0.192	0.154	0.120	0.175	–0.055
	6	0.291*	0.188	–0.050	0.183	0.238	0.323*
Carbohydrates intake[a]	3	0.021	–0.082	0.031	0.096	0.110	0.087
	6	0.336*	0.299	0.119	0.037	0.043	0.372*
	6	0.210	0.197	0.137	0.156	0.177	0.294*
Weight–to– age z–score[a]	3	–0.198	–0.165	–0.025	–0.041	–0.067	–0.188
	6	0.129	0.110	0.100	0.014	0.09	0.123

Table 5 – cont.

1	2	3	4	5	6	7	8
Length–to– age z–score[a]	3	–0.025	–0.016	–0.302	–0.071	–0.100	0.002
	6	0.253	0.290	–0.068	0.176	0.149	0.175
BMI z–score[a]	3	–0.237	–0.197	0.166	0.035	0.059	–0.243
	6	–0.069	–0.016	–0.008	0.001	0.024	–0.039
Weight Δ 3–6 months[a]	3	–0.022	0.058	–0.007	–0.255	–0.153	–0.086
	6	0.105	0.019	0.043	0.111	0.118	0.165
Length Δ 3–6 months[a]	3	–0.222	–0.291	–0.002	0.036	0.125	–0.172
	6	0.053	0.064	0.058	0.177	0.130	0.040
BMI Δ 3–6 months[a]	3	0.059	0.166	–0.063	–0.299	–0.298	–0.042
	6	–0.056	–0.102	–0.114	–0.071	–0.037	–0.040

Δ 3–6 months – changes in z-score between the 6th and 3rd month of life; BMI – body mass index; a – Spearman rank correlations; b – gamma correlations; * $p \leq 0.05$; ** $p \leq 0.01$.

correlation was found between breastmilk macronutrient composition and infant BMI, body mass, or length-to-age z-scores, nor changes in those parameters between the third and sixth month of life.

DISCUSSION

The breastmilk energy value and macronutrients concentration observed in our study during the first, third, and sixth month of lactation were similar to those reported in previous studies from Poland (Bzikowska-Jura et al., 2018; Czosnykowska-Łukacka et al., 2018) and in the meta-analysis of studies analyzing associations with maternal nutrition (Adhikari et al., 2021). Energy value, fat, and carbohydrate concentration remained stable over the first six months of lactation, while breastmilk total and crude protein significantly decreased, which was consistent with previous findings (Lönnerdal et al., 2017; Bzikowska-Jura et al., 2018). Notably, the protein concentration remained stable over time, which is related to evolving infant requirements (Lönnerdal et al., 2017). Breastmilk composition also changed over a 24-hour period (Italianer et al., 2020). We observed significant circadian variations in fat and dry mass in the first and third month of lactation (and energy value in the first), but not in the sixth. At night, breastmilk contained a lower amount of fat than in the morning. However, no other macronutrients show circadian variations, which confirms previous findings (Italianer et al., 2020; Caba-Flores et al., 2022). Moreover, as shown in previous studies, the concentration of amino acids, iron and some bioactive factors (e.g. melatonin, cortisol) undergoes circadian variations (Italianer et al., 2020; Caba-Flores et al., 2022). It is hypothesized that circadian rhythmicity in breastmilk composition plays a significant role in healthy infant development, as infants are born with an incompletely functional circadian clock (Italianer et al., 2020). Furthermore, changes in breastmilk could be related to the adaptation to fat metabolism, which in turn could be an important factor in protecting against obesity (Italianer et al., 2020). Hence, it is important to consider these diurnal changes when feeding infants with expressed milk, as breastmilk serves as a prime example of chrononutrition (Italianer et al., 2020; Caba-Flores et al., 2022)

We also assessed maternal dietary intake in the third and sixth months of lactation. Maternal macronutrient intake and the diet's energy value were similar to those observed in a systematic review by Di Masso et al. (2021) but higher than reported by Bzikowska-Jura et al. (2018). On the other hand, we observed a higher intake of vitamins D, A, E, C, Fe, Zn, Na, P, and K, as well as lower intake of vitamins B_1, B_2,

B_3, B_6, and Ca compared with the median reported by Di Masso et al. (2021), whereas the intake of vitamin B_{12}, Mg and I were higher than those reported by Bzikowska-Jura et al. (2018). Despite this, we observed that many breastfeeding mothers did not meet the nutritional requirements, especially for vitamins D, A, E, B_1, folates, Zn, I, Ca, and K, which was also observed in Latvia (Aumeistere et al., 2022). This is highly concerning with regard to increased maternal needs during lactation (Jarosz et al., 2020; Bzikowska-Jura et al., 2023). However, our study confirmed that breastmilk energy value and macronutrient concentrations are relatively unrelated to maternal dietary intake (Bzikowska-Jura et al., 2018; Adhikari et al., 2021), as we reported only several weak correlations with breastmilk composition. Previous studies have shown that only breastmilk fatty acid profile, water-soluble vitamins (except folates and biotin), vitamins A, K, and D, Mg, Se, and I are susceptible to maternal dietary intake and nutritional status (Bzikowska-Jura et al., 2023). Interestingly, meta-regression by Daniel et al. (2021) found a significant positive association between maternal BMI and breastmilk fat (but not energy value nor another macronutrient) in the first six months of lactation. However, in the present study, we observed contradictory results, as maternal pre-pregnancy and current BMI were adversely correlated with breastmilk fat and energy value.

During exclusive breastfeeding, breastmilk is the sole source of nutrients for infants, thus we also examined its association with infant anthropometric growth. However, we did not find a significant association with infant growth. Brockway et al. (2023) in a recent systematic review reported that breastmilk protein was positively associated with infant length, and carbohydrates with weight within the first two years of life. However, the authors emphasized that due to methodological issues with milk collection and analysis, those results should be interpreted with caution.

CONCLUSION

Our study confirms that breastmilk composition changes throughout the day, and breastfeeding is associated with the benefits of chrononutrition. Hence, the diurnal variation in breastmilk composition should be taken into account during feeding with expressed breastmilk or in the development of infant formula. Moreover, we found that many exclusively breastfeeding mothers struggle to meet the dietary requirements. Our study emphasizes the necessity of developing educational programs dedicated to the role of adequate nutrition during lactation. Since the first months of maternity are challenging, developing different strategies to support maternal nutrition (e.g. preparing meals in advance by family members or a high-quality box diet for mothers) could be helpful.

DATA AVAILABILITY

Datasets from the current study are available from the corresponding author upon request.

DECLARATIONS

Ethical Approval

The study was conducted under the Declaration of Helsinki, and approved by the Ethics Committee of the Medical University of Warsaw (Resolution No. AKBE/139/15).

Competing Interests

The authors declare that they have no conflicts of interest.

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