Supplementation with the omega-3 docosahexaenoic acid: influence on the lipid composition and fatty acid profile of human milk

Suplementação com ácido graxo ômega-3 docosahexaenoico: influência sobre a composição lipídica e perfil de ácidos graxos no leite humano

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A B S T R A C T

Objective
This study assessed the impact of supplementing the diet of women during pregnancy and lactation with fish oil containing the omega-3 fatty acid docosahexaenoic acid, and its influence on the composition of human milk.

Methods
The sample comprised 60 women aged 18 to 38 years with appropriate dietary pattern, all of them healthy and nonsmokers. The intervention consisted of a daily supplementation with fish oil capsules that corresponded to a daily intake of 315mg of docosahexaenoic acid and 80mg of eicosapentaenoic acid during the third
trimester of pregnancy and the first three months postpartum. The total fat content and fatty acid profile of their milk were determined by crematocrit and gas chromatography. Descriptive statistics were used for data analysis and the significance level was set at $p<0.05$.

**Results**

There was no statistical difference between the fat contents of the study (fish oil capsules) and control (capsules containing corn starch as filler) groups. However, the milk of women taking fish oil contained higher docosahexaenoic and eicosapentaenoic acid levels 30 and 60 days after delivery. These results demonstrate that high omega-3 intake can influence its concentration in human milk.

**Conclusions**

Given the importance of docosahexaenoic acid in the neonatal period, it is appropriate for pregnant and breastfeeding women to supplement on long-chain polyunsaturated fatty acids, which may be done by adding fish oil to the regular diet.

**Indexing terms**: Docosahexaenoic acids. Pregnant women. Lactation. Milk, human. Supplementation feeding.

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**INTRODUCTION**

The Long-Chain Polyunsaturated Fatty Acids (LC-PUFA) of the omega-3 series, such as Docosahexaenoic Acid (DHA), Eicosapentaenoic Acid (EPA) and Arachidonic Acid (AA), are important components of cell membranes and central nervous system cells, and are essential for fetal development and development during the first months of life$^{1-3}$.

The brain develops during the third trimester of pregnancy, when the active formation of neurons begins, and this increases DHA requirement considerably$^4$. The amount of DHA that the fetus is capable of synthesizing from its precursors is limited, so it is supplied by the
placenta, which takes it from the maternal plasma at a rate of 60-70 mg/day and transfers it to the fetal plasma.

After birth, polyunsaturated fatty acids are transferred from mother to infant through milk since the infant’s liver is immature and still incapable of synthesizing long-chain polyunsaturated fatty acids, which are important for the development of the nervous system. Adequate DHA intake has been associated with better cognitive development, growth and visual acuity.

During pregnancy, some situations are capable of changing the availability of LC-PUFA, such as inappropriate nutrition, intake of fats and oils having a high omega-6 to omega-3 ratio, and multiple and frequent pregnancies, factors that can significantly decrease the body reserves of these acids. The significant demand and accumulation of DHA by the fetus may cause an important reduction in the mother’s body reserve of this acid, justifying its supplementation, especially when pregnancies are frequent or multiple.

After birth, DHA required by the newborn is ensured by the mother’s milk which contains a small but significant amount of DHA (0.2-0.6% of the milk fat) but the amount varies according to the dietary habits of the mother. Maternal DHA intake has a significant impact on its concentration in her plasma and milk. Supplementation with LC-PUFA, especially omega-3, during pregnancy and lactation favors the mental development of the child.

The objective of the present study was to assess the impact of supplementing the diet of Brazilian pregnant and lactating women living in the state of Paraná with the omega-3 fatty acid DHA in the form of fish oil by verifying its influence on the lipid profile of their milk, especially on DHA content.

METHODS

The study consisted of a randomized, placebo-controlled trial involving pregnant women who received prenatal care in an obstetric healthcare facility at the municipality of Ponta Grossa, Paraná. The inclusion criteria were: non-smoker, healthy pregnant women aged 18 to 38 years in the last trimester of pregnancy, no high-risk pregnancy and appropriate dietary patterns. Clinical data were obtained from their medical records. Twenty-four-hour recalls were used for assessing their dietary patterns. They were administered on alternating days during the same week, including a weekend day. Food intake was assessed using 24-hour recalls.
was reported in preestablished cooking units by the pregnant women during the prenatal visits and by lactating women during home visits. A qualitative analysis was done based on the Food Pyramid Guide. This guide recommends the number of portions according to food groups, which are grains (breads, cereals, roots, tubers); vegetables; fruits; meats and milk. During the study period, the recruited women received dietary advice from a dietitian once a month.

The intervention period consisted of the last trimester of pregnancy and the first three months of lactation, since DHA is an important component of the nervous system and retinal membranes of the fetus, and accumulates mainly during the last months of intrauterine life and the first months of life.

The study was done from 2007 to 2008. Its experimental design was approved by the Ethics Committee of the Health Sciences Sector of the Federal University of Paraná, under protocol number 095.SM058/04-07. All participants signed a free and informed consent form before they joined the study.

The casuistic consisted of 80 pregnant women divided into two groups of 40. The minimum sample size was determined according to Triola. The greatest difficulty regarded estimating the variation (standard deviation) of the data. In order to minimize errors in the mean estimates, the standard deviation was calculated from the results obtained by Patin et al. and Jensen et al. The mean standard deviation was considered to be 0.35 and the maximum error margin 0.11. Considering a significance level of 5%, the study would need a sample size of at least 40 pregnant women.

The 80 participants were randomly divided into two groups of 40 women: the study group who received Omega-3 Supplementation (OG) and the Control Group (CG). The intervention consisted of daily supplementation with five gel capsules of fish oil rich in DHA (Anew Co), totaling 1,150mg of fish oil, 315mg of DHA and 80mg of EPA, according to the recommendation for pregnant and lactating women, which is 300mg/day of DHA. The composition of the capsules was confirmed by chromatographic analysis. The CG received gel capsules containing cornstarch as filler.

Each milk sample was qualitatively and quantitatively analyzed three times for determining their total fat content and fatty acid profile. The samples were collected as recommended by the Brazilian Pediatric Society (Sociedade Brasileira de Pediatria) 30, 60 (time 60) and 90 (time 90) days after delivery. Collection was done at the participants’ homes by hand expression. The samples were stored in a sterile recipient, transported in a cooler, frozen and thawed in a water bath at 38°C when ready for analysis.

Total fat content was determined by the creamatocrit method adapted for human milk. The omega-3 fatty acids were assessed by gas chromatography using the gas chromatogapher Varian, model 3900. The chromatographic conditions were: programmed column temperature; initial temperature of 120°C/5min, heating from 120°C to 220°C (3°C/min) and from 220°C to 235°C (1°C/min), staying at 235°C for 12 minutes; the carrier gas was hydrogen at a flow rate of 1mL/min; the make-up gas was nitrogen at a flow rate of 30mL/min; injector temperature: 270°C; detector temperature: 300°C; injection volume: 1µL.

For the chromatographic analysis, the samples were prepared by making small changes to the method proposed by Bligh & Dyer. In the extraction phase, the samples were thawed to about 4°C and homogenized. Each 1mL aliquot of milk received 10mL of methanol, 10mL of chloroform and 1.3mL of water. The mixture was stirred for 20 minutes followed by extraction, whose filtrate in a vacuum Buchner funnel was transferred to a decanting funnel. The filtrate received 10mL of chloroform and 5mL of anhydrous sodium sulfate (2%). After stirring and decantation, the bottom phase was filtered using filter paper with anhydrous sulfate. The sample was dried under nitrogen. For sterification, 5mL...
of potassium hydroxide in methanol were added to the aliquot, keeping it in a water bath at 70°C for 15 minutes. Once cooled, 15mL of the esterification reagent were added, and the solution submitted to another water bath at 70°C for 10 minutes. After esterification, 10mL of hexane and 15mL of distilled water were added and stirred. After decantation, a Pasteur pipette was used for collecting the solvent fraction and transferring it to test tubes followed by filtration with anhydrous sulfate. Lastly, 15mL of water was added, followed by decantation and collection with a Pasteur pipette. The fatty acids were identified by comparing the retention time of the fatty acids of the samples and standards. A total of 37 saturated, monounsaturated and polyunsaturated fatty acid standards were used (Supelco 37 Component FAME Mix - 47885-U). Fatty acid quantification was done by normalization of the area and the results were expressed in grams per 100g of sample.

All data were expressed by descriptive statistics (mean and standard deviation). Next, boxplot diagrams were used for finding possible outliers, which were excluded. Hypothesis testing using analysis of variance was used for verifying the possibility of differences between the means and later, the Tukey test was used for identifying possible significant differences between the means. The significance level for all calculations was set at 5% (p<0.05). The software Minitab version 15 was used for all the statistical tests.

**RESULTS**

A group of 60 women completed the study, 25 from the OG and 25 from the CG. These women had a mean age of 25 years and their education level varied from elementary school (34%; n=20) to high school (37%, n=22). Most had a mean family in come of two to four minimum wages (57%; n=34). All of them had appropriate dietary patterns for pregnant and lactating women during the entire intervention period (Table 1).

Table 2 shows the lipid profiles of the milk of the two groups. The only significant difference found in DHA content occurred for the intervention group between times OGT30 and OGT90 (p=0.026). The DHA content of the milk of the control group did not vary during the entire study period (p=0.939). The total fat content of the two groups also did not differ significantly (p=0.390).

Table 3 shows the fatty acid profiles of the groups OG and CG (total percentage of fatty acids) 30, 60 and 90 days after delivery. The DHA and EPA contents (Table 4) of the milk of the mothers in the study group (OG) did not differ significantly during the study period (DHA, p=0.368; EPA, p=768). The DHA and EPA contents of the milk of the mothers in the control group

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**Table 1.** Dietary pattern of the pregnant and lactating women assessed during the last trimester of pregnancy and first three months of lactation. Ponta Grossa (PR), Brazil, 2007-2008.

| Group | Milk | Meat | Grains | Vegetables | Fruits |
|-------|------|------|--------|------------|--------|
| Recommended | 3 | 2 | 9 | 4 | 3 |
| Dietary pattern during pregnancy | 3 | 2 | 9 | 4 | 3 |
| Dietary pattern during lactation | 3 | 2 | 10 | 4 | 3 |

*Philippi et al.*

**Table 2.** Mean total lipids* for the study group (OG) and Control Group (CG) on different occasions. Ponta Grossa (PR), Brazil, 2007-2008.

| Variables | Mean | Standard deviation |
|-----------|------|-------------------|
| OGT30** | 3.2000 | 0.6121 |
| OGT60 | 2.7000 | 0.9832 |
| OGT90** | 2.2625 | 0.7891 |
| CGT30 | 2.7846 | 0.7519 |
| CGT60 | 2.7364 | 0.5957 |
| CGT90 | 2.7100 | 0.8258 |

*expressed as g% of the total fatty acids.

**significant difference between OGT30 and OGT90.

OGT30, OGT60 and OGT90 - Mean total lipids of the study group 30, 60 and 90 days after delivery, respectively.

CGT30, CGT60 and CGT90 - Mean total lipids of the control group 30, 60 and 90 days after delivery, respectively.

OG: Group Who Received Omega-3 Supplementation.
(CG) also did not differ significantly during the study period (DHA, \( p=0.298 \); EPA, \( p=0.475 \)).

Although the total fat content of the milk of the two groups (OG and CG) did not differ, the DHA and EPA contents of the milk of the two groups differed significantly (\( p<0.001 \)). The pairs of means that differed statistically (\( p<0.05 \)) were: DHAOG30 and DHACG30 (DHA content of the study and control groups 30 days after delivery) and DHAOG60 and DHACG60 (DHA content of the control and study groups 60 days after delivery).

Table 3  Fatty acid profile of the study human milk* (% of the total fatty acids). Ponta Grossa (PR), Brazil, 2007-2008.

| Fatty acids | Days | 30 d | 60 d | 90 d | 30 d | 60 d | 90 d |
|-------------|------|------|------|------|------|------|------|
| C14:0       |      |      |      |      |      |      |      |
| C15:0       |      |      |      |      |      |      |      |
| C16:0       |      |      |      |      |      |      |      |
| C17:0       |      |      |      |      |      |      |      |
| C18:0       |      |      |      |      |      |      |      |
| C18:1       |      |      |      |      |      |      |      |
| C18:2       |      |      |      |      |      |      |      |
| C18:3       |      |      |      |      |      |      |      |
| C18:4       |      |      |      |      |      |      |      |
| C20:0       |      |      |      |      |      |      |      |
| C20:1       |      |      |      |      |      |      |      |
| C20:2       |      |      |      |      |      |      |      |
| C20:3       |      |      |      |      |      |      |      |
| C20:4       |      |      |      |      |      |      |      |
| C20:5       |      |      |      |      |      |      |      |
| C20:6       |      |      |      |      |      |      |      |
| C22:6       |      |      |      |      |      |      |      |

*OG: Study group consisting of volunteers who received fish oil capsules containing omega-3 fatty acids; CG: Control Group consisting of volunteers who received placebo; EPA: Eicosapentaenoic Acid; DHA: Docosahexaenoic Acid.
The participants who remained in the study (n=60) had similar socioeconomic characteristics, and little variation in education level (elementary and high school) and family income (two to four minimum wages). They received nutritional follow-up during the study period and their mean food intake, determined by three dietary recalls, was within the recommendations. Their food intake did not vary significantly throughout the study period.

The mean fat contents at OGT30 were close to those reported by Rona et al.³⁵ and Aksit et al.³⁶, who studied the fat composition of human milk by creamatocrit. Variation of the total fat content of the milk of the study group OG during the lactating period was similar to those found by other studies³⁶,³⁷, showing that fat content decreases over time, possibly because of a reduction in the maternal fat reserves²⁴. However, the total fat contents were within the ranges found in the literature for mature milk³⁸.

The same variation was not found in the CG, who presented similar fat contents 30 and 90 days after delivery. Likewise, there was no variation between the two groups on the different occasions, showing that omega-3 supplementation in the proposed dosage did not influence the total fat content of the milk. This result was expected because the supplementation consisted of 1,150mg of fish oil, and the women had similar dietary patterns.

The fatty acid profile of the two groups presented some variations, but the differences were not uniform. The fat profile found for the CG were similar to those found by Silva et al.³⁹, Cunha et al.⁴⁰ and Patin et al.²¹, while studying the fat profile of the milk of Brazilian women, including EPA and DHA. Trans fatty acids were also found by Kolelko et al.¹⁷ and by authors of other countries whose results were discussed by Costa & Sabarense²⁴.

Omega-6 (C20:2) fatty acid content of the OG during the entire study period was similar to that found by Silva et al.³⁹ and other studies done on human milk in other countries²⁴, showing that supplementation with 300mg/day of omega-3 did not affect the concentration of omega-6, despite the fact that these fatty acids compete for the same metabolic pathways¹¹,¹²,²⁴. Short-chain fatty acids (C4:0) were found only in the milk of the control group CG. These fatty acids were not found in human milk by other studies²¹,³⁹ possibly because a high-carbohydrate diet favors the endogenous synthesis of short- and medium-chain fatty acids, while a diet high in polyunsaturated fatty acids results in a higher concentration of the latter in the milk⁴¹.

Human milk is a natural source of Conjugated Linoleic Acid (CLA) but its concentration was higher 30 and 60 days after delivery in the milk of the study group (OG, 0.30% and 0.33% respectively) than in that of the control group. This difference is associated with variation in CLA intake, since humans do not synthesize this fatty acid⁴².

The docosahexaenoic acid and eicosapentaenoic acid contents of the milk of mothers who received supplementation OG were consistently lower than those found in the milk of breastfeeding mothers without supplementation. However, these differences were not statistically significant.

### Table 4

DHA and EPA contents* in the milk of the study group (OG) and Control Group (CG) on different occasions. Ponta Grossa (PR), Brazil, 2007-2008.

| Variables | Mean  | Mean standard error | Standard deviation |
|-----------|-------|---------------------|--------------------|
| (OG)C22:6 (T30)* | 0.3238 | 0.0365 | 0.1670 |
| (OG)C22:6 (T60)* | 0.3310 | 0.1200 | 0.4330 |
| (OG)C22:6 (T90) | 0.2071 | 0.0412 | 0.1542 |
| (OG)C20:5 (T30) | 0.1095 | 0.0153 | 0.0700 |
| (OG)C20:5 (T60) | 0.0923 | 0.0178 | 0.0641 |
| (OG)C20:5 (T90) | 0.1071 | 0.0195 | 0.0730 |
| (CG)C22:6 (T30) | 0.1040 | 0.0313 | 0.1567 |
| (CG)C22:6 (T60) | 0.0778 | 0.0222 | 0.0667 |
| (CG)C22:6 (T90) | 0.0300 | 0.0153 | 0.0483 |
| (CG)C20:5 (T30) | 0.0840 | 0.0160 | 0.0800 |
| (CG)C20:5 (T60) | 0.0778 | 0.0147 | 0.0441 |
| (CG)C20:5 (T90) | 0.0500 | 0.0224 | 0.0707 |

*expressed as % of the total fatty acids; a statistically different from (CG) C22:6 (DHA); b statistically different from (CG) C22:6.

DHA: Docosahexaenoic Acid; EPA: Eicosapentaenoic Acid; OG: Group Who Received Omega-3 Supplementation.
also different from those of the mothers who did not receive supplementation CG. The DHA and EPA contents of the study group OG were close to those found by Marangoni et al.43, who studied the composition of the milk of Italian women and by Jensen et al.30, who also provided fish oil supplementation to their study sample.

The milk of the group that received fish oil supplementation OG had a significantly higher DHA content 30 and 60 days after delivery than the other group CG. This suggests that higher DHA intake may influence its content in human milk, especially during the first weeks after delivery. This result is in agreement with those of other studies that demonstrated the positive influence of omega-3 supplementation on the milk of mothers taking such supplements5,17,21,28. Lauritzen et al.44 also observed an increase in the omega-3 content of human milk after fish oil supplementation.

The quality of the fat in the mother’s diet may not influence the total fat content of the milk, but influences the fatty acid profile of the milk. This has been observed by the present and other studies21,28. The highest DHA requirement occurs during the last trimester of pregnancy and first months of life. Therefore, a high intake of this nutrient by the mother increases its bioavailability to the fetus by placental transport and to the infant by milk2,17,28.

The significant impact of DHA intake on the composition of human milk reinforces the recommendation of frequent seafood intake, as recommended by Gaete & Atalah6, or fish oil supplementation (300mg/day of DHA). Jensen28 argues that 300mg/day of DHA may increase the amount of this fatty acid in human milk. It is noteworthy that Brazilian women do not have the habit of consuming fish twice a week, which would help them to meet their DHA requirement45.

**CONCLUSION**

The results of the present study showed that supplementation with fish oil providing 300mg/day of DHA in women with appropriate dietary patterns increased the amount of this fatty acid in their milk but did not change the total fat content of the milk. Ideally, pregnant and lactating women should keep their DHA status always high, supplementing if necessary.

**ACKNOWLEDGMENTS**

We thank the company Anew Co. for donating the fish oil capsules. We thank the city hall of Ponta Grossa for authorizing the study in their healthcare facility. And most of all, we thank the mothers who voluntarily participated in the study.

**COLLABORATORS**

EAFQ BORTOLOZO helped to develop the research project and participated in all stages of the study. E SAUER, MS SANTOS and S BAGGIO helped to perform the creamatocrit and chromatographic analyses. G SANTOS JUNIOR planned and performed the statistical analyses. PV FARAGO made the placebo capsules, analyzed the results and helped to write the article. LMB CÂNDIDO helped to plan the study, collect data from the literature, collect milk samples and write the article. LA PILATTI helped to plan the study, analyze the literature data and write the article.

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Received on: 14/7/2011
Final version on: 19/9/2012
Approved on: 2/10/2012