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ORIGINAL ARTICLE

Impact of diet and body composition on donor human milk: a cross-sectional pilot study

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Abstract

Introduction and Objectives: Donor human milk is recommended in Neonatal Intensive Care Units when maternal breast milk is unavailable. This study aimed to explore the association between the eating habits, body mass index, and body composition of human milk donors with the nutritional composition of their donor milk. **Methods:** A cross-sectional observational study involved 65 human milk donors. Sociodemographic, lifestyle, and clinical data were collected by questionnaires administered through interviews. Height, weight, and fat mass were measured and body mass index was calculated. Eating habits were assessed using a Food Frequency Questionnaire. Donor human milk samples were analyzed before and after pasteurization using the Miris Human Milk Analyzer™. **Results:** Thirty-three (50.8%) human milk donors were overweight, with a median fat mass of 33.2%. No significant associations were found between body mass index or fat mass and the nutritional composition of donated milk. However, infants' gestational age showed a positive correlation with the protein content after the donated milk had been pasteurized. The energy of donor human milk before pasteurization was positively associated with carbohydrate intake ($r = 0.272$, $p = 0.029$). The consumption of carbohydrate sources was positively correlated with the energy of donor human milk before ($r = 0.271$, $p = 0.029$) and after pasteurization ($r = 0.248$, $p = 0.046$). Potato intake showed a positive correlation with fat content in donor human milk before ($r = 0.474$, $p < 0.001$) and after the milk had been pasteurized ($r = 0.443$, $p < 0.001$). **Discussion:** These results highlight the importance of monitoring human milk donors' nutrition to optimize their nutritional status and donated milk composition.

Keywords: Body mass index. Donor human milk. Eating habits. Human milk donors. Nutritional composition.

Impacto da dieta e da composição corporal no leite humano doado: um estudo piloto transversal

Resumo

Introdução e Objetivos: O leite humano de dadora é recomendado nas Unidades de Cuidados Intensivos Neonatais quando o leite materno está indisponível. Este estudo explorou a associação entre hábitos alimentares, índice de massa corporal e

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composição corporal de dadoras de leite humano com a composição nutricional de leite doado. **Métodos:** Estudo transversal com 65 dadoras de leite humano. Obtiveram-se dados sociodemográficos, clínicos e de estilo de vida via questionários. Mediu-se a altura, o peso e massa gorda e calculou-se o índice de massa corporal. Avaliaram-se hábitos alimentares pelo Questionário de Frequência Alimentar. Analisaram-se amostras de leite humano de dadora, antes e após pasteurização, pelo *Miris Human Milk Analyzer*™. **Resultados:** Trinta e três (50,8%) dadoras de leite humano apresentavam excesso de peso e mediana de massa gorda de 33,2%. Não foram encontradas associações significativas entre índice de massa corporal e massa gorda das dadoras de leite humano com a composição nutricional do leite doado. A idade gestacional dos recém-nascidos associou-se positivamente ao teor proteico após pasteurização do leite doado. Antes da pasteurização, o valor energético do leite associou-se positivamente à ingestão diária de hidratos de carbono ($r = 0,272$; $p = 0,029$). O consumo de fontes de hidratos de carbono correlacionou-se positivamente com a energia antes ($r = 0,271$; $p = 0,029$) e após pasteurização ($r = 0,248$; $p = 0,046$) do leite doado. A ingestão de batatas associou-se positivamente à gordura antes ($r = 0,474$; $p < 0,001$) e após pasteurização ($r = 0.443$, $p < 0.001$) do leite. **Discussão:** O acompanhamento nutricional das dadoras de leite humano é crucial para otimizar o seu estado nutricional e a composição do leite doado.

Palavras-chave: Composição nutricional. Dadoras de leite humano. Hábitos alimentares. Índice de massa corporal. Leite humano de dadora.

Keypoints

What is known

- Breastfeeding is often a challenge in Neonatal Intensive Care Units.
- Donor human milk is recommended for preterm and infants with a low birth weight, offering protection against health issues.
- Eating habits and body composition can lead to fluctuations in the nutritional composition of human milk.

What is added

- Body mass index and fat mass do not appear to impact the nutritional composition of donated human milk.
- Greater carbohydrate consumption is linked to higher energy in donated milk, crucial for meeting premature infants' high energy needs.
- Ensuring proper nutrition during breastfeeding is crucial, especially when considering the donation of human milk.

Introduction

Maternal breast milk is a natural and adequate food that helps to meet the energy and nutritional needs of any newborn. Today, the beneficial impact of breastfeeding on the health of both infants and mothers is widely recognized[1,2]. Literature has associated breastfeeding with a lower risk of morbidity and mortality, as well as improvements in infants' neurodevelopment. Breastfeeding has also been associated with a lower incidence of breast and ovarian cancer in women[1,3].

The World Health Organization (WHO) currently recommends that infants be exclusively breastfed during the first six months of life and should continue breastfeeding for two years alongside food diversification[4]. However, there may be circumstances where mother's own milk (MOM) is not available or is insufficient to meet infants' needs, which is a common challenge in Neonatal Intensive Care Units[5]. In these cases, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) suggests donor human milk (DHM) as a viable alternative due to the superiority of human milk (HM) characteristics compared to commercial formulas[6]. Using DHM from Human Milk Banks is particularly important for preterm or newborns with a low birth weight, when MOM cannot be used[7].

According to previously randomized clinical trials[8], feeding infants with DHM instead of commercial formulas has several advantages such as the higher protection conferred against necrotizing enterocolitis, bronchopulmonary dysplasia, and different types of infections[9]. Although HM has the nutrients needed for the healthy development of infants up to six months of age, literature suggests that several factors such as the mother's diet and maternal body composition can cause variations in the concentration of certain nutrients[3,10]. In fact, it has been increasingly suggested that maternal overweight may be associated with nutritional composition changes in HM, particularly in terms of fat and lactose levels[11]. However, the impact

Table 1. Characteristics of human milk donors and their infants (n = 65)

Characteristics of human milk donors	
Age, years, median (p25, p75)	33.0 (30.0, 36.0)
School level, n (%) Lower secondary level Secondary level Advanced qualification	 1 (1.5) 13 (20.0) 51 (78.5)
Health professional, n (%)	21 (32.3)
First pregnancy, n (%)	31 (47.7)
Cesarean delivery, n (%)	28 (43.1)
Smoking (at least once during their lifetime), n (%)	16 (24.6)
Regular physical activity, n (%)	27 (41.5)
Sleep hours, h, median (p25, p75)	7.0 (6.0, 8.0)
BMI, kg/m^2*, median (p25, p75) With pre-obesity (25.0-29.9 kg/m^2), n (%) With obesity (≥ 30.0 kg/m^2), n (%)	25.1 (20.8, 27.6) 26 (40.0) 7 (10.8)
Fat mass†, %, median (p25, p75), n = 56	33.2 (27.5, 37.5)
Characteristics of human milk donors' infants	
Male sex, n (%)	29 (44.6)
Preterm infant (< 37 weeks), n (%)	7 (10.8)
Gestational age, weeks, median (p25, p75)	39.0 (38.0, 40.0)
Birth weight, g, median (p25, p75)	3320.0 (2867.5, 3662.5)

**According to WHO classification: World Health Organization. Obesity: preventing and managing the global epidemic. Report on a consultative meeting. Geneva, Switzerland: Author; 1997.*
†Body fat percentage. [website]. Tanita; 2012 – 2022. [cited on: 23 Jun 18]. Available at: https://tanita.eu/understanding-your-measurements/body-fat-percentage.
p25: 25th percentile; p75: 75th percentile; h: hours; BMI: body mass index; kg/m^2: kilogram per square meter; g: grams; %: percentage.

of a mother's diet on her own milk's composition is not well understood, as available information is limited and often inconsistent, primarily due to the small number of studies on this topic and variations in study methodologies[12,13].

As mentioned above, DHM is a highly beneficial choice for preterm infants or newborns with a low birth weight. It is therefore crucial that we further increase our understanding about the factors associated with its nutritional composition in order to promote the optimization of DHM in alignment with the specific nutritional needs of its main recipients[2]. In Portugal, there is a rising interest in personalized fortification of DHM. Studies have already assessed the influence of DHM's nutritional composition on the growth of recipient infants[14]. However, the potential impact of human milk donors' (HMDs') diet on the composition of DHM has not yet been investigated. Also, given the high prevalence of Portuguese women in reproductive age with obesity[15], understanding the possible implications of their eating habits, body mass index (BMI), and body composition on the nutritional composition of DHM is a matter of urgency.

This paper aimed to study the association between the eating habits, BMI, and body composition of HMDs' and the nutritional composition of their donor milk, and to understand if this relationship differs before and after pasteurizing the DHM.

Methods

Study design and sampling

A cross-sectional observational study was conducted between March 2023 and March 2024 at (institution name) in HMDs'. To become an HMDs', women have to follow specific pre-established criteria such as (a) being a healthy lactating woman who, despite exclusively breastfeeding her child, has excess milk, (b) being the mother of an infant under six months old, (c) not smoking or consuming alcoholic drinks or beverages with a high caffeine content and (d) not testing positive for HIV 1 or 2, Hepatitis B or C, Human T-cell Lymphotropic Virus I or II, or Syphilis. A complete description of the inclusion criteria is described elsewhere[16]. Also, all participants must be over 18 years old and cannot have any special dietary needs. At the start of the study, 26 HMDs' were active, and a further 52 were later recruited, resulting in a total of 78 HMDs'. Of these, 65 were included in this study (participation proportion = 83.3%).

Data collection

For the purpose of the study, a questionnaire with sociodemographic, lifestyle, and clinical data was created and applied through an interview during a clinical appointment at (institution name). The nutritional composition of each participant's milk samples was analyzed and recorded at (institution name).

Sociodemographic data included the participants' age as well as their education level, with 11 options ranging from "no formal education" to "doctorate". We further categorized this into three groups: lower secondary level (< 12 years of schooling), secondary level (12 years of schooling), and an advanced qualification (> 12 years of schooling). Profession was asked as an open question and subsequently categorized as either healthcare or non-healthcare professional.

Table 2. Impact of pasteurization on the nutritional composition of donor human milk (n = 65)

Variables	Before pasteurization*	After pasteurization*	Difference (after – before)	p†	Delta%‡
	Median (p25, p75)				
Energy, kcal/100 ml	69.0 (61.5, 75.1)	65.4 (59.5, 71,2)	–3.0 (–4.9, –1.2)	**< 0.001**	–4.5 (–7.0, –1.9)
True protein, g/100 ml	0.8 (0.7, 0.9)	0.8 (0.7, 0.9)	0.0 (–0.1, 0.0)	**0.003**	0.0 (–11.1, 0.0)
Crude protein, g/100 ml	1.0 (0.9, 1.2)	1.0 (0.9, 1.1)	–0.1 (–0.1, 0.0)	**0.002**	–7.1 (–10.0, 0.0)
Fat, g/100 ml	3.5 (2.8, 4.1)	3.1 (2.6, 3.9)	–0.3 (–0.5, –0.1)	**< 0.001**	–8.3 (–12.7, –3.5)
Carbohydrate, g/100 ml	8.1 (8.0, 8.2)	8.0 (7.9, 8.2)	–0.1 (–0.1, 0.0)	**0.005**	–1.2 (–1.3, 0.0)

*Using the Miris HMA™ (Human Milk Analyzer, Miris AB, Uppsala, Sweden).
†Wilcoxon test.
‡The percentage reduction (Delta %) in energy and macronutrients for DHM was calculated using the ratio for the difference in energy and macronutrients before and after pasteurization and the value of energy and macronutrients before pasteurization.
The statistically significant values (with $p < 0.05$) are highlighted in bold.
p25: 25th percentile; p75: 75th percentile; kcal: kilocalories; g: grams; ml: milliliters.

Lifestyle data related to smoking and physical activity were collected with dichotomous questions (yes/no). The participants answered 'yes' if they had ever smoked in their life, and if they regularly do physical activity. Regarding sleeping habits, they were asked about the number of hours of sleep each day during the week and at weekends. The average of these two periods was then used. Eating habits over the previous year were assessed, by interview, through a semi-quantitative Food Frequency Questionnaire (FFQ) validated for the Portuguese adult population[17,18]. Food frequency was indicated by the participants according to nine categories from "never or less than once a month" to "more than six times a day". For each food item, the amount ingested was registered according to the comparison with pre-established average portions. The consumption of each food, in grams, was calculated by multiplying the frequency of consumption by the portion size reported by the participants and, if appropriable, by a seasonal variation factor. The Food Processor Plus software (ESHA Research, Salem, Oregon) was used to convert FFQ items into nutrient intakes expressed in grams[17,18].

Anthropometric measurements were collected according to standard procedures[19]. Height was measured in centimeters (with 0.1 cm of resolution) using a SECA 213 measuring station or, failing that, it was obtained from the height recorded on the citizen's identity card. Body weight, in kilograms (kg), and fat mass (FM), in percentage (%), were determined using the TANITA® TBF-300 bioimpedance scale. BMI was calculated and categorized according to WHO criteria: underweight (< 18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), pre-obesity (25.0-29.9 kg/m^2), and obesity (≥ 30.0 kg/m^2)[20].

The HMDs' obstetric history was evaluated. They were asked about the number of pregnancies and the type of delivery, as well as the birth weight and gestational age of the infant which had resulted from their last pregnancy. Infants were classified as preterm if their gestational age was less than 37 weeks[21].

The nutritional composition of DHM samples was analyzed, before and after pasteurization, using the Miris Human Milk Analyzer™ (Miris AB, Uppsala, Sweden), an analytical innovative technique based on mid-infrared transmission spectroscopy principles[22]. The samples of raw DHM were collected by HMDs' in their own homes after they were instructed on good practices for extracting and cold-storing milk. When the raw DHM arrived at the Human Milk Bank, conformity parameters were checked in the samples (for example, organoleptic characteristics, bottle condition, and the storage temperature range of the raw DHM at the HMD's home). The Miris Human Milk Analyzer™ measured milk samples' true protein, crude protein, fat, and carbohydrate contents, in grams (g) per 100 milliliters (ml), and its energy, in kilocalories (kcal) per 100 ml, using a 3 ml sample of DHM. Prior to analysis, the device was calibrated by the calibration solution Miris Calibration Control Kit™ (Miris AB, Uppsala, Sweden), and the samples were homogenized using the Miris Ultrasonic Processor™ (Miris AB, Uppsala, Sweden) and warmed according to the respective protocol[22].

Statistical analysis

The collected data was compiled in a database using the IBM SPSS® program (Statistical Package for Social Sciences), version 28.0, for the respective statistical analysis. The normality of each quantitative variable

Table 3. Comparison between the gold standard of mature human milk and donor human milk composition before and after pasteurization (n = 65)

Variables	Gold standard mature HM‡	Before pasteurization* Median (p25, p75)	p†	After pasteurization* Median (p25, p75)	p†
Energy, kcal/100 ml	66	69.0 (61.5, 75.1)	**0.036**	65.4 (59.5, 71.2)	0.604
Crude protein, g/100 ml	1.0	1.0 (0.9, 1.2)	**0.040**	1.0 (0.9, 1.1)	0.629
Fat, g/100 ml	3.8	3.5 (2.8, 4.1)	**< 0.001**	3.1 (2.6, 3.9)	**0.015**
Carbohydrate, g/100 ml	7.0	8.1 (8.0, 8.2)	**< 0.001**	8.0 (7.9, 8.2)	**< 0.001**

*Using the Miris HMA™ (Human Milk Analyzer, Miris AB, Uppsala, Sweden).
†One-sample Wilcoxon signed rank test.
‡According to Rêgo et al.[23]
The statistically significant values (with p < 0.05) are highlighted in bold.
HM: human milk; p25: 25th percentile; p75: 75th percentile; kcal: kilocalories; g: grams; ml: milliliters.

was tested using the Kolmogorov-Smirnov test. To streamline the analysis, the median, 25th percentile (p25), and 75th percentile (p75) were used, as certain variables did not follow a normal distribution. Categorical variables were expressed as absolute and relative frequencies.

A comparison between the nutritional composition of the DHM before and after pasteurization was conducted using the Wilcoxon test. The percentual impact (Delta %) of each DHM's component analyzed was calculated by the ratio, in percentage, between the difference of each component before and after pasteurization and the respective value before pasteurization. The energy and macronutrient contents of DHM before and after pasteurization were compared to the gold standard mature HM[23] described in literature, using a one-sample Wilcoxon signed rank test.

Correlations between the DHM's nutritional composition before or after pasteurization and age, sleep hours, BMI, and FM of HMDs, and birth weight and gestational age of their infants were determined using Spearman's correlation coefficients. An identical approach was used to identify the correlations between the HMDs' dietary habits and respective energy and macronutrient intake with the nutritional composition of DHM before and after pasteurization. An alpha level of < 0.05 was set for all analyses.

Ethics

This research was approved by the Ethics Committee of the Centro Hospitalar Universitário de São João/ Faculdade de Medicina da Universidade do Porto (report number 78/2023) and all the ethical principles of the Declaration of Helsinki, Portuguese Law, and the Good Clinical Practice Guidelines were followed. Informed consents were obtained from each participant, authorizing their participation in the study, after presenting all the necessary information and the respective rights regarding their collaboration in this research.

Results

The median age of this sample was 33.0, ranging from 21.0 to 43.0 years old. Fifty-one (78.5%) had an advanced qualification, 47.7% (n = 31) were pregnant for the first time, 43.1% (n = 28) had a cesarean section, and 41.5% (n = 27) practiced regular physical activity and slept a median of 7.0 hours per day. Also, 50.8% (n = 33) were overweight with a median FM value of 33.2% (Table 1). Table 1 also presents the descriptive characteristics of the HMDs' infants.

Table 2 represents the impact of pasteurization on the nutritional composition of DHM. Pasteurization significantly affected the energy and all the macronutrients of DHM (p < 0.05). There was an increased percentage impact on the energy, crude protein, and fat content of DHM (Delta %: −4.5, −7.1, and −8.3, respectively). In table 3, statistically significant differences were observed for energy and all the macronutrients of DHM before pasteurization when compared to the gold standard value[23] for mature human milk (p < 0.05). Additionally, significant differences were noted in both

Table 4. Spearman's correlation between the characteristics of human milk donors and their infants and the nutritional composition of donor human milk (n = 65)

	Donor human milk composition before pasteurization*										Donor human milk composition after pasteurization*									
	Energy (kcal/100 ml)		True protein (g/100 ml)		Crude protein (g/100 ml)		Fat (g/100 ml)		Carbohydrate (g/100 ml)		Energy (kcal/100 ml)		True protein (g/100 ml)		Crude protein (g/100 ml)		Fat (g/100 ml)		Carbohydrate (g/100 ml)	
	R	p	R	p	R	p	R	p	R	p	R	p	R	p	R	p	R	p	R	p
Characteristics of HMDs'																				
Age, years (n = 65)	0.079	0.531	0.114	0.364	0.075	0.552	0.056	0.660	−0.095	0.453	0.038	0.761	0.070	0.580	0.047	0.709	0.030	0.815	−0.046	0.718
Sleep hours, h (n = 65)	0.207	0.098	0.099	0.432	0.109	0.387	0.226	0.070	−0.189	0.131	0.214	0.087	0.143	0.257	0.132	0.294	0.218	0.081	−0.156	0.215
BMI, kg/m^2 (n = 65)	0.033	0.796	0.088	0.488	0.089	0.483	0.023	0.858	−0.013	0.916	−0.003	0.981	0.042	0.739	0.026	0.836	0.014	0.914	−0.185	0.140
Fat mass, % (n = 56)	0.139	0.306	0.191	0.158	0.187	0.167	0.110	0.418	0.022	0.874	0.128	0.348	0.176	0.195	0.165	0.225	0.130	0.341	−0.155	0.253
Characteristics of infants																				
Gestational age, weeks (n = 65)	−0.139	0.269	0.230	0.065	0.210	0.094	−0.130	0.301	−0.115	0.362	−0.138	0.272	**0.260**	**0.036**	0.211	0.092	-0.136	0.281	−0.174	0.166
Birth weight, g (n = 65)	0.166	0.185	−0.009	0.942	−0.018	0.888	0.159	0.206	0.022	0.864	0.106	0.399	−0.064	0.613	−0.087	0.492	0.124	0.324	−0.002	0.990

*Using the Miris HMA™ (Human Milk Analyzer, Miris AB, Uppsala, Sweden).
The values in bold represent statistically significant correlations, with $p < 0.05$.
HMDs': human milk donors; BMI: body mass index; kcal: kilocalories; g: grams; ml: milliliters; h: hours; kg/m^2: kilogram per square meter; %: percentage.

Table 5. Spearman's correlation between human milk donors' energy and macronutrient intake and the nutritional composition of donor human milk (n = 65)

		Donor human milk composition before pasteurization*										Donor human milk composition after pasteurization*									
	Median (p25, p75)	Energy (kcal/100 ml)		True protein (g/100 ml)		Crude protein (g/100 ml)		Fat (g/100 ml)		Carbohydrate (g/100 ml)		Energy (kcal/100 ml)		True protein (g/100 ml)		Crude protein (g/100 ml)		Fat (g/100 ml)		Carbohydrate (g/100 ml)	
		R	p	R	p	R	p	R	p	R	p	R	p	R	p	R	p	R	p	R	p
Energy and macronutrient intake																					
Energy, kcal/day	2147.4 (1554.0, 2514.7)	0.243	0.052	0.128	0.311	0.127	0.315	0.206	0.100	0.068	0.590	0.180	0.152	0.022	0.861	-0.008	0.949	0.192	0.126	−0.093	0.462
Protein, g/day	103.8 (81.7, 131.6)	0.141	0.262	0.009	0.941	0.002	0.989	0.107	0.396	0.113	0.371	0.047	0.710	−0.082	0.518	−0.123	0.331	0.064	0.615	−0.053	0.673
Fat, g/day	81.8 (58.0, 104.1)	0.171	0.173	0.082	0.515	0.064	0.613	0.139	0.270	0.073	0.563	0.105	0.406	−0.064	0.614	−0.102	0.420	0.127	0.313	−0.100	0.428
CHO, g/day	243.2 (182.5, 313.4)	**0.272**	**0.029**	0.177	0.160	0.192	0.126	0.240	0.054	0.026	0.836	0.240	0.054	0.116	0.359	0.102	0.418	0.240	0.054	−0.082	0.514

*Using the Miris HMA™ (Human Milk Analyzer, Miris AB, Uppsala, Sweden).
The values in bold represent statistically significant correlations, with $p < 0.05$.
p25: 25th percentile; p75: 75th percentile; kcal: kilocalories; Kcal/ day: kilocalories per day; g: grams; g/day: grams per day; ml: milliliters; CHO: carbohydrates.

Table 6. Spearman's correlation between human milk donors' eating habits and the nutritional composition of donor human milk (n = 65)

		Donor human milk composition before pasteurization*										Donor human milk composition after pasteurization*											
	Median (p25, p75)	Energy (kcal/100 ml)		True protein (g/100 ml)		Crude protein (g/100 ml)		Fat (g/100 ml)		Carbohydrate (g/100 ml)		Energy (kcal/100 ml)		True protein (g/100 ml)		Crude protein (g/100 ml)		Fat (g/100 ml)		Carbohydrate (g/100 ml)	
Food group intake, g/day		R	p	R	p	R	p	R	p	R	p	R	p	R	p	R	p	R	p	R	p
Dairy[†]	255.7 (65.6, 508.5)	0.038	0.764	−0.029	0.818	−0.073	0.564	0.033	0.793	0.049	0.699	0.036	0.774	0.014	0.914	0.031	0.806	0.028	0.825	0.081	0.520
Meat, fish, and eggs	200.9 (151.9, 259.0)	0.013	0.920	−0.093	0.459	−0.078	0.535	−0.018	0.885	0.182	0.146	−0.102	0.420	−0.171	0.174	−0.230	0.065	−0.090	0.475	0.012	0.922
Eggs	22.2 (14.8, 44.4)	−0.037	0.769	−0.146	0.244	−0.085	0.503	−0.065	0.606	**0.304**	**0.014**	−0.035	0.782	−0.119	0.347	−0.152	0.227	−0.065	0.605	0.205	0.102
Meat[‡]	110.9 (76.6, 132.6)	0.001	0.995	−0.019	0.879	−0.046	0.714	−0.017	0.895	−0.037	0.771	−0.105	0.407	−0.050	0.695	−0.084	0.506	−0.077	0.544	−0.181	0.149
Fish and seafood[§]	61.7 (33.2, 92.9)	0.047	0.712	−0.085	0.501	−0.048	0.705	0.023	0.855	**0.267**	**0.032**	−0.038	0.761	−0.218	0.080	**−0.273**	**0.028**	−0.039	0.758	0.180	0.151
Fruits[¶]	313.3 (204.1, 451.8)	0.088	0.486	0.121	0.337	0.152	0.228	0.089	0.483	−0.186	0.138	0.099	0.433	0.022	0.863	0.005	0.971	0.121	0.337	−0.173	0.168
Vegetables**	179.8 (111.2, 271.8)	0.073	0.561	−0.033	0.793	−0.030	0.814	0.065	0.608	0.013	0.920	0.047	0.711	−0.106	0.399	−0.126	0.318	0.055	0.663	0.032	0.797
Nuts[††]	10.2 (2.5, 30.5)	0.020	0.874	0.164	0.192	0.150	0.234	0.006	0.961	−0.031	0.805	0.020	0.873	0.054	0.667	0.017	0.896	0.036	0.779	−0.111	0.379
Bread, rice, pasta, and potatoes	191.9 (154.8, 259.3)	**0.271**	**0.029**	0.221	0.077	0.192	0.126	0.231	0.064	0.148	0.240	**0.248**	**0.046**	0.177	0.159	0.176	0.161	0.219	0.080	0.047	0.711
Bread[‡‡]	50.3 (33.6,75.7)	−0.012	0.926	0.244	0.050	0.209	0.094	−0.054	0.669	0.008	0.947	−0.050	0.692	0.234	0.061	0.202	0.107	−0.072	0.566	−0.017	0.894
Rice and pasta	85.7 (60.7, 142.9)	0.173	0.168	0.113	0.372	0.107	0.396	0.148	0.238	0.154	0.219	0.155	0.217	0.068	0.590	0.070	0.580	0.128	0.311	0.082	0.517
Potatoes[§§]	50.0 (23.8, 76.2)	**0.474**	**< 0.001**	0.212	0.090	0.168	0.181	**0.443**	**< 0.001**	0.129	0.306	**0.476**	**< 0.001**	0.176	0.160	0.201	0.109	0.462	**< 0.001**	−0.005	0.971
Fats	10.5 (4.7, 16.9)	0.053	0.675	0.021	0.870	−0.015	0.906	0.037	0.771	0.018	0.889	0.014	0.914	−0.119	0.344	−0.129	0.304	0.030	0.811	−0.024	0.852
Saturated fat[¶¶]	1.0 (0.0, 4.7)	0.208	0.096	0.064	0.615	0.027	0.833	0.183	0.144	0.097	0.441	0.148	0.239	0.007	0.959	−0.027	0.833	0.143	0.256	0.014	0.914
Unsaturated fats***	5.8 (2.9, 13.5)	−0.017	0.893	−0.001	0.997	−0.025	0.844	−0.025	0.842	−0.015	0.903	−0.037	0.769	−0.127	0.313	−0.126	0.317	−0.018	0.886	−0.029	0.816

*Using the Miris HMA™ (Human Milk Analyzer, Miris AB, Uppsala, Sweden).
[†]Whole milk, semi-skimmed milk, skimmed milk, yogurt, and cheese.
[‡]Chicken, turkey, rabbit meat, beef, pork, and lamb.
[§]Fatty, lean and canned fish, cod fish, squid, octopus, and shellfish.
[¶]Apples, pears, oranges, tangerines, bananas, kiwis, strawberries, cherries, peaches, plums, melon, watermelon, persimmons, figs, loquats, and grapes.
**White or savoy cabbage, bunch, kale, broccoli, cauliflower, brussels sprouts, turnip greens, spinach, green beans, lettuce, watercress, onion, carrot, turnip, tomato, pepper, and cucumber.
[††]Almonds, hazelnuts, walnuts, pistachios, and peanuts.
[‡‡]White or wholemeal bread, toast, and cornbread.
[§§]Fried, boiled, roasted, stewed, and mashed potatoes.
[¶¶]Butter.
***Olive oil, oil, and margarine.
p25: 25th percentile; p75: 75th percentile; kcal: kilocalories; g: grams; g/day: grams per day; ml: milliliters.
The values in bold represent statistically significant correlations, with $p < 0.05$.

fat (3.1 vs. 3.8 g/100 ml) and carbohydrate contents (8.0 vs. 7.0 g/100 ml) after pasteurization.

Regarding the relationship between demographic, lifestyle, and anthropometric data of HMDs or their infants and the composition of DHM before and after pasteurization, only gestational age was positively associated with the true protein content of pasteurized DHM ($r = 0.260$, $p = 0.036$), as indicated in table 4.

Table 5 characterizes the energy and nutritional profile of the HMDs' diet, indicating the following median intake values: 2147.4 kcal/day, 103.8 g/day, 81.8 g/day, and 243.2 g/day of energy, protein, fat, and carbohydrate intake, respectively. By analyzing the nutritional composition of the DHM, the energy of DHM before pasteurization was positively correlated with daily carbohydrate intake, in grams ($r = 0.272$, $p = 0.029$).

Table 6 describes their eating habits by food group over the last year. A higher consumption of meat was observed compared to fish and seafood (110.9 vs. 61.7 g/day). Daily fruit intake was higher than vegetable consumption (313.3 vs. 179.8 g/day). Rice and pasta were considered the primary sources of carbohydrates, while bread and potatoes were consumed in smaller quantities (85.7 vs. 50.3 and 50.0 g/day, respectively). There was also a higher intake of unsaturated fats compared to saturated fats (5.8 vs. 1.0 g/day, respectively). In terms of correlations between the dietary habits of HMDs and the nutritional composition of DHM before pasteurization, both eggs and fish and seafood consumption showed a positive association with the carbohydrate content of DHM ($r = 0.304$, $p = 0.014$ and $r = 0.267$, $p = 0.032$, respectively). The consumption of carbohydrate sources was positively associated with the energy of DHM ($r = 0.271$, $p = 0.029$). Potato intake showed a positive correlation with both energy and fat DHM content ($r = 0.474$, $p < 0.001$ and $r = 0.443$, $p < 0.001$, respectively). After pasteurization, similar associations were observed for the intake of carbohydrate sources. Fish and seafood consumption had a negative correlation with DHM's crude protein content ($r = -0.273$, $p = 0.028$).

Discussion

In this cross-sectional study, it was found that Holder pasteurization has an impact on the nutritional composition of DHM. Significant differences were observed, particularly in the energy and fat content of DHM, with a higher percentage reduction in energy, fat, and protein, which aligns with previous research findings[24]. The percentage impact on these macronutrients differs from the findings of Quitadamo et al., (energy: −4.5% vs −8.1%; fat: −8.3% vs −14.9%; crude protein: −7.1% vs 2.7%, respectively)[24]. However, it is evident that fat is consistently the macronutrient that is most significantly affected in both studies. These results have practical implications in clinical settings, especially for the individualized fortification of DHM provided to premature newborns or infants with a very low birth weight, who are the primary recipients of DHM[14]. It was also observed that the macronutrient with the greatest deviation from the gold standard for mature HM was carbohydrates, both before and after pasteurization. Notably, carbohydrates appear to be one of the macronutrients least impacted by pasteurization in percentage terms, which is a key point, given their role as essential energy sources, particularly for DHM-fed newborns with heightened energy needs[25]. It is vital to understand that DHM undergoes a pasteurization process before it is distributed to recipient infants. Therefore, when interpreting the relationship between the eating habits of HMDs' and the composition of DHM, it is essential to consider the nutritional implications of this processing step.

In this sample, 50.8% of participants were overweight, which is greater than the prevalence observed in a recent study with Portuguese women at reproductive age[26] (35%) and lower than the prevalence found in Portuguese women according to the *National Food, Nutrition, and Physical Activity Survey 2015-2016* (55%)[15]. Furthermore, the median FM value (33.2%) of our sample exceeded the recommended range for adult women aged from 20 to 39 years (21.0-33.0%)[27]. Generally, no associations were found between the nutritional composition of DHM and HMDs' body composition in this study, but only with HMDs' eating habits.

In this paper, factors such as age and the number of hours of sleep in HMDs', as well as their infants' birth weight, also do not seem to affect the nutritional composition of DHM. Dritsakou et al., revealed a positive relationship between maternal age and HM fat content, while the gestational age and birth weight of their infants exhibited a negative association with the energy, fat, and protein content of HM[28]. The highest levels of carbohydrates were also observed in mature milk from preterm deliveries. One possible explanation for this study not showing associations with these variables may be the small size of the sample and the fact that it only includes mature DHM samples (> two weeks postpartum) while Dritsakou et al., included HM samples from different stages of lactation[28].

Gestational age seems to be positively correlated with the protein content of DHM after pasteurization in this study. However, it is noteworthy that most of the infants were born at more than 37 weeks. As highlighted by Agostoni et al., it is well-established that the nutritional composition of HM differs between preterm and full-term births[25] but, according to previous research conducted by Gidrewicz et al., this disparity decreases as lactation progresses[29]. Also, a longitudinal study[30] that exclusively analyzed DHM samples from HMDs' with full-term deliveries did not find any association between gestational age and the nutritional composition of DHM. It is important to mention that all of these studies only considered samples of raw milk, so the statistically significant association found could be attributed to the effect of pasteurization on protein in DHM. Therefore, more studies are needed to understand the role of these variables in the nutritional composition of DHM.

The literature suggested that lactating women who are overweight or have a higher percentage of FM may produce milk with a higher fat content[11]. In this study, neither BMI nor FM exhibited any association with the nutritional composition of DHM. Therefore, further research is needed to validate our findings, considering the limited number of participants in this study.

Our specific results revealed that increased carbohydrate intake in HMDs' was associated with the energy content of raw DHM. This impact lost statistical significance after DHM pasteurization, likely due to its susceptibility to the pasteurization process. These findings are supported by Binder et al., who also observed that higher dietary macronutrient intake was reflected in higher levels of respective macronutrients in DHM[21], although this paper did not confirm those results. Binder et al. used a 24-hour recall method to assess participants' eating habits, whereas our study utilized an FFQ, which limits the validity of direct comparisons between the two studies.

In a study conducted on Portuguese pregnant women[26], a lower consumption of fish compared to meat was also observed, while vegetable intake exceeded that of fruit, which is contrary to our findings. It is worth noting that this previous study did not distinguish between carbohydrate sources or different types of fats. Thus, our study can contribute to advancing knowledge in this specific field.

This paper indicated an expected positive association between the consumption of carbohydrate sources and DHM's energy both before and after pasteurization. It is of relevance that the association between dietary intake and DHM's energy content may hold relevant implications for the nutrition of preterm infants, who have higher energy and nutritional requirements compared to full-term infants[31]. Surprisingly, a higher intake of potatoes appears to be associated with higher fat content in DHM. However more research is required to fully comprehend this observation. Also, in this study, we found that the consumption of protein sources (meat, fish, and eggs) was inversely associated with the protein content of DHM, with statistical significance observed only for fish and seafood, after pasteurization. A previous study conducted on lactating women in China investigated the association between maternal dietary patterns during lactation and HM macronutrient composition. The authors found a negative correlation between protein content in colostrum and the "high-in-animal-sourced-foods pattern", characterized by high consumption of oils, vegetables, meat/poultry, grains, beans, dairy, and seafood[32]. This same study[32] claimed that, in mature milk, a positive association was observed between the "high-in-animal-sourced-foods pattern" and the carbohydrate concentration of DHM. Interestingly, our results also revealed a positive association between eggs, fish, and seafood consumption and the carbohydrate content of DHM. However, more studies are needed to provide a comprehensive understanding of this topic.

In general, there is a lack of evidence regarding the relationship between HMDs' food intake and the nutritional composition of DHM, making further studies in this area necessary to better understand all of these results.

There are some limitations in this study that should be noted. The first limitation is the small sample size, which may have impacted the ability to detect significant associations. Additionally, being a cross-sectional study makes it impossible to establish a causal relationship. On the other hand, in this study, a validated FFQ for the Portuguese adult population was used and this was the first study of its kind conducted in a Portuguese Human Milk Bank.

Our findings reinforce the importance of nutritional monitoring during the lactation period, particularly in the context of HM donation. It can also contribute to the development of public policies aimed at improving the dietary habits of HMDs', thereby adjusting the nutritional composition of DHM to meet the specific nutritional needs of recipient infants. Based on the high percentage of overweight HMDs and the potential associations between diet and the nutritional composition of DHM demonstrated in this study, we recommend that HMDs receive nutritional guidance and food education. We also propose increasing awareness about the importance of donating HM to premature infants and those with high-risk conditions.

In conclusion, no relationship was found between the BMI or FM of HMDs' and the nutritional composition of their DHM. Carbohydrate intake was positively associated with the energy of DHM. Further research is needed to confirm findings, considering major covariates and larger sample sizes.

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Author contributions

S. Lopes da Silva: Acquisition of data either from patients, research studies, or literature; Analysis or interpretation of data from patients, research results, or literature search; Drafting the article; Critical review of the manuscript for important intellectual content; Final approval of the version to be published; Agreement to be accountable for the accuracy or integrity of the work. B. Teixeira: Conception and design of the study, report, review or other type of work or paper; Analysis or interpretation of data from patients, research results, or literature search; Drafting the article; Critical review of the manuscript for important intellectual content; Final approval of the version to be published; Agreement to be accountable for the accuracy or integrity of the work. S. Pissarra: Acquisition of data either from patients, research studies, or literature; Critical review of the manuscript for important intellectual content; Final approval of the version to be published; Agreement to be accountable for the accuracy or integrity of the work. S. Fraga: Acquisition of data either from patients, research studies, or literature; Critical review of the manuscript for important intellectual content; Final approval of the version to be published; Agreement to be accountable for the accuracy or integrity of the work. R. Moita: Acquisition of data either from patients, research studies, or literature; Critical review of the manuscript for important intellectual content; Final approval of the version to be published; Agreement to be accountable for the accuracy or integrity of the work. H. Soares: Critical review of the manuscript for important intellectual content; Final approval of the version to be published; Agreement to be accountable for the accuracy or integrity of the work. C. Martins: Critical review of the manuscript for important intellectual content; Final approval of the version to be published; Agreement to be accountable for the accuracy or integrity of the work. C. C. Dias: Analysis or interpretation of data from patients, research results, or literature search; Final approval of the version to be published; Agreement to be accountable for the accuracy or integrity of the work. D. e Silva: Conception and design of the study, report, review or other type of work or paper; Acquisition of data either from patients, research studies, or literature; Critical review of the manuscript for important intellectual content; Final approval of the version to be published; Agreement to be accountable for the accuracy or integrity of the work.

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Conflicts of interest

None.

Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The authors have followed their institution's confidentiality protocols, obtained informed consent from patients, and received approval from the Ethics Committee. The SAGER guidelines were followed according to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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