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# Erythrocyte membrane and breast milk fatty acid profile in lactating mothers: relationship with infant erythrocyte membrane fatty acid profile

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## Abstract

During the first thousand days of life, fetus and infant's nutrition depends on mother's diet. Polyunsaturated fatty acids (PUFA) are important substrates in infant neurogenesis. We related erythrocyte membrane (EM) and breast milk fatty acids (FA) profile in lactating mothers with the EM FA profile in exclusively breastfed infants and evaluated maternal fat consumption. We conducted an observational, cross-sectional analytical study. During the 2016–2019 period, milk and blood samples from adult mothers 90 days post-partum and infant's blood were analysed, and FA were determined by GC. A frequency of consumption survey of fatty acids precursor foods and sources was conducted. The sample included forty-five mother–infant EM and forty-five milk samples donated by the same mothers. A low percentage of DHA (0·14 (0·12–0·2)) was found in milk, consistent with mother's low consumption of DHA-rich foods. A significant positive correlation between infant's EM DHA percentage and milk DHA percentage ($r = 0{\cdot}39$; $P$ value 0·008), as well as between infant's EM ω-3 fatty acids sum and milk DHA percentage ($r = 0{\cdot}39$; $P$ value 0·008), was found. When milk had a DHA percentage greater than or equal to 0·20 %, infants had a significant increase in DHA in their EM. Mother's consumption of DHA precursors and sources was NS. The relation between the DHA percentage distribution found in maternal milk, and the DHA percentage distribution found in infant's and mother's EM was proven in this population. Dietary fatty acid intake is associated with the maternal milk lipid distribution and with mothers' and infant's EM fatty acids percentage.

**Keywords:** **Fatty acids diet: Human milk: Polyunsaturated fatty acids: Erythrocytes**

Pregnancy and lactation are physiological stages immersed in the critical first thousand days of life, representing periods of greater vulnerability for the mother, fetus and infant. During these thousand days, nutrients supply to fetus and infant depends on mother's diet. Therefore, a woman's diet and lifestyle, before and during pregnancy, as well as during lactation, constitute a determining factor in infant health, with implications for their adult life[1,2].

In the last 30 years, lipids study has gained great importance due to their role as constituents of cellular membrane structures, hormones and bile salts. In addition, they fulfil reserve metabolic functions, provide energy and participate in the regulation of gene expression in mammals[3]. Traditionally, lipids were considered a source of energy in an infant's dietary requirements. However, their role during brain development is equally noteworthy. Approximately, 50–60 % of brain dry weight is lipid, specifically long-chain PUFA (LCPUFA) not available for energy metabolism[4,5].

Fatty acids, both saturated and unsaturated, are the most relevant structures within lipids. They are part of phospholipids and glycolipids, constituting molecules of the lipid bilayer of all cell membranes. They are also constituents of the myelin sheath. From conception, PUFA participate in neurogenesis, neuronal migration, gliogenesis, synaptogenesis and myelination[6].

The human body can synthesise almost all the fatty acids it needs from palmitic acid, a SFA with sixteen carbons. This synthesis occurs through a combination of elongation, desaturation and hydroxylation mechanisms in the endoplasmic

**Abbreviations:** ARA, arachidonic acid; FAME, fatty acid methyl esters; LCPUFA, long-chain PUFA; EM, erythrocyte membrane.

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reticulum and mitochondria, except for essential fatty acids[7]. Palmitic acid participates in processes such as palmitoylation, gliogenesis, synaptogenesis and myelination[8]. It is also an important component of breast milk, representing approximately 25 % of the lipids in its composition, of which 60–85 % is found in the sn-2 position of TGA[9].

PUFA synthesis involves a complex process that includes desaturations (incorporation of double bonds) and elongations (lengthening of the chain) of linoleic acid or*α*-linolenic acid (*α*LNA), with the participation of desaturase and elongase enzymes. *n*-3 (ω-3) and *n*-6 (ω-6) PUFA compete for the same enzymes in the synthesis pathway of LCPUFA[10]. LCPUFA are critical for infant growth and development, particularly arachidonic acid (ARA) and DHA. During gestation, DHA and ARA are deposited in the retina, brain and central nervous system[11–13]. It is suggested that approximately 67 mg of ω-3 fatty acids accumulate per day in fetal tissue, during the third trimester of gestation[14].

Tissue levels of fatty acids in a woman, during pregnancy and lactation, are directly related to diet, capacity for reserves and metabolic utilisation of fatty acids. Therefore, the diet and fatty acid metabolism of women, during these stages, plays a crucial role in determining LCPUFA levels present in erythrocytes and breast milk. The availability of LCPUFA for the infant is directly related to the transfer of these fatty acids from the mother to her offspring, first through the placenta and then through breast-feeding[15]. The concentration of LCPUFA in breast milk varies across different cultures and dietary habits[16].

Studies conducted with stable isotope tracers found that only between 1 % and 4 % of *α*LNA from the diet is converted into DHA. Therefore, considering that only a small amount of ω-3 LCPUFAs can be synthesised from the dietary precursor, some women need a dietary source of ω-3 LCPUFA, during pregnancy and lactation, to meet both maternal and infant requirements[17].

The American Dietetic Association and the Dietitians of Canada[18] recommend at least 500 mg/d of ω-3 LCPUFA for all healthy adults, including pregnant and lactating women. The European Commission and the International Society for the Study of Fatty Acids and Lipids recommend that pregnant and lactating women consume a minimum of 200 mg of DHA per day[19]. These recommendations would be adequate to achieve a content of at least 0·3 % DHA (relative to the total fatty acids in breast milk), the minimum amount necessary for an exclusively breastfed infant to receive a supplementation of 100 mg DHA/day, as recommended by the European Food Safety Authority in 2014[20].

During reproduction and breast-feeding, maternal nutrition is crucial for achieving a healthy body composition phenotype in offspring, emphasising the importance of this period in programming health and preventing later diseases in life[21]. A healthy diet during lactation ensures adequate maternal nutritional status and optimal concentrations of fatty acids in human milk, which are necessary for infant development[22].

The aim of the study was to relate the fatty acid profile in erythrocyte membrane (EM) and breast milk of lactating mothers with the fatty acid profile of EM of exclusively breastfed 3-month-old infants.

## Materials and methods

### Study design and protocol

Observational, analytical cross-sectional study. Mothers who are breast-feeding and their 3-month-old infants, attending check-ups at the Mother and Child Health Observatory of the IDIP, HIAEP 'Sor María Ludovica' from La Plata, Ministry of Health/CIC-PBA, in the period 2016–2019, were included.

Healthy mothers over 18 years of age, who agreed to participate in the study and healthy infants with birth weight ≥ 2500 g, born at term (> 37 weeks of gestation), normal fetal-neonatal history, exclusively breastfed for the first 3 months of age, were included. We used a nonprobability form of sampling.

The study protocol was approved by IDIP's Institutional Research Review Board and registered in the Ministry of Health of the province of Buenos Aires. Project registration: NO-202209223322-GDEBA-DPEGSFFMSALGP. It was performed in accordance with the ethical standards laid down in the 1948 Universal Declaration of Human Rights, the Nuremberg Code and the 1964 Declaration of Helsinki and successive revisions and amendments. Special attention was paid to compliance with National Law 25326 on Personal Data Protection, its regulatory decree, and other regulations that complement it. The collection of personal data was carried out in accordance with Article 3°.

Lactating women signed an informed consent for themselves and their children, agreeing to participate in the study.

### Anthropometric nutritional status

Weight and height were evaluated in children. Weight was measured with digital electronic scale (Tanita UM-061, precision 0·1 g, Tanita Corporation of America, Inc.) and height with portable stadiometer (precision 0·5 cm, SECA, UK). Height-for-Age (H/A), Weight-for-age (W/A) and weight-for-height (W/H) indicators were constructed and analysed using the cut-off points of international references[23].

Mothers were assessed using the BMI expressed in kg/m$^2$. Weights and measurements were carried out according to WHO recommendations[24].

### Biochemical determinations

*Sample collection.* Venous blood samples (2·5 ml for mothers and 1 ml for children) were taken at 3 months of age during the control visits at the Health Observatory and collected in tubes containing 5 % ethylene diamine tetra acetic acid, pH 7. Within 30 min of extraction, blood was centrifuged to separate plasma from the erythrocytes. The pellet was washed with sodium chloride 0·9 %, centrifuged three times and kept at −70°C until processing.

Breast milk was collected between 09.00 and 00.00. Complete breast emptying was achieved with a mini electric breast pump (MEDELA Inc.) with sterile automatic breast milk pumper and vacuum regulator, polystyrene suction funnels and screw-top bottles adapted to suction funnels for direct milk collection. The bottles and the suction funnels were autoclaved before use. Milk samples were collected in 25 ml bottles,

immediately aliquoted in 10 ml tubes using sterile material, frozen and stored at −70°C for later analysis[25,26].

*Fat content.* Milk fat content was determined using the creamatocrit method[27]. To express the content per gram of fat, the following formula was used:

$$\text{Fat(g/l)} = (\text{creamatocrit(\%)} - 0{\cdot}59)/0{\cdot}146$$

### Milk fatty acid analysis

Fatty acids were determined using the Folch method[28]. They were extracted from 500 µl of milk with a mixture of chloroform/methanol (v/v, 2:1). After lipid extraction, the chloroform phase was evaporated under nitrogen current; fatty acids were saponified with potassium hydroxide in 10 % methanol and acidified with hydrochloric acid to neutralise. Fatty acid methyl esters (FAME) were extracted twice with n-hexane and obtained after trans-methylation of total lipids using 10 % boron trifluoride-methanol solution for 1 h at 80°C[29]. They were analysed with gas chromatography-flame ionization detection using a 60-m capillary column (DB-23; Agilent Technologies) calibrated against a standard containing thirty-seven FAME (chain length range, 4–24 carbon atoms) (Supelco 37 Component FAME Mix; Supelco). Quantification was performed using the peak areas automatically retrieved from an integrator coupled to the chromatograph (GC System, 7890A Series, Agilent Technologies). FA results were expressed as percentages of total FA detected.

### Erythrocytes membrane fatty acid analysis

Erythrocyte membrane fatty acid composition was determined by the extraction of membrane lipids with a mixture of chloroform/methanol (2/1, v/v)[28]. Partition was performed with 20 % v/v distilled water. The upper methanolic phase was discarded. The remaining chloroformic phase was evaporated to dryness under a nitrogen stream. FAME were obtained after trans-methylation by using boron trifluoride 10 % methanol[29]. FAME were analysed with gas flame chromatography (Agilent 7890). Chromatographic identification of fatty acid peaks was made with standard commercial procedures (methyl esters 99 % purity; C4-C24 Sigma Aldrich) injected under the same conditions as in samples. Quantification was performed using the peak areas automatically retrieved from an integrator coupled to the chromatograph. The fatty acid results were expressed as percentages of total fatty acids detected.

*Mothers dietary intake.* The intake was evaluated using a monthly qualitative consumption frequency questionnaire (simple selection) taking into account the local consumption habits and previous studies performed by our group in lactating mothers[30,31]. They were asked about the type of oil they consumed and the intake of ω-3 precursor foods or sources (precursors: type of oil, specific consumption of canola oil, flaxseed and pumpkin seeds; source of DHA: fatty fish (salmon, tuna, herring and trout) and nuts. They were also questioned to find out if they were supplemented with fatty acids. Data were analysed using the Food Analysis and Registration System (SARA, acronym in Spanish) food chemical composition tables and software[32].

### Assessment of unsatisfied basic need

Unsatisfied basic needs were defined according to the methodology used by the National Institute of Statistics and Censuses (INDEC for its Spanish acronym)[33]. Households with unsatisfied basic needs had at least one of the following deprivation indicators: housing (poor, substandard housing, excluding house or department); sanitary conditions (without toilet); overcrowding (more than three people in a room); school attendance (6–12 years old school age children not attending school); subsistence capacity (four or more people per family member employed and head of household with incomplete third grade).

### Sample size

Sample size was estimated to obtain a 90 % power and a 95 % CI in order to detect 0·5 correlation between mother and infant fatty acid levels. Thus, the required sample size was thirty-eight mothers and thirty-eight infants[34].

### Statistical analysis

Data were processed with Microsoft R 3.5.1 version. Erythrocyte membrane fatty acids were grouped as follows: SFA, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0; MUFA, 16:1n7, 18:1n9 cis and trans, 20:1n9, 22:1n9, 24:1n9; PUFA were divided into ω-6 (18:2n6 cis and trans, 18:3n6, 20:2n6, 20:3n6, 20:4n6, 22:2n6) and ω-3 (18:3n3, 20:3n3; 20:5n3, 22:6n3). Fatty acid levels were reported as percentage of total fatty acids analysed.

Since the average DHA value in our population's milk samples[31] is well below the global average reported by Brenna *et al.* (0·32 (SD 0·22))[35], we arbitrarily decided to use the third quartile of the DHA distribution in our milk samples as a cut-off point. The distribution of DHA values in our samples was skewed, with a significant proportion showing lower DHA levels. Using the third quartile as a cut-off point adequately captures the upper range of the distribution, which is critical for understanding factors associated with higher DHA concentrations. We used this cut-off point to compare the DHA percentage in the erythrocyte membranes between infants consuming milk with DHA above or below this threshold.

Qualitative variables were reported as frequencies and percentages. The normality of each variable was evaluated with the Shapiro–Wilk test. Variables with normal distribution were reported as mean (standard deviation), while non-parametric data were reported as median (interquartile range). Student's *t* tests or Mann–Whitney tests were used for group comparisons, as appropriate. Pearson or Spearman correlations were used to analyse between fatty acid percentages correlations, depending on variable's distribution.

In all cases, results with a significance level of $P < 0{\cdot}05$ were considered statistically significant.

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**Table 1.** Erythrocyte membrane of mothers and infant's percentage fatty acids composition (Median values and interquartile ranges; mean values and standard deviations)

| | Mothers | | | | Infants | | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acids% | Median | IQR | Mean | SD | Median | IQR | Mean | SD |
| 14:0 | | | 0·77 | 0·28 | | | 0·68 | 0·23 |
| 16:0 | 24·98 | 22·85–28·22 | | | 25·08 | 23·68–26·78 | | |
| 18:0 | 21·02 | 19·00–23·42 | | | 20·42 | 19·20–22·13 | | |
| 18:1n9 cis | 14·60 | 13·69–15·88 | | | 13·09 | 12·18–13·80 | | |
| 18:2n6 trans | | | 0·91 | 0·13 | | | 1·06 | 0·25 |
| 18:2n6 cis | | | 13·03 | 1·66 | | | 11·22 | 1·85 |
| 20:3n6 | | | 2·01 | 0,55 | | | 2,16 | 0·41 |
| 20:4n6 (ARA) | 15·69 | 11·61–16·80 | | | 18·71 | 16·74–20·45 | | |
| 24:1n9 | | | 1·69 | 0·56 | | | 1·74 | 0·36 |
| 22:6n3 (DHA) | | | 2·54 | 1·04 | | | 3·84 | 1·04 |
| SFAs | 45·80 | 42·98–53·34 | | | 47·03 | 44·89–50·34 | | |
| MUFA | 18·61 | 16·79–20·19 | | | 14·79 | 13·94–15·51 | | |
| PUFA | 34·74 | 27·86–37·98 | | | 37·45 | 35·19–39·59 | | |
| ∑ω-6 | 31·95 | 25·85–34·50 | | | 33·33 | 31·75–35·80 | | |
| ∑ω-3 | | | 2·54 | 1·04 | | | 3·79 | 1·04 |

∑ω-6, sum *n*-6; ∑ω-3, sum *n*-3; SD, standard deviation; IQR, interquartile range.

## Results

A total of forty-five mother–baby dyads and forty-five donated milk samples were enrolled in the study. Mother's median age (interquartile range) was 25 years (21–31). They offered exclusive breast-feeding at the time of study enrolment. Infant's median age was 3·19 (3·06–3·45) months, and 51 % of the infants were female. Mother's median BMI was 26·14 (23·90–31·87), with 76 % completed primary education and 68 % with unmet basic needs.

Weight 0·37 (–0·61–1·41) and height (0·48 (SD 1·10)) Z-scores for children were found within the normal range for sex and chronological age.

Percentage fatty acids composition of mother's EM, infant's EM and maternal milk was determined. The results are shown in Table 1, where the percentage of each fatty acid is presented and grouped according to its type of saturation (SAF, MUFA and PUFA), as well as of ω-6 and ω-3 fatty acids sum.

Mean breast-feeding length was 93 (84–95) days. Median fat content was 28·8 (16·35–40·5) g/l. Donated milk fatty acids' percentage composition is shown in Table 2.

Median DHA content in breast milk was 0·14 % (interquartile range 0·12–0·21), only three samples exceeded the 0·30% recommended. Infants who consumed milk with a DHA content lower than 0·20% (third quartile) had a median of 3·30 (2·80–4·41) % of DHA in their EM, while those consuming milk with a percentage higher than 0·20% had a median of 4·56 (4·20–5·21), with a *P*-value of 0·004.

However, when mother's EM DHA percentage was evaluated and grouped according to whether their milks had a DHA content greater or less than 0·20 %, no significant differences were found, a *P*-value of 0·129.

Mother's dietary intake was determined through a monthly consumption frequency questionnaire (simple selection). Total lipids consumption was 54·08 (SD 30·88) %. The discriminated intake of the different types of fatty acids can be observed in Table 3.

**Table 2.** Mother´s milk percentage of fatty acids composition (Median values and interquartile ranges; mean values and standard deviations)

| Breast milk fatty acids % | Median | IQR | Mean | SD |
|---|---|---|---|---|
| 10:0 | | | 0·72 | 0·23 |
| 12:0 | | | 4·61 | 1·74 |
| 13:0 | 0·03 | 0·02–0·03 | | |
| 14:0 | | | 5·88 | 2·08 |
| 14:1n9 | | | 0·25 | 0·11 |
| 15:0 | | | 0·32 | 0·09 |
| 15:1n10 | | | 0·09 | 0·03 |
| 16:0 | | | 20·78 | 2·55 |
| 16:1n9 | | | 2·17 | 0·63 |
| 17:0 | | | 0·53 | 0·14 |
| 17:1n10 | | | 0·36 | 0·11 |
| 18:0 | 8·58 | 7·66–9·31 | | |
| 18:1n9 trans | 0·91 | 0·35–1·04 | | |
| 18:1n9 cis | 31·58 | 29·36;33·11 | | |
| 18:2n6 trans | 0·06 | 0·05–0·12 | | |
| 18:2n6 cis | | | 20·31 | 5·17 |
| 18:3n6 | 0·13 | 0·09–0·17 | | |
| 18:3n3 | 0·62 | 0·41–0·96 | | |
| 20:0 | | | 0·21 | 0·04 |
| 20:1n11 | 0·29 | 0·27–0·32 | | |
| 20:2n6 | | | 0·40 | 0·09 |
| 20:3n6 | | | 0·47 | 0·11 |
| 20:4n6 (ARA) | | | 0·46 | 0·09 |
| 20:3n3 | | | 0·04 | 0·01 |
| 22:0 | | | 0·06 | 0·02 |
| 20:5n3 | | | 0·05 | 0·02 |
| 22:1n9 | 0·06 | 0·06–0·07 | | |
| 22:2n6 | 0·05 | 0·04–0·05 | | |
| 24:1n9 | 0·1 | 0·06–0·11 | | |
| 22:6n3 (DHA) | 0·14 | 0·12–0·2 | | |
| SFAs | | | 36·32 | 3·87 |
| MUFAs | 34·87 | 31·63–36·57 | | |
| PUFAs | | | 22·83 | 5·55 |
| ∑ω-6 | | | 21·89 | 5·29 |
| ∑ω-3 | 0·79 | 0·56–1·11 | | |

∑ω-6, sum *n*-6; ∑ω-3, sum *n*-3; IQR, interquartile range.

The DHA precursors and sources consumption was not significant. Only 12 % of mothers ate fish in the previous month (hake only, but no oily fish). None of them was supplemented

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**Table 3.** Fatty acids percentage composition in mother's dietary intake (median values and interquartile ranges)

| Fatty acids % | Median | IQR |
|---|---|---|
| SFA | 10·54 | 6·83–17·89 |
| MFA | 14·46 | 8·92–20·08 |
| PUFA | 5·65 | 2·27–8·62 |
| LA | 4·82 | 1·73–8·34 |
| α LNA | 0·32 | 0·20–0·51 |
| ARA | 0·07 | 0·03–0·14 |
| DHA | 0·01 | 0·00–0·04 |

LA, linoleic acid; α LNA, alpha-linolenic acid; ARA, arachidonic acid; IQR, interquartile range.

with or consumed food rich in linolenic acid, such as canola oil, flax seeds and/or pumpkin seeds and dried fruits. Sunflower oil, a source of ω-6, was the only oil consumed.

Correlations between fatty acids percentages present in the EM of mothers and infants, as well as between EM and milk, were performed. We found a significant positive correlation between infant's EM DHA percentage and milk DHA percentage ($r = 0{\cdot}39$; $P$ value 0·008); as well as between infant's EM ω-3 fatty acids sum and milk DHA percentage ($r = 0{\cdot}39$; $P$-value 0·008). Although there was not statistically significant correlation, a trend was observed between DHA levels in the mothers' EM and the DHA percentage in their milk ($r = 0{\cdot}28$, $P = 0{\cdot}066$).

A significant positive correlation between the linoleic acid (ω-6 precursor) percentage in the mother's EM and the linoleic acid percentage in the infant's EM ($r = 0{\cdot}44$, $P = 0{\cdot}003$) was also found.

## Discussion

The main finding of this study was the low percentage of DHA in mother's milk (0·14 (0·12–0·2)), consistent with the low consumption of DHA-rich foods; only three of the samples exceeded the 0·3 % threshold established as the global cut-off[35].

A significant positive correlation was found between the DHA content in milk and DHA content in the infant's EM. When milk samples had a DHA percentage equal to or greater than 0·20 %, infants showed a significant increase in DHA content in their EM.

The availability of essential PUFA for infants is directly related to their transfer from the mother through breast milk[15,36]. In medium- or low-incomes countries, the consumption of essential fatty acids, especially ω-3, in pregnant and lactating women is inadequate[37,38]. We observed a low intake of DHA from natural sources, such as fish or seafood, and none of the studied mothers were supplemented. There was a higher intake of foods rich in ω-6 fatty acids, especially oils like sunflower oil, which could reduce the mother's ability to transfer DHA to her offspring during lactation[39]. Consistent with our findings, studies by Kim [40] and Judge[41] observed a high consumption of ω-6 fatty acids, which could limit the biosynthesis of ω-3. As ω-6 and ω-3 compete for the same enzymes in their respective synthesis pathways, this could interfere with the ω-3 PUFA metabolism.

Mother's EM PUFA levels observed in our study, especially DHA (2·54 (SD 1·04)), were below values found in the region. Studies conducted by Barrera C *et al.*[15] in Chile (5·80 (SD 0·04)), Krasevec JM *et al.*[42] in Cuba (6·80 (SD 1·24)) and Pontes PV *et al.*[43] in Brazil (4·67 (SD 0·5)) had higher values. The low bioconversion rate of α LNL to DHA, observed through stable isotope tracer studies, shows that only 1–4 % of dietary α LNL is converted to DHA. It is known that conversion of α LNL to DHA increases during pregnancy, but maternal supplementation with α LNL during pregnancy was not enough to increase blood DHA concentration in pregnant women or their newborns[17]. Therefore, DHA should be provided through the diet, since its concentration in breast milk is not constant and varies with maternal intake[44]. Therefore, the low intake of preformed DHA in our population would correlate with the low percentage of DHA found.

The WHO recommends an intake of 300 mg/d of DHA and 200 mg/d of EPA during pregnancy and lactation. Based on the data indicating low consumption of marine products, rich in ω-3, in the maternal studied population, supplementation with ω-3 PUFA is essential to meet the requirements during pregnancy and lactation. It provides numerous benefits for brain and visual development and extending during the first years of life[45].

Milk analysed in our study presented DHA levels similar to those found by Gaete *et al.*[46], in the milk of Chilean mothers with exclusive breast-feeding, during the fourth week post-partum (0·16 (SD 0·05) %); and Bosch *et al.*[47], who studied Venezuelan mothers, with 8 weeks of breast-feeding and low socio-economic level (0·17 (SD 0·09)). These populations had similar characteristics to ours: carbohydrate-rich diets and poor in fats.

Although our milk samples were below the worldwide average (0·32 %) reported by Brenna *et al.*[35], when the amount of DHA was equal to, or greater than 0·20 % (third quartile), infant's DHA in EM increased significantly. The relative amount of DHA in erythrocyte membranes correlates with DHA content in the brain[36]; thus, this finding could have a positive impact on neurogenesis, synaptogenesis and myelination in exclusively breastfed infants, as demonstrated by other authors[48,49].

Regarding ARA, we have observed that values found in breast milk are similar to those reported by Brenna *et al.*[35]. According to data, from sixty-five studies, on breast milk from 2474 women, ARA mean concentration was 0·47 (SD 0·13) %. According to Del Prado *et al.*, the level of ARA in breast milk is much more stable than the level of DHA. This stability in breast milk is biologically important because it provides preformed ARA consistently at a time when brain growth and development are critical. Most breast milk ARA does not come from dietary linoleic acid but from maternal ARA reserves[50]. However, a moderate association was found between the mother's EM linoleic acid and the infants' EM linoleic acid percentage, providing a direct evidence of maternal dietary ω-6 essential fatty acid intake.

We found some differences between ARA values in the EM of mothers and infants compared with the literature. Torres[51] study in Brazilian women presents lower mean values of ARA

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(11·52 (SD 1·79) %) in membranes, similar to the study done by Barrera C[15] in Chilean women (11·8 (SD 1·3) %). The same occurs with the mean of ARA in infant's EM; values reported by Pontes[43] in Brazilian infants (ARA = 13·77 (SD 3·7)) and Jakobik[52] in Hungarian infants (10·38 (SD 3·59)) were lower.

The general pattern of SFA composition in breast milk and in the EM of the studied mothers was similar to that reported by Torres and colleagues[51] in their study conducted in a population of Brazilian lactating women (Breastmilk = 16:0 (19·7 (SD 1·7)); 18:0 (6·28 (SD 1·07))/EM = 16:0 (24·9 (SD 2·49)); 18:0 (13·2 (SD 1·88))). However, we observed differences with Barrera *et al.* study[15].Their results of palmitic acid levels were higher; this could be attributed to differences in the types of foods consumed (Breastmilk = 16:0 (25·1 (SD 2·8)); EM = 16:0 (31·4 (SD 3·2))).

The EM SAF values for infants in our study align with those from a 2016 study[53] conducted at the same institution, where breast-feeding mothers had similar feeding patterns and socio-economic status (16:0 = 22·5 (SD 3·00); 18:0 = 19·94 (SD 3·04)). Additionally, our results are consistent with findings by Jakobik *et al.*[52] in their study of Hungarian infants' membrane composition (16:0 = 26·46 (SD 4·47); 18:0 = 21·16 (SD 4·35)) and Pontes[43] in their research on Brazilian infants born at term (16:0 = 27·67 (SD 2·1); 18:0 = 19·27 (SD 1·4)).

The limitation of this study was that, despite knowing that there are many sources of *n*-6 and *n*-3, the questionnaire selected was based on food availability in the Argentine market. Additionally, the survey was designed based on the purchasing power of the population and previous surveys.

Low-income women attending public health care hospitals, in a region of Argentina, have DHA percentages below international recommendations. We support the idea of mother's supplementation during the last trimester of pregnancy and first 6 months of breast-feeding[17]. ω-6 PUFA and SAFs seem to be less influenced by the diet according to our results and other authors[51].

## Conclusion

The DHA percentage distribution found in maternal milk correlates with this fatty acid percentage distribution in both mothers and infant's EM. An increase above 0·20 % in the maternal milk DHA percentage resulted in a significant increase in the same fatty acid in the infant's EM.

Dietary fatty acid intake is associated with the mother's EM fatty acid percentage distribution and the maternal milk lipid composition. Low consumption of DHA-rich food sources and lack of supplementation resulted in a low percentage of ω-3 in both maternal milk and EM.

We found a correlation between ω-6 fatty acids precursor (linoleic acid) in the maternal EM and infant's EM. However, no correlation was found with its breast milk composition. Further studies will be necessary to explain these findings.

No correlation was found between SFA in either maternal EM or between the infant's EM and human milk.

This study reaffirms the need to generate more evidence on the nutritional status of PUFA in breast-feeding mothers. This will help to develop public supplementation policies in countries of the region that do not yet have such measures, including ours.

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The authors' contributions are as follows: S.V and H.F.G conceived the study; V.F performed the statistical analyses and S.V, A.M, M.S and H.F.G interpreted the data, wrote the manuscript, reviewing and editing. All authors have read and agreed to the final version of the manuscript.

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