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Characterization of the macronutrient composition of human milk from mothers of premature babies at the Montevideo's Clinical Hospital

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Abstract


The composition of breast milk varies according to several factors, lipids being the most variable components. In Uruguay, there are few studies on the composition of human milk and its lipid profile. This study aimed to characterize the macronutrients composition and lipid profile of milk from 25 mothers with preterm newborns. Milk was obtained from the three stages of lactation: colostrum, transitional milk and mature milk.

The macronutrient composition was determined with Lactoscan SP, the fat content by Rose-Gottlieb method, and the lipid profile by Gas Chromatography. The average macronutrient composition was: protein 1.21 g/100mL, lactose 7.44 g/100mL, and total fat 3.0 g/100mL. The lipid profile showed 46.35% saturated FAs; 34.33% monounsaturated FAs. The major polyunsaturated FAs were linoleic (13.18%) and linolenic (0.66%). The omega-3 FA content was 1.20% on average.

In conclusion, the average protein and total fat contents are within the values described in the literature, while the average lactose content was higher. With respect to the lipid profile, the prevalent FAs were saturated with an average higher than that indicated in the bibliography. As described in the literature, the most abundant was palmitic acid; the average monounsaturated FA was lower than described. In relation to the polyunsaturated FA values, the linoleic acid was similar to that indicated in the literature, and the linolenic acid was lower. The essential fatty acids and omega-3 derivatives present in milk depend on the maternal diet, so it is important to consider this for possible interventions to improve milk composition.

Keywords: composition, human milk, fatty acid profile, preterm

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Caracterización de la composición en macronutrientes de leche humana de madres de prematuros del Hospital de Clínicas de Montevideo

Resumen

La composición de la leche materna varía según diversos factores, siendo los lípidos los componentes más variables. En Uruguay, existen pocos estudios sobre la composición de la leche materna y su perfil lipídico. Este estudio tuvo como objetivo caracterizar la composición de macronutrientes y el perfil lipídico de la leche de 25 madres con recién nacidos prematuros. La leche se obtuvo de las tres etapas de la lactancia: calostro, leche de transición y leche madura.

La composición de macronutrientes se determinó con Lactoscan SP, el contenido de grasa mediante el método de Rose-Gottlieb y el perfil lipídico mediante cromatografía de gases. La composición promedio de macronutrientes fue: proteína 1,21 g/100 ml, lactosa 7,44 g/100 ml, grasa total 3,09 g/100 ml; el perfil lipídico mostró 46,35 % de ácidos grasos saturados y 34,33 % de ácidos grasos monoinsaturados. Los principales ácidos grasos poliinsaturados fueron el linoleico (13,18 %) y el linolénico (0,66 %). El contenido de ácidos grasos omega-3 fue de 1,20 % en promedio.

En conclusión, los contenidos promedio de proteína y grasa total se encuentran dentro de los valores descritos en la literatura, mientras que el contenido promedio de lactosa fue mayor. Con respecto al perfil lipídico, los AG prevalentes fueron saturados con un promedio mayor al indicado en la bibliografía. Como se describe en la literatura, el más abundante fue el ácido palmítico; el AG monoinsaturado promedio fue menor al descrito. En relación con los valores de AG poliinsaturados, el ácido linoleico fue similar al indicado en la literatura; el ácido linolénico fue menor. Los ácidos grasos esenciales y los derivados omega-3 presentes en la leche dependen de la dieta materna, por lo que es importante considerar esto para posibles intervenciones que mejoren la composición de la leche.

Palabras clave: composición, leche humana, perfil de ácidos grasos, pretérmino

Caracterização da composição de macronutrientes do leite humano de mães de bebês prematuros do Hospital de Clínicas de Montevideú

Resumo

A composição do leite materno varia de acordo com vários fatores, sendo os lipídios os componentes mais variáveis. No Uruguai, são poucos os estudos sobre a composição do leite humano e seu perfil em ácidos graxos (AG). O objetivo deste estudo foi caracterizar a composição de macronutrientes e o perfil lipídico do leite de 25 mães com recém-nascidos prematuros. O leite foi obtido a partir das três fases da lactação: colostro, leite de transição e leite maduro.

A composição em macronutrientes foi determinada com Lactoscan SP, o conteúdo de lipídios por o método Rose-Gottlieb e o perfil em ácidos graxos por cromatografia de gases. A composição média de macronutrientes foi: 1,21 g/100mL de proteínas, lactose 7,44 g/100mL, gordura total 3,09 g/100mL; o perfil lipídico apresentou 46,35% de AG saturados; 34,33% de AG monoinsaturados. Os AG poliinsaturados majoritários foram o linoleico (13,18%) e linolênico (0,66%). O conteúdo de AG ômega 3 foi de 1,20% em média.

Pode-se concluir que os conteúdos médios de proteínas e gordura total estão dentro dos valores descritos na bibliografia, o teor médio de lactose foi superior. Com relação ao perfil lipídico, os AG prevalentes foram os AG saturados com uma média maior do que a de outros trabalhos e o mais abundante foi o ácido palmítico; a média de AG monoinsaturados foi menor do que a descrita na literatura. Em relação aos AG poliinsaturados, principalmente os essenciais e os derivados ômega 3 presentes no leite dependem da dieta materna, por isso é importante considerar isso para possíveis intervenções que melhorem a saúde do lactente.

Palavras-chave: composição, leite humano, perfil de ácidos graxos, prematuro

1. Introduction

Human milk is the best food for newborns, providing them with all the nutrients and bioactive compounds necessary for their growth and development⁽¹⁾. In this regard, the World Health Organization (WHO) recommends exclusive breastfeeding for all infants during the first 6 months of life, followed by continued breastfeeding up to 2 years of age, alongside complementary feeding. Breastfeeding is one of the most effective interventions for preventing infant morbidity and mortality, offering benefits not only for the newborn but also for the mother⁽²⁾.

Milk changes its composition and adapts to the needs of the infant⁽³⁾. It can vary due to various factors, such as the stage of lactation, time of day, maternal nutritional status and diet, gestational age, genetic factors, and others, with lipids being one of the most variable components⁽⁴⁾.

Premature or preterm babies are those born before completing 37 weeks of gestation. The different categories of prematurity are established based on the number of weeks completed. Thus, the WHO defines extremely premature babies as those born before 28 weeks of gestation, very premature babies as those born before 32 weeks, and moderately to late babies as those born before 37 weeks⁽⁵⁾.

Human milk is also the best choice for premature babies, as it is the best tolerated, provides bioactive, immunological and microbiological components that promote the development of the gastrointestinal tract and the immune system, and prevents diseases associated with prematurity such as necrotizing enterocolitis⁽⁴⁾⁽⁶⁾. Even with these benefits, evidence suggests that the nutrient supply of breast milk is not always sufficient to allow the correct growth of the premature baby, so in many cases it is necessary to resort to fortifying the milk. Considering the individual variability of each mother's milk, it is important to have more specific data on the composition of preterm milk and how it changes over time in order to carry out a more individualized fortification in each case⁽⁷⁾.

Regarding the composition of preterm milk, the literature reports higher protein content; however, the extent of the difference depends on the stage of lactation⁽⁸⁾. In the review by Gates and others⁽⁷⁾, several studies describe protein concentrations that can reach 4 g/100 mL in the first days postpartum and drop to half or less at 4 weeks. Regarding fat content, this increases to almost double in the first 2 weeks, going from values close to 2 g/100 mL in early colostrum (0 to 3 days postpartum) to values of almost 4 g/100 mL in mature milk. Meanwhile, the reported lactose values showed large variations but with concentrations not less than 5.4 g/100 mL⁽⁷⁾.

Therefore, this study aimed to characterize the composition of human milk in terms of macronutrients and the lipid profile of milk from mothers with preterm newborns. Understanding the composition of human milk allows for the individualization and adaptation of energy and nutrient intake according to the nutritional needs of preterm infants, thereby contributing to improved prognosis.

2. Materials and Methods

The analysis of human milk was conducted on samples from 25 women, aged 18 to 45 years, who were mothers of premature infants hospitalized in the Neonatology Unit of the Clinical Hospital Dr. Manuel Quintela in Montevideo, Uruguay. The samples were collected between September 2023 and July 2024. A non-probability, convenience sampling method was used.

This work was approved by the Ethics Committee of the Clinical Hospital, project registration No. 8213197 dated 05/10/2023.

Each mother was asked for a single milk sample. Depending on the postpartum day, it could be: colostrum up to 7 days postpartum (8 samples), transitional milk up to 15 days postpartum (12 samples), and mature milk after 15 days postpartum (5 samples). Samples of 20 mL were requested, which were obtained by manual or mechanical extraction by the mother. A 5 mL sample of fresh or refrigerated milk was used to measure macronutrients in the Lactoscan, the rest was stored in a freezer at -80 °C until further analysis.

Lactoscan (Milkotronic Ltd., Nova Zagora, Bulgaria) was used to quantify milk macronutrients. For the quantification of milk FAs, lipid extraction was performed using the Rose-Gottlieb technique⁽⁹⁾⁽¹⁰⁾.

The procedure for extracting fatty acids began by weighing about 3 g of milk in a screw-top tube (tube 1). Then, 0.5 mL of ammonium hydroxide was added and vortexed for approximately 1 minute. Next, 3 mL of 95% ethanol was added and vortexed for approximately one minute. Subsequently, 7.5 mL of diethyl ether was added and vortexed for approximately 1 minute. After that, 7.5 mL of petroleum ether was added and vortexed for approximately 1 minute. Once all reagents had been added, the samples were stirred with a magnetic stirrer for 45 minutes to 1 hour. After this time, they were centrifuged at 3500 rpm for 10 minutes at room temperature. In each case the organic phase was transferred to a previously weighed screw-top tube 2 (the solvent volume was reduced). Once this step was completed, tube 1 was washed with 9 mL of a diethyl ether-petroleum ether mixture (1/1 v/v). It was centrifuged at 3500 rpm for 10 minutes and the organic phase was transferred with a Pasteur pipette to tube 2. The tube 1 was washed once more, and finally it was taken to dryness by venting with nitrogen (in a bath at 40 °C) until constant mass. This procedure was performed in triplicate for each sample.

The percentage of fat was calculated by weight difference, likewise the coefficient of variation between the two replicas was calculated, and in those samples that obtained a coefficient of variation of less than 10%, the percentage of fat reported resulted from the average between both. Subsequently, the extracted fatty material was derivatized so that all the starting fatty material was transformed into a mixture of methyl esters. To perform the derivatization of the fatty material, the plastic tube containing the extracted fatty material was taken and 1 mL of hexane was added. It was shaken in a vortex and the content was transferred to a previously weighed vial using a Pasteur pipette. Nitrogen venting was performed until constant mass was achieved; approximately 70 to 80 mg of fatty material was obtained. Next, 2 mL of hexane and 0.1 mL of 2 N methanolic KOH were added. It was stirred magnetically for 10 minutes. Lastly, it was placed in a centrifuge tube and a spatula tip of anhydrous Na₂SO₄ was added. It was centrifuged, and the sample was extracted (organic phase where the methyl esters of the fatty acids were found).

Finally, the fatty acid profile analysis was performed using a Shimadzu 2014 gas chromatograph equipped with FID and fitted with a Supelco SP-2560 100-meter capillary column. The temperature program used was as follows: initial temperature of 90 °C for 2 minutes, increase to 175 °C at a speed of 20 °C/min and hold for 35 minutes at that final temperature; temperature rise from 175 °C to 240 °C at a speed of 15 °C/min and hold at that final temperature for 25 minutes.

For statistical analysis, Graph Prism 9 software was used. To describe the quantitative variables, the mean was used, with its respective standard deviation (SD). The analysis of the quantitative variables was carried out using the T-test or the Mann-Whitney test, depending on whether or not they had a normal distribution according to the Shapiro-Wilk test. The statistical significance was defined as 95% confidence and indicated in the figures with asterisks (* $p \leq 0.05$).

3. Results

Table 1 shows the sample of the 25 mothers studied. Most (18) had preterm newborns born at or after 32 weeks; most (21) had incomplete high school/UTU or higher educational level; 13 mothers were classified as having both underweight (4 had a BMI < 18.5 kg/m²) and overweight (9 had a BMI > 24.9 kg/m²); and 22 were 35 years of age or younger (**Table 1**).

Table 1. Characteristics of mothers

VARIABLES	RESULTS	n=25
<i>Age</i>	18 to 25 years	12
	26 to 35 years	10
	Older than 35	3
<i>Educational level</i>	Complete primary	4
	Incomplete high school	18
	Complete high school	2
	Complete university	1
<i>Number of children</i>	1 to 2	18
	3 to 4	5
	5 or more	2
<i>Pregestational BMI</i>	Underweight (< 18.5)	4
	Normal weight (18.5 – 24.9)	9
	Overweight (25 – 29.9)	5
	Obesity (≥ 30)	4
	No data	3
<i>Gestational age</i>	Moderate to late preterm (32 to less than 37 weeks)	18
	Very preterm (28 to less than 32 weeks)	7

With respect to the average macronutrient composition according to the stage of breast milk, no significant differences ($p > 0.05$) were found in lactose content (7.61 g/100 mL, 7.36 g/100 mL, and 7.34 g/100 mL in colostrum, transition milk, and mature milk, respectively). Differences in protein content (1.24 g/100 mL, 1.20 g/100 mL, and 1.19 g/100 mL in colostrum, transition milk, and mature milk, respectively) were also not significant ($p > 0.05$). However, the differences in fat content between colostrum and mature ($p = 0.0015$), and transition and mature milk ($p = 0.0013$) were significant (2.43 g/100 mL, 2.68 g/100 mL, and 4.01 g/100 mL in colostrum, transition milk, and mature milk, respectively) (**Figure 1**).

In the analysis of milk composition, the overall results were as follows: protein ranged from 0.90 to 1.72 g/100 mL (average 1.21 g/100 mL), lactose ranged from 5.55 to 10.54 g/100 mL (average 7.44 g/100 mL), and total fat ranged from 1.73 to 5.30 g/100 mL (average 3.09 g/100 mL).

The lipid profile showed SFA (saturated fatty acids) ranging from 36.93% to 55.75% (average 46.35%, SD = 5.48), with palmitic acid (C16:0) being the most abundant, averaging 21.56%. MUFA (monounsaturated fatty acids) ranged from 26.11% to 42.03% (average 34.33%, SD = 3.87), with oleic acid (C18:1) averaging 31.41%. PUFA (polyunsaturated fatty acids) ranged from 9.04% to 21.66% (average 15.82%, SD = 3.09), the majority of which were linoleic acid (C18:2), averaging 13.18%, and linolenic acid (C18:3), averaging 0.66%. The omega-3

FA content was 1.20% on average (SD = 0.36). The contents of vaccenic acid (TVA) and conjugated linoleic acid (CLA) were 0.58% and 0.36%, respectively (**Table 2**).

No correlation was found when studying the correlation between gestational age and the composition of transitional milk in terms of macronutrients and lipid profile.

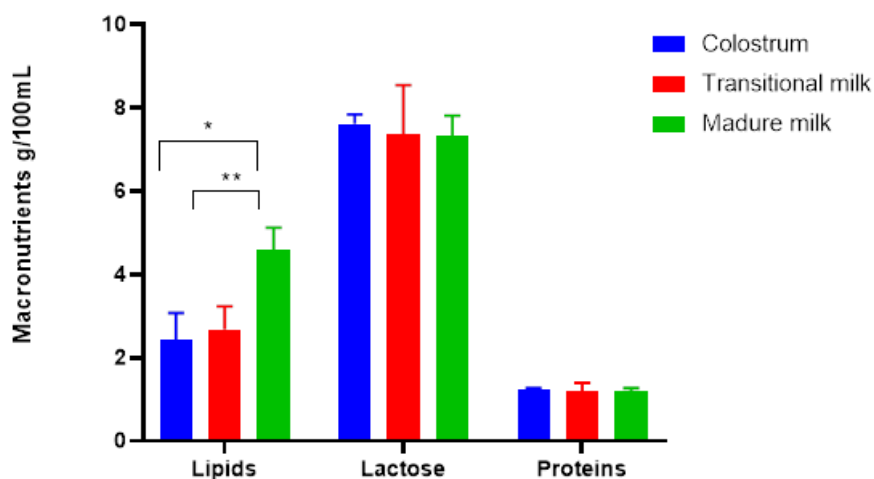


Figure 1. Differences in the average composition of macronutrients according to stages of breast milk. (*) $p = 0.0015$, (**) $p = 0.0013$, according to t-test

Table 2. Lipid profile of human milk by stage and total milk samples

	ALL STAGES	COLOSTRUM	TRANSITIONAL MILK	MATURE MILK
FAs	mean \pm SD	mean \pm SD	mean \pm SD	mean \pm SD
SFA %	46.35 \pm 5.48	42.52 \pm 5.37	49.35 \pm 4.03	45.29 \pm 5.22
10:0	1.52 \pm 0.64	0.93 \pm 0.36	1.86 \pm 0.62	1.63 \pm 0.39
12:0	6.94 \pm 2.58	4.24 \pm 1.26	8.44 \pm 2.10	7.64 \pm 1.73
14:0	8.62 \pm 2.47	6.72 \pm 2.02	9.96 \pm 2.16	8.44 \pm 1.99
16:0	21.56 \pm 2.39	22.82 \pm 2.62	21.45 \pm 1.62	19.82 \pm 2.84
17:0	0.35 \pm 0.09	0.39 \pm 0.13	0.34 \pm 0.05	0.32 \pm 0.08
18:0	5.94 \pm 1.04	5.97 \pm 1.66	5.87 \pm 0.67	6.05 \pm 0.60
MUFA %	34.33 \pm 3.87	36.27 \pm 3.06	32.48 \pm 3.33	35.64 \pm 4.82
16:1n7	1.97 \pm 0.67	2.38 \pm 0.83	1.85 \pm 0.47	1.62 \pm 0.59
18:1 cis	31.41 \pm 3.73	32.79 \pm 3.02	29.72 \pm 2.97	33.24 \pm 5.17
PUFA %	15.82 \pm 3.09	17.27 \pm 3.41	14.70 \pm 2.62	16.21 \pm 3.12
18:3n3	0.66 \pm 0.20	0.62 \pm 0.14	0.68 \pm 0.26	0.67 \pm 0.15
20:5n3	0.15 \pm 0.17	0.22 \pm 0.27	0.13 \pm 0.07	0.08 \pm 0.07
22:6n3	0.27 \pm 0.11	0.26 \pm 0.08	0.30 \pm 0.13	0.23 \pm 0.13
18:2 cis	13.18 \pm 3.01	14.55 \pm 3.27	11.96 \pm 2.51	13.90 \pm 3.09
omega-3 fatty acids %	1.20 \pm 0.36	1.26 \pm 0.47	1.23 \pm 0.33	1.04 \pm 0.21
TVA	0.58 \pm 0.33	0.68 \pm 0.43	0.67 \pm 0.21	0.21 \pm 0.12
CLA	0.36 \pm 0.10	0.40 \pm 0.14	0.34 \pm 0.07	0.34 \pm 0.10

SFA: Saturated fatty acids; MUFA: Monounsaturated cis fatty acids; PUFA: Polyunsaturated cis fatty acids; CLA: Conjugated linoleic acid

4. Discussion

Regarding the characteristics of the mothers, we highlight the factors that influence the lipid content in breast milk, such as BMI (Body Mass Index). In our group, more than half have a pre-pregnancy BMI outside the normal range, either due to underweight or overweight or obesity, and more than $\frac{2}{3}$ of mothers have low parity.

Previous studies in Uruguay by Martell et al. found an average concentration of fat of 4.0 (SD 1.92) g/100 mL, lactose of 6.25 (SD 0.61) g/100 mL and protein of 1.63 (SD 0.41) g/100 mL; however, the work does not clarify whether it is preterm or term milk or the stage of lactation⁽¹¹⁾. On the other hand, Irazusta and others⁽¹²⁾, in mature milk samples from a human milk bank, found a lipid concentration of 4.95 g/100 mL (interquartile range 4.66-6.51 g/100mL), proteins 1.28 g/100mL (interquartile range 1.21-1.41 g/100mL) and lactose 6.18 g/100mL (interquartile range 6.15-6.24 g/100mL)⁽¹²⁾.

Comparing our work with that of Mayans and Martell⁽¹¹⁾, it is important to highlight that the values found regarding fat content turned out to be similar to those of the mature milk in our work, with average fat values of 4.01 (SD 1.36) g/100 mL; likewise, in our experience the values of lactose turned out to be higher (7.34 g/100 mL, SD 0.47), while those of protein turned out to be lower (1.19 g/100 mL, SD 0.08)⁽¹¹⁾. Otherwise, when compared with Irazusta and others⁽¹²⁾, the lipid content of our samples was lower, the protein content was similar, and the lactose content of our samples was higher⁽¹²⁾.

With respect to the milk of mothers of premature babies, the meta-analysis by Gidrewicz and Fenton⁽⁸⁾ describes a protein composition according to different studies of 1.7 to 2.7 g/100 mL for colostrum, which turns out to be higher than the average found in our study; while in the transition milk, the value of 1.5 g/100 mL is similar to that obtained in our study (**Table 2**). Regarding mature milk, it was similar (mean of 1.19 g/100 mL) to that indicated in other studies at similar postpartum days, with a value of 1.4 g/100 mL. Concerning lactose, the same meta-analysis presents results between 5.1 and 6.3 g/100 mL, 5.7 g/100 mL and 6.0 g/100 mL for colostrum, transitional and mature milk from similar postpartum days, respectively; in all cases, values were lower than those obtained in our study (**Table 2**). In relation to lipids, in the same meta-analysis, values range from 2.2 to 3.0 g/100 mL for colostrum, 3.5 g/100 mL for transition and mature milk, while in our study the lipid content corresponds at 2.43 g/100 mL, 2.68 g/100 mL and 4.01 g/100 mL in colostrum, transition and mature, respectively (**Table 2**).

These differences could be attributed to methodological factors, including the time of day and stage of lactation at which samples were collected, the type of milk analysed, the number of freeze-thaw cycles, the time elapsed between sample collection and analysis, and the analytical technique used⁽¹³⁾. Other factors influencing milk composition include maternal diet, geographical region, lactation stage, and gestational age⁽⁴⁾.

Regarding methodological aspects, Mayans and Martell⁽¹¹⁾ collected samples via manual expression. Macronutrients were quantified using a spectrum absorption-based method (Milko Scan 133), while lipid quantification was performed using the crematocrit method. However, it is not specified if it is milk from premature or full-term babies and neither the type of milk.

On the other hand, Irazusta and others⁽¹²⁾ used 16 pools of mature milk (5 to 10 mothers per sample), and, as in our case, macronutrients were quantified with Lactoscan⁽¹²⁾.

In the meta-analysis by Gidrewicz and Fenton⁽⁸⁾, protein determination was carried out using two methods: one estimating protein content based on total nitrogen assumption and another measuring true protein by excluding non-protein nitrogen. The included studies collected milk samples over a 24-hour period without specifying the

technique used for lipid or lactose measurement. This meta-analysis considered term and preterm milk at different lactation stages, but only included data from North America, Europe, Australia, Israel, and Japan.

Thus, the differences observed between our results and previous studies can be primarily attributed to methodological variations and, in the case of the meta-analysis, also to geographical factors.

In turn, we also compared our results with the review by Gates and others⁽⁷⁾. In this case, regarding protein content, our results did not show such a high protein concentration in relation to several studies mentioned in the review (highest values reached of 4 g/100 mL). Regarding fat content, our study showed a similar trend to that reported in the review, with values close to 2 g/100 mL in colostrum (2.43 g/100 mL in our case) and doubling its concentration in mature milk up to 4 g/100 mL (4.01 g/100 mL in our study). When comparing the lactose values indicated in the review, there is agreement in the sense that the values found are greater than 5.4 g/100 mL; as regards the great variability reported in the review, this was not the case in our case where the values were quite constant (7.61 g/100 mL, 7.36 g/100 mL and 7.34 g/100 mL in the different stages of lactation)⁽⁷⁾.

Regarding the lipid profile, we compared our work with a Canadian CHILD cohort⁽⁴⁾ and a European cohort⁽¹⁴⁾. It is important to highlight that although both cohorts were milk from mothers of full-term babies, the lipid profile of the milk was very similar to that in our study with milk from mothers of preterm babies. In both studies, similar values of SFA are described in the order of 40% (CHILD 39.75% and European cohort between 40 and 43%, approximately), which turn out to be lower than those found in our sample with an average of 46.35%. In all cases, the most abundant is palmitic acid, all of which are in the order of 20% (CHILD 20.90%, European cohort 22-25% and our study an average of 21.56%). In the case of MUFA, the average for CHILD is 43.06%, for the European cohort between approximately 40 and 43%, while in our case the value obtained is below both studies with 34.33%. Oleic acid is the most abundant in all cases (CHILD 37.05%, European cohort 34-36% and our study an average of 31.41%). With respect to PUFA, linoleic acid is the most abundant in all cases (CHILD 13.62%, European cohort 12-14% and our study an average of 13.18%); those of the omega-3 family represent 1.20%, in this sense it is highlighted that in our study the value obtained is a little lower than that obtained in the other studies (CHILD 2.39%, European cohort 1.39-1.61%).

It is highlighted that the fat content turns out to be different according to the stage of lactation, in this sense we found that mature milk contains a significantly higher content of total lipids compared to transitional milk and colostrum, which is in agreement with what was found in previously published studies⁽¹⁴⁾⁽¹⁵⁾.

Our findings showed a significantly lower concentration of capric and lauric saturated fatty acids (C10:0 and C12:0) in colostrum compared to transitional and mature milk. Similar results were reported in the European cohort for capric acid (0.45 ± 0.34 , 1.63 ± 0.42 , 1.53 ± 0.34 g/100 g of total FA, in colostrum, transitional and mature milk, respectively) and lauric acid (2.50 ± 1.29 , 5.97 ± 2.01 , 5.30 ± 1.65 g/100 g of total FA, in colostrum, transitional and mature milk, respectively), although their concentrations in our study were higher. Regarding myristic acid (C14:0), we observed a significant increase in its concentration in transitional and mature milk compared to colostrum. This finding was not reported in the European cohort, where concentrations were similar across all three stages (5.40 ± 1.51 , 6.35 ± 1.97 , 5.71 ± 1.76 g/100 g of total FA, in colostrum, transitional and mature milk, respectively). As for PUFA, their concentration in colostrum was significantly higher compared to transitional milk, but not to mature milk. In contrast, in the European cohort, PUFA levels were similar across all three stages (16.26 ± 3.37 , 16.16 ± 4.37 , 16.27 ± 4.81 g/100 g of total FA, in colostrum, transitional and mature milk, respectively)⁽¹³⁾.

Regarding the lipid profile, the most abundant are SFA, followed by MUFA and PUFA. It is important to highlight that SFA provides energy, participates in the absorption of fat-soluble vitamins (A, D, E and K), as well as being essential for growth and development. Medium-chain SFA are easily absorbed and contribute to the energy

supply⁽¹⁶⁾. Regarding MUFA, oleic acid, abundant in the human milk of this group of premature babies, is an efficient source of energy, favours the absorption of fat-soluble vitamins, as well as regulating various cellular processes and intracellular communication⁽¹⁷⁾.

The contents of PUFA found in the milk samples analysed agree with those of other studies. This group includes the essential fatty acids linoleic (omega-6) and alpha-linolenic acid (omega-3), which are of great importance for neuronal and visual development. These fatty acids are precursors to arachidonic acid (ARA: n6) and docosahexaenoic acid (DHA: n3), which have been shown to positively influence the cognitive and visual development of babies. Breast milk is a key source of these fatty acids and they depend on the maternal diet⁽¹⁸⁾⁽¹⁹⁾.

Another important aspect to consider is the influence that the mother's diet has on the quality of the fat in her milk. The fatty acids present in milk fat come from *de novo* synthesis in the mammary gland itself, from body reserves (adipose tissue) and from maternal intake⁽¹³⁾. Part of the body reserves also come indirectly from the mother's diet, therefore, although it was not the object of this study, the lipid profile obtained in this population of mothers could be reflecting certain aspects of the quality of the fats in the Uruguayan dietary pattern⁽²⁰⁾. In this sense, it is important to highlight the high intake of red meat (beef) as well as dairy products that provide especially SFA; the consumption of seed and grain oils such as sunflower, corn and soybean, which contribute to the supply of fatty acids, especially the essential fatty acid linoleic acid (omega-6 family). On the other hand, lower values were obtained with respect to MUFA, perhaps in response to lower consumption, about other populations, of pork and olive oil, which are rich in these FA, as well as a lower average value of PUFA of the omega-3 family, which include as the most relevant the essential FA alpha-linolenic, present in foods such as nuts, chia seeds, flax seeds and canola oil among others, and EPA and DHA, present in fatty fish and shellfish, which are also low in consumption in the Uruguayan population. Taking into account the relevance of PUFA of the omega-3 family for the development of the newborn, they should be part of the maternal diet to obtain a better lipid profile in the milk.

Finally, as shown in a previous study⁽⁸⁾, the differences between premature milk and term milk are more pronounced in the first postpartum days. Premature milk exhibits higher protein content than term milk; these differences decrease as the postnatal days progress. On the other hand, lactose and lipid levels follow a similar pattern of variation over time in both term and premature milk. However, the lipid profile between preterm milk and term milk remains the same⁽¹⁹⁾.

5. Conclusions

The macronutrient content and lipid profile of breast milk from mothers of premature babies admitted to the University Hospital of Uruguay were studied. In this sense, the milk samples corresponded to different stages of lactation. This study provides a reference profile of macronutrients with a fatty acid profile of 25 samples of human milk from mothers of premature babies. It reports a quantitative description of the protein, lactose and lipid profile at different stages of lactation.

It was determined that the lactose and protein content is similar in the colostrum, transitional milk and mature milk groups. However, a 65% increase in lipid content is observed in mature milk compared to colostrum, and a 50% increase compared to transitional milk.

The lipid profile of the breast milk content of this group of mothers was studied. The higher content of SFA stands out, compared to other studies, probably reflecting the characteristics of the Uruguayan diet rich in red meat and whole dairy products. Also relevant is the content of PUFAs, especially linoleic acid (omega-6), similar to that

found in other studies. Regarding omega-3 fatty acids, the levels found were lower than those reported by other authors, which could reflect the low consumption of foods rich in these fatty acids in the Uruguayan population.

Knowing the composition of human milk helps us personalise the caloric and nutrient intake of each premature infant. It would be interesting in the future to be able to study the effect of the mother's diet on the composition of milk, as a starting point for possible interventions.

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Transparency of data

Data not available: The data set that supports the results of this study is not publicly available.

Author contribution statement

CS: Formal Analysis, Conceptualization, Research, Writing – Preparation of Original Draft, Writing – Review & Editing.

LT: Formal Analysis, Research.

MR: Formal Analysis, Research.

MR: Conceptualization, Acquisition of Funding, Research, Methodology, Project Administration, Supervision, Writing – Preparation of Original Draft, Writing – Review & Editing.

IV: Research, Methodology, Supervision, Writing – Preparation of Original Draft, Writing – Review & Editing.

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