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Analysis of the mature milk composition of women giving birth prematurely and on time

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ABSTRACT
Introduction: The composition of breast milk is not consistent; its variability is determined by the nutritional needs of the baby: duration of pregnancy, phases of a single feeding, lactation period, variability of fat concentration.
The aim: To assess the energy and essential nutrients content in mature milk from mothers delivering preterm and full-term infants.
Material and methods: 75 women participated in the study, at 4 weeks postpartum. The study sample was divided into three groups according to the duration of pregnancy: G1>=32 weeks of pregnancy, G2) 33-36 weeks of pregnancy, G3) <=37 weeks of pregnancy. The analysis of breast milk composition was performed with the Miris Human Milk Analysis analyser.
Results: The pregnancy duration of a sample of women participating in the study does not differentiate the nutritional composition and energy content in breast milk (p>0.05) analysed in the fourth week of lactation.
Conclusions: The assessment of the milk from women who gave birth on time and prematurely showed no significant differences in protein, fats, carbohydrates and energy content considering the same stage of lactation. However, it should be emphasised that not only is human milk considered to be a nutrient but also functional food. Due to the content of enzymes, hormones and growth factors, breast milk has a protective function. It is a part of treatment for premature babies. Nevertheless, the results of our study indicate the need for further research measuring the macronutrients of human milk in order to individualize more the nutritional approach for a particularly demanding group of newborns, which may help optimize their growth and development.
Key words: breast milk analysis, breastfeeding, preterm milk, mature milk

Introduction
Breast milk is composed of appropriately selected, in terms of quantity and quality, nutrients and bioactive ingredients which adapt to the needs of the developing child. The composition of breast milk is unique. The basic components of breast milk include protein, fats, carbohydrates, bioactive components, vitamins, water, macronutrients and minerals. The components of breast milk have a building, protective, immunomodulatory function, which reduces the morbidity and mortality rate in newborns and infants [1]. The composition of breast

milk is not consistent; its variability is determined by the nutritional needs of the baby. The literature on the subject indicates thathuman milk composition is affected by: the duration of pregnancy, the phases of a single feeding, the lactation period, the diet of the mother who breastfeeds and the variability of fat concentration [1, 2]. In the first few days after birth, colostrum is secreted, referred to as *„a thick, yellow fluid"*. Colostrum owes its colour to the high beta-carotenecontent. It is assumed that in the first few days after birth a newborn drinks between 2 and 10 ml of breastmilk at once during feeding,which gives 50-100 ml of colostrum per day.Not only does colostrum differ from mature milk in taste and smell, but also in composition and caloric content. It contains more protein, vitamins A and E, carotene, sodium, chloride, potassium, zinc, and less lactose, fats and water-soluble vitamins [3]. The average energy content in colostrum is lower than in mature milk, at 58-67 kcal/dL. Colostrum also contains a very high concentration of antibodies (especially sIgA class) and leukocytes, which is very important for the proper functioning of the newborn's immune system. This milk appears between the 7th and 14th day. Studies show that transitional milk has a significantly higher energy content than colostrum, which is related to its variable composition. Transitional milk contains increased concentration of fats, lactose and water-soluble vitamins, but decreased levels of immunoglobulins, protein and fat-soluble vitamins [1]. Mature milk appears about two weeks postpartum. Mature milk volume is significantly higher in comparison with colostrum and transitional milk. The analysis of mature milk composition shows an increase in fats and lactose, and a decrease in protein. There is also an increase in the energy content of mature milk, which depends on the fat content [1, 3]. The results of the study show that fat concentration in milk is higher in overweight and obese mothers. The average caloric content of mature milk is 65-75 kcal/dL, but the literature on the subject presents studies with an energy range between 43.9 and 115 kcal/dL [3]. In addition, the milk composition of mothers whose babies were born prematurely differs from that of women who gave birth on time. The composition of preterm milk adapts to the needs of the newborn's immature body, in particular for the proper development of the immune, nervous and digestive systems. It is characterised by a higher content of protein, fats, minerals (potassium, sodium, iron, chlorides, magnesium, phosphorus, zinc, calcium) and a lower concentration of lactose. The high content of immunoglobulins (especially the sIgA class), growth factors, hormones, immunomodulators, lactoferrin and anti-infective factors is crucial for the immune system. Milk from mothers delivering prematurely has a higher energy content (58 - 70 kcal/100 ml) compared to milk - colostrum from women (48 - 64 kcal/100 ml) who gave birth on time[1, 4].

The aim of the study

To assess the energy and essential nutrients content of mature milk from mothers delivering preterm and full-term infants.

Material and methods

The study was conductedfrom April 2019 to January 2020. The study was approved by the Bioethics Committee of the Nicolaus Copernicus University in Toruń at the Ludwik Rydygier Collegium Medicum in Bydgoszcz *(KB 121/2019)*. Women after natural delivery and caesarean section admitted at the Clinic of Obstetrics, Feminine Diseases and Gynaecology-Oncology, the Department of Obstetrics and Pregnancy Pathology, in dr Jan Biziel University Hospital No. 2 in Bydgoszcz, and women after childbirth in other medical facilities who were willing to participate in the study via social media were included in the study. The actual study was carried out in the 4th week after delivery. The selection of study participants was purposeful. Participation in the research project was voluntary. Prior to participating in the study, the respondents were informed about the purpose andthe course of the study and about the opportunity to ask questions and withdraw from the research at any time. Then each participant provided written informed consent.

The study inclusion criteria

The other study inclusion criteria included: breastfeeding or feeding the child expressed human milk using a bottle / tube, good, logical verbal contact with the participant of the study, physical and mental condition allowing to express breast milk on their own.

Initially 165 women were included in the study. However, 75 puerperae were included in the actual study, which was carried out in the fourth week after delivery. 80 women were excluded from participating in the research project due to lactation suppression and feeding the baby modified milk. The majority of women (68 women) did not breastfeed or express breast milk due to: nipple soreness, recurrent milk stasis, incorrect breastfeeding technique, lack of family support, general postpartum fatigue. In the study sample, four women stopped breastfeeding for medical reasons. Furthermore, 8 women were not included in the study due to the death of their baby (born before 32 weeks of pregnancy). The study sample was divided into three groups (G1, G2, G3) in terms of the duration of pregnancy: 25 patients whose children were born before32 weeks of pregnancy (G1), 25 patients whose children were born between 33 and 36 weeks of pregnancy (G2) and 25 patients whose children were born after 37 weeks of gestation (G3).

Human milk analysis.

The analysis of human milk composition was performed by means of the Miris Human Milk Analyzer (HMA) in Human Milk Bank at the Ludwik Rydygier Provincial Polyclinic Hospital in Torun. The device measures the caloric content (kcal/100ml) in human milk and allows to determine the levels of carbohydrates (g/100ml), protein (g/100ml), fat (g/100ml) and dry matter (g/100ml) in human milk. Milk collected from four time periods during the day (06:00-12:00, 12:00-18:00, 18:00-24:00 and 24:00 - 06:00) was tested,collecting a portion of milk at the beginning (5 ml) and at the end of feeding (5 ml) was taken into account. In order to ensure the overall quality of the sample, the milk, after having been collected, was frozen and stored at -20°C. Prior to the start of the analysis of milk composition, the consistency of each sample was checked, including the temperature of milk storage, signs of any damage to the container with the milk sample or whether the container had been unsealed. The process of milk thawing for the analysis was slow - the milk was left overnight in the refrigerator (4 ° C).

Statistical methods

The statistical analysis was carried out using the PQStat package, 1.8.0.338 version. The results of milk composition in terms of pregnancy duration were compared by means of the one-way analysis of variance, the Tukey's post-hoc test and the Fisher's linear trend (if the distributions did not deviate significantly from the theoretical normal distribution) or by means of the Kruskal-Wallis test, the Dunn's post-hoc test with Bonferroni correction and the Jonckheere-Terpstra trend test (if the distributions deviated significantly from the theoretical normal distribution). The test probability at the level of $p < 0.05$ was considered significant.

Results

Socio-demographic data of the study sample

The mean age of the study sample of women was nearly 31 years, with a distribution median of 31 years and a standard deviation of 5 years. The youngest participant was 20 years old and the oldest one was 46 years old. The majority of the 75 respondents had higher education *(57.33%)* and secondary education *(30.67%)*. Only one woman *(1.33%)* declared primary education. Women with vocational *(8%)* and lower secondary school education *(2.67%)* were in minority. The vast majority of the respondents *(69.33%)* lived in the city, only one in three *(30.67%)* lived in the countryside. Nearly 99% of the patients were in a relationship on the day of the study. Most of them were married *(65.33%)*. One third of the respondents declared cohabitation. Only one respondent was single *(1.33%)*. The study sample *(n = 75)* described their socioeconomic conditions as "good". Almost three quarters of the respondents worked. Every fourth patient *(28%)* was unemployed.

Clinical data of the study sample of women

The vast majority of the surveyed women were multiparous *(60%)*, 40% of the participants gave birth for the first time. All patients gave birth to a single baby. Only one third of the surveyed women*(33.33%)* gave birth on their due date. 50 women *(66.66%)*had a preterm labour, mothers of late preterm babies (33-36 weeks of pregnancy) constituted half of this group *(33.33%)*. The remaining 33.33% of women gave birth before 32 weeks of pregnancy. 50.67% of the respondents gave birth via caesarean section. In 34 women *(45.33%)* the course of pregnancy was complicated by the following conditions: gestational diabetes *(n=12)*, thyroid disease *(n=5)*, hypertension *(n=5)*, placental pathologies *(n=4)*, intrauterine growth retardation *(n=2)*, oligohydramnios *(n=2)*, polyhydramnios *(n=2)*, cholestasis of pregnancy *(n=1)*, thrombocytopenia *(n=1)*. Every third participant *(33.33%)* had no history of preterm birth. The vast majority *(78.67%)* of participants *(n=59)* had no history of miscarriage. 13 patients had experienced one miscarriage *(17.33%)*, 1 woman had had two miscarriages *(1.33%)* and 2 study participants had had three miscarries *(2.67%)*.

Assessment of protein content in breast milk in terms of the duration of pregnancy in the study sample of women 4 weeks postpartum.

The study attempted to determine whether there was a difference in the mature milk protein content between women who gave birth prematurely and those who gave birth on time. As the probability distribution differed significantly from the theoretical normal distribution, the Jonckheere-Terpstra trend test was used, which found no significant difference between mature milk protein content and the duration of pregnancy in the study sample of women (G1, G2, G3).

The highest numeric value for the mean total protein content in breast milk was obtained by women whose pregnancy finished between 33 and completed 36 weeks pregnancy (G2).

Table 1. Comparison of protein content in human milk [g/100ml] in terms of the duration of pregnancy in the sample of women four weeks postpartum

		DURATION OF PREGNANCY		
		<32T weeks pregnancy (G1)	33 – 36 weeks pregnancy (G2)	>37 weeks pregnancy (G3)
Arithmetic mean		1,56	1,72	1,59
Median		1,5	1,5	1,5
Standard deviation		0,26	0,71	0,25
Minimum		1,1	1,2	1,2
Maximum		2,2	5	2,4
Lower quartile		1,4	1,5	1,4
Upper quartile		1,7	1,6	1,7
Shapiro-Wilka test	W	0,97	0,44	0,85
	Z	-0,48	5,6	2,96
	p	0,68	<0,0001	0,0016
Kruskala-Wallisa test	df	2		
	H	0,36		
	p	0,84		
POST-HOC (Dunn Bonferroni)	<32		1	1
	33 - 36	1		1
	>37	1	1	
Jonckheere-Terpstra test	Z	0,22		
	p	0,83		

Assessment of breast milk fat content in terms of the duration of pregnancy in the study sample of women four weeks postpartum

Due to the equal number of women in each group: G1 (n=25), G2 (n=25), G3 (n=25), a linear test (Fisher's LSD) was used to determine the difference between breast milk fat content and the duration of pregnancy in the study sample of women (G1, G2, G3). As a result, no significant difference (p=0.2374) was found between the studied parameters.

Table 2.Comparison of fat content in human milk [g/100ml] in terms of the duration of pregnancy in the study sample of women four weeks postpartum

		DURATION OF PREGNANCY		
		<32 weeks pregnancy (G1)	33 – 36 weeks pregnancy (G2)	>37 weeks pregnancy (G3)
Arithmetic mean		3,46	3,84	3,55
Median		3,4	3,7	3,6
Standard deviation		0,75	0,97	1,23
Minimum		2,2	2	1,3
Maximum		4,9	6,2	6,2
Lower quartile		2,8	3,2	2,7
Upper quartile		4	4,4	4,3
Shapiro-Wilka test	W	0,96	0,97	0,98
	Z	0,09	-0,44	-0,79
	p	0,46	0,67	0,79
Browna-Forsythea test	F	1,76		
	p	0,18		
ANOVA	η^2	0,039		
	F	1,46		
	p	0,24		
POST-HOC (Tukey HSD)	<32		0,75	0,21
	33 - 36	0,75		0,59
	>37	0,21	0,59	
Linear trend (Fisher LSD)	F	0,52		
	p	0,24		

Assessment of carbohydrate content in breast milk in terms of the duration of pregnancy in the study sample of women four weeks postpartum

The study attempted to determine whether the content of carbohydrates in mature milk differs due to the duration of pregnancy in the study sample of women (G1, G2, G3). In order to verify that the distribution of the studied random variable follows a normal distribution, the Shapiro-Wilk test was used. A statistically significant value was obtained in each group (G1, G2, G3), as shown in Table 3. The Kruskal-Wallis test, the Dunn's post-hoc test with Bonferroni correction and the Jonckheere-Terpstra trend test were also used to study the difference between carbohydrates content in breast milk and the duration of pregnancy. As a result, no significant difference was found between carbohydrates content in mature milk and the duration of pregnancy in the study sample of women (G1, G2, G3).

Table 3.Comparison of carbohydrates content in human milk [g/100ml] in terms of the duration of pregnancy in the study sample of women four weeks postpartum

		DURATION OF PREGNANCY		
		<32 weeks pregnancy (G1)	33 – 36 weeks pregnancy (G2)	>37 weeks pregnancy (G3)
Arithmetic mean		7,9	7,65	7,85
Median		8	7,7	8
Standard deviation		0,57	0,84	1,03
Minimum		6,2	4,2	3,3
Maximum		8,7	8,6	8,8
Lower quartile		7,6	7,5	7,8
Upper quartile		8,3	8,1	8,3
Shapiro-Wilka test	W	0,91	0,69	0,57
	Z	1,83	4,41	5,06
	p	0,0335	<0,0001	<0,0001
Kruskala-Wallisa test	df	2		
	H	2,9		
	p	0,24		
POST-HOC (Dunn Bonferroni)	<32		0,73	1
	33 - 36	0,73		0,29
	>37	1	0,29	
Jonckheere-Terpstra test	Z	0,51		
	p	0,61		

Assessment of dry matter content in breast milk in terms of the duration of pregnancy in the study sample of women four weeks postpartum

Based on the Jonckheere-Terpstra test, no significant difference was found between dry matter content in mature milk and the duration of pregnancy in the study sample of women (G1, G2, G3).

Table 4.Comparison of dry matter content in human milk[g/100ml] in terms of the duration of pregnancy in the study sample of women four weeks postpartum

		DURATION OF PREGNANCY		
		<32 weeks pregnancy (G1)	33 – 36 weeks pregnancy (G2)	>37 weeks pregnancy (G3)
Arithmetic mean		13,06	13,4	13,28
Median		13	13,1	13,5
Standard deviation		1,14	1,13	1,82
Minimum		10,7	11,8	6,9
Maximum		15,1	16,7	16,8
Lower quartile		12,5	12,5	12,6
Upper quartile		13,7	14	14
Shapiro-Wilka test	W	0,94	0,92	0,87
	Z	0,94	1,53	2,61
	p	0,17	0,06	0,0046
Kruskala-Wallisa test	df	2		
	H	0,8		
	p	0,67		
POST-HOC (Dunn Bonferroni)	<32		1	1
	33 - 36	1		1
	>37	1	1	
Jonckheere-Terpstra test	Z	0,54		
	p	0,59		

Assessment of energy content in breast milk in terms of the duration of pregnancy of the sample of women four weeks postpartum

The literature on the subject indicates that the caloric content of human milk increases along with the duration of lactation [1]. The study attemptedto determine whether there was a relationship between the caloric content in breast milk and the duration of pregnancy. The composition of the mature milk collected by the sample of women during the 24-hour milk collection four weeks after delivery was analysed. Based on the performed statistical analysis, it is observed that the energy content of mature milk is similar in the study sample of women (G1, G2, G3).

Table 5. Comparison of energy content in human milk[g/100ml] in terms of the duration of pregnancy in the study sample of women four weeks postpartum

		DURATION OF PREGNANCY		
		<32 weeks pregnancy (G1)	33 – 36 weeks pregnancy (G2)	>37 weeks pregnancy (G3)
Arithmetic mean		71,88	75,48	74,44
Median		70	73	74
Standard deviation		8,11	9,28	11,74
Minimum		55	58	50
Maximum		89	99	100
Lower quartile		67	70	67
Upper quartile		78	81	80
Shapiro-Wilka test	W	0,98	0,97	0,98
	Z	-1,48	-0,01	-1,63
	P	0,93	0,5	0,95
Browna-Forsythea test	F	0,8		
	P	0,46		
ANOVA	η^2	0,03		
	F	1,05		
	P	0,36		
POST-HOC (Tukey HSD)	<32		0,32	0,75
	33 – 36	0,32		0,75
	>37	0,75	0,75	
Linear trend (Fisher LSD)	F	0,53		
	P	0,24		

Discussion

The literature on the subject indicates that scientists around the world are constantly analysing the composition of breast milk. The conducted research conducted provides a lot of new insights into the properties and composition of breast milk. Based on the results of the conducted studies, researchers report that the duration of pregnancy is one of the factors that differentiate the composition of breast milk [5, 6, 7]. Underwood also indicates in his research that breast milk from mothers of preterm babies differs from breast milk from mothers of babies born on time [8]. The results show that initially preterm milk has a higher content of proteins, fats, free amino acids and sodium than milk from women giving birth on time. However, the content of the above-mentioned components decreases within the first few weeks [9,10]. Bauer et al., who evaluated the composition of breast milk from the first to the eighth week postpartum also found higher levels of protein, carbohydrates, fats and calories in breast milk samples from mothers of preterm babies in comparison with breast milk samples from mothers of babies born on time [6]. Some researchers ascribe the differences in the composition of breast milk to the lower milk volume produced by women who gave birth before the 37 weeks of pregnancy. On the other hand, higher protein levels in preterm milk may also be a response of the preterm infant's body to the protein requirement [11]. However, in the presented study, the energy content and basic composition of breast milk collected 4 weeks after delivery from women who gave birth prematurely before 32 weeks of pregnancy (G1), between 33 and 36 weeks of pregnancy (G2) were not statistically significantly different from the milk samples from women who gave birth on time, after 37 weeks of pregnancy (G3). Similar results are presented in the study by Chrustek et al. The researchers analysed breast milk samples that came from the 4th/5th week of lactation from women who gave birth before 37 weeks of pregnancy, as well as from women who gave birth after 37 weeks of pregnancy. The medians of total protein content and caloric content of breast milk in both groups were identical and were respectively: 1.5 g/100ml and 74 kcal/100 ml. In the group of women giving birth before 37 weeks of pregnancy, the median of fat content in breast milk was 3.6 g/100 ml, carbohydrates 7.85 g/100 ml and dry matter 13.3 g/100ml, while in the group of women giving birth after 37 weeks of pregnancy, the median was: 3.7 g/100ml, 8.1 g/100ml and 13.5 g/100ml, respectively [12]. The results of the study by Fischer Fumeaux et al. who compared the macronutrient composition of the milk of mothers of extremely preterm infants (28 1/6 to 32 6/6 weeks of pregnancy) with the milk of mothers whose children were born on time (37 1/6 to 41 6/6 weeks of pregnancy) also seem interesting. While evaluating 500 breast milk samples, both the postnatal age of the children and the postmenstrual agewere taken into account.Human milk was analysed at 12 time points for four months for mothers of preterm infants and at 8 time points for two months for mothers who gave birth on time. The results of the study show that the protein content in the milk of women of both samples, depending on postnatal age, gradually decreased from birth to four months from 2.2 ± 0.3 g/100 ml to 1.5 ± 0.5 g/100 ml in the milk of women who gave birth prematurely and from 2.5 ± 0.8 g/100 ml to 1.7 ± 0.3 g/100 ml in the milk of women who gave birth on time, respectively. Nevertheless, significant statistical differences occurred when comparing the protein content of the milk of the study sample of women in terms of postmenstrual age. The greatest differences in protein content in breast milk occurred at 39 weeks (gestational age) for milk from women who gave birth on time with values up to 1.7 times higher than values for milk from women who gave birth prematurely considering 39thweek (postmenstrual age). On the other hand, in the first two weeks of lactation, the milk of women who gave birth prematurely hada higher energy content and contained significantly higher levels of fats, whereaswhen the next stage of lactation (from the third to the eighth week) was taken into account, a higher energy content and higher levels of fats were observed in the milk from women who gave birth on time. The lactose content of breast milk of both study samples remained stable and comparable at similar stages of lactation with a statistically insignificant trend towards higher lactose content in the milk from women who gave birth prematurely compared to the milk from women who gave birth on time [13]. The literature on the subject also indicates studies in which higher concentrations of lactose in the first month of lactation were found in the milk of women who gave birth on time compared to the milk of women who gave birth prematurely [14, 15]. Numerous researchers presented the differences between preterm milk and milk from women who gave birth on time - indicating the higher nutritional value of milk from mothers of preterm infants [5, 6, 7, 16]. Nevertheless, the results of our studies show more similarities than differences in the breast milk composition of the individual samples (G1, G2, G3), which was also observed by Fischer Fumeaux et al. in their study [13].

It should be emphasised that in the conducted study the milk sampling procedure was carefully planned and carried out in order to minimise errors resulting from physiological factors and consequently affecting the composition of breast milk. Additional strengths of this study include the use of advanced techniques to assess breast milk according to the recommended protocol, which minimised possible errors in breast milk composition.

The study has some limitations as well. First of all, the study was conducted among a small number of participants, so further large-scale studies in diverse populations are warranted. Secondly, the study did not take into account the maternal and infant factors that affect human milk composition. It is confirmed by research conducted by, among others, Fischer Fumeaux et al. who showed that the newborn gender is a factor determining the variability of breast milk. Higher fat and energy contents were observed in the milk of women who gave birth to male babies compared to the milk of women who gave birth to female babies. It was shown both in the analysis of milk from mothers of preterm babies and babies born on term [13].

Conclusions

The literature review shows that breast milk is optimal food for a baby regardless of the duration of pregnancy. The study results confirm the great value of human milk and the sense of feeding babies born prematurely with breast milk. Not only is human milk considered to be a nutrient but also functional food. Due to the content of enzymes, hormones and growth factors, human milk has a protective function. It is a part of treatment for babies born prematurely and/or with health problems. The results of our study indicate the need for further research measuring the macronutrients of human milk in order to individualize more the nutritional approach for a particularly demanding group of newborns, which may help optimize their growth and development. As the literature on the subject indicates, it is also important to remember about studies analysing other maternal and infant factors which influence breast milk composition, including the offspring gender.

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