Dietary and breast milk *trans* fatty acids seen in Croatian breastfeeding women from Adriatic region

GRETA KREŠIĆ – MIHELA DUJMOVIĆ – MILENA L. MANDIĆ – IVANČICA DELAŠ

Summary

This paper aims to provide the information on the ratio of *trans* fatty acids (TFA) in breast milk obtained from 83 Croatian breastfeeding women at 3 months postpartum, and to relate that information to dietary TFA intake. The diet was evaluated using two consecutive 24-hour recalls, while breast milk fatty acid composition was determined using gas chromatography. The obtained results showed daily TFA intake to be 2.0 g or 0.8% daily energy intake. The major sources of TFA were bakery, confectionery and fried food (35.4%), together with dairy products (21.7%) and margarine (19.9%). Women having a high level of *trans* isomers in their milk consumed significantly higher amounts of the above products. The ratio of TFA in total fatty acids was 2.3%, and was inversely correlated to the ratio of arachidonic ($r = -0.440$, $p = 0.040$), linoleic ($r = -0.275$, $p = 0.025$) and α-linolenic ($r = -0.213$, $p = 0.038$) acids. It could be concluded that Croatian breastfeeding women should be advised on daily intake of foods which were identified as TFA sources, since TFA ratio in milk reflects mothers' current diet and could negatively affect essential fatty acids metabolism.

Keywords

breast milk; breastfeeding women; diet; *trans* fatty acids

Trans fatty acids (TFA) are unsaturated fatty acids containing one or more isolated (i.e. non-conjugated) double bonds in *trans*-configuration, the conjugated linoleic acid thereby being the exception. Two major dietary sources of TFA are industrial hydrogenation of vegetable oils and microbial hydrogenation of polyunsaturated fatty acids taking place in animal rumen. Sources of natural TFA are milk, dairy products and meat. Ruminant fats generally contain TFA in the ratio of 1% to 8% of total fatty acids, with C18:1*t*11 (vaccenic acid) being the predominant *trans* C18:1 isomer [1]. On the other hand, due to the presence of partially hydrogenated vegetable oils, industrially processed foodstuffs can contain up to 50% TFA, mainly elaidic acid (C18:1*t*9) and octadecenoic acid (C18:1*t*10) [1]. In the last decade, the effect of TFA on human health has become an important area of research, while evidence on TFA-related increase in cardiovascular and cancer risk stemming from epidemiological and clinical studies was recently reviewed by GEBAUER et al. [2].

From a paediatric perspective, atherogenic effect of TFA is not a concern; one should rather focus on their potential interference with the metabolism of long-chain polyunsaturated fatty acids (LCPUFA) and body composition. Special attention has been paid to the possible impairment of the conversion of essential fatty acids, linoleic acid (C18:2*n*-6) and α-linolenic acid (C18:3*n*-3), to their long-chain metabolites mediated in humans by *trans* isomers. Precisely, a possible inhibitory effect has been established for TFA on delta-6-desaturase and/or delta-5-desaturase, the enzymes included into the metabolic pathways of arachidonic acid (C20:4*n*-6) and docosahexaenoic acid (C22:6*n*-3) synthesis [3]. This impairment is an issue due to the role of breast milk LCPUFA in

Greta Krešić, Mihela Dujmović, Department of Food and Nutrition, Faculty of Tourism and Hospitality Management, University of Rijeka, Primorska 42, P. O. Box 97, 51410 Opatija, Croatia.
Milena L. Mandić, Department of Food and Nutrition Research, Faculty of Food Technology, University of Osijek, Franje Kuhača 20, 31000 Osijek, Croatia.
Ivančica Delaš, Department of Chemistry and Biochemistry, School of Medicine, University of Zagreb, Šalata 3, 10 000 Zagreb, Croatia.

Corresponding author:
Greta Krešić, tel.: ++385 51 294 714; fax: ++385 51 292 965, e-mail: Greta.Kresic@fthm.hr

early anatomic and functional development of the central nervous system [4]. Additionally, ANDERSON et al. [5] recently showed a positive correlation between mothers' TFA intake and the development of excess adipose tissue in both mothers and breastfeeding babies. Taking into account that the level of *trans* fatty acids and LCPUFA in breast milk mirrors mother's dietary intake, monitoring of the latter with special attention being paid to these two components and their supply to children via breast milk becomes an issue important to address [6].

Although studies on breast milk fatty acid composition and dietary intake of breastfeeding women residing in different countries all over the globe are numerous, the research conducted insofar in the Mediterranean region is fragmentary and mainly concerns Greece, Spain and Italy [7–9]. However, none of these works have specially emphasized the *trans* fatty acid problem. It is therefore fair to state that breast milk composition of the Mediterranean women still represents an under-investigated issue. To the best of our knowledge and with the exception of the above-stated, such studies have neither been done in Croatian, nor in other female populations occupying the Mediterranean region. That is exactly the reason why we chose a group of Croatian women populating the coastal Adriatic region, i.e. the northern part of the Mediterranean basin, to be our study sample.

In view of the above, this work was conducted with the aim to provide information on the ratio of TFA in total fatty acids in milk of breastfeeding women populating the Mediterranean Croatia and to relate these results to their dietary records. Within the frame of the study, the relationship between TFA and polyunsaturated fatty acids (PUFA) in the breast milk was given special attention. We hypothesized that the fatty acid (in particular TFA) composition of the breast milk these women excrete, is distinctive in its characteristics, which are possibly attributable to their dietary patterns.

MATERIALS AND METHODS

Subjects and breast milk sampling

This study involved 83 full breastfeeding mothers from the Croatian Primorsko-Goranska County. The women were volunteers recruited in paediatric clinics 3 months ± 1 week postpartum via word-of-mouth and based on the criteria listed below. Women aged 18 to 40, who have given birth to healthy, full-term babies having a birth weight of >2500 g, were considered eligible. Women suffering from any metabolic disorder were excluded from the study. The women were asked about their feeding practices, full breastfeeding thereby being defined as an almost exclusive breastfeeding allowing for some non-milk supplemental liquids (e.g. water or water-based drinks such as sweetened and flavoured water, teas and infusions), fruit juice, oral rehydration salt solution, drops and vitamins, minerals and medicines given in form of syrup [10]. The researchers arranged an appointment with every participant at their own homes in order to collect diet records and milk samples.

The data about the mothers' dietary intake were collected using two consecutive 24-hour recalls (including one weekend day), which were collected by a trained researcher during a home visit. The types and quantities of consumed foods were entered into a computer programme based upon the Croatian Food Composition Tables [11] and partly upon data brought by Danish food tables [12]. Danish food tables served as a source of data on the amount of a particular fatty acid in food. Average nutrient intakes were compared to the Dietary Reference Intakes (DRI) for breastfeeding adult women [13].

During the visit to mothers' homes, the researchers also collected breast milk samples. An amount of approximately 5 ml of breast milk was manually collected from each mother at the end of breastfeeding, i.e. between 10 and 12 a.m. The samples were immediately frozen in sterile plastic tubes and stored at –20 °C pending analysis. Analyses were performed within 3 months of collection.

This study was conducted according to the guidelines laid down under the Helsinki Declaration, while all the procedures were pre-approved by the Board of Ethics of the Faculty of Food Technology, University of Osijek, Croatia. After being thoroughly informed about the purpose, requirements and procedure of the study, all women signed informed consents.

Fatty acid analysis

Milk samples were thawed at room temperature and tempered in a mixer (TechnoKartell TK3S, Silverwater, Australia) before analysis. Lipids were extracted using the method described by BLIGH and DYER [14], while the preparation of fatty acid methyl esters (FAME) made use of the method described by HARTMAN and LAGO [15]. Fatty acid composition was determined using a gas chromatograph (SRI 8610C, SRI Instruments, Torrance, California, USA) equipped with

a flame ionization detector and (78% cyanopropyl) methylpolysiloxane capillary column 007-23 (Quadrex Corporation, Woodbridge, California, USA), 60 m × 0.25 mm × 0.25 μm. Hydrogen was used as a carrier gas at a flow rate of 22 ml·min^{-1}. The initial column temperature of 160 °C was kept for 10 min and then raised up to 230 °C at a rate of 10 °C·min^{-1}; the final temperature was maintained for 20 min. Injector and detector temperatures were 160 °C and 250 °C, respectively. Separated fatty acid methyl esters were identified by virtue of comparing the retention times with those obtained with pure standards (Supelco 37 Component FAME Mix in methylene chloride, EC Number 200-838-9; Supelco, Bellafonte, Pennsylvania, USA). Data were collected and processed using PeakSimple 3D software, version 2.97 (SRI instruments), while the results are reported as mass ratio of total fatty acids.

Statistical analysis

The normality of data distribution was tested using the Kolmogorov-Smirnov test; since the data were normally distributed, the results are expressed as means ± standard deviations. Correlation analysis was conducted with the aim to establish the strength of association between the ratio of TFA in milk and the mothers' dietary intake of TFA coming from main food sources. Additionally, within the frame of the statistical analysis, correlation analysis between each (C16:1*t* or C18:1*t*) TFA and other fatty acids, as well as between the sum of C16:1*t* and C18:1*t* and individual fatty acids, was established using the Pearson's correlation coefficient. Statistica 8.1 software (StatSoft, Tulsa, Oklahoma, USA) was used for statistical analysis.

RESULTS AND DISCUSSION

Characteristics descriptive of 83 breastfeeding Croatian women who participated in the study are shown in Tab. 1. The majority of the participants were primiparous (76%). The average duration of their education was 13.5 years, with 44.6% of the participating women holding university degrees. Although, according to the data of the Croatian Bureau of Statistics [16], 19.7% of women aged 25–34, and 17.5% of women aged 35–44 have university degree, if it is taken into account the fact that women who participated in study were volunteers, it is to be expected that more educated women are more motivated to participate, what was confirmed within our study sample.

According to dietary records, the diet of Croatian breastfeeding women at three months postpartum was hypo-caloric (77% DRI), with a share of energy derived from proteins, saccharides and lipids of 14.5%, 50.4% and 35.1%, respectively (Tab. 2). The hypo-caloric intake noted among our breastfeeding women was not of concern since it can probably be attributed to over-assessed recommendations for dietary intake applicable to this population, as reported previously [17]. The ratio of saturated : monounsaturated : polyunsaturated fatty acids intake was 1 : 1.04 : 0.48, with the daily intake of TFA of about 2 g, ranging from 1.1 g to 6.3 g. The share of energy coming from TFA represented less than 1% of the total daily energy intake (Tab. 2) and about 2.5% of the total daily fat intake (data not shown). Daily TFA intakes seen across our participants are in agreement with the reports on mean percentage contributions of TFA to fatty acid composition of the Mediterranean diet. In European countries, mean daily intakes of TFA range from minimal

Tab. 1. Characteristics of Croatian breastfeeding women.

	Mean ± standard deviation
Age at delivery [years]	31.8 ± 4.6
Weight [kg]	69.0 ± 13.5
Height [cm]	168.3 ± 5.5
Parity (number of children)	1.5 ± 0.7
Education [years]	13.5 ± 2.1
Body mass index [kg·m^{-2}]	24.3 ± 4.3

n = 83.

Tab. 2. Daily energy and nutrient intakes of Croatian breastfeeding women at three months postpartum.

Parameter	Nutrient intake	Daily energy intake [%]
Energy	9193.8 ± 2387.7 kJ	77.0 ± 19.9[a]
Proteins	81.4 ± 25.5 g	14.5 ± 2.9[b]
Saccharides	266.4 ± 74.9 g	50.4 ± 6.9[b]
Total lipids	81.3 ± 21.7 g	35.1 ± 6.4[b]
SFA	32.0 ± 8.7 g	13.9 ± 1.8[b]
MUFA *cis*	31.2 ± 8.7 g	14.4 ± 3.5[b]
PUFA	16.1 ± 8.2 g	6.8 ± 2.8[b]
TFA	2.0 ± 0.9 g	0.8 ± 0.3[b]

Values are expressed as mean ± standard deviation, n = 83. a – ratio of dietary reference intake; b – ratio of daily energy intake; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; TFA – *trans* fatty acids.

Tab. 3. Major sources of *trans* fatty acids in the diet of Croatian breastfeeding women at three months postpartum

Food groups	Daily intake of TFA [g·d^{-1}]	Contribution to total daily intake of TFA [%]
Bakery, confectionery, fried foods	0.73 ± 0.31	35.4
Dairy products	0.43 ± 0.19	21.7
Margarine	0.39 ± 0.17	19.9
Bread	0.17 ± 0.10	8.9
Red meat	0.12 ± 0.05	6.2
Sausages, salami	0.08 ± 0.03	4.2
Poultry, chicken	0.07 ± 0.03	3.1
Fish	0.002 ± 0.001	0.6
Total	2.00 ± 0.87	100.0

Values are expressed as mean ± standard deviation, $n = 83$. TFA – *trans* fatty acids.

intakes of 1.4–2.1 g or 0.5–0.7% of energy intake seen in Mediterranean countries (i.e. Greece, Italy, Spain and Portugal) to 2.1–2.8 g or 0.7–1.3% of energy intake seen in Germany, Sweden, Denmark and France. The highest TFA daily intakes of 4.3–5.4 g or 1.6–2.0% of energy intake were recorded in the Netherlands and Iceland [1]. The majority of Croatian breastfeeding women (54.2%) had a daily TFA intake of less than 2 g. In addition, 43.4% of our participants had a daily TFA intake in the range of 2–4 g. In only two participants (2.4%) a daily intake greater than 4 g was recorded (data not shown). The current recommendation on TFA intake advises not to consume them in quantities which surpass 1% of the total daily calories [18]. In North America and some European countries, a recent decrease in *trans* fatty acid diet content was observed, presumably due to modifications of the commercially available fats or due to changes in consumer choices. Producers established in European countries have already decreased TFA contents in their products, with many table spreads and snack foods being reformulated [1]. Additionally, it has recently been shown that concentration of *trans* fatty acids in milk and muscle can be varied through dietary fatty acid manipulation in lactating cows [19].

The major sources of *trans* isomers in the mothers' diets were bakery, confectionary and fried foods (35.4%). The second most represented sources were dairy products (21.7%), followed by margarine (19.9%) (Tab. 3). Similarly, bakery products, confectionery and snacks were the major sources of TFA in the diet of breastfeeding women on a typical westernized diet [20, 21]. Although human metabolism is able to elongate and desaturate the ingested *trans* isomeric fatty acids and transform them into longer-chain and less saturated products, it is unable to synthesize *trans* fatty acid isomers de novo. Consequently, *trans* fatty acid isomers detected in human milk must have their origin in the maternal diet [6]. According to data reported worldwide, TFA intake coming from the consumption of the ruminant products such as meat, milk and butter, represents the smaller but constant percentage of total TFA, while the proportion of TFA originating from processed food, bakery, snacks and fast food is continuously decreasing worldwide [22–24]. Due to detrimental health effects of TFA, several countries, e.g. Canada and USA, adopted the declaration of TFA content on food labels as mandatory [25]. In Europe, Denmark was the first country that implemented in 2003 the legislation restricting the proportion of TFA derived by industrial procedures in all food products and ready-to-eat meals to a maximum of 2% of total fat content [26]. Regretfully, Croatia has insofar failed to enforce any legislation governing the amount of TFA in food whatsoever, making TFA intake monitoring considerably difficult, since it can easily be underestimated if analysed solely using food composition tables coming from countries with strict legislation.

Although our analytical method allowed for a sensitive analysis of 21 fatty acids in breast milk samples using gas chromatography, in order to make the interpretation of the results easier, Tab. 4 shows only the proportion of certain fatty acids, expressed as a percentage of total fatty acids. Out of 32.6% of total saturated fatty acids, the majority were long chain saturated fatty acids (LCSFA) (23.3%), while the rest were medium chain saturated fatty acids (MCSFA) (9.3%). The saturated fatty acids level established in the breast milk of Croatian breastfeeding women was in agreement with worldwide reports on the mean

percentage of saturated fatty acids in total fatty acids [21, 27, 28]. Due to a diet characterized by a moderate intake of saccharides, the milk obtained from the studied women had a lower proportion of MCSFA in comparison to the milk of women from developing countries. Diet rich in saccharides (> 70% daily energy intake), common in developing countries, gives rise to MCSFA in breast milk [29]. Oleic acid, which is considered to be an important source of energy for a breast-fed infant, was detected in the highest proportion (43.0% of total fatty acids) (Tab. 4). Since olive oil was one of the dominant fats used in food preparation, the value of oleic acid fraction in total fatty acids in milk which reflects mothers' diet was higher as compared to the usual value of 25–30% obtained by other researchers [21, 27]. Another possible reason for higher oleic acid proportion could be the higher SCD (stearoyl-coenzyme A desaturase) activity. SCD is a rate-limiting enzyme playing role in the synthesis of monounsaturated fatty acids (oleic acid) from saturated fatty acids (stearic acid). Therefore, its increased activity could cause a higher de novo oleic acid synthesis. Total essential fatty acids approximated to 17.5%, out of which linoleic acid was predominant, with the share of 16.3%. LCPUFA, among which the most important were eicosapentaenoic (C20:5*n*-3) and docosahexaenoic acid (C22:6*n*-3), were detected in the proportion of 0.1% and 0.2%, respectively. Among PUFA, the predominant ones were fatty acids from *n*-6 group (18.2%) as compared to *n*-3 group (1.7%) (Tab. 4).The value of total PUFA fraction was higher than that detected in European population (10–16.6%), with the predominance of PUFA from *n*-6 series. The ratio of total *n*-6 to *n*-3 unsaturated fatty acids (11 : 1) was in accordance with the recommended ratio in the range from 5 to 15. The ratio of two major LCPUFA: docosahexaenoic acid and arachidonic acid in human milk is of particular importance since short-term diets clearly influence the LCPUFA breast milk content, while evidence shows that habitual intake has an influence as well [30]. The proportion of arachidonic acid (0.4%) in the milk of Croatian women was lower than that revealed by meta-analysis of global data (0.5%) [25]. Similar situation was with the proportion of docosahexaenoic acid (0.2%), which was also by 0.1% lower than the worldwide average [31] (Tab. 4). Of concern is the fact that the proportion of docosahexaenoic acid found in the breast milk of Croatian women was the lowest in the Mediterranean region [7–9].

The average proportion of TFA in total fatty acids was 2.3%, ranging from 1.1% to 5.1%. This value is similar to the averages established in other countries, varying from 0.5% to 7.2% [1]. Only a few studies reported that human milk from countries with traditionally high TFA consumption (i.e. Canada and USA) contained about 7% of TFA [32]. However, the mean TFA breast milk values reported for European countries, i.e. Poland (2.7%), Italy (2.7%), Czech Republic (2.1%), France (2%) and Turkey (2.1%), were all similar to the values seen in our study [21]. The differences in values of total TFA fraction in breast milk established in various parts of the world may be attributed to the differences in the diets of the women in question. *Trans* isomeric octadecanoic acid (C18:1*t*) represented the main *trans* isomer group (2.3%) reflecting the composition of the maternal diet. Relatively small but detectable amounts of *trans* C16:1 (0.1%) indicated that *trans* fatty acids in the maternal diet also came from commercially hydrogenated fish oils added to confectionery together with hydrogenated vegetable oils [33].

Tab. 4. Proportions of fatty acids in total fatty acids in the breast milk of Croatian women at three months postpartum.

Fatty acids	Ratio [%]
C16:1 *trans*	0.1 ± 0.0
C18:1 *trans*	2.3 ± 0.2
Total TFA	2.3 ± 0.2
Total SFA	32.6 ± 1.9
Total LCSFA	23.3 ± 1.8
Total MCSFA	9.3 ± 0.4
C18:1 *n*-9 (oleic acid)	43.0 ± 2.0
C18:3 *n*-3 (α-linolenic acid)	1.4 ± 0.0
C18:2 *n*-6 (linoleic acid)	16.3 ± 0.6
Total essential fatty acids[a]	17.5 ± 0.4
C20:5 *n*-3 (eicosapentaenoic acid)	0.1 ± 0.0
C22:6 *n*-3 (docosahexaenoic acid)	0.2 ± 0.0
C20:4 *n*-6 (arachidonic acid)	0.4 ± 0.0
Total LCPUFA	1.2 ± 0.1
Total *n*-3 PUFA[b]	1.7 ± 0.1
Total *n*-6 PUFA[c]	18.2 ± 0.6

Values are expressed as mean ± standard deviation, $n = 83$.
TFA – *trans* fatty acids, SFA – saturated fatty acids. LCSFA – long chain saturated fatty acids (C16:0, C17:0, C18:0, C20:0, C22:0, C24:0), MCSFA – medium chain saturated fatty acids (C12:0, C14:0), LCPUFA – long chain polyunsaturated fatty acids (C20:3*n*-6, C22:4*n*-6, C22:5*n*-6, C20:5*n*-3, C22:6*n*-3, C20:4 *n*-6), PUFA – polyunsaturated fatty acids.
a – include C18:3*n*-3 and C18:2*n*-6; b – include C18:3*n*-3, C20:5*n*-3, C22:5*n*-3, C22:6*n*-3; c – include C18:2*n*-6, C20:3*n*-6, C20:4*n*-6, C22:4*n*-6, C22:5*n*-6.

Tab. 5. Correlation between *trans* fatty acid intake from the certain food groups and the proportion of *trans* fatty acids in total fatty acids in breast milk.

Food groups	*r*	*p*-value
Bakery, confectionery, fried foods	0.314	0.039
Dairy products	0.285	0.014
Margarine	0.251	0.047
Sausages, salami	0.222	0.044
Red meat	0.126	ns
Poultry, chicken	0.125	ns
Fish	0.099	ns
Bread	0.098	ns

$n = 83$.
r – Pearson's correlation coefficient, ns – not significant.

The influence of breastfeeding mothers' diet on the proportion of TFA in total fatty acids in milk is shown in Tab. 5. The results of the statistical analysis revealed a weak albeit significant positive correlation between bakery, confectionery and fried food intakes ($r = 0.314$; $p = 0.039$), the intake of dairy products ($r = 0.285$, $p = 0.014$), margarine ($r = 0.251$, $p = 0.047$) and sausages ($r = 0.222$, $p = 0.044$), known to be TFA sources, and the proportion of TFA in milk. This suggests that the proportion of TFA in human milk reflected the current maternal TFA intake and that mothers who had a high level of *trans* isomers in their milk consumed significantly higher amounts of TFA-containing food. We have recently shown the correlation of similar strength between the dietary intake of some other fatty acids and their proportion in breast milk [34].

A negative and statistically significant correlation was found between total TFA and principal fatty acids in human milk. This correlation was the strongest for arachidonic acid ($r = –0.440$, $p = 0.040$), and weak albeit significant for linoleic acid ($r = –0.275$, $p = 0.025$) and α-linolenic acid ($r = –0.213$, $p = 0.038$). A very weak correlation was established between eicosapentaenoic acid ($r = –0.065$, $p = 0.026$) or docosahexaenoic acid ($r = –0.032$, $p < 0.001$) and total TFA proportion. There were no significant inverse correlations between *trans* hexadecenoic acid (C16:1*t*) and the investigated *n*-3 and *n*-6 fatty acids. Total TFA showed a weak significant inverse correlation to the total *n*-3 PUFA ($r = –0.264$, $p = 0.041$) and total *n*-6 PUFA ($r = –0.122$, $p = 0.029$) (Tab. 6).

From a nutritional standpoint, one of the outputs of this study is the demonstration of a significant inverse correlation between the isomer of 18-carbon fatty acid and total TFA, as well as that between the biologically important essential fatty acids and various PUFA. Although the correlation was weak, taking into account the fact that breast milk level of PUFA detected across our study sample is the lowest in the Mediterranean, a potentially detrimental TFA effect is important to address. The results on the correlation between TFA and various PUFA in human milk are contradictory at

Tab. 6. Correlation between *trans*-isomeric and principal fatty acids in the breast milk of Croatian women.

Fatty acids	C16:1*t*		C18:1*t*		Total TFA	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value
Total SFA	0.005	ns	0.081	ns	0.075	ns
C18:1 *n*-9 (oleic acid)	0.022	ns	0.145	ns	0.107	ns
C18:3 *n*-3 (α-linolenic acid)	–0.009	ns	–0.243	0.041	–0.213	0.038
C18:2 *n*-6 (linoleic acid)	–0.004	ns	–0.312	0.030	–0.275	0.025
Total essential fatty acids[a]	–0.107	ns	–0.293	0.038	–0.266	0.031
C20:5 *n*-3 (eicosapentaenoic acid)	–0.040	ns	–0.074	0.036	–0.065	0.026
C22:6 *n*-3 (docosahexaenoic acid)	–0.011	ns	–0.061	< 0.001	–0.032	< 0.001
C20:4 *n*-6 (arachidonic acid)	0.072	ns	–0.491	0.041	–0.440	0.040
Total LCPUFA	–0.012	ns	–0.326	0.048	–0.306	0.037
Total *n*-3 PUFA[b]	–0.003	ns	–0.288	0.039	–0.264	0.041
Total *n*-6 PUFA[c]	–0.002	ns	–0.102	0.044	–0.122	0.029

$n = 83$.
TFA – trans fatty acids, SFA – saturated fatty acids, LCPUFA – long chain polyunsaturated fatty acids (C20:3*n*-6, C22:4*n*-6, C22:5*n*-6, C20:5*n*-3, C22:6*n*-3, C20:4*n*-6), PUFA – polyunsaturated fatty acids, *r* – Pearson's correlation coefficient, ns – not significant.
a – include C18:3*n*-3 and C18:2*n*-6; b – include C18:3*n*-3, C20:5*n*-3, C22:6*n*-3; c – include C18:2*n*-6, C20:3*n*-6, C20:4*n*-6, C22:4*n*-6, C22:5*n*-6.

best. In the study conducted by MOSLEY et al. [35], no correlation between TFA and *n*-6 or *n*-3 PUFA was found. However, SZABO et al. [32] found an inverse correlation between C18:1*t* and C18:2*t*, as well as between *n*-6 and *n*-3 PUFA, including C18:2*n*-6, C20:4*n*-6, C18:3*n*-3 and C22:6*n*-3. Similar to these results, the strongest correlation revealed by our research was that between total TFA and arachidonic acid. An inverse correlation between TFA and LCPUFA may be explained by the impairing effect TFA on the synthesis of 18:2*n*-6 and 18:3*n*-3 LCPUFA [3]. Studies on a porcine model confirmed that high exposure to TFA inhibited the incorporation of LCPUFA into phospholipids of arterial cells [36]. The putative effect of 18C TFA in terms of inhibiting the conversion of essential fatty acids into their longer-chain metabolites may explain the fact that the negative correlation between total TFA and arachidonic acid, such as between C18:1*t* and arachidonic acid, found in our study, was stronger than that between TFA and linoleic acid and α-linolenic acid.

Our study provides data on dietary TFA intake and the proportion of TFA in breast milk in Croatia for the first time. These results should also fill the gap in knowledge and reduce the scarcity of data on countries with a Mediterranean dietary pattern. In our study, dietary intakes were recorded and milk samples were collected without interfering with the lives of the breastfeeding women. A possible limitation of our study may be caused by the fact that participants were well educated, highly motivated and belonged to the well-to-do population group. As a further limitation could be considered that the knowledge about TFA levels in processed food available in Croatia is very limited, and further efforts are needed to update the national Food Composition Tables.

CONCLUSION

The present study provides information about TFA intake of Croatian breastfeeding women, mirrored in their milk, for the first time and therefore makes a valuable contribution to the scarce data describing the Mediterranean region. Although the dietary intake of TFA and their proportion in breast milk can be considered adequate, of concern is the established statistically significant inverse correlation between TFA in breast milk and arachidonic acid. Although the correlation established between TFA and other PUFA was weak, it should not be neglected. Lactating mothers should be advised to avoid foods that are identified as major sources of TFA, not just because of their own health but also because of the potential *trans* isomers-related impairment of essential fatty acid metabolism. Despite the weak effect, TFA intake and consequently its proportion in breast milk should be monitored carefully, since the proportion of PUFA in the milk of Croatian women was established to be among the lowest in the Mediterranean region.

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