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Correlation between macronutrient content and donation characteristics in Croatian human milk bank

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Abstract

Objectives: Optimal macronutrient (protein, fat, carbohydrate) values in human milk (HM) are essential for the healthy growth of infants, particularly those with very low birth weight. This study aimed to investigate the correlations between macronutrient content in HM and the characteristics of donations in the Croatian HM bank.

Methods: A total of 211 pools of HM from 51 donors were included. Analysis of HM was performed on MIRIS Human Milk Analyzer (Miris AB, Uppsala, Sweden). Prior to routine use, a brief verification of MIRIS was performed (between-run and within-run precision).

Results: In precision study the coefficients of variation were all below 10 %, as well as bias, with the exception of the between-run for crude proteins high control level (bias was 12 %). Storage temperature in the donor's home positively correlated with fat (p=0.004) and energy content (p=0.009) and negatively correlated with carbohydrates (p=0.003). The duration of lactation (p<0.001) and the duration of HM storage (in donor's home p=0.022; in the bank p=0.001; total duration of HM storage p<0.001) negatively correlated with proteins in HM. Statistically significant difference was obtained for all components (fat p=0.008, proteins p=0.006, carbohydrates p<0.001, energy content p=0.002) when comparing infant's sex and milk macronutrients, with male infants having higher values.

Conclusions: This study demonstrates that various factors can affect the macronutrient content of donated HM. It is important to monitor all factors that may impact on the quality of HM.

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Keywords: human milk; macronutrients; protein; fat; carbohydrate; donation characteristics

Introduction

It is well established that a mother's own milk is important for an infant's growth and development, particularly during the first six months of life, as endorsed by the World Health Organization [1, 2]. From a nutritional standpoint, infancy is a critical and vulnerable period due to the immaturity of tissues and organs involved in nutrient metabolism [3]. Human milk (HM) is highly variable in macronutrient content, with changes occurring at different stages of lactation (e.g. the concentration of lactose in colostrum is lower than in mature milk), and variations between mothers, within the same mother, throughout the day, and even during a single feeding session [1]. Optimal macronutrient composition (protein, fat, and carbohydrate) in HM is crucial for the healthy growth of infants, especially those with very low birth weight and premature infants, as HM reduces the risk of digestive intolerance, necrotizing enterocolitis, late onset sepsis, bronchopulmonary dysplasia, and retinopathy of prematurity [4]. However, some mothers are unable to provide their own milk for their infants for various reasons (e.g. insufficient milk supply) [4, 5]. In such cases, particularly for premature infants in neonatal intensive care units, HM from HM bank is the best possible substitute. It is important to emphasize that HM banks provide safe and high-quality donated HM. This was the rationale for establishing the Human Milk Bank at the Croatian Tissue and Cell Bank, University Hospital Centre Zagreb [6].

The aim of this study was to investigate the associations between donor characteristics, storage conditions of donated HM and macronutrient content in HM.

Materials and methods

This study was conducted at the Human Milk Bank of the Croatian Tissue and Cell Bank, University Hospital Center Zagreb, Croatia, in the period from December 2019 to October 2020. Prior to donating HM, each donor completed a

questionnaire regarding her health status, childbirth and infant's health, risk behaviors, travelling history, dietary habits, smoking, alcohol consumption, medication and illegal drug use. Exclusion criteria for donors included: infectious diseases (e.g. hepatitis, syphilis, encephalitis, endocarditis), neurological diseases (e.g. multiple sclerosis, Huntington's diseases, Guillain-Barré syndrome), organ and cell transplantation, collagen tissue diseases, use of specified medications, smoking, alcohol and drug use. HM in Croatian milk bank can be donated until the end of the infant's first year.

If the donor met all criteria for milk donation, she received a milk collection set and written instructions on milk expression, personal hygiene, labeling of milk containers, handling and storage of milk at home. The set included a data logger for continuous recording of the storage temperature. During the donation period each donor maintained a diary of milk expression and a safety assessment with data on her health and milk storage conditions.

In this study, a total of 211 pools of HM from 51 donor mothers of 23 male and 30 female children were included, four of whom were born from twin pregnancies. Six donors gave birth before 37 weeks of gestation, which is considered a premature delivery and two of them donated preterm milk that was collected in the first four weeks after premature delivery.

Collection of HM at donor's home

Mothers who donate HM have lactation that exceeds their own child's needs and were willing to donate at least 2 L of surplus milk. They were instructed to practice exceptional hygiene and careful expressing, labeling and storage of donated milk.

Donors received thirty sterile bisphenol A (BPA) free collection containers of 130 mL capacity, respective labels, a diary of expression, a health questionnaire, informed consent, instructions for expression and storage of milk, and a data logger. The data logger was placed in the freezer with the first filled container and removed at the time when milk was transferred to the bank. It recorded the temperature every 60 min. The temperature curve of the milk storage condition at the donor's home was checked as part of entrance control within the milk bank.

Donors were instructed to collect milk in the same container throughout the collection day and to freeze it within 24 h of the first milk expression, keeping it in the refrigerator in the meantime. Some mothers had more than 130 mL of surplus milk per day, so they filled multiple containers. The milk was stored for a maximum of two months in the donor's kitchen freezer, either combined with a refrigerator or a stand-alone freezer. Each collection container was labeled with the donor's name and surname, date and time of milk expression and freezing. Containers were not overfilled to allow space for the expansion of frozen milk.

Mothers who had already stored surplus milk before contacting a milk bank or becoming a donor usually stored milk in the BPA-free plastic bags, which were accepted into the bank if appropriate labeling, hygiene and storage procedures were followed.

Processing of the HM in the bank

Upon arrival at the bank, HM was stored in a refrigerator at −30 °C until pasteurization. The day before pasteurization, the containers with HM were placed in a refrigerator at +4 °C, to thaw gradually. HM from the same mother was pooled on the day of pasteurization in a grade D clean room (in cabinet with grade A of air quality). One pool contained approximately 1.5 L of donated milk (median 1.45 L, 95 %CI for the median 1.3–1.6, range 0.4–2.2 L). Samples for determining nutritional values and microbial quality testing were taken aseptically before pasteurization from each pool. Holder pasteurization was used for the pasteurization process [7].

Analysis of the HM samples

Analysis of HM was performed on MIRIS Human Milk Analyzer (Miris AB, Uppsala, Sweden), which measures macronutrients – fat, carbohydrates and proteins using mid-infrared spectrometry technology, and calculates the energy content of HM [8–10]. For protein analysis, MIRIS measures true proteins, which are a measure of only proteins in food, while crude proteins are a measure of all nitrogen sources and include non-protein nitrogen.

Before analysis, all samples, as well as commercial controls (Miris Calibration Control Kit, Miris AB, Uppsala, Sweden) and Miris Check solution (Miris AB, Uppsala, Sweden), were warmed to +40 °C in a water thermostatic bath. The first step in the analysis process was checking the analyzer using Miris Check solution, which validates the linearity, zero settings and performs analyzer cleaning. If the set criteria were met, commercial control samples were analyzed. In the daily routine, commercial control samples at two levels were used and when controls met the set criteria, routine HM samples were analyzed. Three mL of sample was required for analysis on a MIRIS analyzer.

Before measurement, samples were homogenized using an ultrasonic processor (Miris AB, Uppsala, Sweden) with parameters set to 75 % amplitude and 1.5 s/mL of milk.

Verification of HM analyzer MIRIS

Before introduction into routine laboratory work, a short verification of the Miris analyzer was performed, including determination of between-run and within-run precision. For between-run precision commercial control samples at two concentration levels (Miris Calibration Control 1, Miris Calibration Control 2; lot 250801) were analyzed in triplicate over five days [11]. Within-run precision was performed by analyzing 10 replicate measurements of one routine residual milk sample. Bias was calculated using data from between-run precision, using the following equation:

$$\text{Bias}\ (\%) = (\text{Mean value} - \text{Target value})/\text{Target value} \times 100.$$

All samples used in the study were from routine work and the study was conducted according to the principles of the Declaration of Helsinki and under the terms of all relevant legislation. Institutional ethics committee approval was obtained for this study (8.1.-24/112-2; 02/013-JG).

Statistical analysis

Statistical analysis was carried out using the MedCalc statistical software, version 19.5.2. (MedCalc, Ostend, Belgium). Data distribution normality was assessed using the Shapiro-Wilk test. Depending on data normality distribution, results were expressed as means and standard deviations, or median and interquartile ranges. Statistical analysis of results with an abnormal distribution was performed by Kruskal-Wallis or Mann-Whitney, depending on the number of analyzed groups.

Results for within-run and between-run precision were expressed as mean, standard deviation (SD) and coefficients of variations (CVs).

Samples were divided into subgroups according to donor's age (4 groups: ≤25 years, 25 < age ≤ 30 years, 30 < age ≤ 35 years, age >35 years), donor's body mass index (BMI) (3 groups: BMI ≤25 kg/m^2, 25 < BMI ≤ 30 kg/m^2, BMI >30 kg/m^2), duration of lactation in weeks at time of HM donation (4 groups: ≤4 weeks, 4 weeks < age ≤ 12 weeks, 13 weeks < age ≤ 24 weeks, age >24 weeks), infant sex, type of HM storage container (bag or bottle), and storage temperature at donor's home (3 groups: <–25 °C temperature, –24 °C ≤ temperature < –18 °C, temperature >–18 °C). A Spearman rank correlation coefficient was used to estimate the correlation between donor's characteristics, storage conditions and HM macronutrients. A $p<0.05$ was considered statistically significant.

Multiple regression analysis was used to examine the influence of donor's characteristics and storage conditions on donated HM macronutrient components if they were statistically significant in univariate analysis. A $p<0.05$ was considered statistically significant.

Results

The characteristics of donors and donated HM are presented in Table 1.

The results of precision study are presented in Table 2. For between-run precision CVs were all <10 %. Additionally, bias for both control levels was ≤10 %, except for crude proteins at the high control level (bias was 12 %). For within-run precision, CVs for all assays were <10 %.

Table 1: Characteristics of donors and donated human milk (51 donors; 211 human milk pools).

	Mean/ median[a]	Standard deviation/95 % CI of the median[b]	Range
Donor's age, years	32	4	22–40
Donor's body mass index at first donation, kg/m^2	24.0	3.0	18.7–33.3
Duration of lactation, weeks	12[a]	8–16[b]	1–49
Volume of pool of donated milk, mL	1,450[a]	1,300–1,600[b]	400–2,200
Volume of pool of donated milk in bags, mL	1,250[a]	1,050–1,605[b]	400–2,200
Volume of pool of donated milk in bottles, mL	1,550[a]	1,419–1,650[b]	450–2,200
Duration of milk storage in donor's home, days	28[a]	25.8–29.2[b]	3–83
Temperature of milk storage in donor's home, °C	−21.0[a]	−21.0 to (−20.0)[b]	−34.0 to (−12.5)
Duration of milk storage in milk bank before pasteurization, days	11[a]	8–14[b]	1–56
Composition of human milk pools			
Carbohydrates, g/100 mL	8.2[a]	8.1–8.2[b]	6.9–8.6
True proteins, g/100 mL	1.0[a]	0.9–1.0[b]	0.5–2.0
Fat, g/100 mL	3.69	1.02	1.4–6.4
Energy content, kcal/mL	72.3	10.0	50–99

[a]Median; [b]95 % CI, of the median; CI, confidence interval.

Table 2: Results of precision study on the MIRIS analyzer.

Between-run precision					Bias, %
Assay	**Control level**	**Mean**	**SD**	**CV, %**	
Fat, g/100 mL	LOW	3.6	0.10	2.8	2.9
	HIGH	6.6	0.05	0.7	0
Carbohydrates, g/100 mL	LOW	7.7	0.06	0.8	0
	HIGH	8.4	0.08	1.0	2.4
Crude proteins, g/100 mL	LOW	1.3	0.05	3.9	8.3
	HIGH	2.8	0.05	1.8	**12.0**
True proteins, g/100 mL	LOW	1.1	0.05	4.8	10.0
	HIGH	2.2	0.08	3.7	0

Within-run precision (n=10)			
Assay	**Mean**	**SD**	**CV, %**
Fat, g/100 mL	5.4	0.3	5.0
Carbohydrates, g/100 mL	8.9	0.2	2.3
Crude proteins, g/100 mL	1.1	0.1	6.3
True proteins, g/100 mL	0.9	0.1	7.8
Energy content, kcal/100 mL	89.8	2.7	3.0

SD, standard deviation; CV, coefficient of variation. Bold values indicate results that did not meet the set criteria.

When examining the correlation, no association was found between donor's age, body weight, BMI or volume of pool of donated HM with macronutrient values in donated milk. The temperature of milk storage in donor's home positively correlated with fat (rho 0.209, p=0.004) and energy content (rho 0.180, p=0.009) and negatively correlated with carbohydrate content (rho −0.213, p=0.003). The duration of lactation (rho −0.738, p<0.001) and duration of milk storage (in donor's home: rho −0.158, p=0.022; in the bank: rho −0.224, p=0.001; total duration of HM storage rho −0.253, p<0.001) negatively correlated with protein content in HM. Other correlation data are shown in Table 3. Multivariate analysis using multiple regression indicated that the duration of lactation (coefficient −0.012, standard error 0.001, p<0.001) affects protein content in HM.

When comparing data on the association between an infant's sex and macronutrient values, statistically significant differences were observed for all components (fat p=0.008, proteins p=0.006, carbohydrates p<0.001, energy content p=0.002). The HM of donors with male infants had higher values of all macronutrients compared to donors with female infants (Figure 1). Additionally, a statistically significant difference was found between groups when comparing the duration of lactation with macronutrient content in HM for fat (p=0.036), proteins (p<0.001) and energy content (p=0.010) (Figure 2).

When comparing the type of storage container before pasteurization (bottle vs. bag), a statistically significant difference was observed only for protein concentration (p<0.001).

Regarding the temperature of milk storage in donor's home, statistically significant differences were observed between groups for all macronutrients except proteins (fat p=0.038, carbohydrates p<0.001, energy content p=0.043).

When examining the associations of a mother's BMI and age on HM macronutrient content, statistically significant differences were found only for carbohydrates and the donor's BMI and age (p=0.041 for BMI; p=0.012 for age).

Discussion

The significance of HM usage is well established. However, despite HM being considered homogenous, there is

Table 3: Spearman rank correlation coefficient between donor characteristics, storage conditions and human milk macronutrient components.

Donor characteristics and storage conditions	Units	Fat		Carbohydrates		Proteins		Energy content	
		Spearman's rho	p-Value	Spearman's rho	p-Value	Spearman's rho	p-Value	Spearman's rho	p-Value
Mothers age	years	−0.009	0.891	−0.024	0.725	0.074	0.284	−0.004	0.960
Mothers body weight	kg	−0.061	0.379	0.056	0.417	0.013	0.850	−0.069	0.316
Mothers BMI	kg/m^2	−0.035	0.615	0.039	0.571	0.011	0.873	−0.050	0.473
Duration of lactation	weeks	0.048	0.486	0.002	0.981	−0.738	**<0.001**	−0.041	0.551
Volume of donated milk	mL	−0.080	0.247	−0.004	0.953	0.070	0.309	−0.076	0.271
Temperature of milk storage in donor's home	°C	0.209	**0.004**	−0.213	**0.003**	0.065	0.370	0.18	**0.009**
Duration of milk storage in donor's home	days	−0.059	0.393	−0.013	0.849	−0.158	**0.022**	−0.067	0.336
Duration of milk storage in bank before pasteurization	days	−0.088	0.204	−0.073	0.292	−0.224	**0.001**	−0.100	0.149
Duration of milk storage in donor's home and in bank before pasteurization	days	−0.120	0.083	−0.088	0.204	−0.253	**<0.001**	−0.129	0.062

Bold values represent statistically significant p-values.

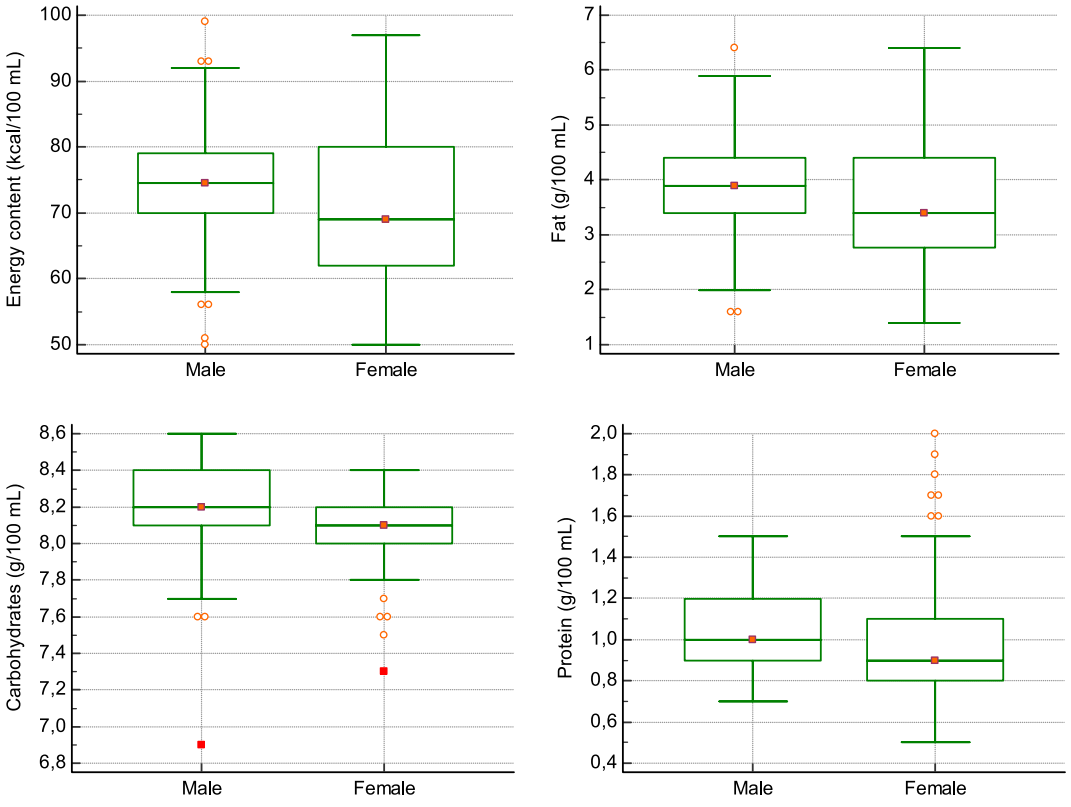

Figure 1: Influence of infant's sex on the macronutrients of the donated human milk.

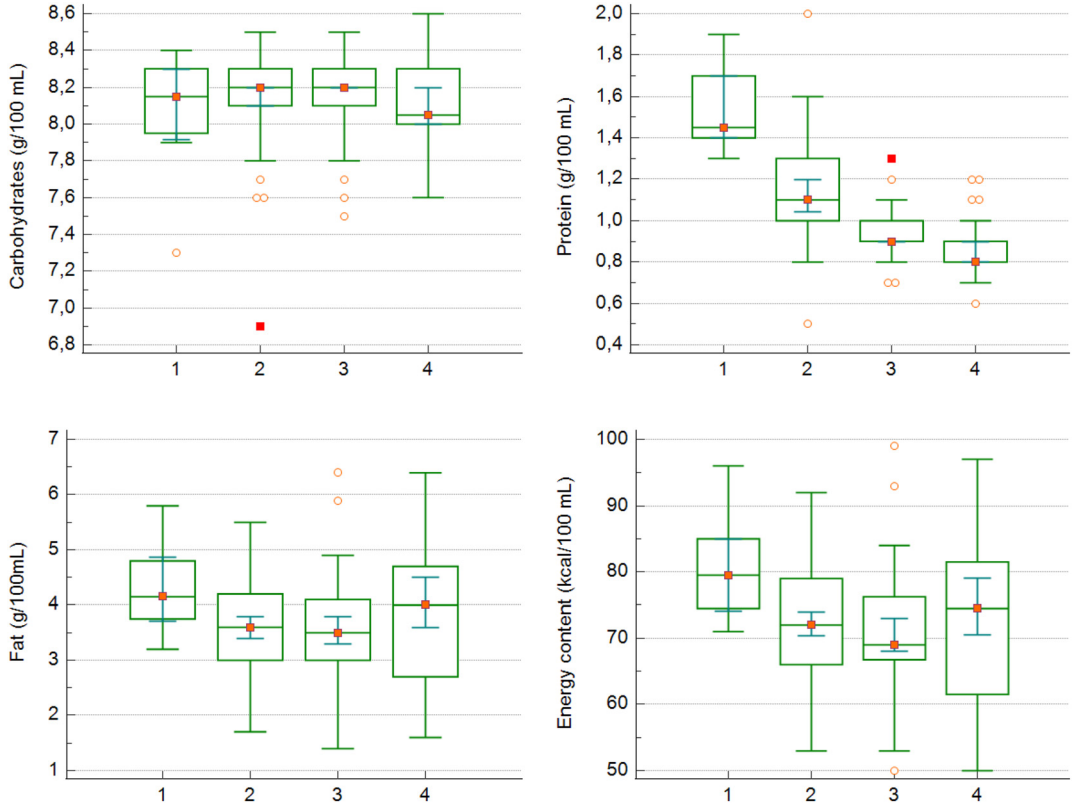

Figure 2: Influence of duration of lactation (weeks) on the macronutrients of the donated human milk (1: ≤4 weeks, 2: 4 weeks < lactation ≤12 weeks, 3: 12 weeks < lactation ≤24 weeks, 4: lactation >24 weeks).

substantial variability in macronutrient values related to different donor's characteristics and storage conditions [12]. Optimizing infant feeding necessitates determining the nutritional content of HM [8]. According to good laboratory practice, validation or verification of methods must be performed before implementation in routine work. In this study we assessed the accuracy of the MIRIS analyzer. CV's between-run and within-run precision were below 10 %, as well as bias, except for crude proteins at the high control level. In our milk bank we don't use crude proteins (which are also known as total proteins) as part of milk quality assessment, so we do not consider this data to be clinically significant. In the analysis of the influence of donor's characteristics (age, weight and BMI) on the macronutrient components of the HM, we observed no significant differences, indicating that the examined factors did not influence macronutrient levels. Bzikovska et al. presented similar results in their study for maternal BMI, finding no correlation between protein and carbohydrate concentrations and BMI [1]. However, when we classified donors into subgroups according to BMI and age, we found differences between groups for carbohydrate concentration in HM. Contrary to our results, Bachour and colleagues found an increase in lipid concentration in the milk of mothers over 35 years of age and statistically significant difference between different BMI groups and protein concentration of the HM [13].

When comparing the association between infant sex and HM macronutrients, our results showed that sex influenced all measured macronutrients. In study of Păduraru et al. is stated that infant sex influences only protein content, which is higher in mothers of male infants [12]. Similar results for protein values were obtained in our study. Furthermore, Fischer Fumeaux et al. found that HM from mothers of male infants was more concentrated in fat and energy compared to mothers of female infants [14].

Duration of lactation affects the composition of HM. In this study we also observed that duration of lactation affected protein content. It is a known fact that there is a difference in the composition of HM between preterm and term milk, with preterm containing higher concentration of proteins, fat and energy [15, 16]. Our results showed that donated preterm milk and term milk in the first 4 weeks of lactation had higher concentrations of fat, proteins and energy compared to milk expressed after 4 weeks of lactation (Figure 2).

Literature states that fats are the most variable macronutrients of HM [1]. In this study neither the donor's age, body weight, BMI, volume of pool of donated HM, nor the duration of milk storage (whether in the donor's home or in the milk bank) affected fat concentration in donated HM, only storage temperature did affect fat concentration (Table 3).

According to guidelines for the quality and safety of HM, the most common method of HM preservation is freezing at temperature below −20 °C [17]. Proper storage and monitoring HM temperature are crucial to preserving nutritional quality during storage. Since donated milk has been accepted both in bags and bottles, the effect of the type of container on nutritional content was analyzed. The results showed a statistically significant difference between the type of storage containers for protein concentration in donated HM, with higher protein values in bags. This result may be related to the fact that the HM from mothers of preterm and infants younger than 4 weeks was donated more often in bags than in bottles, and vice versa for older subgroups of infants.

A limitation of this study is the small number of premature HM pools, as only 6 of 51 donors were mothers of premature infants, and just 4.73 % of donated HM pools were premature milk.

Conclusions

This study demonstrates that various factors can affect the macronutrient composition of donated HM, including the infant's sex, duration of lactation, duration and temperature of HM storage, and the type of storage containers. Therefore, it is important to monitor all factors that may impact the quality of HM, and to determine its macronutrient values. This allows for the adjusted fortification of HM, if necessary, to enhance the nutrition of premature infants.

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Research ethics: The study was approved by the University Hospital Centre Zagreb Ethics Committee in June 2024 (8.1-24/112-2; 02/013-JG) and conducted according to the principles of the Declaration of Helsinki.

Informed consent: Not applicable.

Author contributions: Concept and design: VR and JN; acquisition, analysis and interpretation of data: VR and JN; drafting the article: VR; revising it critically for important intellectual content: JN, IB and BGC; approved final version of the manuscript: VR, JN, BGC, and IB. All authors have read the manuscript and approved it as well as responsible authorities at the institution where the work has been carried out. All authors attest to the validity and legitimacy of the data and its interpretation and agreed to its submission in Journal. Research described in the study has not been published before and it is not under consideration for publication anywhere else. All authors listed have contributed sufficiently to the project to be included as authors.

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