

## **Evaluation of polyunsaturated fatty acids content in the three stages of breast milk: colostrum, transitional and mature milk of a nursing mother submitted to Roux-en-Y gastric bypass surgery two months prior to pregnancy: Case study**

**Avaliação do teor de ácidos graxos poliinsaturados nos três estágios do leite materno: colostro, leite de transição e leite maduro de nutriz submetida à cirurgia de bypass gástrico em Y-de-Roux dois meses antes da gravidez: Estudo de caso**

**Evaluación del contenido de ácidos grasos poliinsaturados en las tres etapas de la leche materna: calostro, leche de transición y leche madura de una madre lactante intervenida de bypass gástrico en Y de Roux dos meses antes del embarazo: Estudio de caso**

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### **Abstract**

The purpose of this case study was to investigate polyunsaturated fatty acids in colostrum, transitional milk and mature milk of a nursing mother attended to Roux-en-Y gastric bypass two months prior to pregnancy. For this, was collected 2ml (colostrum), 4ml (transitional milk) and 4ml (mature milk) samples obtained by manual expression of the breast that was not sucked in the last feeding, in the morning and placed in Eppendorfs-type microtubes. The fatty acid composition was performed by means of gas chromatography. The mean proportions of fat in colostrum, transition milk and mature milk were: eicosapentaenoic acid: 0.06%; 0.05% and 0.06%; docosahexaenoic acid: 0.12%, 0.08% and 0.09%; arachidonic acid: 0.58%, 0.37% and 0.42%, respectively; trans fatty from the industrial process: 0.11%, 0.08% and 0.12% and trans fatty from ruminant sources (0.09%, 0.10% and 0.12%, respectively). The relevance of this case study lies in the fact that it shares knowledge about on the fatty acid composition of breast milk from a nursing mother post Roux-en-Y gastric bypass. The mean linoleic acid and alpha-linolenic acid found in the mature milk was below the means found in studies carried out in Brazil from eutrophic women who did not underwent bariatric surgery. In this study, trans fatty acids were found in maternal milk but considered low values. As a relevant

recommendation for future work, it is suggested that research be carried out with a larger population undergoing such a procedure to investigate the composition of polyunsaturated fatty acids in breast milk.

**Keywords:** Bariatric surgery; Human milk; Fatty acid.

### Resumo

O objetivo deste estudo de caso foi investigar os ácidos graxos poliinsaturados no colostro, leite de transição e leite maduro de uma nutriz submetida a bypass gástrico em Y-de-Roux dois meses antes da gravidez. Para isso, foram coletadas amostras de 2ml (colostro), 4ml (leite de transição) e 4ml (leite maduro) obtidas por ordenha manual da mama que não foi sugada na última mamada, no período da manhã e colocadas em microtubos tipo Eppendorfs. A composição dos ácidos graxos foi realizada por meio de cromatografia gasosa. As proporções médias de gordura no colostro, leite de transição e leite maduro foram: ácido eicosapentaenóico: 0,06%; 0,05% e 0,06%; ácido docosahexaenóico: 0,12%, 0,08% e 0,09%; ácido araquidônico: 0,58%, 0,37% e 0,42%, respectivamente; gordura trans de processo industrial: 0,11%, 0,08% e 0,12% e gordura trans de origem ruminante (0,09%, 0,10% e 0,12%, respectivamente). A relevância deste estudo de caso reside no fato de compartilhar conhecimento sobre a composição de ácidos graxos do leite materno de uma nutriz, pós bypass gástrico em Y-de-Roux. As médias de ácido linoleico e ácido alfa-linolênico encontradas no leite maduro ficaram abaixo das médias encontradas em estudos realizados no Brasil com mulheres eutróficas que não realizaram CB. Neste estudo, os ácidos graxos trans foram encontrados no leite materno, mas em valores considerados baixos. Como recomendação relevante para trabalhos futuros, sugere-se a realização de pesquisas com uma população maior submetida a tal procedimento para investigar a composição de ácidos graxos poliinsaturados no leite materno.

**Palavras-chave:** Cirurgia bariátrica; Leite humano; Ácido graxo.

### Resumen

El propósito de este estudio de caso fue investigar los ácidos grasos poliinsaturados en el calostro, la leche de transición y la leche madura de una madre lactante que se sometió a un bypass gástrico en Y de Roux dos meses antes del embarazo. Para ello se recolectaron muestras de 2ml (calostro), 4ml (leche de transición) y 4ml (leche madura), obtenidas por ordeño manual de la mama que no fue succionada en la última toma, en la mañana, y colocadas en recipientes tipo Eppendorf. microtubos. La composición de ácidos grasos se realizó mediante cromatografía de gases. Las proporciones medias de grasa en calostro, leche de transición y madura fueron: ácido eicosapentaenoico: 0,06%; 0,05% y 0,06%; ácido docosahexaenoico: 0,12%, 0,08% y 0,09%; ácido araquidónico: 0,58%, 0,37% y 0,42%, respectivamente; grasas trans de proceso industrial: 0,11%, 0,08% y 0,12% y grasas trans de ruminantes (0,09%, 0,10% y 0,12%, respectivamente). La relevancia de este estudio de caso radica en que comparte conocimientos sobre la composición de ácidos grasos de leche de una madre lactante, después de un bypass gástrico en Y de Roux. Los promedios de ácido linoleico y ácido alfa-linolénico encontrados en la leche madura fueron inferiores a los promedios encontrados en estudios realizados en Brasil con mujeres eutróficas que no se sometieron a BS. este estudio se encontraron ácidos grasos trans en la leche materna, pero en valores considerados bajos. Como recomendación relevante para trabajos futuros, se sugiere realizar investigaciones con una población mayor sometida a tal procedimiento para investigar la composición de poliinsaturados ácidos grasos en la leche materna.

**Palabras clave:** Cirugía bariátrica; Leche materna; Ácidos grasos.

## 1. Introduction

Currently, bariatric surgery (BS) is considered the most effective tool in the control and treatment of severe obesity (Zilberstein et al., 2019).

The main type of BS performed in Brazil, considered the gold standard, is the Roux-en-Y gastric bypass (RYGB) due to its safety and, mainly, its effectiveness (SBCBM, 2017).

Due to alteration of the gastrointestinal transit that impairs the pathways of absorption and/or food intake, post-BS nutritional deficiencies are common (Zaparolli, et al., 2018), both in the short and long term, which can cause severe clinical impacts (Ledoux, et al., 2020). According to Gascoin et al. (2017) micronutrient deficiencies increase after BS. Furthermore, exocrine pancreatic insufficiency is very common after RYGB (Uribarri-Gonzalez, et al., 2021).

Forbes et al. (2016) investigated the impact of BS on essential fatty acid (EFA) status in non-pregnant women undergoing RYGB. However, no studies on quantify polyunsaturated fatty acids in colostrum, transitional milk and mature milk of a nursing mother with previous bariatric surgery were found.

The objective of the case study was to quantify polyunsaturated fatty acids in colostrum (COL), transitional milk (TM) and mature milk (MM) of a nursing mother submitted to Roux-en-Y gastric bypass two months prior to pregnancy.

## 2. Methodology

This article is a case study. According to Pereira et al. (2018), a case study is a description and analysis, as detailed as possible, of a case that presented some particularity that makes it special and can bring a wealth of data and information in order to contribute to knowledge in the area in which one is used. In this type of study, patient can be seen as a case with its particularities.

In this study the patient signed the Free and Informed Consent Term to participate in the research and stated that she agreed with the publication of the study. The research project was approved by the Research Ethics Committee of the National Institute for Women, Children and Adolescents' Health Fernandes Figueira / Oswaldo Cruz Foundation - IFF / Fiocruz, under number CAAE: 56999422.9.0000.5269, following resolution 466/2012.

COL (2ml), TM (4ml) and MM (4ml) samples were obtained by manual expression of the breast that was not sucked in the last feeding, in the morning and placed in Eppendorfs-type microtubes.

All breast milk (BM) samples were immediately stored in a -20°C freezer and subsequently transported on ice to the Nutritional Biochemistry Laboratory of the Josué de Castro Institute of Nutrition/Federal University of Rio de Janeiro - INJC/UFRJ, where they remained stored at -80°C, until the separation of lipids and quantification of fatty acids (FAs).

For the analysis of fatty acids (FAs), the techniques described by Folch et al. (1957) and Lepage & Roy (1986).

FAs methyl esters (FAME) were quantified on the Perkin Elmer Autosystem XL Chromatograph, as described in another study (Costa et al., 2011). FAs concentrations were expressed as the percentage of the total area comprising all FAs peaks (% of total FAME).

For statistical analysis, we estimated percentages, means and standard deviations.

## 3. Case Study

This is a 29-year-old woman who underwent Roux-en-Y gastric bypass (RYGB) two months prior to pregnancy.

At 30 weeks of gestation, she began receiving nutritional care at the Nutrition outpatient clinic of the IFF / Fiocruz.

Table 1 shows maternal and newborn characteristics.

**Table 1** – Maternal and newborn characteristics recorded in the protocol of the Nutrition outpatient clinic of a National Institute, Rio de Janeiro-RJ. January-March, 2018.

<b>Maternal characteristics</b>	
Age at the time of pregnancy (years)	29
Bariatric surgery type	Roux-en-Y <i>Bypass</i>
BMI before surgery (kg/m <sup>2</sup> )	40,89
Pre-pregnancy BMI (kg/m <sup>2</sup> )	26,04
BMI before labor (kg/m <sup>2</sup> )	26,55
Weight gain during pregnancy (kg)	1,33
Time between second pregnancy and start of current pregnancy (months)	12
Time between surgery and onset of pregnancy (months)	02
Length of pregnancy (weeks)	38
Ballard (weeks)	39
Delivery type	Cesarean
Maternal complications	
- Preoperative diagnosis: PHD + iteractivity	
- Postoperative diagnosis: PHD + iteractivity + uterine rupture with newborn covered by uterine serous film	
Calorie-protein supplementation use	No
<b>Newborn characteristics</b>	
Sex	Female
Birth weight (g)	3.280
Birth length (cm)	46,4
Head circumference (cm)	35,4
Apgar (1/5 minute)	6/9

BMI - body mass index; PHD – Perinatal Hemolytic Disease. Source: Elaborated by the authors (2023).

#### 4. Results and Discussion

Analysis results of the composition (%) of polyunsaturated FAs in samples of COL, TM and MM of the nursing mother are shown in Table 2.

**Table 2** - Composition of fatty acids (% of total FAME) in samples of colostrum, transitional milk and mature milk of nursing. Rio de Janeiro - RJ. January-March, 2018.

PUFA (PUFA and LC-PUFA)	COLOSTRUM (mean±SD)	TRANSITIONAL MILK (mean±SD)	MATURE MILK (mean±SD)
<b>TFA (processed foods)</b> <b>(C18:2 8t; C18:2 11t; C18:2 9t)</b>	0,11±0,03	0,08±0,03	0,12±0,03
<b>CLA</b> <b>C18:2 9c 11t</b>	0,09±0,01	0,10±0,01	0,12±0,01
<b>LNA</b> <b>C18:2 n-6</b>	10,49±0,29	7,8±0,23	12,65±0,63
<b>ALA</b> <b>C18:3n-3</b>	0,74±0,03	0,38±0,02	0,77±0,05
<b>C20:2 n-6</b>	0,48±0,01	0,25±0,01	0,37±0,02
<b>C20:3 n-6</b>	0,37±0,02	0,25±0,02	0,35±0,02
<b>ARA</b> <b>C20:4n-6</b>	0,58±0,01	0,37±0,01	0,42±0,02
<b>C22:2 n-6</b>	0,09±0,01	0,04±0,01	0,05±0,01
<b>EPA</b> <b>C20:5n-3</b>	0,06±0,01	0,05±0,01	0,06±0,01
<b>C22:3 n-6</b>	0,13±0,01	0,06±0,01	0,07±0,01
<b>C22:4 n-6</b>	0,18±0,01	0,10±0,01	0,07±0,01
<b>C22:5 n-6</b>	0,02±0,01	0,01±0,01	0,01±0,01
<b>C22:5 n-3</b>	0,07±0,01	0,05±0,01	0,06±0,01
<b>DHA</b> <b>C22:6n-3</b>	0,12±0,01	0,08±0,01	0,09±0,01
<b>Total TFA (%)</b>	<b>0,2</b>	<b>0,18</b>	<b>0,24</b>
<b>Total PUFA (%)</b>	<b>13,53</b>	<b>9,62</b>	<b>15,21</b>

All values are means ± standard deviation of the mean, PUFA - polyunsaturated fatty acids, LC-PUFA - long-chain polyunsaturated fatty acids, TFA - trans fatty acids, LNA - linoleic acid, ALA - alpha-linolenic acid, ARA - arachidonic acid, EPA - eicosapentaenoic acid, DHA - docosahexaenoic acid, CLA - conjugated linolenic acid. Source: Elaborated by the authors (2023).

Due to the fact that we did not find studies that evaluated polyunsaturated fatty acids (PUFA) and long-chain polyunsaturated fatty acids (LC-PUFA) in BM of women who underwent BS, we compared the results with data available in the scientific literature of non-operated women.

A study conducted by Forbes et al. (2016), investigated the impact of BS on EFA status in non-pregnant women undergoing RYGB. The study demonstrated that after surgery, a transient increase in 20:4n-6 (+18%) and a decrease in 20:3n-6 were observed in 1 (-47%) and 6 months (-47%). Similar changes were seen in n-3 fatty acids after RYGB, including a transient increase in 22:6n-3 (+11%) and reductions in 20:5n-3 (-79 and -67% at 1 and 6 months, respectively). EFA status improved after surgery in the RYGB group.

BM is the food of choice for infants due to its ability to promote digestion and absorption of vitamins, the metabolic functions attributed to its ideal composition of linoleic acid (LNA), alpha-linolenic acid (ALA), arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which play an important role in optimal development. nutritional and immunological (Falcão, 2020).

Regarding ALA in the COL, Berenhauer et al. (2012) found a value like that found in our study. In a study carried out in Brazil, Freitas et al. (2019) found values of LNA and ALA in the MM higher than those found in our case report: LNA

(13.90%) and ALA (1.04%). Berenhauser et al. (2012) also found higher ALA value in the MM: 0.86%. Castro et al. (2021), investigating the LNA content in BM, found in COL: 26.52%, TM: 22.34% and 22.26% in MM and ALA in COL: 1.71%, TM: 1.12% and 1.57% in the MM, values much higher than those in our case report. This difference in results suggests that malabsorption of these lipids may have occurred, since BC significantly alters the dynamics of the digestive process. Exocrine pancreatic reserve is very common after RYGB (Uribarri-Gonzalez, et al., 2021).

Furthermore, studies show that, according to dietary, genetic, sociodemographic, health, and environmental factors and throughout the three stages of production: COL, TM, and MM, different types of milk are successively produced, with different compositions (Miliku, et al., 2019).

The ARA had the highest content in the COL phase and the lowest content in the TM and MM phases, which was also observed in the study by Castro et al. (2021).

In the present study, it was found that the concentration of EPA in the MM (0.06%) was lower than that observed in the study by Giufrida et al. (2022) and Castro et al. (2021) who found 0.32% and 0.17%, respectively. Miliku et al. (2019) found a content of 0.08%

Regarding DHA, Castro et al. (2021) found much higher values than our report, 0.44% in COL, 0.30% in TM and 0.19% in MM. DHA content in breast milk reflects variations in maternal intake of this FA. Populations with a high intake of fish such as tuna, anchovies, sardines and salmon, among others, have a high content of this DHA in breast milk (Kim, et al., 2017).

Maternal adipose tissue can serve as a reservoir of PUFAs for BM as well as plasma which also supplies PUFAs to BM (Giufrida, et al., 2022). It should be noted that essential fatty acids (EFAs) are polyunsaturated fatty acids that must be obtained from the diet (Forbes, et al., 2016). If both adipose tissue and plasma PUFAs reflect dietary intake, it is necessary to provide these PUFAs during pregnancy or even before conception and lactation to ensure availability to mothers and sufficient supply to the baby via BM. Perhaps, the participant in this study had no reservations due to the lack of an adequate diet prior to pregnancy, due to common dietary errors in obesity, as well as nutritional deficiencies that occur due to the presence of food intolerances and/or changes in intestinal absorption after BS (Aguas-Ayesa, et al., 2023; Mechanick, et al., 2020).

de la Garza Puentes et al. (2019) showed that breast milk of mothers with a high BMI presented decreased ALA and DHA concentrations than milk from normal weight nursing mothers. This finding corroborates what was observed in our study, when comparing the levels of ARA and DHA found in the BM samples of the nursing mother studied with that of Castro et al. (2021), who used COL, TM and MM samples from eutrophic women. It is important to point out that the nursing mother in the study was still in weight loss due to performing the BS only two months before the beginning of the pregnancy and presenting a nutritional diagnosis of overweight. Thus, our findings support the hypothesis that the nutritional status of the nursing mother influences the n-3 CL-PUFA lipid composition of MM.

It is noteworthy that DHA and ARA are present in the phospholipids of cell membranes and directly influence neuronal development, visual acuity and the baby's immune system (Freitas, et al., 2019).

There are two sources of *TFA*s in diets: industrial and ruminant (Costa, et al., 2016). Total *TRAs* contributed with 0.20% in COL, 0.18% in TM and 0.24% in MM. Of this percentage, the average of *TFA* from ruminant source (CLA) was 0.09% in COL, 0.10% in TM and 0.12% in MM, higher values than those found in the study by Berenhauser et al. (2012) who analyzed COL and MM and found an average of 0.06% for both the COL and MM phases. The CLA content can be used as a marker of dairy fat and meat intake, since it is found almost exclusively in ruminant fat. This finding corroborates the high milk intake reported by the participant.

The average *TFA* from the industrial process in the COL, TM and MM samples were 0.11%, 0.08% and 0.12%, respectively. Study carried out by Aumeistere et al. (2021) in European women also observed levels of 0.10% of these FAs in

BM, considered low values. This percentage found in our study may have occurred because the study participant received nutritional guidance while attending the nutrition/prenatal outpatient clinic and did not consume large amounts of industrialized foods such as margarine, vegetable creams, cookies, chips, ice cream, pastries, which are sources of *TFA*, according to the report during the consultation.

Excessive consumption of *TFA* from industrialized foods by mothers can affect the growth and development of newborns, in addition to causing potential negative effects on metabolism by inhibiting the desaturation of LNA into ARA and ALA into DHA (Costa, et al., 2011).

## 5. Final Considerations

The relevance of this study case lies in the fact that it shares knowledge about on the fatty acid composition of BM from a nursing mother post RYGB. The mean LNA and ALA found in the MM was below the means found in studies carried out in Brazil from eutrophic women who did not underwent BS. In this study, TFAs were found in maternal milk but considered low values. The lack of other studies on the profile of PUFAs in the BM of women undergoing BS limited the comparison of the results found. As a relevant recommendation for future work, it is suggested that research be carried out with a larger population undergoing such a procedure to investigate the composition of polyunsaturated fatty acids in breast milk as well as analysis of maternal plasma phospholipids.

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