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ORIGINAL ARTICLE

trans Fatty Acids in Colostrum, Mature Milk and Diet of Lactating Adolescents

Roseli de Souza Santos da Costa[1,2] · Flavia da Silva Santos[1] · Daniela de Barros Mucci[1] · Tânia Vignuda de Souza[3] · Fátima Lucia de Carvalho Sardinha[1] · Célia Regina Moutinho de Miranda Chaves[2] · Maria das Graças Tavares do Carmo[1]

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Abstract The purpose of this study was to investigate the *trans* fatty acids (TFA) content and distribution in colostrum, mature milk, and diet of adolescent mothers, after TFA declaration in food labels became mandatory in Brazil. Participants were healthy adolescents (*n* 54, 15–19 years, 1–90 days postpartum) practicing exclusive breastfeeding. Milk samples were collected 3 days after delivery (colostrum) and in the third month postpartum (mature milk) by hand expression. The fatty acid composition of the milk samples was determined by gas chromatography. TFA intake corresponded to 1.23 % of total energy value. Total 18:2 TFA accounted for less than 0.5 % of the energy intake. The amount of total 18:1 TFA (mean ± SEM) was 1.9 % ± 0.14 in colostrum and 1.5 % ± 0.2 in mature milk. The total content of n-3 PUFA was inversely correlated with the total content of 18:1 TFA in colostrum. Both in colostrum and in mature milk, vaccenic acid (11t-18:1) was found to be the most abundant 18:1 *trans* isomer, followed by elaidic acid (9t-18:1), whereas rumenic acid (9c,11t-18:2 CLA) was the predominant 18:2 *trans* isomer. In conclusion, the levels of TFA of industrial sources found in the mother's diet and breast milk (colostrum and mature milk) showed a decrease in relation to those observed in studies conducted prior to the TFA labeling resolution in Brazil. However, the current low intake levels of n-3 LCPUFA and DHA content in the milk of lactating adolescents may be insufficient for supporting adequate neurological development of the infants.

Keywords Adolescent mother · Human milk · Colostrum · *Trans* fatty acids

Abbreviations

ALA	Alpha-linolenic acid (18:3n-3)
ANVISA	National Sanitary Surveillance Agency
ARA	Arachidonic acid (20:4n-6)
BMI/A	Body mass index for age
c	*cis*
CLA	Conjugated linoleic acid
DHA	Docosahexaenoic acid (22:6n-3)
EFA	Essential fatty acid(s)
EPA	Eicosapentaenoic acid (20:5n-3)
FA	Fatty acid(s)
FAME	Fatty acid methyl ester(s)
FID	Flame ionization detector
Fiocruz	Oswaldo Cruz Foundation
HM	Human milk
IFF	National Institute of Women, Children and Adolescents Health Fernandes Figueira
LCPUFA	Long-chain polyunsaturated fatty acid(s)
LNA	Linoleic acid (18:2n-6)
LNBINJC/UFRJ	Laboratory of Nutritional Biochemistry of the Institute of Nutrition Josué de Castro/Federal University of Rio de Janeiro
MUFA	Monounsaturated fatty acid(s)

✉ Roseli de Souza Santos da Costa
rsscosta@iff.fiocruz.br

[1] Instituto de Nutrição Josué de Castro da Universidade Federal do Rio de Janeiro. Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

[2] Instituto Nacional da Saúde da Mulher, da Criança e do Adolescente Fernandes Figueira, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

[3] Escola de Enfermagem Anna Nery, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

PHVO	Partially hydrogenated vegetable oil(s)
PUFA	Polyunsaturated fatty acid(s)
SFA	Saturated fatty acid(s)
t	*trans*
TFA	*trans* Fatty acid(s)
WHO	World Health Organization

Introduction

In Brazil, changes in the urban population patterns of feeding, in recent years, include a higher consumption of meat, dairy, refined sugars and processed products. Moreover, since the 1960s, with the rise in production of partially hydrogenated vegetable fat, a substantial increase in consumption of products containing *trans* fatty acids (TFA) has been observed in the country over the years, especially among adolescents [1].

There are two sources of TFA in diets: industrial and ruminant. Most dietary TFA comes from partial hydrogenation of vegetable oils by the industry. The major industrially produced monounsaturated TFA, found in processed food products, are 9 *trans* (*t*)-18:1 (elaidic acid) and 10*t*-18:1 [2]. However, industrial *trans* fats also contain a considerable amount of 11*t*-18:1 (vaccenic acid), usually more than 20 % [3]. Industrial production of linoleic acid *trans* isomers also yields a mixture of 9,11- and 10,12-18:2 isomers [2].

Ruminant TFA are synthetized in the rumens of cows and sheep by ruminant bacterial isomerases that can convert the *cis* (c) double bonds of dietary fat into a *trans* configuration [4]. Therefore, meat and dairy products contain small amounts of naturally occurring TFA, in which 11t-18:1 is the major 18:1 TFA in ruminant fats, resulting in a low 9*t*/11*t*-index (<1) [4, 5]. In addition, ruminant fats contain small amounts of 9c,11*t*-18:2 conjugated linoleic acid (CLA), which can also be produced from ingested vaccenic acid in animals and in humans [6]. It should be noted that consumption of TFA from partial hydrogenation (industrial sources), but not from ruminant sources, has been related to negative health effects [7–9], although studies in humans have been inconclusive on this aspect [10, 11].

Since the TFA found in human milk (HM) come from the mother's diet and her body supplies [12, 13], it has been suggested that the profile of dietary fatty acids (FA) consumed by the mother is a determining factor for the FA composition of the milk produced by her. The presence of monounsaturated TFA from industrial sources in HM may be a concern because of their possible adverse effects on infant development [7]. Infant tissues incorporate TFA from HM and these isomers are known to affect the essential fatty acids (EFA) metabolism and impair the biosynthesis of longer-chain, more unsaturated derivatives, such as arachidonic acid (ARA) and docosahexaenoic acid (DHA) [7, 8, 14]. Indeed, studies show that the concentration of TFA found in milk and in newborns is inversely proportional to their levels of long-chain polyunsaturated fatty acids (LCPUFA) [7, 15, 16]. Considering that ARA is fundamental for fetal growth [7, 14] and DHA, in particular, plays a critical role in brain and retina development during pregnancy and the early years of life [14, 17], maternal intake of industrial *trans* fats may be detrimental to offspring health.

The World Health Organization (WHO), supported by scientific evidence showing the harmful effects of industrial TFA, has made recommendations limiting their consumption to less than 1 % of daily total energy intake, in order to prevent chronic diseases [18]. Most public health agencies have implemented labeling or ingredient restrictions of *trans* fats, including Brazil. In 2003, the National Sanitary Surveillance Agency (ANVISA) made declaration of TFA amount obligatory on the nutritional labels of processed foods commercialized in Brazil and, after 2006, several industrialized products in the country were declared absent of *trans* fats [19].

Studies determining the amount of EFA and LCPUFA in breast milk and blood samples of Brazilian adolescent mothers have been conducted [20], including some by our group [21, 22]. However, data on the FA composition of HM are still quite conflicting and a Brazilian study associating maternal diet with the content and distribution of TFA in colostrum and mature milk from lactating adolescents, especially after declaration of TFA in food labels became mandatory, could be valuable for public policy. Considering the above mentioned, the purpose of this study was to investigate the content of TFA in colostrum, mature milk and diet of adolescent mothers living in the city of Rio de Janeiro, State of Rio de Janeiro, Brazil.

Materials and Methods

This was a cross-sectional study of a convenience sample of lactating adolescents, conducted from September 2005 to June 2008, in the Maternity Ward of the National Institute of Women, Children and Adolescents Health Fernandes Figueira (IFF), a unit of the Oswaldo Cruz Foundation (Fiocruz), in Rio de Janeiro (RJ), Brazil. The study was approved by the Ethics in Research Committee of IFF. Initially, 84 mothers aged 15–19 years who delivered their babies at term and practiced breastfeeding were recruited. From the initial group, only 54 mothers who were exclusively breastfeeding their infants, from June 2007 to June 2008, remained in the study for human milk fatty acid analysis. The follow-up group was representative of the initial group of adolescents, since there were no statistically

significant differences. Adolescent mothers with pregnancy complications or other diseases, as well as mothers of infants with any evidence of metabolic or physical abnormalities were not included in the study. All the adolescents and their parents gave written informed consent prior to the beginning of the research.

Food Intake, Anthropometric Measurements and Sample Collection

Information on maternal food consumption was collected on the third month postpartum, according to the maternity routine schedule. Data from maternal dietary intake were obtained by two methods: qualitative food frequency questionnaire, which evaluated the number of times specific foods or food groups were consumed, according to frequency categories [1]; and 24-h recall. Data from the food records were analyzed using NutWin Software®—Nutrition Support Program version 1.5 [23], with the inclusion of information on the nutritional composition of food sources of TFA, obtained from the Brazilian Table of Food Composition [24].

Breast milk samples (5 mL) were collected once in the morning (08.00 a.m. to 10.00 a.m.) 1–3 days after delivery (colostrum) and on the third month postpartum (mature milk) from all volunteers. The breast that had not been sucked in the last feeding was emptied of milk by hand expression, insuring that fore-milk, mid-milk, and hind-milk were all collected in the container. Immediately afterwards, the content was thoroughly mixed by inversion in order to obtain a uniform sample. All samples were briefly kept in a freezer at −20 °C, then transported on ice to the Laboratory of Nutritional Biochemistry of the Institute of Nutrition Josué de Castro/Federal University of Rio de Janeiro (LNBINJC/UFRJ), where they remained stored at −80 °C until lipid separation and quantification of FA. Analyses were performed within 6 months of collection.

Data of newborns including date of birth and gestational age, according to Ballard *et al*. [25], birth weight, length, head circumference and weight/gestational age index were collected from medical records. Infant anthropometric measurements of weight, length, and head circumference were obtained on the third month postpartum, according to the recommendations of the Brazilian Ministry of Health [26].

The weight of unclothed infants (in grams) was measured using a calibrated digital-type scale (Filizola) with a sensitivity of 10 g. The length (expressed in centimeters) was measured with the baby in the supine position, using a wooden stadiometer, from the crown to the heel. The head circumference (expressed in cm) was obtained using a tape measure placed in the region of the supra-orbital ridges and the largest frontooccipital diameter. The nutritional status of infants was evaluated by the weight-for-age and height-for-age indices. The criteria for classification were those recommended by the WHO [27] and the Brazilian Ministry of Health [28].

Maternal weight and height were evaluated according to the recommendations of the Brazilian Ministry of Health [29]. Teenage girls were measured while barefoot without excess clothing and weight was obtained with a platform-type scale (Filizola) with 100 g subdivisions and a maximum load of 150 kg. The scale used for measuring height was fixed to the balance on a vertical shaft, with 0.5 cm subdivisions and an extension range of 95–195 cm. Nutritional status was assessed by maternal body mass index-for-age (BMI/A) and the cutoff points established in z scores for adolescents, according to the recommendations of WHO [30, 31] and adapted for Brazil [28].

Per capita income was assessed according to indicators and core data from Brazil, which classified families with a monthly per capita income up to half the minimum wage in the "poverty" state [32].

Fatty Acid Analysis

Lipids in milk samples were extracted and purified in a chloroform:methanol (2:1) solution [33]. Total lipid extracts were saponified and fatty acids were methylated with 2 mL methanol:toluene 4:1 (v/v) solution and 200 µL acetyl chloride cold addition, following the Lepage and Roy method [34]. The mixtures were subjected to methanolysis at 80 °C over a period of 2.5 h. Fatty acid methyl esters (FAME) were separated and quantified on a Perkin Elmer Autosystem XL chromatograph equipped with an autoinjector, a split/splitless capillary injection system, and a flame ionization detector (FID) as described elsewhere [35]. Samples were injected (1 µL) with a split ratio of 1:4 and FAME were separated on a capillary column SP 2560 (biscyanopropyl-polysiloxane, 100 m × 0.25 mm ID, 0.20 µm film thickness; Supelco, Bellefonte, PA, USA). Hydrogen was used as carrier gas (0.75 mL/min), Nitrogen as make up gas (25 mL/min) and FID flow rates were 30 mL/min, for hydrogen, and 400 mL/min, synthetic air. Detector and injector temperatures were set to 250 °C and column temperature gradient started at 100 °C (3 °C/min) up to 140 °C, followed by 140 °C (0,5 °C/min) up to 170 °C and lastly 170 °C up to 220 °C (3.2 °C/min), staying at this final temperature for 35 min. Methyl ester standards (GLC-463 Reference Standard containing 52 FAME mixture (purity >99 %) and trans-mix GLC 481 (purity >99 %) Nu-Chek Prep, Inc., Elysian, MN, USA) were used to identify peaks and determine response factors for integration with a Turbochrom software (Perkin Elmer, USA). This procedure enables separation of the major 18:1 *trans* isomers and also indicates that very small co-eluted

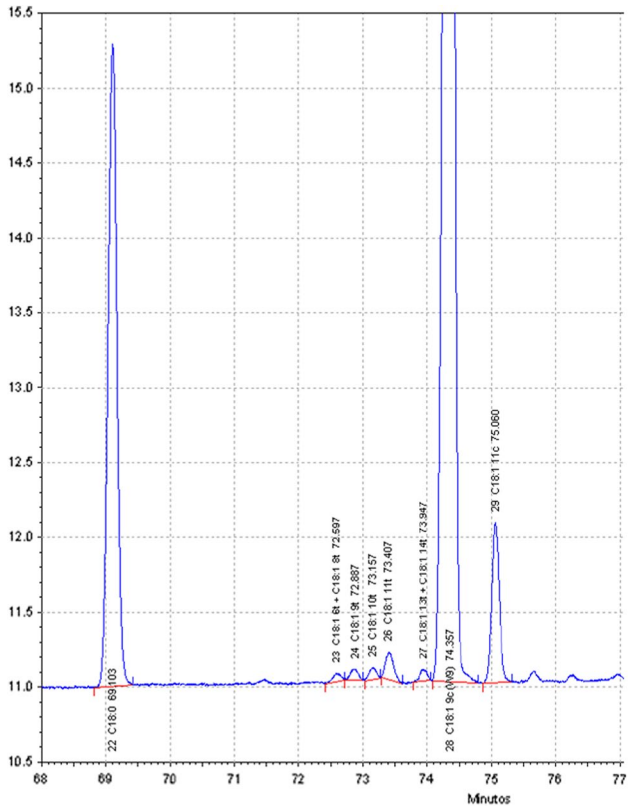

Fig. 1 Significant part of the chromatogram of mature milk showing the zone of 18:1 *trans* fatty acid methyl esters (see "Materials and Methods" for operating conditions)

peaks of 18:1 *trans* isomers are present in the milk fat (Fig. 1). The total *trans* 18:1 isomers included: 6/8*t*-, 9*t*-, 10*t*-, 11*t*-, 13/14*t*-18:1. The 12*t*-, 15*t*-, 16*t*-, 6/8*c*- and 10*c*-isomers were not included, because separation was not feasible. The gas chromatography analysis was performed without a pre-separation of the *cis* and *trans* 18:1 FA by Ag^{+}-TLC, therefore an under- or overestimation of 13/14*t*- and 15*t*- due to coelution with 9*c*-18:1 is possible. In addition, 6/8*c*- and 14*c*- could coelute with 13/14*c*- and 16*t*, respectively, however, these *cis* 18:1 isomers are considered negligible in milk fat [36]. Furthermore, conjugated linoleic acids 9*c*,11*t*-; 10*t*,12*c*-; 9*t*,11*t*-18:2 and 9*t*,12*t*-18:2 isomers were identified in samples. The CLA peaks were identified by comparison with the retention time of the reference standard, where 9c,11*t*-18:2 (rumenic acid) was identified as the major CLA isomer, as reported in other study of Brazilian lactating women [37]. However, an overestimation of 9c,11*t*-CLA content due to coelution with 7*t*,9*c*-; 9*c*,11*t*- and 8*t*,10*c*-CLA could have occurred. Fatty acid concentrations were expressed as the percentage of the total area comprising all FA peaks (% of total FAME).

Statistical Analysis

Data analysis was carried out using the Statistical Package Program for Social Sciences (version 15.0 for Windows; SPSS Inc., Chicago, USA). To determine the correlation between the study variables, the Spearman correlation coefficient was used. The differences between the concentrations of FA in colostrum and mature milk were then compared using the Wilcoxon test. The association between the concentration of total TFA (dependent variable) and the investigated factors (independent variables) was analyzed by multivariate regression. We used a selection process in which the independent variables and potential confounders (maternal weight gain, nutritional status before pregnancy, adequacy of weight gain during pregnancy, birth weight, birth length, head circumference at birth, gestational age, linoleic acid, alpha-linolenic acid, polyunsaturated fatty acids (PUFA), monounsaturated fatty acids, EFA, total n-6 PUFA, ARA, eicosapentaenoic acid, DHA, 18:2 TFA) were added to the model according to their significance (*P*). An *P* value <0.05 was considered statistically significant. Results are presented as means ± standard errors of the means.

Results

Regarding maternal nutritional status, only 11 % of mothers had a pre-pregnancy BMI/A within the normal range, but in the third month postpartum most of them (86.8 %) had an adequate BMI/A. The gestational weight gain was above the recommendation in 42 % of cases. The majority of participants (71.1 %) had an income per capita below the minimum wage and 33.3 % were in the poverty state. Only 9.3 % of mothers did not attend high school. Most newborns (87 %) had appropriate weight/gestational age and 7.4 % were premature. The average birth weight and length were 3140 ± 0.45 g and 50.41 ± 2.47 cm, respectively. The average length of gestation was 39.13 ± 1.37 weeks (Table 1).

According to the qualitative food frequency questionnaire answered in the third month postpartum, at least once a week, participants consumed processed foods, such as cakes, snacks, cream crackers biscuits, margarine, bread, chocolate, sweet bread, creamy ice cream, sandwich cookies, sweet biscuits, creamy candy and instant noodles. Only 20 % of adolescent mothers reported habitual intake of fish. Most reported regular consumption of chicken (83 %), bovine milk (90 %) and beef (74 %). Regular consumption of margarine was reported by 83 % of the mothers.

The average total energy intake of adolescent mothers was 2169.40 ± 134.30 kcal/day, ranging from a minimum of 830 kcal/day to a maximum of 5444 kcal/

Table 1 General characteristics of lactating Brazilian adolescent and newborns studied ($n = 54$)

Variables *n* %	*n*	%
Maternal		
Age (years)		
15–17	40	74.1
18–19	14	25.9
Marital status		
Unmarried	40	74.1
Married	14	25.9
Education (years)		
Up to 8 years	05	9.3
Between 9 and 11 years	49	90.7
Family income per capita (MS)[a]		
Up to 0.5	18	33.3
0.51–0.99	20	37.0
1.0–2.0	04	5.0
Unable to provide	12	22.2
Pre-pregnancy nutritional status		
Low BMI/A	07	13.0
Surveillance for low BMI/A	38	70.4
Appropriate BMI/A	06	11.1
Surveillance for high BMI/A	03	5.6
Nutritional status at 3 months postpartum		
Surveillance for low BMI/A	04	7.4
Appropriate BMI/A	47	87.0
Surveillance for high BMI/A	03	5.6
Adequacy of gestational weight gain		
Low	16	29.6
Adequate	15	27.8
Excessive	23	42.6
Newborns		
Birth weight (g)		
<2500	02	3.7
2500–4000	50	92.6
>4000	02	3.7
Head circumference (cm)		
<35	34	63.0
≥35	20	37.0
Correlation weight/gestational age		
SGA	05	9.3
AGA	47	87.0
LGA	02	3.7
Gestational age		
<37 weeks	04	7.4
>37 weeks	50	92.6

BMI/A Body Mass Index/Age, *SGA* small for gestational age, *AGA* appropriate for gestational age, *LGA* large for gestational age, *MS* minimum salary

[a] Current minimum salary: 2005 (R$ 300.00/$121.46); 2006 (R$ 350.00/$194.44); 2007 (R$ 380.00/$170.40); 2008 (R$ 415.00/$228.00)

Table 2 Dietary intake of lactating Brazilian adolescents at 3 months post-partum

Energy and fatty acids	Mean ± SEM ($n = 54$)
EI (kcal)	2169.40 ± 134.30
Total fat (% EI)	23.75 ± 0.76
SFA (% EI)	8.78 ± 0.47
MUFA (% EI)	8.64 ± 0.38
PUFA (% EI)	3.81 ± 0.30
n-3 (%)	0.37 ± 0.02
n-6 (%)	3.29 ± 0.28
n-6:n-3	9.27 ± 0.71
ARA (%)	0.06 ± 0.01
DHA (%)	0.04 ± 0.02
PUFA:SFA	0.51 ± 0.06
18:1 *trans* fatty acids (g)[a]	2.94 ± 0.51
18:2 *trans* fatty acids (g)[a]	0.13 ± 0.03

All values are means ± standard errors of the mean

EI energy intake, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *n-3* alpha-linolenic acid, *n-6* linoleic acid, *ARA* arachidonic acid, *DHA* docosahexaenoic acid, *n-6*:*n-3* linoleic acid:alpha-linolenic acid ratio, *PUFA*:*SFA* polyunsaturated fatty acids:saturated fatty acids ratio

[a] Nutritional composition of food sources of *trans* fatty acids, obtained from the Brazilian Table of Food Composition (TACO/NEPA 2011)

day. Carbohydrates and lipids consumption contributed to approximately 59 and 24 % of the total energy intake, respectively. The average intake of total TFA was 3.07 g, corresponding to 1.23 % of total energy value (Table 2).

Most samples of colostrum (89 %) and mature milk (94 %) contained 18:1 TFA. *trans* Isomers of 18:2 were found in 30 and 67 % of colostrum and mature milk samples, respectively. As shown in Table 3, the sum of saturated fatty acids (SFA) increased significantly ($P < 0.001$) from colostrum to mature milk, with an increase in 10:0, 12:0, 14:0 ($P < 0.001$) and 15:0 ($P < 0.05$), and a significant decline in 16:0 and 18:0 levels during this same period of time ($P < 0.001$). While 16:1 *cis* and *trans*, 22:1 and 24:1 remained constant, 14:1 and 20:1 significantly increased ($P < 0.001$), and 18:1 TFA ($P < 0.05$), 18:1n-9 *cis* and total monounsaturated fatty acids ($P < 0.001$) significantly declined from colostrum to mature milk. No differences were observed between the concentrations of 20:3n-3 and 20:5n-3 (EPA, eicosapentaenoic acid) in colostrum and mature milk. However, total 18:2 TFA ($P < 0.05$), the n-6 EFA linoleic acid (LNA, 18:2n-6 *cis*; $P < 0.001$) and total n-6 PUFA ($P < 0.05$) significantly increased from colostrum to mature milk, whereas 20:4n-6 (ARA), the n-3 EFA alpha-linolenic acid (ALA, 18:3n-3; $P < 0.001$), 22:6n-3 (DHA) and total n-3 PUFA declined during this same period of time ($P < 0.05$).

Table 3 Composition of fatty acids (% of total FAME) in samples of colostrum and mature milk of lactating adolescents

Fatty acids	Colostrum (% of total FAME) $n = 54$	Mature milk (% of total FAME) $n = 54$	P
SFA			
10:0	0.17 ± 0.05 (0.00–1.52)	1.17 ± 0.07 (0.00–2.23)	<0.001
12:0	1.93 ± 0.19 (0.00–7.80)	6.39 ± 0.28 (1.81–10.13)	<0.001
14:0	4.64 ± 0.22 (2.27–9.96)	7.07 ± 0.28 (0.15–11.85)	<0.001
Total *de novo* synthesis[a]	6.74 ± 0.42 (2.65–19.13)	14.63 ± 0.60 (5.12–25.26)	<0.001
15:0	0.19 ± 0.06 (0.00–3.00)	0.22 ± 0.02 (0.00–0.68)	0.02
16:0	26.09 ± 0.49 (15.00–35.00)	21.60 ± 0.45 (16.12–35.30)	<0.001
18:0	7.87 ± 0.24 (4.73–13.76)	6.96 ± 0.19 (3.73–10.50)	<0.001
Total SFA	40.89 ± 0.48 (34.80–56.29)	43.41 ± 0.39 (33.78–63.68)	<0.001
MUFA			
14:1	0.04 ± 0.01 (0.00–0.40)	0.18 ± 0.03 (0.00–1.58)	<0.001
Total C16:1 TFA[b]	0.09 ± 0.03 (0.00–0.62)	0.05 ± 0.02 (0.00–0.52)	0.74
16:1 *cis*	1.53 ± 0.11 (0.00–4.93)	1.75 ± 0.12 (0.00–5.56)	0.06
18:1n-9 *cis*	31.74 ± 0.48 (17.1–35.85)	28.26 ± 0.65 (0.03–34.30)	<0.001
Total C18:1 TFA[c]	1.91 ± 0.14 (0.00–4.29)	1.55 ± 0.20 (0.00–9.39)	0.01
20:1n-9	0.79 ± 0.07 (0.00–2.84)	1.05 ± 0.08 (0.00–2.22)	<0.001
22:1n-9	0.03 ± 0.01 (0.00–0.43)	0.02 ± 0.01 (0.00–0.42)	0.95
24:1n-9	0.39 ± 0.1 (0.00–3.37)	0.15 ± 0.07 (0.00–3.62)	0.06
Total MUFA	37.52 ± 0.45 (21.06–38.04)	33.01 ± 0.70 (2.31–38.85)	<0.001
PUFA			
Total 18:2 TFA[d]	0.55 ± 0.01 (0.00–1.06)	0.68 ± 0.01 (0.00–1.26)	<0.05
18:2n-6 *cis* (LNA)	18.05 ± 0.39 (9.90–24.73)	21.82 ± 0.63 (11.30–32.00)	<0.001
20:4n-6 (ARA)	1.12 ± 0.07 (0.00–2.40)	0.43 ± 0.03 (0.00–1.10)	<0.001
Total n-6 PUFA	19,85 ± 0.40 (11.32–25.19)	22.80 ± 0.63 (11.77–22.45)	<0.05
18:3n-3 (ALA)	0.65 ± 1.30 (0.00–6.75)	0.48 ± 0.04 (0.00–1.85)	<0.001
20:3n-3	0.09 ± 0.07 (0.06–0.27)	0.07 ± 0.01 (0.00–0.57)	0.76
20:5n-3 (EPA)	0.08 ± 0.02 (0.00–0.90)	0.06 ± 0.01 (0.00–0.17)	0.34
22:6n-3 (DHA)	0.92 ± 1.18 (0.00–6.50)	0.17 ± 0.03 (0.00–1.20)	<0.05
Total n-3 PUFA	1.74 ± 0.28 (0.00–12.36)	0.78 ± 0.06 (0.00–2.52)	<0.05
Total PUFA	21.59 ± 0.52 (12.73–30.90)	23.58 ± 0.64 (12.43–34.00)	0.51

All values are means ± standard errors of the mean, limits in parentheses

FAME fatty acid methyl esters, *SFA* saturated fatty acids, *TFA trans* fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *LNA* linoleic acid, *ALA* alpha-linolenic acid, *ARA* arachidonic acid, EPA eicosapentaenoic acid, *DHA* docosahexaenoic acid

[a] Total *de novo* synthesis is the sum of 10:0, 12:0, 14:0

[b] Total 16:1 TFA includes 9*t*-, 11*t*-16:1

[c] Total 18:1 TFA includes 6*t*/8*t*-, 9*t*-, 10*t*-, 11*t*-, 13*t*/14*t*-18:1

[d] Total 18:2 TFA includes 9*t*,12*t*-, 9c,11*t*-, 10*t*,12*c*-, 9*t*,11*t*-18:2

The most abundant TFA present in maternal milk are described in Table 4. The mean total *trans* content (18:1 and 18:2-linoelaidic acid) in colostrum was 2.16 ± 0.19 *versus* 1.65 ± 0.13 % in mature milk. Moreover, in both colostrum and mature milk, vaccenic acid (11t-18:1) and elaidic acid (9t-18:1) were present in detectable amounts, whereas the rumenic acid (9*c*,11*t*-18:2) + other *cis*/*trans* 18:2 were the prevalent CLA isomers (Table 4). In both milk samples, 9*t*-18:1 and 10*t*-18:1 levels were about half of the 11*t*-18:1 concentration, leading to 9*t*/11*t* and 10*t*/11*t* index values <1. The content of 9*t*-18:1, 10*t*-18:1 and 11*t*-18:1 in human milk lipids were higher in colostrum compared to mature milk ($P < 0.05$). The levels of total CLA did not differ between milk samples, although rumenic acid concentration significantly increased in mature milk ($P < 0.05$).

Data analysis regarding fatty acid correlation with characteristics of adolescent mothers and infants showed that there was no association between the general characteristics of adolescent mothers (total maternal weight gain, pre-pregnancy BMI/A, maternal nutritional status before pregnancy, family income per capita, intake of LNA, ALA, ARA, DHA, 18:2 TFA and the composition of 18:1 TFA in

Table 4 Individual *trans* isomers of 18:1 and 18:2 fatty acids (% of total FAME) in samples of colostrum and mature milk of lactating adolescents

Fatty acids	Colostrum (% of total FAME) $n = 54$	Mature milk (% of total FAME) $n = 54$	P
Total *trans* fatty acids[a]	2.16 ± 0.19	1.65 ± 0.013	<0.05
Total 16:1 *trans* isomers	0.09 ± 0.02	0.05 ± 0.02	0.74
9*t*-16:1	0.03 ± 0.02	0.02 ± 0.02	0.53
11*t*-16:1	0.06 ± 0.02	0.03 ± 0.02	0.23
Total 18:1 *trans* isomers	1.91 ± 0.14	1.55 ± 0.20	0.01
9*t*-18:1 elaidic acid	0.39 ± 0.08	0.26 ± 0.06	0.01
10*t*-18:1	0.28 ± 0.03	0.19 ± 0.05	<0.05
11*t*-18:1 vaccenic acid	0.76 ± 0.08	0.67 ± 0.05	<0.05
13*t*- and 14*t*-18:1	0.25 ± 0.02	0.22 ± 0.02	0.35
6*t*- and 8*t*-18:1	0.23 ± 0.01	0.21 ± 0.04	0.66
Total 18:2 *trans* isomers	0.55 ± 0.01	0.68 ± 0.01	0.01
9*t*,12*t*-18:2 linoelaidic acid	0.11 ± 0.04	0.10 ± 0.02	0.49
Total conjugated linoleic acids (CLA)	0.44 ± 0.12	0.58 ± 0.10	0.55
9*c*,11*t*-18:2 rumenic acid + other *cis*/*trans* 18:2	0.40 ± 0.04	0.53 ± 0.08	<0.05
9*t*,11*t*-18:2	0.01 ± 0.02	0.03 ± 0.02	0.94
10*t*,12c-18:2	0.03 ± 0.02	0.02 ± 0.02	0.48
Elaidic acid/vaccenic acid index	0.51 ± 0.03	0.38 ± 0.02	0.01
10*t*-18:1/vaccenic acid index	0.36 ± 0.00	0.28 ± 0.01	0.05

All values are means ± standard errors of the mean

FAME fatty acid methyl esters

[a] Total *trans* fatty acids include: total *t*-16:1, 6*t*-/8*t*-18:1, 13*t*-/14*t*-18:1, 11*t*-18:1, 9*t*-18:1, 10*t*-18:1 and *t*,*t*-18:2

Table 5 Results of multivariate regression with the predictor variable of total 18:1 *trans* fatty acids concentration in colostrum

Variable	Exp(β)	EP	P
n-3 LCPUFA	−0.085	−2.31	0.025

milk) and general characteristics of infants (head circumference, gestational age, birth weight, etc.). In the multivariate regression model, we observed that the n-3 LCPUFA content in colostrum was negatively associated with its concentration of total 18:1 TFA ($P = 0.025$, Table 5).

Discussion

The fatty acid composition of the maternal diet, in the present study, does not differ from that observed in another study with adolescent mothers that attended four public institutions in Rio de Janeiro/Brazil [20]. The average energy and fat intakes found in this study were 2169 kcal/day and 58 g/day, respectively, corresponding to a mean contribution of fat to energy intake (24 %) that is considered adequate. However, the intake of n-6 and n-3 PUFA was below the acceptable limit [18]. Food intake analysis showed a high consumption of n-6 PUFA food sources, such as soybean oil, as opposed to n-3 PUFA sources, like flaxseed and fish, resulting in a dietary n-6/n-3 ratio of 9.27, which corresponds to the upper limit of the acceptable range [38]. Diets containing a high n-6/n-3 ratio have been associated with impaired endogenous conversion of the n-3 EFA alpha-linolenic acid to EPA and DHA. Considering the critical role of n-3 LCPUFA supply, through maternal milk, for optimal infant neurological development [39], the low frequency of n-3 LCPUFA food sources and high n-6/n-3 ratio, observed in the diet of the adolescent mothers, could result in poorer milk FA profile and offspring health.

So far, studies on TFA intake have been primarily focused on industrial sources, which have been associated with adverse effects on infant development [7, 14, 16, 22]. These negative effects are mainly attributed to isomers artificially produced in partially hydrogenated vegetable oils (PHVO), such as 6/7/8*t*-18:1, 9*t*-18:1 and 10*t*-18:1 [40], and are weakly related to TFA of natural sources, found in ruminant fats, such as vaccenic (11*t*-18:1) and rumenic (9*c*,11*t*-18:2) acids [41].

At present, insufficient data are available concerning the isomeric distribution of 18:1 TFA from different food sources in Brazil, and human dietary intake of these individual isomers is generally unknown. Nonetheless, we were able to indirectly assess the total intake of 18:1 TFA, which presented an average value of 2.94 g/day, corresponding

to 1.21 % of the daily energy intake, as well as 18:2 TFA, which represented less than 0.5 % of the energy intake. This estimated TFA consumption is slightly above the maximum recommendation of less than 1 % of total energy intake [18], even though total dietary fat was low. It should be noted that the Brazilian Table of Food Composition [24], used to determine TFA in the diet, comprises food products that were analyzed prior to the TFA labeling resolution in Brazil, possibly leading to overestimation of industrial *trans* fat consumption. Thus, considering data from the food intake questionnaires and the TFA distribution found in maternal milk samples, it is hypothesized that mainly ruminant but also industrial sources may have considerably contributed to the TFA dietary intake, including bovine milk, beef, margarine, cream crackers, bread, cookies (filled and unfilled), stuffed buns and creamy sweets.

Recent evidence in Brazil revealed a reduction in TFA contents in processed foods, such as biscuits [42]. In fact, the *trans* intake found in the present study was less than the one reported by pregnant adolescents living in the United States (2.8 %) [4] and Costa Rica (4.35 %) [43]. In 2003, a study estimating TFA intake was carried out by de Castro *et al*. [44] on a sample of 2298 male and female subjects, including 803 adolescents, living in São Paulo, Southeastern Brazil. The authors found a mean total TFA intake of 7.4 g/day or 3.0 % of total energy intake, considering the sum of hexadecenoic acid (7*t*-16:1), elaidic acid (9*t*-18:1) and linoelaidic acid (9*c*,12*t*-, 9*t*,12*c*- and 9*t*,12*t*-18:2). These data suggest that the consumption of industrial *trans* fat in Brazil may have actually declined, after the enforcement of the mandatory declaration of TFA content on labels of manufactured products. However, it should be pointed out that nutritional information on food labels can be estimated based on the original ingredients, using food composition tables, instead of direct analysis of processed food products. Since distribution of TFA isomers considerably varies among different hydrogenated fats and food categories, and TFA content might be influenced by food processing and preparation, the exact intake of TFA is difficult to estimate and may not be accurately stated in food labels.

Maternal TFA exposure can also be indirectly estimated by determining FA composition of maternal biological samples, including breast milk [39]. In this study, the mean total 18:1 TFA concentration found in both milk samples (colostrum and mature milk) of Brazilian adolescent mothers did not exceed 2 % (Table 3). This result is in marked contrast to those from several Western countries that reported total TFA averages of 3.8–7.2 % in human milk [45–47]. Regarding changes over time in Brazil, the values found in the present study were lower than the ones from an earlier study performed by our group [15], in which data collection occurred between 2001 and 2003. Our previous results and a study by Silva *et al*. [48], that evaluated the content of TFA in the milk of Brazilian women before the mandatory labeling of TFA, showed slightly higher mean values of total TFA (2.34 % in colostrum and 2.19 % in mature milk and 2.36 % in mature milk, respectively). In a different study, performed between 2009 and 2010 in São Paulo, Southeastern Brazil [49], with adult women living far from the coastal area, the content of TFA found in mature milk was 2.05 %, a result similar to that reported in the present study. Therefore, the introduction of mandatory legislation regarding TFA content in foods, in several countries, seems to have promoted a decline in total TFA content in human milk, including in Brazil.

Changes in milk composition according to the time of the lactation period may reflect adaptations to the nutritional needs of the baby as well as the increased capacity of the child's digestive system and metabolism [50]. In the present study, as time increased, there was a significant reduction in the levels of TFA, total n-3 PUFA, ARA and DHA, while higher levels of LNA were observed in mature milk compared to colostrum. Other studies have found similar changes in the FA composition as function of time over the lactation period [15, 51]. High proportions of LCPUFA in milk immediately after birth meet the infant needs during the first week of life, when total breast milk consumption is still low and the requirements of these FA is high [52]. Furthermore, higher FA levels in colostrum may be associated with the increased rate of lipolysis in maternal adipose tissue to meet part of her energy needs and promote an effective milk production during early lactation [53].

Human milk FA, which are implicated in infant neurological development, are extremely sensitive to maternal nutrition. Particular attention is given to the n-3 PUFA because of the dependence of humans on their dietary sources and the presence of high amounts of DHA in the brain and retina [39]. In general, the FA composition of HM from Brazilian women is similar to that of women from other countries [50, 54]. However, milk DHA content is lower in most studies involving Brazilian women [20, 48, 49, 55] when compared to mean international data [54, 56]. These results are consistent with the low intake of dietary sources of n-3 LCPUFA in Brazilian lactating adolescents and their DHA status, evaluated in the present study and corroborated by others studies on Brazilian adolescents [20, 57]. In fact, some authors reported that n-3 LCPUFA concentrations in plasma are more dependent on maternal dietary intake than on endogenous production [58, 59]. Unfortunately, the impact of inadequate dietary intake of LCPUFA by adolescent mothers on infant development has not been well evaluated in Brazil.

Evidence suggests that different TFA isomers from varied food sources may elicit different effects [59, 60]. The potential impairment of EFA metabolism to their long-chain n-3 and n-6 metabolites by specific *trans* isomers, in humans,

deserves special attention. For instance, regarding n-3 LCPUFA content in fetal plasma, a negative correlation was found only for 9*t*-18:1 (elaidic acid), but not for 11*t*-18:1 (vaccenic acid) [61]. In contrast, in the present study, no significant correlation was observed between 9*t*-18:1 nor 11*t*-18:1 and n-3 LCPUFA in milk, although, in colostrum, the concentration of n-3 LCPUFA was inversely correlated with total TFA. Recently, another study carried out by our group [21] found a significant positive association between total n-3 PUFA in plasma of the adolescent mothers and newborn weight and head circumference. In the present study, there were no statistically significant associations between ARA and DHA levels in milk and growth parameters in the newborns.

The 9*c*,11*t* CLA (rumenic acid) content of breast milk may be used as a marker of dairy fat intake, since it is found almost exclusively in ruminant fat [62]. On the other hand, some TFA such as vaccenic acid (11*t*-18:1) are known to be present in both natural fat of ruminant origin and PHVO [5]. Moreover, vaccenic acid is a metabolic precursor of 9*c*,11*t* CLA [5, 61, 62]. Therefore, it is very difficult to evaluate the relative intake of ruminant and industrial sources of TFA through the analysis of *trans* isomers present in biological samples. A previous biomarker study has shown that diets rich in dairy products are strongly associated with low 9*t*/11*t*-18:1 ratio in human milk (ratio <1), whereas diets low in dairy products (and probably higher in PHVO-originated TFA) lead to a high ratio (>1) [63]. In our study, both in colostrum and in mature milk, 11*t*-18:1 vaccenic acid was found to be the most abundant 18:1 isomer, followed by 9*t*-18:1 elaidic acid, whereas 9c,11t CLA rumenic acid was the predominant 18:2 isomer. Our study also found 9t/11t-18:1 ratios of 0.51 and 0.38; and 10*t*/11t-18:1 ratios of 0.36 and 0.28, in colostrum and mature milk, respectively (Table 4). These results are similar to those reported in human milk samples from Netherlands and German women, in which 11*t*-18:1 was the major 18:1 isomer, with a 9*t*/11*t*-18:1 ratio of 0.54 and a 10*t*/11*t*-18:1 ratio of 0.47 [62, 64], indicating high dairy intake.

A recent study has shown that high concentrations of vaccenic acid in maternal plasma during the first trimester of pregnancy may protect against the development of atopic eczema in 1-year-old infants [59]. In addition, a direct correlation between rumenic acid and n-3 LCPUFA in maternal plasma was observed in this study [59], thereby confirming the variable effects of TFA, depending on isomeric specificity. These results point out the need for further studies that examine the long term effects of specific dietary TFA in more detail, as well as the metabolism of individual isomers during the gestational and postnatal periods.

Limitations to our study include: (a) the relatively small sample size; (b) the use of a convenience sample, both factors negatively affecting the extrapolation of the results to the general population and (c) as in our previous studies [15, 21, 22, 35], we did not include specific analytical techniques to confirm the identity of specific *trans* isomers, as reported by others [62, 64]. Although we cannot eliminate the possibility that overlap of *cis* and *trans* isomers may have occurred to some extent, the gas chromatography methodology employed in this study was able to properly separate the 9*t*-,10*t*- and 11*t*-18:1 isomers, which are the major TFA in partially hydrogenated oils and dairy fats [2, 5], as well as 9*c*,11*t*-18:2 CLA (rumenic acid) in milk fat of Brazilian women [37]. Finally, the Brazilian Food Composition Table that was used to estimate TFA in maternal diet includes food products that were analyzed before TFA labeling became mandatory, and does not comprise information on individual *trans* isomers, but only the sum of all TFA, thus not providing the detailed information that would be required to evaluate the relative intake of industrial and ruminant *trans* fats.

In conclusion, this study on adolescent mothers living in the city of Rio de Janeiro, State of Rio de Janeiro, Brazil, further evidences the impact of maternal dietary intake on the fatty acids composition of human milk. The 18:1 and 18:2 *trans* fatty acids concentrations observed in milk samples and maternal diet reflected the intake of meat and dairy products from ruminants, as well as processed foods containing partially hydrogenated fats. Furthermore, the amounts of *trans* fatty acids found in the mother's diet and breast milk (colostrum and mature milk) showed a decrease in relation to those reported by studies prior to the Brazilian resolution that made the statement of *trans* fat content on food labels mandatory. However, the current low intake of n-3 PUFA food sources and consequent small amounts of n-3 LCPUFA in milk samples of Brazilian adolescent mothers might still be of public health concern.

References

1. Rodrigues PRM, Pereira RA, Cunha DB, Sichieri R, Ferreira MG, Vilela AAF, Gonçalves-Silva RM (2012) Factors associated with dietary patterns in adolescents: a school-based study in Cuiabá. Mato Grosso Rev Bras Epidemiol 15(3):662–674
2. Emken EA (1984) Nutrition and biochemistry of *trans* and positional fatty acid isomers in hydrogenated oils. Annu Rev Nutr 4:339–376
3. Hulshof KF, van Erp-Baart MA, Anttolainen M, Becker W, Church SM, Couet C, Hermann-Kunz E, Kesteloot H, Leth T, Martins I, Moreiras O, Moschandreas J, Pizzoferrato L, Rimestad AH, Thorgeirsdottir H, van Amelsvoort JM, Aro A, Kafatos AG, Lanzmann-Petithory D, van Poppel G (1999) Intake of fatty acids in western Europe with emphasis on *trans* fatty acids: the TRANSFAIR Study. Eur J Clin Nutr 53(2):143–157
4. Craig-Schmidt MC (2006) World-wide consumption of *trans* fatty acids. Atheroscler Suppl 7:1–4
5. Kuhnt K, Baehr M, Rohrer C, Jahreis G (2011) *Trans* fatty acid isomers and the trans-9/trans-11 index in fat containing foods. Eur J Lipid Sci Technol 113:1281–1292
6. Turpeinen AM, Mutanen M, Aro A, Salminen I, Basu S et al (2002) Bioconversion of vaccenic acid to conjugated linoleic acid in humans. Am J Clin Nutr 76:504–510

7. Craig-Schmidt MC (2001) Isomeric fatty acids: evaluating status and implications for maternal and child health. Lipids 36:997–1006
8. Kummerow FA, Zhou Q, Mahfouz MM, Smiricky MR, Grieshop CM, Schaeffer DJ (2004) *Trans* fatty acids in hydrogenated fat inhibited the synthesis of the polyunsaturated fatty acids in the phospholipid of arterial cells. Life Sci 74:2707–2723
9. Motard-Belanger A, Charest A, Grenier G, Paquin P, Chouinard Y *et al* (2008) Study of the effect of *trans* fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. Am J Clin Nutr 87:593–599
10. Brouwer IA, Wanders AJ, Katan MB (2010) Effect of animal and industrial *trans* fatty Acids on HDL and LDL cholesterol levels in humans—a quantitative review. PLoS One 5(3):e9434
11. Chardigny JM, Destaillats F, Malpuech-Brugere C, Moulin J, Bauman DE *et al* (2008) Do *trans* fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the *trans* fatty acids collaboration (TRANSFACT) study. Am J Clin Nutr 87:558–566
12. Samur G, Topcu A, Turan S (2009) *Trans* fatty acids and fatty acid composition of mature breast milk in Turkish women and their association with maternal diet's. Lipids 44(5):405–413
13. Demmelmair H, Baumheuer M, Koletzko B, Dokoupil K, Kratl G (1998) Metabolism of U13C-labeled linoleic acid in lactating women. J Lipid Res 39:1389–1396
14. Hornstra G, van Eijsden M, Dirix C, Bonsel G (2006) *Trans* fatty acids and birth outcome: some first results of the MEFAB and ABCD cohorts. Atheroscler Suppl 7:21–23
15. Tinoco SMB, Sichieri R, Setta CL, Moura AS, Tavares do Carmo MG (2008) *Trans* fatty acids from milk of Brazilian mothers of premature infants. J Paediatr Child Health 44(1–2):50–56
16. Elias SL, Innis SM (2001) Infant plasma *trans*, n-6, and n-3 fatty acids and conjugated linoleic acids are related to maternal plasma fatty acids, length of gestation, and birth weight and length. Am J Clin Nutr 73:807–814
17. Innis SM (2007) Dietary n-3 fatty acids and brain development. J Nutr 137:855–859
18. World Health Organization (2003) Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series No 916. Geneva, WHO, 2003
19. ANVISA. The National Sanitary Surveillance Agency. Ministry of Health. Foods e Obligatory Nutritional Labelling of Foods. RDC Resolution no. 360. http://www.anvisa.gov.br/legis/resol/2003/rdc/360_03rdc.htm. Accessed 12 Apr 2008
20. Meneses F, Torres AG, Trugo NM (2008) Essential and long-chain polyunsaturated fatty acid status and fatty acid composition of breast milk of lactating adolescents. Br J Nutr 100(5):1029–1037
21. Oliveira ORC, Santana MG, Santos FS, Conceição FD, Sardinha FLC, Veiga GV, Tavares do Carmo MG (2012) Composition of fatty acids in the maternal and umbilical cord plasma of adolescent and adult mothers: relationship with anthropometric parameters of newborn. Lipids Health Dis 11(1):157–165
22. Santos FS, Chaves CRMM, Costa RSS, Oliveira ORC, Santana MG, Conceição FD, Sardinha FLC, Veiga GV, Tavares do Carmo MG (2012) Status of *cis* and *trans* fatty acids in Brazilian adolescent mothers and their newborns. J Pediatr Adolesc Gynecol 25:270–276
23. UNIFESP—Universidade Federal de São Paulo. Escola Paulista de Medicina (2002) Programa de apoio a nutrição (NUTWIN): programa de computador, versão 1.5. São Paulo, Brazil
24. Tabela brasileira de composição de alimentos/NEPA–UNICAMP.-4. ed. rev. e ampl. -- Campinas: NEPAUNICAMP (2011), p 161. http://www.unicamp.br/nepa/taco/tabela.php?ativo=tabela. Accessed 15 Oct 2011
25. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R (1991) New Ballard score, expanded to include extremely premature infants. J Pediatr 119(3):417–423
26. Brasil. Ministério da Saúde (2004) Vigilância Alimentar e Nutricional–SISVAN: orientações básicas para a coleta e análise de dados antropométricos em serviços de saúde. Série A. Normas e Manuais Técnicos. Ministério da Saúde, Brasília
27. WHO (2009) Anthro for personal computers, version 3: software for assessing growth and development of the world's children. WHO, Geneva. http://www.who.int/childgrowth/software/en/. Accessed 18 Oct 2009
28. Ministério da Saúde, Brasil (2008) Secretaria de Atenção à Saúde. Departamento de Atenção Básica. Protocolos do Sistema de Vigilância Alimentar e Nutricional–SISVAN na assistência à saúde/Ministério da Saúde, Secretaria de Atenção à Saúde. Departamento de Atenção Básica–Brasília: Ministério da Saúde il.–(Série B. Textos Básicos de Saúde)
29. Ministério da Saúde (2005) Departamento de Ações Programáticas Estratégicas. Área Técnica de Saúde da Mulher, Brasil. Pré-natal e puerpério: atenção qualificada e humanizada: manual técnico. Brasília, DF. (Série A: Normas e Manuais técnicos)
30. World Health Organization (WHO) (2007) Growth reference data for 5–19 years. http://www.who.int/growthref/who2007_bmi_for_age_field/en/index.htmL. Accessed 05 June 2009
31. WHO (2009) AnthroPlus for personal computers Manual: Software for assessing growth of the world's children and adolescents. WHO, Geneva. http://www.who.int/growthref/tools/en/. Accessed 05 June 2009
32. Brasil (2008) Ministério da Saúde. Indicadores e Dados Básicos—Brasil—2008b IDB-2008. http://tabnet.datasus.gov.br/cgi/idb2000/fqb05.htm. Accessed 08 Aug 2009
33. Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509
34. Lepage G, Roy CC (1986) Direct transesterification of all classes of lipid in a one etep reaction. J Lipid Res 27(1):114–120
35. Costa RSS, Santos FS, Conceição FD, Saunders C, Sardinha FLC, Chaves CRMM, Tavares do Carmo MG (2011) Plasma levels of *trans*-fatty acids are low in exclusively breastfed infants of adolescent mothers. Lipids 46:537–543
36. Destaillats F, Golay PA, Joffre F, Wispelaere M, Hug B, Giuffrida F, Fauconnot L, Dionisi F (2007) Comparison of available analytical methods to measure *trans*-octadecenoic acid isomeric profile and content by gas–liquid chromatography in milk fat. J Chromatogr A 1145:222–228
37. Torres AG, Ney JG, Meneses F, Trugo NMF (2006) Polyunsaturated fatty acids and conjugated linoleic acid isomers in breast milk are associated with plasma non-esterified and erythrocyte membrane fatty acid composition in lactating women. Br J Nutr 95:517–524
38. Institute of Medicine (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. National Academy Press, Washington
39. Innis SM (2014) Impact of maternal diet on human milk composition and neurological development of infants. Am J Clin Nutr 99(suppl):734S–741S
40. Cook H, Emken EA (1990) Geometric and positional fatty acid isomers interact differently with desaturation and elongation of linoleic and linolenic acids in cultured glioma cells. Biochem Cell Biol 68:653–660
41. Rosenthal MD, Whitehurst MC (1983) Selective effects of isomeric *cis* and *trans* fatty acids on fatty acyl D9 and D6 desaturation by human skin fibroblasts. Biochim Biophys Acta 753:450–459
42. Dias FSL, Passos MEA, Tavares do Carmo MG, Lopes MLM, Mesquita VLV (2015) Fatty acid profile of biscuits and salty

snacks consumed by Brazilian college students. Food Chem 171:351–355
43. Monge-Rojas R, Campos H, Rojas XF (2005) Saturated and *cis*- and *trans*-unsaturated fatty acids intake in rural and urban costa rican adolescents. J Am Coll Nutr 24(4):286–293
44. Castro MA, Barros RR, Bueno MB, Cesar CLG, Fisberg RM (2009) *Trans* fatty acid intake among the population of the city of Sao Paulo, Southeastern Brazil. Rev Saude Publ 43:991–997
45. Mosley EE, Wright AL, McGuire MK, McGuire MA (2005) *Trans* fatty acids in milk produced by women in the United States. Am J Clin Nutr 82:1292–1297
46. Friesen R, Innis SM (2006) *Trans* fatty acids in human milk in Canada declined with the introduction of trans fat food labeling. J Nutr 136:2558–2561
47. Koletzko B, Mrotzek M, Bremer HJ (1988) Fatty acid composition of mature human milk in Germany. Am J Clin Nutr 47:954–959
48. Silva MH, Silva MT, Brandão SC, Gomes JC, Peternelli LA, Franceschini SC (2005) Fatty acid composition of mature breast milk in Brazilian women. Food Chem 93:297–303
49. Nishimura RY, Castro GSF, Jordão Junior AA, Sartorelli DS (2013) Breast milk fatty acid composition of women living far from the coastal area in Brazil. J Pediatr (Rio J) 89(3):263–268
50. Donangelo CM, Trugo NMF (2003) Lactation. Human milk composition and nutritional value. In: Trugo LC, Finglas PM (eds) Encyclopedia of food sciences and nutrition, 20th edn, vol 6. Academic Press, Elsevier Science, pp 3449–3458
51. Sala-Vila A, Castellote AI, Rodriguez-Palmero M, Campoy C, Lopez-Sabater MC (2005) Lipid composition in human breast milk from Granada (Spain): changes during lactation. Nutrition. 21(4):467–473
52. Genzel-Boroviczény O, Wahle J, Koletzko B (1997) Fatty acid composition of human milk during the 1st month after term and preterm delivery. Eur J Pediatr 156(2):142–147
53. Herrera E, Ortega-Senovilla H (2010) Maternal lipid metabolism during normal pregnancy and its implications to fetal development. Clin Lipidol 5:899–911
54. Innis SM (2007) Human milk maternal dietary lipids and infant development. Proc Nutr Soc. 66(3):397–404
55. Torres AG, Trugo NMF (2009) Evidence of inadequate docosahexaenoic acid status in Brazilian pregnant and lactating women. Rev Saúde Pública 43(2):359–368
56. Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, Arterburn LN (2007) Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. Am J Clin Nutr 85(6):1457–1464
57. da Cunha J, Macedo da Costa TH, Ito MK (2005) Influences of maternal dietary intake and suckling on breast milk lipid and fatty acid composition in low-income women from Brasilia, Brazil. Early Hum Dev 81:303–311
58. Donahue SM, Rifas-Shiman SL, Olsen SF, Gold DR, Gillman MW, Oken E (2009) Associations of maternal prenatal dietary intake of n-3 and n-6 fatty acids with maternal and umbilical cord blood levels. Prostaglandins Leukot Essent Fatty Acids 80:289–296
59. Chisaguano AM, Montes R, Castellote AI, Morales E, Júlvez J, Vioque J, Sunyer J, López-Sabater MC (2014) Elaidic, vaccenic, and rumenic acid status during pregnancy: association with maternal plasmatic LC-PUFAs and atopic manifestations in infants. Pediatr Res 76(5):470–476
60. Kuhnt K, Wagner A, Kraft J, Basu S, Jahreis G (2006) Dietary supplementation with 11trans- and 12trans-18:1 and oxidative stress in humans. Am J Clin Nutr 84:981–988
61. Enke U, Jaudszus A, Schleussner E, Seyfarth L, Jahreis G, Kuhnt K (2011) Fatty acid distribution of cord and maternal blood in human pregnancy: special focus on individual *trans* fatty acids and conjugated linoleic acids. Lipids Health Dis 10:247
62. Mueller A, Thijs C, Rist L, Simões-Wust AP, Huber M, Steinhart H (2010) *Trans* fatty acids in human milk are an indicator of different maternal dietary sources containing *trans* fatty acids. Lipids 45:245–251
63. Rist L, Mueller A, Barthel C, Snijders B, Jansen M, Simoes-Wust AP, Huber M, Kummeling I, von Mandach U, Steinhart H, Thijs C (2007) Influence of organic diet on the amount of conjugated linoleic acids in breast milk of lactating women in the Netherlands. Br J Nutr 97:735–743
64. Precht D, Molkentin J (1999) C18:1, C18:2 and C18:3 *trans* and *cis* fatty acid isomers including conjugated cis delta 9, trans delta 11 linoleic acid (CLA) as well as total fat composition of German human milk lipids. Nahrung 43:233–244