Energy content of milk from nursing mothers of premature infants and its relationship with caloric intake and maternal sociodemographic characteristics

Conteúdo energético do leite de nutrizes de lactentes prematuros e sua relação com a ingestão calórica e com características sociodemográficas maternas

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ABSTRACT
Aim: To evaluate the energy content of milk of nursing mothers of premature infants and identify whether there is association with caloric intake and maternal sociodemographic variables. **Methods**: analytical, cross-sectional study with milk donated by 18 nursing mothers of premature infants admitted to the Neonatal Intensive Care Unit of the National Institute of Woman, Child and Adolescent Health - IFF/Fiocruz. The energy content of breast milk was evaluated by crematocrit, by the human milk bank. To assess maternal food consumption, the Food Frequency Questionnaire was used and sociodemographic data were obtained from interviews with the nursing mothers. Data were evaluated using the SPSS 22.0 software.**Results**: It was found that 89% of the milk collected was normocaloric, with an average energy content of 597.2 ± 79.1 kcal/L and that most of the milk expressed was colostrum. The average caloric intake of the nursing mothers was 2554 ± 413.1 kcal/day and the consumption of polyunsaturated fats, which corresponded to 3.5% of the total energy intake, was below the recommendation. There was no association between the energy content of human milk and the studied variables, but there was a tendency for nursing mothers living outside the city of Rio de Janeiro to have a higher caloric content in milk (p = 0.06).**Conclusion**: The human milk samples were normocaloric, maternal food consumption was inadequate for polyunsaturated fats and there was no association of sociodemographic variables or maternal caloric intake with the energy content of human milk.

Keywords: human milk, prematurity, human milk bank, nutritional status.

RESUMO
Objetivo: Avaliar o conteúdo energético do leite de nutrizes de lactentes prematuros e identificar se existe associação com a ingestão calórica e com variáveis sociodemográficas maternas. **Métodos:** estudo transversal analítico com leite doado por 18 nutrizes de lactentes prematuros internados na Unidade de Terapia Intensiva Neonatal do Instituto Nacional de Saúde da Mulher, da Criança e do Adolescente Fernandes Figueira - IFF / Fiocruz. O conteúdo energético do leite materno foi avaliado por crematócrito, pelo banco de leite humano. Para avaliar o consumo alimentar materno foi utilizado o Questionário de Freqüência de Consumo Alimentar e dados sociodemográficos foram obtidos a partir de entrevista com as nutrizes. Os dados foram avaliados com auxílio do software SPSS 22.0 **Resultados:** Verificou-se que 89% do leite coletado foi normocalórico, com teor energético médio de 597,2 ± 79,1 kcal/L e que a maior parte do leite ordenhado foi colostro. A ingestão calórica média das nutrizes foi de 2554 ± 413,1 kcal/dia e o consumo de gorduras poliinsaturadas, que correspondeu a 3,5% do valor energético total ingerido, esteve abaixo da recomendação. Não foi encontrada associação do conteúdo energético do leite humano com as variáveis estudadas, mas se observou tendência das nutrizes residentes fora da cidade do Rio de Janeiro apresentarem maior teor calórico no leite (p = 0,06). **Conclusão**: As amostras de leite humano foram normocalóricas, o consumo alimentar materno foi inadequado para gorduras

poliinsaturadas e não foi observada associação das variaveis sociodemográficas ou da ingestão calórica materna com o conteúdo energético do leite humano.

Palavras-chave: leite humano, prematuridade, banco de leite humano, estado nutricional
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1 INTRODUCTION

Human milk (HM) gives infants all the necessary nutrients of excellent quality. Breastfeeding is the most natural and the best food for the baby because it offers all the nutrients needed for exclusive feeding in the first six months of life and complements up to two years or more (1). It acts as an immunity agent, supplies the biological and psychological needs of infants and promotes the bonding between mother and infant (2-4).

Increasing evidence currently shows that short-and long-term benefits of HM feeding for preterm newborns (PTNB). Feeding preterm infants HM is reported to reduce significantly the rates of infection, necrotizing enterocolitis, and mortality, while improving neurocognitive and cardiovascular outcomes at the long-term (5-9).

Preterm is defined as babies born alive before 37 weeks of pregnancy are completed. Based on gestational age there are sub-categories of preterm birth: extremely preterm (less than 28 weeks), very preterm (28 to 32 weeks) and moderate to late preterm (32 to 37 weeks) (10).

These newborns present extreme immaturity, in addition to presenting little reserve of carbohydrate and lipids, and therefore, nutrition should be adjusted to each circumstance and not only adapted to birth weight (11).

Researches has shown little success in exclusive breastfeeding in premature newborns (12-13). An effective and possible solution is to collect the mother's own milk and store it for later use. One of the strategies to promote breastfeeding and contribute with the renewed adoption of this practice for newborns that have to be hospitalized was the Brazilian Network of Human Milk Banks [R*ede Brasileira de Bancos de Leite Humano*, (REDEBLH)]. In addition to promoting, supporting and protecting breastfeeding, Human Milk Banks (HMBs) collect process and distribute human milk to premature newborns and infants with nutritional disorders and allergy to heterologous proteins (14). For the control of the quality of pasteurized milk, the creamatocrit, which determines the amount of fat and the energy content of expressed human milk is currently adopted (15).

Evidence shows the influence of diet on the composition of breast milk. The dietary habits, composition of the maternal diet and lactation period constitute one of the main factors that modulate the lipid fraction, described in the literature (16-18).

Considering importance of human milk in PTNB nutrition as well as the influence of maternal diet on the composition of human milk, this study aims to evaluate the dietary intake and energy content of milk of mothers of preterm newborns seen at a center Located in the city of Rio de Janeiro.

2 METHODS

The quantitative, descriptive, cross-sectional study was carried out on the subjects of preterm infants hospitalized at the Neonatal Intensive Care Unit (NICU) and who donated human milk at the Human Milk Bank of Brazil, of National Institute of Woman, Child and Adolescent Health Fernandes Figueira - IFF/Fiocruz (IFF/Fiocruz), located in the city of Rio de Janeiro, Brazil. All adult participant signed the Free and Informed Consent Term (FICT) and those under the age of 18 signed the Free and Informed Assent Term (FIAT), as well as having had the FICT signed by the person responsible for their participation in the research, and the study was approved by the Research Ethics Committee of the National Institute of Woman, Child and Adolescent Health - IFF/Fiocruz, CAAE 53376916.6.0000.5269016.

Sampling was done for convenience, which is, composed of individuals who meet the entry criteria and are easily accessible to the researcher (19). Twenty-one registered nursing mothers were invited to participate in the study. Of these, 18 remained in the follow-up. There were three losses, one for maternal withdrawal and two for soiling in milk, which made it impossible to analyze the fat and calorie content by the crematocrit method.

The data sources used were primary and secondary. The primaries consisted of interviews with the donor mothers and the secondary ones consisted of the registers of the mothers existing in the HMB.

A semi-structured questionnaire was used, consisting of objective and subjective questions regarding age, marital status, schooling, occupational situation, family income, current clinical history; Anthropometric evaluation; Caloric and lipid content of the milk as well as lactation period, in which the answers were annotated by the researcher. The interview was carried out at the Human Milk Bank, in the joint housing ward and in the

waiting rooms of the IFF/Fiocruz. Data were grouped and presented in tables using non-parametric statistics.

The analysis of the caloric content of the milk was obtained from secondary sources, that is to say, by means of records of the results of the examinations of analysis of the caloric content obtained by the crematocrit, available in the Bank of Human Milk of the IFF. The creamatocrit technique consists in centrifuging milk for 15 minutes to separate serum from cream. Using a ruler, the length of the cream column (mm and the total column are measured. These values are used in the formulas described below to calculate the amount of cream, fat and caloric content (kcal/L) (20).

- % cream – cream column (mm) x 100 ÷ total column (mm)
- % fat = (% cream – 0.59) ÷ 1.46
- kcal = % cream x 66.8 + 290

The milk samples were classified according to the energy content in Hypocaloric (<400 kcal / dl); (> 400 kcal / dl and <700 kcal / dl) and Hypercaloric (> 700 kcal / dl). To evaluate dietary habits, a validated and standardized instrument was used: a Food Frequency Questionnaire (FFQ), in which quantitative and qualitative information about habitual food patterns was obtained, with data on foods and amounts consumed by mothers on a daily, weekly, monthly or never consume.

The collected data were digitalized and processed electronically by the SPSS software (Statistical Package for the Social Sciences), version 22.0. The data collected regarding maternal nutrition, from the frequency questionnaire, were entered and analyzed in the program Avanutri, version 4.0.

The descriptive analysis for continuous variables with normal distribution were reported as means and standard deviations. When the normality was not verified, the median, minimum and maximum values were presented. The Shapiro-Wilk test was used to verify the assumption of normality. The Student's t-test or The Mann-Whitney test were used to compare measurements between two groups. The analysis of variance (ANOVA) or the Kruskal-Wallis tests were used to compare three groups. The software used in the data analysis was SPSS, version 22 and the level of significance was 5%.

3 RESULTS AND DISCUSSION

The nursing mothers characteristics are displayed in Table 1. The results showed that the majority were between the ages of 19 and 35 years (61%). The majority are in the reproductive age group, with the average age of women being 27.5 years. From the

reproductive point of view, the age range between 21 and 30 years is considered optimal, since it presents lower perinatal risk (21). About 61% of the mothers were 11 years or more of education. National studies on breastfeeding show that mothers with higher education exclusively breastfeed for longer, perhaps for the possibility of greater access to information on the advantages of breastfeeding (22-23).

Only one woman reported having a family income less than a minimum wage and 44.4% (n = 8) reported a family income of one to two minimum wages. It should be noted that the average number of people living in the same household among the nursing mothers surveyed was 3.06 ± 1.35. Low family income is an indicator of an adverse environment and is associated with a higher chance of preterm birth (24). Among the 18 infants studied, 83.3% presented adequate housing conditions, considering as adequate, in Brazil, that household served by supply of piped water, adequate sanitary sewage and collection of regular waste served by cleaning service or bucket (3x/week), all linked to the general public network. The majority of the mothers were employed (44.4%) and mothers who were housewives were 39.8%. Only 16.7% were students (teenage mothers). It should be noted that some of these mothers, besides having to share their time with the work, still kept the breastmilk donations to the HMB, for use in the diet of their children. This situation may be due to the quality of the work developed by the HMB as well as by the professionals who support and promote greater adherence of women to the practice of breastfeeding and donation. The majority of nursing mothers (55%) live in the city of Rio de Janeiro. Caminha et al. (25) concluded that one of the factors positively related to the practice of breastfeeding is to reside in the metropolitan region.

Regarding the pre-gestational nutritional status, 10 (55.6%) mothers of PTNB presented adequate nutritional status. The average pre-gestational weight was 56.95 ± 13.25 kg. The average body mass index was 22.86 ± 4.87 kg/m², similar to that found by Costa (26) in a prospective study, in which the population had body mass index (BMI) of 22.3 kg / m² (± 3.5). In the study by Silva et al (27) it can be seen that significant part of mothers that presented complications during the gestational period was overweight and the percentage of preterm births was more prevalent in women classified as obese.

According to Fildler and Koletzko (28) and Anderson et al (29), lipid content and type of fatty acid in human milk can be modulated by several factors, such as nutritional status and maternal food intake. It should be emphasized that the interpretation of the BMI value obtained during this puerperium phase should be made with caution, since there is no exclusive classification for infants. Therefore, the recommended values for

adult women are used as parameters, which place the nursing mothers of the study in situations of overweight or obesity (30-35).

Several authors such as Stotland et al (36), Hedderson et al (32), Oken et al (33), Crane et al (34) and Diouf et al (35) report that nutritional status and adequate maternal weight gain are important factors for the successful outcome of pregnancy as well as for long-term health maintenance. The authors cite that both obesity and maternal weight gain above or below recommendations increase the risks for adverse outcomes, such as: increased neonatal morbidity and higher incidence of obesity, overweight and metabolic disorders in childhood and adolescence as well as higher rates of Low birth weight (<2.500g) and small infants for gestational age (SGA).

Table 1. Sociodemographic and nutritional characteristics of nursing mothers, mothers of premature babies, donors of human milk at the Human Milk Bank of the National Institute of Woman, Child and Adolescent Health Fernandes Figueira - Rio de Janeiro, 2016.

Variables	n=18	%
Age group (years)		
14 - 18	4	22.2
19 - 35	11	61.1
>35	3	16.7
Schooling (years of study)		
4 a 7	3	16.7
8 a 10	4	22.2
11 or more	11	61.1
Place of residence		
Municipality of RJ	10	55.6
Outside of Municipality of RJ	8	44.4
Housing conditions		
Proper	15	83.3
Inadequate	3	16.7
Monthly family income (minimum wages)		
≤ 1	1	5.6
1 to 2	8	44.4
2 to 4	5	27.8
> 4	3	16.7
Do not know how to inform	1	5.6
Occupation		
From home	7	38.9
Work out	8	44.4
Student	3	16.7
Marital status		
Single	9	50.0
Married/stable marriage	9	50.0
Skin color		
White	4	22.2
Not white	14	77.8
Pre-gestational nutritional status - BMI (kg/m²)		
Low weight (< 18.5)	4	22.2
Eutrophic (18.5 a 24.99)	10	55.6
Overweight and obesity (>24.99)	4	22.2

MW - minimum wage force in 2016 (R$ 880.00)

As shown in Table 2, the most prevalent lactation period in our study was colostrum, representing 50% of the mothers (Table 2). This fact can be explained by the collection period, which generally happened until the third postpartum day. According to caloric content, 16 (88.9%) were considered as normocaloric and 2 (11.1%) hypercaloric, in relation to the human milk caloric content of PTNB mothers. The difficulty in obtaining hypercaloric milk has described in study in which, most of the milk collected was caloric, but not hypercaloric (36). Hypocaloric milk was not found in the sample. These findings may be explained by donor characteristics and their breastfeeding stage. It may also be explained by the time when milk was collected, whether in the beginning or end of the breastfeeding session, because human milk fat varies according to that (37-38). As the caloric content was recorded from secondary sources of HMB, it was not possible to identify at what time the sample was collected.

Table 2. Lactation period and caloric content of the human milk of nursing mothers, mothers of premature babies, donors of human milk at the Human Milk Bank of the National Institute of Woman, Child and Adolescent Health Fernandes Figueira - Rio de Janeiro, 2016.

Variables	n=18	%
Lactation period		
Colostrum (up to the 7th day of production)	9	50.0
Transition milk (8° to 14° day of production)	4	22.2
Mature milk (> 14° production)	5	27.8
Caloric content of human milk		
Normocaloric (> 400 kcal/L - <700 kcal/L)	16	88.9
Hypercaloric (≥700 kcal/L)	2	11.1

The mean caloric content of the milks analyzed was 597.16 ± 79.05 kcal/L. The dietary intake of the nursing mothers shows that the average daily total energy consumption was 2554 ± 413 kcal (Table 3). Studies conducted with mothers in exclusive breastfeeding presented lower consumption values: 1800 kcal/day (39), 2233 kcal/day (9) and 2287 kcal/day (40). It is recommended for women of reproductive age and with light activity, consumption of 2200 kcal/day, plus the energy supplement of 500 kcal/day for infants in the first six months (41).

The relative distribution of the macronutrients in the diet was considered adequate, in relation to the total energy value (TEV), using as reference the recommended values of Acceptable Macronutrients Distribution Range (AMDR): carbohydrates: 45 to 65%, proteins: 10 to 30% and lipids: 20 to 35% of the TEV (42).

The total dietary lipid intake of the investigated mothers contributed approximately 25% of the total daily intake of energy. However, the mean intake of polyunsaturated fatty acids (PUFAs - 3.35%) is below acceptable limits. It is recommended that intake of PUFA be between 6-10% of daily energy consumption (43).

In the study by Costa et al (44) and Azeredo (45), the PUFA values also presented below the recommendations, being 3.81% and 4.63%, respectively. The low intake of PUFAs and PUFAs from the n-3 series can be attributed to the low consumption of fish such as tuna, anchovy, sardines and salmon that are food sources of these nutrients (46). The Brazilian diet is characterized by low fish consumption and high consumption of vegetable oils (rich in n-6), and this type of diet results in the high n-6/n-3 ratio, which affects the endogenous conversion of A-linolenic acid in EPA and DHA (47).

According to the V Brazilian Guideline on Dyslipidemias and Prevention of Atherosclerosis cholesterol consumption should be less than 200 mg and saturated fat less than 7% (48). Based on the recommended value, we observed that the values of total cholesterol were above that indicated (309.65 ± 139). According to Rodrigues et al (49), it is common to identify among adolescents a standard food similar to the "western standard", including fast foods, soft drinks, sweets, cookies, chips, processed meats, sauces and salty snacks. Being these foods with high cholesterol content. Increased consumption of the foods described above by adolescent and adult nursing mothers in the present study may explain increased levels of serum cholesterol.

Table 3. Caloric content, fat percentage and total energetic value of dietary intake of nursing mothers, mothers of preterm infants, donors of human milk at the Human Milk Bank of the National Institute of Woman, Child and Adolescent Health Fernandes Figueira.- Rio de Janeiro, 2016.

Variables	Mean ± standard deviation (*n*=18)
Caloric content of milk (kcal/L)	597.16±79.05
% of milk fat of PTNB mothers	2.46±0.89
Total maternal energy value (kcal/day)	2554±413
Total lipids (%)	24.71±3.23
Cholesterol (mg)	309.65±139
Saturated (%)	5.36±1.79
Monounsaturated (%)	5.00±1.80
Polyunsaturated fatty acids (PUFA) (%)	3.35±1.23
Carbohydrate (%)	59.03±4.64
Protein (%)	16.23±2.79

All values are mean ± standard deviation of the mean.

When analyzing the relationship between variables: place of residence, marital status and skin color with the caloric content of the human milk of the studied mothers, it was verified that there was no statistical significance (Table 4). Despite the non-significance of the aforementioned variables, this study provided evidence that mothers living outside the city of Rio de Janeiro, in general, have higher milk caloric content.

Table 4: Caloric content according to sociodemographic characteristics of the mothers' preterm babies, donors of human milk at the Human Milk Bank of the National Institute of Woman, Child and Adolescent Health Fernandes Figueira - Rio de Janeiro, 2016.

Variables	Caloric content of HM (kcal/L)	*P - value*
Place of residence		
Municipality of RJ	566.30 ± 51.2	0.061*
Outside Municipality of RJ	635.75 ± 93.51	
Marital status		
Single	623.11 ± 75.56	0.171*
Married/stable marriage	571.22 ±25.95	
Skin color		
White	539 (525-646)	0.371§
No white	593 (474-786)	

Values expressed as mean (± standard deviation) or median (minimum and maximum). * Student t test. § Mann-Whitney test.

The results described in Table 5 show no significant correlation between the studied variables. However, it is observed that women classified as low weight (BMI <18.5 kg/m²) presented lower mean percentage of human milk fat, followed by those classified as overweight and obese, compared to those considered as being eutrophic. The results suggest that the inadequacy of nutritional status interferes with the percentage of milk fat. It is known that both lipid content and the type of fatty acid in human milk can be modulated by several factors, such as nutritional status and maternal food intake (50-52) (29). It is also observed that, when analyzing the nutritional status with dietary intake, overweight and obese women presented lower dietary intake when compared to those of low weight or eutrophic. According to Westerterp and Goris (53), the variation between actual and reported consumption by obese patients may vary by about 41%, representing a hypothesis for the disagreement between the nutritional status of the patients and the mean of the TEV investigated. These findings may also be due to the reduced sample size of the present study.

It should also be taken into account that the food frequency questionnaire has some limitations, since it depends on the interviewee's eating habits and memory, as well as a list of standardized foods that may underestimate or overestimate the total caloric value. The most appropriate method for dietary assessment would be the weighing of food. However, it is a more costly and more invasive method than the others (52). It is emphasized that factors such as diet complexity, eating habits, information quality, age, body image, interviewee's memory, beliefs, behavior, culture and socioeconomic status as well as exposure factors are variables that interfere and make it very difficult to act. To record the intake of an individual, without influencing it (53).

Table 5: Percentage of milk fat, dietary intake and age according to the nutritional status of mothers, preterm babies, donors of human milk at the Human Milk Bank of the National Institute of Woman, Child and Adolescent Health Fernandes Figueira - Rio de Janeiro, 2016.

Variables	Nutritional status			
	Low weight (<18.5kg/m²)	Eutrophic (18.5-24.99kg/m²)	Overweight/obese (>24.99kg/m²)	*P - value*
Milk fat (%)	2.21±0.91	2.62±0.90	2.32±1.0	0.719*
Dietary Intake (kcal)	2429 (2141-2946)	2860 (1744-3161)	2387 (2178-2624)	0.614§
Maternal age (years)	21±2.94	27.10±10.02	35±8.98	0.113*

Values expressed as mean ± standard deviation or median (minimum and maximum). * ANOVA test § Kruskal Wallis test.

There was a higher dietary intake of mothers whose milk caloric content was considered hypercaloric. However, this difference was not statistically significant (Table 6). According to Costa and Sabarense (18), milk lipid modulation is not due to isolated effects, but due to several factors intrinsic and extrinsic to the mother, which act concomitantly and make difficult the evaluation of such modulation. It is noteworthy that few studies evaluate the modulation of fatty acids in human milk as a function of the maternal consumption of these nutrients. In this sense, there should be a careful evaluation of maternal nutrition. Regarding the influence of maternal nutrition, Anderson et al (29) reports that the consumption of *trans* fatty acids (TFA) reduces the total content of milk lipids and the consequent caloric content of human milk.

Table 6: Caloric content of milk according to dietary intake of nursing mothers, mothers of premature babies, donors of human milk at the Human Milk Bank of the National Institute of Woman, Child and Adolescent Health Fernandes Figueira - Rio de Janeiro, 2016.

Variables	Dietary intake (kcal/day)	*P - value*
Caloric content of human milk		
Normocaloric (>400 <700 kcal/L)	2611.09 (1754.98 – 3161.09)	0.732§
Hypercaloric (≥700 kcal/L)	2651.13 (2178.09 – 3124.17)	

Values expressed in median (minimum and maximum). § Mann-Whitney test.

4 CONCLUSION

At the end of this study, we verified that most of the milk collected from mothers of preterm infants is normocaloric. The most prevalent lactation period in our study was colostrum.

In spite of the non-significance of the study, the study provided evidence that mothers living outside the city of Rio de Janeiro, in general, have higher caloric content

milk. The same can be noticed when correlating nutritional status with dietary intake. The results show that there is no significant correlation between the studied variables. However, it can be observed that women who presented low weight, overweight and obesity had lower dietary intake when compared to eutrophic, suggesting that the inadequacy of nutritional status interferes with the percentage of milk fat.

Limitations/Strengths of the study

The main limitation of this study is the number of participants, reflecting the small number of donors, mothers of preterm newborns enrolled in the HMB. In addition, a limitation of this study that may have influenced the results is the possibility that mothers did not report correct food consumption data, which may lead the participant to reduce consumption to simplify the task of reporting the food consumed. Another limitation would be the fact that was not possible to identify at what time the sample was collected, in the beginning or end of the breastfeeding session, as the caloric content was recorded from secondary sources of HMB, besides the fact that it is transverse type research. From the results identified in the research, it is suggested that new studies with a larger sample number be done so that the data can be confronted, and although the majority of mothers present adequacy of pre-gestational nutritional status, the results indicate the importance of implementing actions that favor this adequacy, particularly during the lactation period; future studies should take into account the stage of lactation of mothers, since the caloric content is influenced by the postpartum period and the time of feeding, since the posterior milk is richer in lipids; carrying out a detailed evaluation of the nutrition of the mothers, including values related to the TFA and long chain polyunsaturated fatty acid (LC-PUFA) of the n-3 and n-6 series as well as the food survey associated with the FFQ, such as the 24h recall. Finally, create protocols to better investigate the dietary intake and nutritional status of human milk donors.

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