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Pilot study of the effect of EPA + DHA supplementation on the fatty acid profile of erythrocytes and breast milk of lactating women from Sonsón, Colombia

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ABSTRACT

Objective: The objective of this study was to evaluate changes in the concentrations of EPA and DHA in the erythrocytes and breast milk of a group of lactating women in the municipality of Sonsón (Antioquia) before and after receiving supplementation with these fatty acids for three months.
Design: In a quasi-experimental study, 11 lactating women were evaluated before and after EPA (100 mg) and DHA (250 mg) supplementation for three months. The consumption of omega-3 food sources was determined by simple frequency, anthropometry (weight, height) was performed, and the fatty acid profiles of erythrocytes and breast milk were determined with gas chromatography.
Environment: Sonsón, Colombia.
Participants: A group of lactating women in the municipality of Sonsón (Antioquia).
Results: Low consumption of foods that are sources of omega-3 fatty acids was found, as was low EPA and DHA content in erythrocytes and breast milk at the beginning of the study period. After supplementation, there was no significant change for EPA, however, there was a significant increase in DHA in both erythrocytes and breast milk; in addition, there was a decrease in the omega-6/omega-3 ratio.
Conclusions: Supplementation with 250 mg of DHA increased its concentration in the blood and breast milk to levels approaching the recommended average DHA of 0.3%, where benefits have been seen for the mother.

1. Introduction

Nutritional status and maternal and child health generate considerable public health interest worldwide, since nutritional deficiencies during gestation and lactation have adverse effects on the health of the mother and the development of children in the first years of life, which in turn affects the well-being of individuals and the progress of nations (UNICEF, 2009).

Breast milk is known to be the ideal food for newborns (Kim et al., 2017), and its unique composition of nutrients and other substances has been associated with adequate growth and development in breastfed children. Breast milk fat fulfils important functions, such as providing a source of energy, transporting fat-soluble vitamins and serving other regulatory and structural purposes. Its regulatory and structural functions depend on its polyunsaturated fatty acid (PUFA) content (Kim et al., 2017). Maternal malnutrition has been related to changes in the fatty acid profile of milk, particularly in terms of linoleic (LA-18:2 *n*-6), arachidonic (ARA-20:4 *n*-6), alpha-linolenic (ALA-18:3 *n*-3), eicosapentaenoic (EPA-20:5 *n*-3) and docosahexaenoic (DHA-22:6 *n-3*) fatty acids. In addition, the content of breast milk varies within and among populations according to their dietary intake and is reflected in the blood levels of *n*-6 and *n*-3 fatty acids in children fed breast milk (Innis, 2007).

DHA-22:6 *n*-3 is the main fatty acid in the brain and retina and is essential for neurodevelopment during the fetal period and the first years of life. Additionally, it contributes to maternal health because it has been associated with decreases in preterm delivery and postpartum depression and lower cardiovascular risk in mothers (Marangoni et al., 2016).

The World Health Organization (WHO) and the Food and Agriculture

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Organization of the United Nations (FAO) recommend a minimum consumption of 300 mg/day EPA-20:5 *n*-3 + DHA-22:6 *n-3* for pregnant and lactating mothers, at least 200 mg/day of which should correspond to DHA-22:6 *n-3* (FAO and FINUT, 2008; B ü y ü kuslu et al., 2017). The sources of ALA-18:3 *n-3* (metabolic precursor of EPA-20:5 *n-3* and DHA-22:6 *n-3*) are mainly of plant origin and include nuts, flax, chia and hemp and soybean oils (Vannice and Rasmussen, 2014), while EPA-20:5 *n-3* and DHA-22:6 *n-3* are found in shellfish and fish such as tuna, mackerel, salmon, sardine, horse mackerel, herring and smelt (FAO and FINUT, 2008). These foods are scarcely present in the Colombian diet, are not part of the food culture and are expensive. Women of childbearing age, pregnant women, breastfeeding women and children under five years of age are advised to avoid consuming fatty fishes, such as those mentioned above, due to the bioaccumulation of heavy metals such as methyl-mercury (MeHg) (Díaz-Gómez et al., 2013). The ingestion of MeHg through fish and seafood is currently a public health problem due to its toxicity to the foetal neurological system and to developing infants (Raimann et al., 2014).

The Western diet is generally low in DHA-22:6 *n-3*; therefore, supplementation during pregnancy and lactation has been suggested as a source of these nutrients (Valenzuela et al., 2025). Supplementation is a relatively inexpensive alternative for supplying EPA-20:5 *n-3* and DHA-22:6 *n-3* (FAO and FINUT, 2008). Supplementation with DHA-22:6 *n-3* during pregnancy and/or lactation can quickly improve its concentration in blood and breast milk; however, the ideal situation is for the mother to have an optimal level of this nutrient prior to pregnancy to ensure that organs such as the brain and maternal adipose tissue have a good reserve (Luxwolda et al., 2014).

The objective of this research was to evaluate changes in the concentrations of EPA-20:5 *n-3* and DHA-22:6 *n-3* in the blood and breast milk of a group of lactating women in the municipality of Sonsón (Antioquia) before and after receiving supplementation with these fatty acids for three months.

2. Methods

2.1. Subjects

For this nonrandomized clinical trial, the database of pregnant women treated at the State Social Enterprise San Juan de Dios Hospital of the municipality of Sonsón, Antioquia, was searched. Those who were close to their delivery date were contacted, and the conditions of the study were shared with them. The inclusion criteria were as follows: healthy women residing in urban or rural areas near the municipality who were between 18 and 35 years of age, had a full-term delivery and had established exclusive breastfeeding and intended to continue breastfeeding for the first six months of the infant's life. Women who, during their pregnancy, had gestational diabetes, hypertension, preeclampsia or serious complications of childbirth, autoimmune disease, multiple gestations, more than two children, anaemia, low weight or obesity and who consumed supplements containing EPA-20:5 n-3 and/or DHA-22:6 n-3, drugs, cigarettes or alcohol were excluded. The participants received a home visit to remind them of the objectives and procedures, obtain informed consent, collect sociodemographic information and perform an anthropometric evaluation. The study was carried out in accordance with the codes of ethics and complied with the Declaration of Helsinki for studies with humans, and it was approved by the ethics committees of the ESE San Juan de Dios Hospital, with a certificate dated February 19, 2019, and those of the University Research Headquarters of the University of Antioquia, with approval certificate 19-93-833 of April 25, 2019. In Fig. 1, the process used to obtain the sample of 11 women is detailed.

2.2. Supplementation

Each woman was given a fish oil supplement containing 250 mg DHA-22:6 *n-3* and 100 mg EPA-20:5 *n-3*. With the supplement, the participants were given instructions to take one tablet daily with one of the main meals; in addition, a form was provided for recording daily consumption. To improve adherence to treatment, a text message was sent daily or via WhatsApp that included notes on breastfeeding and a reminder to take the supplement. At each home visit, the participants were asked about symptoms associated with the supplement, leftover tablets and consumption records were collected, and another 30-day supply of the supplement was provided.

2.3. Anthropometric evaluation

Weight (kg) and height (m) were measured to obtain the body mass index (BMI; kg/m^2) according to internationally used equipment and techniques (Lohman et al., 1992) after the training and standardization of the nutritionist in charge. The participating women were evaluated at admission and once a month until the end of the three-month study period.

The measurements were taken and classified according to the criteria of Resolution 2465 of 2016 of the Ministry of Health and Social Protection of Colombia (Ministerio de Salud y Protección Social, 2016).

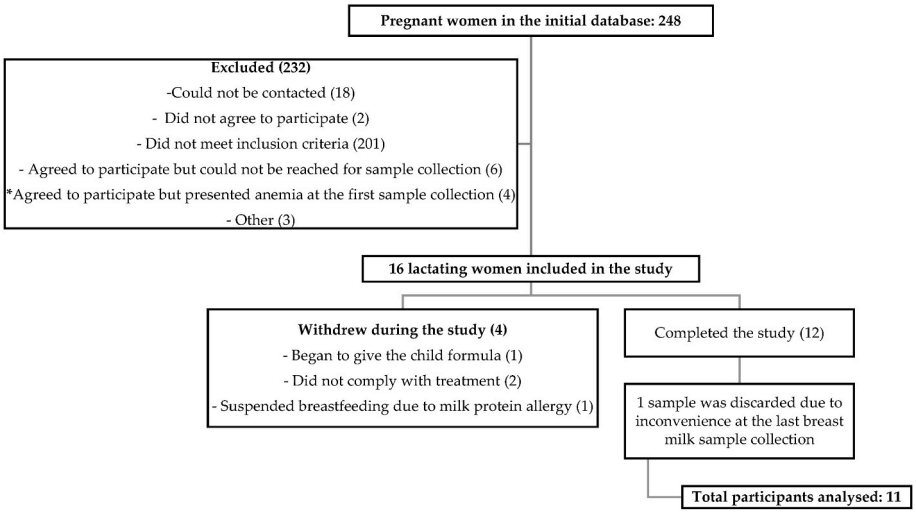

Fig. 1. Recruitment of lactating women.

2.4. Evaluation of the consumption of food sources of omega-3

A simple frequency count of omega-3 food sources was developed. Using this tool, the women were asked how often they consumed such foods: daily, weekly, monthly or occasionally. The tool was applied by a nutritionist or a trained nutrition student and was administered at the beginning and end of the supplementation period.

2.5. Blood sample collection

The women attended the hospital after 8–10 h of fasting, and 11 mL venous blood was extracted by puncture of the antecubital vein into two tubes: a 4 mL tube and a 7 mL tube. The sample in the 4-mL tube was used for determination of the complete blood count and was processed immediately at Hospital in Sonsón. If the haemoglobin result was less than 12 g/dL, the mother did not continue with the study and was referred for anaemia treatment; otherwise, she continued with the process.

The 7 mL tube was stored under refrigeration (2–8 °C) until it was transferred on the same day to the city of Medellin for analysis of fatty acids in erythrocytes at the laboratory. Samples were obtained at the beginning and end of the supplementation period.

2.6. Breast milk sample collection

The mothers were asked from which breast the baby had fed at the last feeding and were asked to hold the baby to the other breasts for sampling. The baby was allowed to suckle for an average of 5 min, and then the participants extracted 5 mL of milk manually into sterile jars. The samples were stored under refrigeration between 2 and 4 °C and were transported with ice packs to the city of Medellin on the same day for fatty acid content analysis at the laboratory, where the samples were stored at −80 °C until analysis. Samples were obtained at the beginning and end of the supplementation period.

2.7. Fatty acid analysis

2.7.1. Extraction of erythrocytes from maternal blood

Erythrocytes were extracted immediately upon their arrival at the laboratory as follows. Whole blood was centrifuged at 1500 rpm for 10 min to separate the plasma from the red blood cells and the buffy coat. Then, 1 mL of 0.9% saline solution was added to the red blood cells, and the sample was centrifuged again at 1500 rpm for 10 min. This process was repeated two more times. Finally, the sample was stored at −80 °C for a maximum of one day for processing (Blincoe, 1974).

2.7.2. Extraction of fatty acids from erythrocytes and breast milk

Fatty acids were extracted from erythrocytes and breast milk based on the Folch method (Folch et al., 1957): 40 μL of internal standard (nonadecanoic acid, 5 mg/mL) was added to 100 μL of milk and 100 μL erythrocytes in separate Pyrex tubes with a screw cap. Then, 2 mL of chloroform/methanol mixture (2:1) was added, and the mixture was vortexed for 1 min. Then, 1 mL of 0.9% sodium chloride was added, and the mixture was vortexed for 1 min and then centrifuged for 5 min at 3500 rpm to separate the organic and aqueous phases. The organic phase was carefully aspirated with a Pasteur pipette and transferred to another Pyrex tube; 2 mL of chloroform was added to the aqueous phase, and the extraction process was repeated again. The organic phases were then collected, and the chloroform was evaporated in a water bath.

2.7.3. Derivatization of fatty acids

To the extract obtained from the erythrocytes and breast milk, 1 mL of hexane and 1 mL of boron trifluoride (BF_3) in 20% methanol were added, and the tube was capped. The solutions were mixed, and the tubes were placed in a water bath at 80–90 °C for 1 h. The tubes were allowed to cool to room temperature, 5 mL of saturated sodium chloride solution was added, the phases were separated, and the upper (organic) phase was collected and transferred to an Eppendorf tube containing a pinch of anhydrous sodium sulfate. Two hundred microlitres was removed and transferred to a vial for gas chromatography analysis (Kang and Wang, 2005).

2.7.4. Gas chromatography analysis

The lipid extracts of erythrocytes and breast milk were analysed by gas chromatography using an Agilent 6890N chromatograph with a flame ionization detector (FID); a TR-CN100 capillary column 60 m × 250 μm × 0.20 μm ID; a split/splitless injector with a split ratio of 100:1, an injection volume of 1.0 μL and an injector temperature of 260 °C; an oven temperature programmed to start at 90 °C × 7 min, increase at a rate of 5 °C/min up to 240 °C and remain there for 15 min; a detector temperature of 300 °C; and He as the carrier gas at a flow of 1.1 mL/min. For the identification of the fatty acids, the retention times of the samples were compared with a standard (FAME Mix of 37 components: C4 – C24, Supelco). Fatty acids were quantified by percentage normalization (Kang and Wang, 2005).

2.8. Statistical analysis

For the descriptive analysis, absolute and relative frequencies and summary indicators such as the arithmetic mean, standard deviation, median, interquartile range (IR), minimum and maximum values were used. The normality of continuous variables was established using the Shapiro–Wilk test.

The food consumption analysis was performed using descriptive measures, and the change in the consumption pattern between the first and second measurements was evaluated using double-entry tables. The significance of this change was determined with marginal homogeneity tests and the marginal homogeneity chi-square association test using the Stuart and Maxwell extension of the McNemar test.

To evaluate the relationship between supplement consumption and the concentrations of EPA-20:5 *n-3* and DHA-22:6 *n-3* in maternal erythrocytes and breast milk before and after supplementation, paired Student's *t*-test or the exact Wilcoxon signed-rank test was applied. These tests were complemented with estimation of the effect size and its respective 95% confidence intervals with Cohen's d measures or with the matched pairs rank biserial correlation.

A multivariate repeated measures design was implemented using the generalized linear model (GLM) procedure to adjust for the effect of some fatty acids in erythrocytes and breast milk by multiple factors and covariates. These determinations were complemented by the measurement of the partial Eta squared as a magnitude of the effect.

In all cases, results with a p value < 0.05 were considered statistically significant. Large effect sizes were indicated by a value > 0.80 for Cohen's d test, ≥ 0.50 for the matched pairs rank biserial correlation and >0.14 for the partial eta-squared test (statsExpressions, 2020). The descriptive and inferential statistical analysis was performed with the Statistical Package for the Social Sciences, SPSS® V 24.0, and the statistical analyses for comparisons and for determining the effect size were performed with the program R Studio® V 4.0.

3. Results

3.1. Maternal characteristics

The median age and weeks of gestation of the breastfeeding women were 28 [9] years and 39 [1] weeks, respectively. The participants were mainly of low socioeconomic status (64%), 73% had higher education, and 55% indicated that this was their first child. Forty percent of the mothers reported that during previous pregnancies, they had gestational anaemia; however, upon entering the study, test results verified that none of them had anaemia. The average supplement consumption was 83 ± 7 capsules. Eighty-two percent indicated that they had not

experienced any adverse symptoms, while 18% reported nausea or headache in the first days of supplement use but said that this did not affect their consumption of the supplement.

Table 1 shows the participants' anthropometric data before and after supplementation. The average height was 1.59 ± 0.05 m, and the average weight gained during pregnancy was 11.2 ± 3.4 kg. Fifty-five percent of the breastfeeding women had an adequate BMI at the beginning and the end of the study.

3.2. Consumption of food sources of omega-3

The evaluation of the consumption of foods that are sources of omega-3 showed that most of the breastfeeding women did not consume such foods. Table 2 shows the evaluated foods with the highest frequencies of consumption; however, low percentages of women included these foods in their daily or weekly diet. The most frequently consumed foods were tuna in oil, which was consumed weekly by 36% of the participants at the beginning and the end of the study period, and canned sardines, which were initially consumed monthly by 36% of the participants at the beginning of the study, but by the end of the study period, they were consumed occasionally by 64% of the women evaluated. No statistically significant differences in the reported consumption of food sources of omega-3 were found between the first and second evaluations.

3.3. Fatty acid composition of erythrocytes

The composition of the fatty acids extracted from erythrocytes at the beginning and end of the supplementation period is shown in Table 3. At both time points, saturated fatty acids (SFAs) had the highest concentration, followed by PUFAs and monounsaturated fatty acids (MFAs). Among the fatty acids, palmitic acid-16:0, stearic acid-18:0 and oleic acid-18:1 *n*-9 had the highest concentrations. There were no significant changes in the concentrations of arachidonic-20:4 *n*-6 and EPA-20:5 *n-3* fatty acids after supplementation. However, a significant change in DHA-20:6 *n*-3 with a large effect size of −1.135 was noted; its concentration increased by 20.5%, from 3.5514 ± 0.3957% to 4.2844. ± 0.7138% (p = 0.007). The total omega-6 fatty acids did not change significantly after supplementation; in contrast, the total omega-3 fatty acids changed significantly, increasing from 3.7752 ± 0.5096% to 4.6065. ± 0.7477% with a large effect size of −1.292 (p = 0.007). These differences in changes were reflected in the *n*-6/*n*-3 ratio, which decreased significantly from 7:1 to 6:1 (p = 0.038), with a large effect size of 0.949.

3.4. Fatty acid composition of breast milk

Table 4 shows the composition of the fatty acids extracted from breast milk at the beginning and end of supplementation. The average fat contents before and after supplementation were 6.8 (2.2) g/100 g and 7.5 (4.7) g/100 g, respectively, without significant differences.

The proportion of SFAs was the highest, followed by MFAs and finally PUFAs, with no significant difference between the first and second measurements. The fatty acids with the highest concentrations were palmitic acid-16:0, oleic acid-18:1 *n*-9 and linoleic acid-18:2 *n*-6 at both measurements, with no significant differences. Similarly, no significant differences in the concentration of EPA-20:5 *n-3* were observed. However, a significant change with a large effect size was observed for ALA-18:3 *n-3*, arachidonic-20:4 *n*-6 and DHA-22:6 *n-3* fatty acids: the concentration of the first increased by 45%, from 0.8709 ± 0.3170% to 1.2655 ± 0.4830%, with an effect size of −0.943 (p = 0.037); the concentration of the second decreased by 25% from 0.5535 ± 0, 0774% to 0.4157 ± 0.1115%, with an effect size of 1.404 (p = 0.003); and the concentration of the third increased by 57%, from 0.1867 [0.0499]% to 0.2930 [0.0713]%, with an effect size of −0.859 (p = 0.002). These changes in fatty acid concentrations were reflected in the total concentration of omega-3 fatty acids, which increased significantly from 1.1464 ± 0.3301% to 1.6670 ± 0.5106, with an effect size of −1.196 (p = 0.011), and in the *n-6/n-3* ratio, which decreased significantly from 15:1 to 12:1 (p = 0.001), with a large effect size of 1.633.

Table 5 shows the changes in the concentrations of some fatty acids in erythrocytes and breast milk adjusted for the following variables: the number of children, weight gain during pregnancy and education level. The changes in ARA-20:4 *n-6* in erythrocytes and in EPA-20:5 *n-3* in breast milk became significant, with large adjusted effect size, indicating that these variables can influence the final concentrations of these fatty acids. In contrast, no change was found in the p values of the fatty acids ARA-20:4 *n-6* in breast milk and DHA-20:6 *n-3* and EPA-20:5 *n-3* in erythrocytes after adjustment, indicating that these variables did not influence the change in the concentration of these fatty acids before and after supplementation.

Table 1
Anthropometric variables of breastfeeding women.

	Mean ± SD		
Height (m)	1.59 ± 0.05		
Weight gained during pregnancy (kg)	11.2 ± 3.4		
	Before [a]	**After** [b]	**p value** [c]
Weight (kg)	65.1 ± 7.7	63.5 ± 8.4	0.074
BMI (kg/m^2)	25.6 ± 2.3	24.9 ± 2.8	0.083

[a] Initial baseline measurement.
[b] Final measurement 3 months later; BMI: Body mass index.
[c] The p value was calculated with Student's *t*-test for related samples. Data are presented as mean ± standard deviation.

Table 2
Frequency of consumption of selected food sources of omega-3 before and after supplementation in breastfeeding women.

Frequency of consumption	Period *	Frequency of consumption	Percentage	p value †,‡
Canned tuna with oil	Initial	Do not consume	18%	0.853
		Weekly	36%	
		Monthly	18%	
		Occasionally	27%	
	Final	Do not consume	18%	
		Weekly	36%	
		Monthly	27%	
		Occasionally	18%	
Canned sardines	Initial	Do not consume	27%	0.221[2]
		Monthly	36%	
		Occasionally	36%	
	Final	Do not consume	27%	
		Monthly	9%	
		Occasionally	64%	
Rainbow trout	Initial	Do not consume	55%	0.777
		Occasionally	45%	
	Final	Do not consume	45%	
		Monthly	18%	
		Occasionally	36%	
Almonds	Initial	Do not consume	55%	0.159
		Weekly	9%	
		Monthly	9%	
		Occasionally	27%	
	Final	Do not consume	18%	
		Weekly	27%	
		Occasionally	55%	

*Period: Initial = first evaluation, conducted before supplementation began; Final = second evaluation, conducted after the supplementation period. The p value was calculated with the tests of †marginal homogeneity and ‡marginal homogeneity chi-square associations with the Stuart and Maxwell extension of the McNemar test.

4. Discussion

Most of the participating women presented adequate weight gain

Table 3
Fatty acid profile in erythrocytes before and after supplementation (w%).

Fatty acid	Before[a],[†]	After [a],[†]	p value[‡]	Effect size [c], [p] (95% CI)
Decanoic-10:0	0.0000 [0.2823] (0.0000; 0.8732)	0.0000 [0.0000] (0.0000; 0.0000)	0.125	0.552[p] (0.302–0.727)
Myristic-14:0	0.4088 [0.4748] (0.0000; 0.5403)	0.0000 [0.0000] (0.0000; 0.3217)	**0.016**	**0.715[p] (0.552 to 0.850)**
Palmitic-16:0	30.7791 ± 1.671	31.0222 ± 2.9572	0.815	−0.096[c] (−0.760 to 0.569)
Palmitoleic-16:1 *n-7*	0.3529 [0.5200] (0.0000; 0.5718)	0.0000 [0.2475] (0.0000; 0.2992)	**0.037**	**0.630[p] (0.162 to 0.885)**
Heptadecanoic-17:0	0.4003 [0.1855] (0.0000; 0.6598)	0.0000 [0.4072] (0.0000; 0.4870)	0.092	0.537[p] (0.015–0.847)
Stearic-18:0	16.8905 ± 1.5773	19.4683 ± 1.3660	**0.001**	**−1.747[c] (-3.126 to -0.367)**
Oleic-18:1 *n-9*	20.5987 ± 2.4406	16.7425 ± 3.8765	**0.013**	**1.138[c] (0.322 to 1.954)**
Linoleic-18:2 *n-6*	12.6932 ± 2.0544	13.4242 ± 1.4840	0.351	−0.399[c] (−1.063 to 0.267)
Eicosadienoic-20:2 *n-6*	0.0000 [0.3547] (0.0000; 0.4853)	0.0000 [0.2856] (0.0000; 0.4569)	0.281	0.410[p] (−0.204 to 0.721)
Eicosatrienoic-20:3 *n-6*	2.1279 ± 0.5272	1.4281 ± 0.8769	**0.037**	**0.879[c] (0.254 to 1.504)**
Behenic-22:0	0.0000 [0.0000] (0.0000; 0.2609)	0.0000 [0.0000] (0.0000; 0.3039)	0.500	−0.404[p] (−0.624 to −0.302)
Arachidonic-20:4 *n-6*	12.0322 ± 0.8259	12.8348 ± 1.2713	0.097	−0.754[c] (−1.860 to 0.351)
EPA-20:5 *n-3*	0.0000 [0.3842] (0.0000; 0.5808)	0.3679 [0.5808] (0.0000; 0.6069)	0.516	−0.211[p] (−0.757 to 0.383)
DHA-22:6 *n-3*	3.5869 ± 0.3935	4.3331 ± 0.6963	**0.007**	**−1.315[c] (-2.501 to -0.129)**
SFAs	48.5603 ± 1.8732	50.7576 ± 3.7529	0.103	−0.724[c] (−1.621 to 0.174)
MFAs	20.9225 ± 2.3876	16.8535 ± 3.9406	**0.010**	**1.185[c] (0.359 to 2.011)**
PUFAs	30.8024 ± 1.9923	32.8889 ± 2.7436	0.138	−0.660[c] (−1.586 to 0.267)
Total *n*-6	26.8533 ± 2.0693	27.6870 ± 2.4425	0.398	−0.368[c] (−1.207 to 0.471)
Total *n*-3	3.7752 ± 0.5096	4.6065 ± 0.7477	**0.007**	**−1.292[c] (-2.405 to -0.179)**
n-6/n-3	7.2565 ± 1.2537	6.1481 ± 1.0736	**0.038**	**0.949[c] (-0.029 to 1.927)**

[a] Median [interquartile range] (minimum value; maximum value); [†]mean ± standard deviation. [‡]The p value was calculated with Student's *t*-test or the exact rank test with Wilcoxon sign according to the distribution of the variables. The effect size was calculated with the following tests: [c]Cohen's d or [p]rank correlation; 95% CI: confidence interval. The results shown in bold had significant differences and moderate to large effect sizes. EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; SFAs: saturated fatty acids; MFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; Total *n-6*: total omega-6 fatty acids; Total *n-3*: total omega-3 fatty acids; *n-6/n-3*: ratio of omega-6 fatty acids to omega-3 fatty acids.

Table 4
Fatty acid profile in breast milk before and after supplementation (w%).

Fatty acid	Before*,[†]	After*,[†]	p value[‡]	Effect size [c], [p] (95% CI)
Caproic-6:0	0.0183 ± 0.0077	0.0414 ± 0.0086	**< 0.001**	**−2.821[c] (-4.986 to -0.657)**
Caprylic-8:0	0.1039 ± 0.0357	0.1210 ± 0.0286	0.229	−0.528[c] (−1.345 to 0.290)
Decanoic-10:0	0.9005 ± 0.2731	1.0870 ± 0.2097	0.089	−0.762[c] (−1.654 to 0.129)
Lauric-12:0	3.8707 ± 1.4027	4.4615 ± 0.9999	0.270	−0.481[c] (−1.305 to 0.343)
Tridecanoic-13:0	0.0247 ± 0.0086	0.0265 ± 0.0121	0.685	−0.168[c] (−0.715 to 0.379)
Myristic-14:0	4.8540 ± 1.4659	5.0770 ± 1.5779	0.735	−0.146[c] (−0.834 to 0.541)
Myristoleic-14:1	0.1539 ± 0.0630	0.1378 ± 0.0687	0.574	0.243[c] (−0.302 to 0.789)
Pentadecanoic-15:0	0.2864 ± 0.0913	0.2756 ± 0.1109	0.806	0.106[c] (−0.620 to 0.831)
Palmitic-16:0	26.5010 ± 2.9186	24.6358 ± 2.7557	0.139	0.657[c] (−0.168 to 1.482)
Palmitoleic-16:1 *n-7*	2.4789 ± 0.9073	1.7927 ± 0.4898	**0.043**	**0.881[c] (0.113 to 1.648)**
Heptadecanoic-17:0	0.3307 ± 0.0874	0.3007 ± 0.0661	0.375	0.387[c] (−0.507 to 1.281)
Stearic-18:0	7.4394 ± 1.4208	7.18889 ± 0.4913	0.590	0.236[c] (−0.673 to 1.146)
Oleic-18:1 *n-9*	33.4993 ± 3.0993	31.9734 ± 3.3344	0.280	0.473[c] (−0.159 to 1.105)
Linoleic-18:2 *n-6*	16.2616 ± 3.9286	19.2180 ± 5.1934	0.149	−0.638[c] (−1.478 to 0.203)
g-linolenic-18:3 *n-6*	0.0956 [0.0578] (0.0265; 0.2367)	0.0000 [0.0259] (0.0000; 0.0794)	**0.007**	**0.778[p] (0.482 to 0.889)**
Arachidic-20:0	0.2238 [0.0254] (0.1623; 0.3149)	0.3091 [0.0924] (0.2154; 0.3772)	**0.002**	**−0.859[p] (-0.889 to -0.727)**
α-linolenic-18:3 *n-3*	0.8709 ± 0.3170	1.2655 ± 0.4830	**0.037**	**−0.943[c] (-1.794 to -0.093)**
eicosenoic-20:1 *n-9*	0.2779 [0.3885] (0.0000; 0.4331)	0.3290 [0.0649] (0.2475; 0.4801)	0.278	−0.350[p] (−0.754 to 0.298)
Eicosadienoic-20:2 *n-6*	0.3981 ± 0.0899	0.2648 ± 0.0627	**0.013**	**1.172[c] (0.055 to 2.290)**
Eicosatrienoic-20:3 *n-6*	0.4960 ± 0.1310	0.3300 ± 0.0854	**0.003**	**1.393[c] (0.658 to 2.128)**
Behenic-22:0	0.0411 [0.0621] (0.0000; 0.0880)	0.0900 [0.0399] (0.0606; 0.1398)	**0.001**	**−0.883[p] (-0.892 to -0.886)**
Eicosatrienoic-20:3 *n-3*	0.0341 ± 0.0226	0.0366 ± 0.0076	0.737	−0.144[c] (−1.009 to 0.720)
Arachidonic-20:4 *n-6*	0.5535 ± 0.0774	0.4157 ± 0.1115	**0.003**	**1.404[c] (0.456 to 2.352)**

(*continued on next page*)

Table 4 (*continued*)

Fatty acid	Before*,†	After*,†	p value‡	Effect size c, p (95% CI)
Erucic-22:1 *n*-9	0.0788 [0.1344] (0.0000; 0.1699)	0.0943 [0.0456] (0.0715; 0.2546)	0.123	−0.482p (−0.808 to 0.081)
Docosadienoic-22:2	0.0322 [0.0533] (0.0000; 0.0797)	0.0293 [0.0106] (0.0000; 0.0862)	0.898	−0.054p (−0.644 to 0.513)
EPA-20:5 *n-3*	0.0485 ± 0.0239	0.0574 ± 0.0150	0.307	−0.450c (−1.388 to 0.489)
Lignoceric-24:0	0.0274 [0.0524] (0.0000; 0.0683)	0.0470 [0.0310] (0.0210; 0.0940)	0.067	−0.564p (−0.862 to 0.027)
DHA-22:6 *n-3*	0.1867 [0.0499] (0.1578; 0.2544)	0.2930 [0.0713] (0.1936; 0.5378)	**0.002**	**−0.859p (-0.889 to -0.727)**
SFAs	45.2920 ± 5.4845	43.3894 ± 5.2981	0.418	0.353c (−0.308 to 1.013)
MFAs	35.2276 [6.9650] (30.1810; 42.1464)	32.8087 [5.6281] (30.3967; 40.4510)	0.240	0.401p (−0.189 to 0.889)
PUFAs	19.1308 ± 4.1650	22.6160 ± 5.8547	0.125	−0.676c (−1.465 to 0.113)
TG (g/100 g)	6.8000 [2.2771] (4.5269; 13.5964)	7.5451 [4.7562] (1.8997; 13.8011)	0.999	0.000p (−0.567 to 0.567)
Total *n-6*	17.4117 ± 3.8606	19.9764 ± 5.1935	0.205	−0.557c (−1.395 to 0.281)
Total *n-3*	1.1464 ± 0.3301	1.6670 ± 0.5106	**0.011**	**−1.196c (-2.220 to -0.171)**
n-6/n-3	15.4792 ± 2.0289	12.2839 ± 1.8729	**0.001**	**1.633c (0.742 to 2.523)**

*Mean ± standard deviation; †median [interquartile range] (minimum value; maximum value). ‡The p value was calculated with Student's *t*-test or the exact rank test with Wilcoxon sign according to the distribution of the variables. The effect size was calculated with the following tests: cCohen's d or prank correlation; 95% CI: confidence interval. The results shown in bold had significant differences and moderate to large effect sizes. EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; SFAs: saturated fatty acids; MFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; GT: total fat in grams per 100 g of breast milk; Total *n-6*: total omega-6 fatty acids; Total *n-3*: total omega-3 fatty acids; *n-6/n-3*: ratio of omega-6 fatty acids to omega-3 fatty acids.

during pregnancy, and a high percentage had BMIs that remained within normal values. Excessive weight gain during pregnancy or a BMI above the adequacy range can negatively affect the composition of fatty acids such as DHA-22:6 *n-3* in breast milk (Barrera et al., 2018).

Sixty-four percent of the mothers had a low socioeconomic status, which can influence the acquisition and consumption of omega-3 source foods. As reported, the average consumption of DHA-22:6 *n*-3 in Western countries is 70–200 mg/day, and in some cases, the average intake is lower (30–70 mg/day) (Makrides, 2009; Sherry et al., 2015). To meet the recommended consumption of 200 mg/day DHA-20:6 *n*-3 from food sources alone, 150 g–350 g of fatty fish and shellfish would need to be consumed weekly, which is equivalent to 2–3 servings of approximately 100 g (Sherry et al., 2015; Agnieszka et al., 2019).

The simple frequency measure that was applied showed that the consumption of foods that are sources of omega-3 was low. The women included only some of these foods in their diet and did so sporadically, and no significant changes were noted between the two measurements;

Table 5
General linear model of repeated measures of some fatty acids in erythrocytes and breast milk.

Fatty acid	p value [a]	Effect size [a]	Adjusted p value[b]	Adjusted effect size [c]
ARA-20:4 *n-6*_E	0.097	−0.754	<0.0001	0.974
DHA-22:6 *n-3*_E	0.007	−1.315	0.002	0.816
DHA-22:6 *n-3*_LM	0.002	−0.859	0.006	0.747
ARA-20:4 *n-6*_LM	0.003	1.404	0.020	0.623
EPA-20:5 *n-3*_LM	0.307	−0.450	0.021	0.619
EPA-20:5 *n-3*_E	0.516	−0.211	0.436	0.104

[a] The p value and effect size were taken from Tables 3 and 4

[b] p value adjusted for the following variables: number of children, weight gain during pregnancy and education level.

[c] Adjusted effect size calculated with the partial Eta squared. ARA-20:4 *n-6*_E: arachidonic acid in erythrocytes; DHA-22:6 *n-3*_E: docosahexaenoic acid in erythrocytes; DHA-22:6 *n-3*_LM: docosahexaenoic acid in breast milk; ARA-20:4 *n-6*_LH: arachidonic acid in breast milk; EPA-20:5 *n-3*_LM: eicosapentaenoic acid in breast milk; EPA-20:5 *n-3*_E: eicosapentaenoic acid in erythrocytes.

therefore, we can say that the women in this study did not consume the recommended daily amounts of EPA-20:5 *n-3* and DHA-22:6 *n-3* from their diet, which is consistent with other studies: in Colombia, according to the 2015 National Nutritional Survey (Encuesta Nacional de Situación Nutricional - ENSIN), 65.3% of the adult population consumes fish and shellfish, but the frequency is less than one serving per week (ICBF, 2015); furthermore, in the 2019 food and nutritional profile of Antioquia, fish ranked 48th among the foods consumed, and its consumption was reported by only 6.1% of the population (de Antioquia, 2019).

Erythrocytes are a good biomarker of the habitual intake of fatty acids such as DHA-22:6 *n-3* and are not influenced by endogenous synthesis (Torres and Ney, 2006). Our study found that in erythrocytes, the fatty acids with the highest concentrations were palmitic acid-16:0, stearic acid-18:0 and oleic acid-18:1 *n*-9, which coincides with studies such as that by Walker et al. (2015).

The EPA-20:5 *n*-3 levels in the erythrocytes of the women in our study were minimal at the beginning of the study period, but after supplementation, they increased to an average concentration of 0.3679%. These values approached those reported in studies conducted in South Africa and Brazil, where the average concentrations of this fatty acid in women within the first two months of breastfeeding, without supplementation, were 0.4% and 0.33%, respectively (Siziba et al., 2020; Torres and Trugo, 2009), but they remain very far from the values reported in countries such as Chile, where the average is 1.03% (Barrera et al., 2018). Although the change between measurements was not significant in this study, EPA-20:5 *n-3* did show a slight increase, which is important because it is a precursor of DHA-20:6 *n-3* and ensures that this fatty acid can continue to be generated.

The concentration of DHA-20:6 *n*-3 in erythrocytes increased significantly between the two measurements. At baseline, the concentration was similar to that mentioned in studies from South Africa (3.8%) and Chile (3.96%) (Barrera et al., 2018; Siziba et al., 2020). In studies monitoring the concentration of fatty acids in the erythrocytes and plasma of lactating women who were not taking supplements, a decreasing trend in the DHA-20:6 *n-3* concentration was observed (Luxwolda et al., 2014; Jackson and Harris, 2016). A different situation was reported in studies that used fish oil-based supplements; those studies found positive and statistically significant changes in the concentrations of EPA-20:5 *n*-3 and DHA-20:6 *n*-3 in the erythrocytes and plasma of breastfeeding women (Walker et al., 2015; Jensen et al., 2000).

Breast milk fatty acids can be obtained via mobilization of maternal reserves, mainly adipose tissue; from the endogenous synthesis in the liver and breast tissue; and from dietary sources (Luxwolda et al., 2014; Siziba et al., 2020; Amaral et al., 2017). Our study found that the fatty acids with the highest concentrations in milk were palmitic acid-16:0, oleic acid-18:1 *n*-9 and linoleic acid-18:2 *n*-6, which is consistent with studies of Spanish (Sánchez-Hernández et al., 2019), Greek (Antonakou et al., 2013) and Chilean (Barrera et al., 2018) women.

The PUFA content in breast milk has been suggested to reflect both long-term and short-term eating habits. PUFAs vary considerably among countries, populations and even individuals, especially DHA-20:6 *n-3*, which has been shown to have an adequate dose–response ratio and comprises <0.1% to >1.0% of the total fatty acids (Brenna et al., 2007). This variability depends on the usual intake of foods such as fatty fish and other marine products or the consumption of supplements (Amaral et al., 2017).

A DHA-20:6 *n*-3 content of 8.0% in maternal erythrocytes has been determined to correspond to a 1% content of DHA-20:6 *n*-3 in breast milk (Luxwolda et al., 2014). A DHA content greater than 6% in erythrocytes has been found in populations with high and frequent consumption of food sources of EPA-20:5 *n-3* and DHA-20:6 *n-3*, as is the case of some tribes in Tanzania and populations of countries such as Japan, China and the Philippines; this condition provides women with adequate adipocyte and hepatic reserves of these fatty acids and, during lactation, higher DHA-20: 6 *n*-3 contents in their breast milk (Luxwolda et al., 2014; Koletzko et al., 2014). However, for the Western population, these percentages are very difficult to obtain due to the low consumption of food sources of omega-3 and the imbalance in the consumption of omega-6 and omega-3.

The recommendation of different international organizations to consume a minimum of 200 mg per day of DHA-20:6 *n-3* would lead to a concentration of 0.3% in breast milk, at which beneficial effects on the health of the baby are observed (Jackson and Harris, 2016; Koletzko et al., 2014). Our study provided 250 mg of DHA and 100 mg of EPA at a dose that is within the parameters recommended by the FAO/WHO and found that at the beginning of the study, before receiving supplementation, the participants had a median DHA-20:6 *n*-3 concentration in breast milk of 0.19%. This value is similar to that found in a study conducted in the United States, which reported a median of 0.18% (Juber et al., 2016), and is below those reported in other studies conducted in Greece and Spain among women during similar postpartum periods who did not receive supplementation, which reported mean DHA-20:6 *n*-3 concentrations of 0.55% (Antonakou et al., 2013) and 0.39% (S á nchez-Hern á ndez et al., 2019), respectively.

After supplementation, the median DHA-20:6 *n-3* concentration in breast milk increased by 57%–0.29%, approaching the recommended cut-off point and the world average reported by Brenna et al. (2007), who, in an analysis of various studies conducted throughout the world, found an average DHA-20:6 *n-3* concentration of 0.32 ± 0.22% in breast milk, with a range of 0.06–1.4%. This change confirms the importance of EPA-20:5 *n-3* and DHA-20:6 *n-3* supplementation in countries with low development and low availability of, access to and/or consumption of foods of marine origin, since without the consumption of those foods, the DHA-20:6 *n*-3 content in breast milk tends to decrease, compromising the supply to the baby and the reserves of the mother (Barrera et al., 2018; Fidler et al., 2000).

The increase in these omega-3 fatty acids in erythrocytes and breast milk in this study is reflected in the total *n*-3, which increased significantly and in turn influenced the *n-6/n-3* ratio, which decreased significantly after supplementation from 7:1 to 6:1 in erythrocytes and from 15:1 to 12:1 in breast milk. According to a hypothesis presented by Ailhaud et al. a reduced proportion of *n-6/n-3* fatty acids in the maternal diet can help limit the early growth of adipose tissue in the offspring and can therefore represent a new strategy for the primary prevention of childhood obesity (Much et al., 2013; Ailhaud et al., 2007). In addition, an imbalance in the consumption of these fatty acids affects the action of the Δ5 and Δ6 desaturases, which decreases the efficiency of DHA-20:6 *n-3* production from ALA-18:3 *n-3* (Barrera et al., 2018).

Multiple factors can modify the fatty acid content in breast milk, especially that of DHA-20:6 *n*-3 (Fu et al., 2016), including excess weight gain during pregnancy and a lack of micronutrients such as zinc, magnesium, calcium and vitamins B6 and C, which can negatively affect the action of desaturases Δ5 and Δ6 (Barrera et al., 2018). The number of pregnancies, alcohol consumption, tobacco use and age can also affect the availability and reserve of long-chain fatty acids (Barrera et al., 2018; Vergilio Visentainer et al., 2016). The repeated measures model was adjusted for some of these variables and was applied to the PUFAs of interest, including DHA-20:6 *n-3* in erythrocytes and breast milk; the results indicated that these variables did not affect the change in these PUFAs after supplementation and did not influence the final study results for this fatty acid.

Among the limitations of the study are the nonquantitative evaluation of food consumption due to the lack of information about the fatty acid contents of the foods; the lack of a control group and therefore nonrandomization of the study; and the size of the sample, which does not allow generalization of the results to the entire Colombian population. Despite these limitations, our study is the first to report data on the nutritional status of DHA-20:6 *n*-3 in two specimen types in breastfeeding women in Colombia. Another strength was the monitoring of the daily intake of the supplement, which helped maintain adherence to treatment during the three months of the study. We were also able to ensure that the babies were exclusively breastfed for at least four months, which was the average duration of the investigation.

5. Conclusions

This study allows us to conclude that in this population group a more adequate profile of fatty acids was presented with the use of the supplement, improving the concentration of DHA both in erythrocytes and in breast milk, it also decreases the n-6/n-3 ratio, which has positive effects on the mother, who maintains more adequate levels in her blood and, in turn, may pass on amounts of DHA that are considered adequate to her child through breastfeeding.

This is the first study in Colombia to evaluate supplementation with EPA + DHA at the doses recommended by the FAO/WHO and to report a significant change in the concentration of DHA-20:6 *n-3* in both erythrocytes and milk to levels approaching the reported global average and the recommended DHA-20:6 *n-3* concentration of 0.3%. This finding is important because at this minimal concentration, benefits for the brain, cognitive, visual and immunomodulation development of the baby begin to be observed.

Supplementation with EPA-20:5 *n-3* and DHA-20:6 *n-3* at recommended doses should be promoted for women during gestation and lactation, especially in populations where the consumption of food sources of omega-3 and foods of marine origin is limited.

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Authorship

All authors of this study contributed equally to the processes of conceptualization, analysis, research, methodology, project management, validation, writing, review and editing.

Ethical considerations

The study was carried out in accordance with the codes of ethics and

complied with the Declaration of Helsinki for studies with humans and was approved by the ethics committees of the ESE San Juan de Dios Hospital, with a certificate dated February 19, 2019, and those of the University Research Headquarters of the University of Antioquia, with approval certificate 19-93-833 of April 25, 2019.

CRediT authorship contribution statement

Alejandra Valencia-Naranjo: Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Luz M. Manjarres-Correa:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Juliana A. Bermúdez-Cardona:** Methodology, Investigation, Visualization, Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: This research was funded by Laboratorios Laproff SA, which donated fresh and in-kind resources (supplement) equivalent to 33% of the total value of the research. The other 67% of fresh and in-kind resources for the research were granted by the University of Antioquia.

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