Relationship between Mediterranean diet and breast milk fatty acid profile: a study in breastfeeding women in Croatia

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ORIGINAL PAPER

Relationship between Mediterranean diet and breast milk fatty acid profile: a study in breastfeeding women in Croatia

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Abstract The composition of breast milk secreted by Mediterranean women is still under-investigated. Due to the specific Mediterranean diet, the fatty acid profile of such milk could be distinctive. The objective of this study was to assess the relationship between maternal diet and fatty acid profile of the mature milk obtained from 83 breastfeeding women residing in the coastal Croatia, lactating for 5–25 weeks. Their diet was evaluated using two consecutive 24-h recalls, while the fatty acid milk content was determined using gas chromatography. Among the dietary intake of saturated, monounsaturated and polyunsaturated dietary fatty acids, the most represented were palmitic (21.70 g.day^{-1}), oleic (29.20 g.day^{-1}) and linoleic acid (13.81 g.day^{-1}), with the ratio of total dietary *n*−6/*n*−3 fatty acids of 12.01. In milk, the most represented primary monounsaturated fatty acid was oleic acid (39.63%), while the most represented saturated fatty acid was palmitic acid (20.65%). The share of linoleic, α-linolenic and docosahexaenoic fatty acid was 17.28%, 1.41% and 0.21%, respectively. The correlation between dietary fatty acids and their breast milk concentrations was established for docosahexaenoic (r=0.54, P<0.001), linoleic (r=0.24, P=0.032), palmitic (r=0.18, P=0.021) and oleic acid (r=0.21, P=0.024). In conclusion, the issue of concern is the sub-optimal dietary intake of *n*−3 long-chain polyunsaturated fatty acids, resulting in their low breast milk concentrations, especially that of docosahexaenoic acid. Given its biological importance, the impact of fatty acid profile on infant health should be further investigated.

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1 Introduction

Human breast milk provides an optimal infant nutrition, with the majority of its energy value being provided by fatty acids. Although breast milk composition on the whole can be considered relatively stable, fatty acid component of maternal diet mirrors in the fatty acid content of her breast milk (Innis 2007; Urwin et al. 2012).

Unsaturated fatty acids of a chain length greater than 20 carbon atoms, defined as long-chain polyunsaturated fatty acids (LC PUFAs), that is to say, docosahexaenoic acid (DHA, C22:6*n*−3) and arachidonic acid (AA, C20:4*n*−6), are essential for foetal and neonatal growth and neurodevelopment (Bernardi et al. 2012). Essential fatty acids such as linoleic (LA, C18:2*n*−6) and α-linolenic acid (ALA, C18:3*n*−3), which pose as the precursors of LC PUFAs, cannot be secreted by mammalian cells, so that the entire LC PUFA breast milk content originates from maternal diet, either directly or post-storage or further metabolism of the consumed food in maternal tissues (Agostoni 2010; Innis 2007).

Due to the typical Western diet, characterised by an increased intake of *n*−6 fatty acids and a low intake of foodstuffs rich in *n*−3 LC PUFAs, starting from the 1990s up to the 2000s, the breast milk secreted by women of western origin had been characterised by an increase in LA and a decrease in DHA content, as compared to its composition witnessed from the 1950s up to the 1980s. As suggested, the predominance of *n*−6 over *n*−3 fatty acids in breast milk has a negative impact on long-term infant health outcomes (as witnessed by an increased incidence of asthma, allergies and obesity). Consequently, LC PUFAs supply via breast milk is important to address (Gibson et al. 2011).

Contrary to the typical Western diet, the Mediterranean dietary pattern has been shifted into research focus due to its fatty acids profile characterised by a low intake of saturated, *n*−6 and *trans* fatty acids, as well as with a moderate intake of plant and marine *n*−3 fatty acids (De Lorgeril and Salen 2012). Mediterranean dietary pattern favouring the consumption of fruit and vegetables, whole grains, legumes, nuts, seeds and low-fat dairy products can be highly recommended to any given population (Kontou et al. 2011).

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 (Antonakou et al. 2012; Sala-Vila et al. 2005; Scopesi et al. 2001). It is therefore fair to state that the breast milk composition of 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.

Although an in-depth dietary intake analysis, especially that of dietary fatty acid profiles, has not been done, an epidemiological study conducted in Croatia has shown that women residing in the coastal part of Croatia stick to a dietary

pattern featured by the elements of Mediterranean diet being healthier than that observed by women residing in the northern and central Croatia (Doko-Jelinić et al. 2009). In view of the above, we have deliberately chosen a group of Croatian women populating the Adriatic coastal region, i.e. the northern part of the Mediterranean basin, for our study sample. We hypothesised that the fatty acid composition of the breast milk these women secret is distinctive in its characteristics, possibly attributable to their dietary intakes. We launched this study in hopes that the obtained results would be a welcomed supplement to otherwise scarce data on dietary and breast milk fatty acid profiles seen in women from this part of the Mediterranean.

The objective of this study was to gain insight into the dietary fatty acid intake and to assess the relationship between maternal dietary intake and the mature milk fatty acid profile seen in women populating the Mediterranean Croatia. Due to its biological importance for both mothers and babies, a special attention was paid to LC PUFAs intake and their breast milk content.

2 Subjects and methods

2.1 Subjects and breast milk sampling

The study enrolled 83 full breastfeeding Croatian women from Primorsko-Goranska County. The women were volunteers recruited in paediatric clinics via word of mouth. The study sample was recruited based on the criteria listed below. Women aged 18–40, who have given birth to healthy, full-term babies having a birth weight of >2,500 g at least a month ago, were considered eligible. The women were asked about their feeding practices; full breastfeeding was 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, infusions); fruit juice; oral rehydration salt solution; drops; and vitamins, minerals and medicines given in form of a syrup (WHO 1991). The recruited women were in their 5th to 25th lactation week. Women suffering from any metabolic disorders were excluded from the study. Breast milk samples were collected at the mothers' homes during the researcher's visit. The amount of approximately 5 mL of milk was expressed manually from one breast of each mother at the end of the breastfeeding session, somewhere between 10 and 12 A.M. The milk was stored in previously sterilised containers, transported to food analysis laboratory in an ice-cooled box and frozen at −20 °C for further analysis. During the researcher's visit, the mother's body weight (in kilogram) was measured using Body Composition Monitor Omron BF500 (Omron, Medizintechnik, Mannheim, Germany) in line with the procedure described by Bosy-Westphal et al. (2008). Woman's height was self-reported. Body mass index (BMI) was calculated as weight (kg)/height (m^2).

2.2 Fatty acids analysis

Milk samples were defrosted at room temperature and tempered in a mixer (TechnoKartell TK3S, Silverwater, Australia) before analysis. Lipids were extracted

by a modified method described by Blight and Dyer (1959). A 1.5-mL milk sample was mixed with 9.5-mL solvent mixture (methanol/chloroform/water, 2:1:0.8, *v*/*v*). The mixture was agitated for 30 min, followed by the addition of 2.5 mL chloroform and 2.5 mL of 2% anhydrous sodium sulphate solution. The final mixture had a final proportion of methanol/chloroform/water of 2:2:1.8. This mixture was agitated for 2 min and centrifuged at 3,000×*g* for 15 min. The organic phase was separated using an injection needle. Chloroform (5 mL) was added to the remaining phase and again centrifuged under the same conditions. The organic phase was combined with the previous one (a total of two extractions). Anhydrous sodium sulphate was added to the extract of total fats and left overnight. The extract was then filtered into pre-weighted test tubes and evaporated to dryness in a vacuum evaporator (Unicryo MC 2 L, Munich, Germany). The preparation of the fatty acid methyl esters was conducted by the method described by Hartman and Lago (1986). Briefly, to lipid extracts 1 mL of NaOH solution in methanol (c=1 mol.L^{-1}) was added in a screw caped tube. Tubes were incubated at 70 °C for 30 min, then 2 mL of BF_3 were added and the tubes were incubated for another 10 min at 70 °C. After cooling to room temperature, 2 mL of hexane containing 0.2% BHT and 1 mL of 20% NH_4Cl were added. The resulting suspension was centrifuged for 15 min at 3,000 rpm, organic phase was collected and left over anhydrous sodium sulphate overnight. Sodium sulphate was removed by filtration and the solution evaporated to dryness. Samples were dissolved in chloroform, and the composition of fatty acid methyl esters was analysed by a gas chromatograph (SRI 8610 C, SRI Instruments, Torrance, USA) equipped with a flame ionisation detector, and (78% cyanopropyl) methylpolysiloxane capillary column (007–23, Quadrex Corporation, Woodbridge, 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, then raised to 230 °C at a rate of 10 °C min^{-1}, and the final temperature was maintained for 20 min. Injector and detector temperatures were 160 and 250 °C, respectively. Separated fatty acid methyl esters were identified by comparing the retention times with those obtained by pure standards (Supelco Inc., Bellafonte, PA, USA). Data were collected and processed using PeakSimple 3D software, version 2.97 (SRI instruments, Torrance, USA), and the results are reported as the weight percent of total fatty acids.

2.3 Diet evaluation

Data on dietary intake of the selected breastfeeding mothers were collected using two consecutive 24-h recalls (on food intake during a working.day and during a weekend day). During the visit, a trained researcher recorded all food and beverages the mother had consumed. The women were also asked to show their kitchen utensils (i.e. spoons, cups, glasses, plates) and were encouraged to assess the amounts of the consumed food using those utensils. In order to make the above quantification easier, a photo catalogue displaying typical food rations (Senta et al. 2004) was used as well. In addition, the multi-pass protocol was applied (Johnson et al. 2008). Within this protocol, the respondents first provide a list of all food eaten on the previous day using any recall strategy they desire (i.e. the food should not necessarily be listed in a chronological order of consumption). The interviewer then obtains a more detailed

list by probing for additions to this food and by giving the respondents an opportunity to recall the consumption of food items initially omitted from the list. Late-report food items had been rechecked by review for their accuracy and subsequently entered into the recall. All of this aided to a more precise evaluation of the amount of food consumed.

Types and quantities of the consumed food were then computed so as to calculate daily energy, macronutrient and fatty acid intakes. The software utilised to this effect is based on the Croatian Food Composition Tables (Kaić-Rak and Antonić 1990) and partly on the data brought by other food tables (Møller et al. 2005). The average nutrient intakes were compared to the Dietary Reference Intakes (DRI) and the Acceptable Macronutrient Distribution Ranges applicable to the lactating adult women (Institute of Medicine 2002).

2.4 Statistical analysis

All data were presented as mean, median and range. Association between fatty acids of interest and their intake and breast milk concentration were analysed using linear regression. For the data suspected to be overly influential, Cook's distance was calculated (Neter et al. 1996). If confirmed, these data were omitted from the analysis, while the regressions were re-calculated. A P value<0.05 was considered to be significant. Statistica 8.1 (StatSoft. Inc. Tulsa, OK, USA) software was used for statistical analysis.

2.5 Ethical approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the Board of Ethics of the Faculty of Food Technology, University of Osijek. All women signed informed consent after being thoroughly informed of the purpose, requirements and procedure of the study.

3 Results

3.1 Study sample demography and anthropometrics

The majority of the women invited to participate in this research were primiparous. Most of the women willing to take part in the study were highly educated (almost 94% of the participants have finished high school or graduated from university; Table 1). As for the study sample, anthropometrics, on the occasion of dietary data and milk samples collection, 1 of our volunteers appeared severely overweight (BMI >35 kg/m^2), 11 were mildly overweight (BMI, 30–35 kg/m^2) and 3 were mildly underweight (BMI, 18–20 kg/m^2).

3.2 Diets and fatty acid intakes

When it comes to the diets of the women included in this study, a moderate caloric deficit (74.1% DRI) was established (Table 2). The average protein, carbohydrate and

Table 1 Demographic and anthropometric characteristics of the study sample (n=83)

	Mean±SD	Median	Range
Age at delivery (years)	31.80±4.60	32.00	21–40
Weight (kg)	69.04±13.53	68.41	50.20–116.71
Height (cm)	168.36±5.51	168.14	158.0–181.0
Parity (number of children)	1.49±0.72	1.44	1–4
Education (years)	13.54±2.10	13.49	4–20
Body mass index (kg.m^{-2})	24.29±4.32	24.10	17.91–40.25
Gestational weight gain (kg)	16.17±5.34	16.05	6.0–35.5

fat percent shares in total energy intake were 13.3%, 50.6% and 36.0%, respectively. The ratio of saturated (SFA) over monounsaturated fatty acids (MUFA) and PUFA was 1:1.1:0.48. The fats were primarily derived from dairy, vegetable oils and hydrogenated fats (spreads and processed food). The staple food turned out to be milk and dairy, fruit, vegetables and cereals. The average daily intake of fish and fish products was close to 50 g, with a notably high standard deviation indicating a high proportion of breastfeeding women refraining from fish consumption (about 41% of the study participants; Table 3). The participants having a habit of regular fish consumption consume three fish servings per week on the average.

An in-depth analysis of the fatty acid intake confirmed that, when it comes to SFAs, the highest individual intake was that of palmitic acid (PA, C16:0), which amounts to 21.70 g.day^{-1}. As for MUFAs, oleic acid (OA, C18:1n−9), with a daily intake of 29.20 g, takes the lead. LA (consumed in the amount of 13.81 g.day^{-1}) accounted for almost 90% of the total PUFA intake, which was established to be 15.41 g.day^{-1}. The ratio of total daily intake of n−6 over n−3 fatty acids equalled to 12.01 (Table 4).

Table 2 Daily energy and macronutrient intakes of Croatian breastfeeding women (n=83)

Parameter	Mean±SD	Median	Range
Energy (kJ)	8,840±2,565	8,738	3,315–17,504
Energy (% DRI)	74.1±21.4	73.1	27.9–144.4
Proteins (% kJ)	13.3±3.9	13.1	8.8–26.3
Carbohydrates (% kJ)	50.6±6.6	50.5	32.1–69.1
Fats (% kJ)	36.0±6.6	36.2	21.4–55.0
SFA (% kJ)	14.0±2.9	13.8	4.9–23.4
MUFA (% kJ)	15.2±3.7	15.1	6.4–33.3
PUFA (% kJ)	6.7±2.8	6.6	1.5–16.8

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

Table 3 Daily intake of different food seen in Croatian breastfeeding women (n=83)

Food group (g.day^{-1})	Mean±SD	Median	Range	Nonconsumers[a]
Cereals and bakery products	172.7±72.7	165.9	21.7–538.5	0
Fruits	228.0±244.8	207.3	0–1,102.4	4
Vegetables	188.1±104.2	178.4	7.7–551.3	2
Meat and meat products	139.9±92.6	134.8	0–439.89	5
Fish and fish products	48.6±85.9	20.1	0–467.3	34
Eggs	23.8±32.9	13.6	0–179.5	25
Milk and dairy	479.1±283.6	469.4	0–2,101.1	1
Sweets	69.9±56.9	58.2	0–327.9	3
Oils and fats	34.0±19.8	29.9	4.2–104.8	1
Alcohol	20.5±59.8	0	0–400	66

[a] The number of women who does not consume certain type of food at all

3.3 Breast milk fatty acids

Table 5 shows the fatty acid composition of the breast milk lipids constituting the obtained breast milk samples. Fatty acids were grouped as follows: total SFAs, total MUFAs, total n−3 and n−6 PUFAs, total n−3 and n−6 LC PUFAs, and total PUFAs. The breast milk of the Croatian women participating in the study contained the following 12 fatty acids, present in the amounts of less than 1% of the total fatty acid content: C17:0 (0.22%), C20:0 (0.23%), C22:0 (0.10%), C24:0 (0.09%), C20:1 (0.44%), C16:1 *trans* (0.04%), C20:3n−6 (0.37%) C20:4n−6 (0.42%), C22:4n−6 (0.12%), C22:5n−6 (0.14%), C20:5n−3 (0.05%) and C22:6n−3 (0.21%).

OA accounted for the highest share, i.e. 39.63% of the total breast milk fatty acids, while the predominating saturated fatty acid was established to be PA, with the percent share of 20.65% in the total fatty acid content (Table 5). The average total SFA share was 34.95%. *Trans* fatty acids quantified within the frame of this study were 16:1 *trans* and 18:1 *trans*, present in the total amount of 2.46%. About 98% of all detected *trans* fatty acids were 18:1 *trans* that accounted for 2.43% of the total breast milk fatty acid content. Total PUFAs amounted to 20.01% of the total fatty acid content. The content of the essential fatty acid LA equalled to 17.28% (Table 6). Another essential fatty acid, ALA, was present in the share of 1.41%. The ratio of LA over ALA established in the analysed breast milk was 12.24, while the amount of total n−6 PUFAs equalled to 18.40%. The concentration of total LC PUFAs (from n−3 and n−6 series) was 1.30%, in which total n−3 LC PUFAs were represented with 20%. As for n−3 polyunsaturated fatty acids, the highest concentration was that of DHA (0.21%). The ratio of total n−6 over n−3 PUFAs was 11.00, while the ratio of AA over DHA equalled to 2.37 (Table 5).

The results of the regression analysis revealed a positive relationship between certain fatty acid intakes and breast milk fatty acid concentrations. It appears that

Table 4 Dietary intake (gram per day) of individual fatty acids witnessed in Croatian breastfeeding women (n=83)

Fatty acid	Mean±SD	Median	Range
Saturated			
C4:0	0.48±0.30	0.45	0–1.72
C6:0	0.55±0.29	0.54	0–1.43
C8:0	0.34±0.20	0.31	0–1.73
C10:0	0.61±0.54	0.50	0–1.62
C12:0	0.93±0.72	0.78	0–2.64
C14:0	2.33±2.08	1.91	0.2–6.81
C15:0	0.01±0.01	0.01	0–0.15
C16:0	21.70±4.68	20.79	13.81–41.49
C17:0	0.13±0.03	0.12	0–0.23
C18:0	4.80±2.61	4.29	0.89–26.36
C20:0	0.13±0.13	0.09	0–1.17
C22:0	0.09±0.006	0.07	0–0.42
C24:0	0.005±0.01	0.001	0–0.13
Total SFAs	31.69±9.17	30.29	16.39–75.65
Monounsaturated			
C14:1	0.29±0.18	0.25	0–1.23
C16:1	0.89±0.52	0.78	0.12–3.11
C18:1 n−9	29.20±8.94	27.47	14.0–73.9
C18:1 *trans*	1.96±0.81	1.72	0.82–3.44
C20:1	0.27±0.19	0.23	0.02–1.71
C22:1	0.03±0.11	0.02	0–1.16
Total MUFAs	32.84±9.62	29.98	14.26–79.23
Polyunsaturated			
C18:2n−6	13.81±7.67	12.42	1.63–50.89
C18:3n−3	1.27±1.33	1.10	0.18–14.61
C18:4n−3	0.005±0.003	0.004	0–0.03
C20:4n−6	0.05±0.068	0.042	0–0.47
C20:5n−3	0.02±0.02	0.01	0–0.15
C22:5n−3	0.006±0.02	0.001	0–0.15
C22:6n−3	0.12±0.07	0.10	0–0.54
Total n−3	1.49±1.42	1.15	0.3–15.56
Total n−6	13.84±7.70	12.52	1.64–51.02
Total n−6/n−3	12.01±7.44	10.49	1.25–51.09
Total n−6 LC PUFAs	0.05±0.068	0.03	0–0.47
Total n−3 LC PUFAs	0.15±0.11	0.14	0.06–0.17
Total PUFAs	15.41±8.19	14.45	2.03–52.33

SFAs saturated fatty acids, *MUFAs* monounsaturated fatty acids, *PUFAs* polyunsaturated fatty acids, *LC PUFAs* long-chain polyunsaturated fatty acids

Table 5 Fatty acid composition (wt%) of the breast milk secreted by Croatian breastfeeding women (n=83)

Fatty acid	Wt % (mean±SD)	Wt % (median)	Range
Saturated			
C12:0	3.79±0.23	3.63	2.79–4.37
C14:0	5.33±0.86	5.33	3.04–6.95
C16:0	20.65±3.41	20.38	17.00–33.41
C17:0	0.22±0.01	0.21	0.10–0.24
C18:0	4.79±1.43	4.18	1.04–9.40
C20:0	0.23±0.02	0.22	0.19–0.29
C22:0	0.10±0.01	0.11	0.08–0.13
C24:0	0.09±0.04	0.08	0.03–0.17
Total SFAs	34.95±4.95	34.10	26.10–51.07
Monounsaturated			
C16:1	2.52±0.46	2.39	1.85–4.71
C18:1 n−9	39.63±4.25	39.89	25.99–46.16
C20:1	0.44±0.02	0.43	0.41–0.49
Total MUFAs	42.26±3.89	43.10	28.46–49.00
C16: 1 *trans*	0.04±0.01	0.04	0.03–0.04
C18:1 *trans*	2.43±0.39	2.44	1.82–3.88
Total *trans*	2.46±0.39	2.49	1.85–3.92
Polyunsaturated			
C18:2n−6	17.28±1.76	17.18	14.00–21.41
C20: 3n−6	0.37±0.05	0.37	0.04–0.45
C20:4n−6	0.42±0.06	0.42	0.21–0.56
C22:4n−6	0.12±0.02	0.12	0.09–0.16
C22:5n−6	0.14±0.02	0.13	0.11–0.31
Total n−6 PUFAs	18.40±1.95	18.10	15.01–22.71
Total n−6 LC PUFAs	1.04±0.09	1.02	0.69–1.31
C18:3n−3	1.41±0.11	1.39	1.25–1.64
C20:5n−3	0.05±0.01	0.05	0.03–0.06
C22:6n−3	0.21±0.04	0.21	0.08–0.165
Total n−3 PUFAs	1.67±0.16	1.66	1.38–2.62
Total n−3 LC PUFAs	0.26±0.10	0.26	0.11–1.22
Total n−3 and n−6 LC PUFAs	1.30±0.12	1.26	0.95–2.22
Total PUFAs	20.01±1.93	19.95	16.42–24.58
LA:ALA	12.24±0.67	12.14	10.41–14.27
AA/DHA	2.37±1.18	1.95	0.39–5.67
Total n−6/n−3	11.00±0.82	11.02	7.02–13.39

SFAs saturated fatty acids, *MUFAs* monounsaturated fatty acids, *PUFAs* polyunsaturated fatty acids, *LC PUFAs* long-chain polyunsaturated fatty acids

Table 6 Correlation between dietary fatty acid intake (gram per day) of Croatian breastfeeding women (n=83) and fatty acid content (% wt) of the mature breast milk

Fatty acid	Correlation coefficient (r)	P
DHA	0.54	<0.001
AA	0.20	ns
LA	0.24	0.032
PA	0.18	0.021
OA	0.21	0.024

DHA docosahexaenoic acid, *AA* arachidonic acid, *LA* linoleic acid, *PA* palmitic acid, *OA* oleic acid

the maternal DHA intake is strongly related to DHA breast milk content (r=0.54, P<0.001). The association between LA, PA and OA intake and their breast milk concentrations were represented by the following regression coefficients: 0.24 (P=0.032), 0.18 (P=0.021), 0.21 (P=0.024), respectively. The relationship between AA dietary intake and its concentration in the breast milk was not significant (Table 6). As for other fatty acids, a significant association between their breast milk concentrations and dietary intakes failed to be established. Interestingly, the correlation between DHA and AA breast milk content was statistically significant (r=0.127, P=0.0046; Fig. 1).

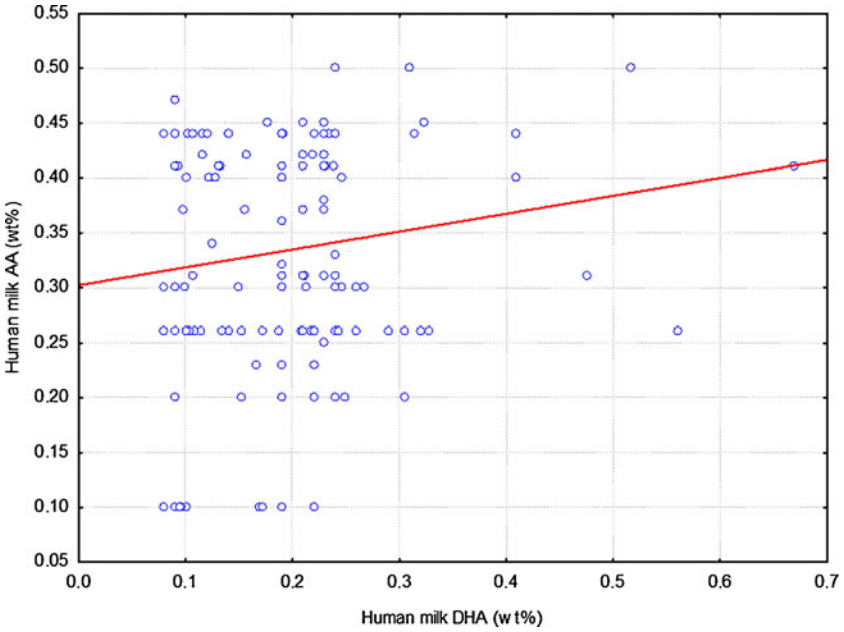

Fig. 1 Correlation between docosahexaenoic fatty acid (C22:6n−3) and arachidonic fatty acid (C20:4n−6) breast milk concentrations seen in Croatian breastfeeding women (n=83, r=0.127, P=0.0046)

4 Discussion

The results of our study show that, in general, fatty acid dietary intake profile seen among full breastfeeding Croatian women conforms to the Mediterranean dietary pattern. Individual fatty acid intakes were characterised by a high MUFA (especially that of OA) and PUFA intake. Due to the high intake of fats rich in *n*−6 PUFA, the milk had a high LA over ALA ratio, while, a low concentration of LC PUFA attributable to a low intake of fish and other food sources rich in *n*−3 LC PUFAs poses as the grounds for concern. Statistically significant relationships between dietary intakes and breast milk concentrations were established for DHA, PA, LA and OA.

Based on the evaluation of dietary intake of Croatian lactating women, it became obvious that their diet is characterised by a slight caloric deficit, a moderate share of carbohydrates and a higher share of fats. The fatty acid dietary profile seen among Croatian women was quite similar to that of women adhering to the traditional Mediterranean diet characterised by a high total fat, low SFA and high MUFA intake (Antonakou et al. 2012; Sala-Vila et al. 2005; Scopesi et al. 2001). Although PUFA intake witnessed in our study sample was even higher than that seen in other Mediterranean countries, the pattern disclosed to be distinctive for Croatia is a lower intake of *n*−3 fatty acids and a higher intake of *n*−6 fatty acids.

The predominance of *n*−6 PUFAs over *n*−3 PUFAs in the diet of our study women is obvious from *n*−6 over *n*−3 PUFA ratio which equals to 12.01. As per recommendations, this ratio should span from 2 to 5 (FAO/WHO 1994). An increased dietary intake of *n*−6 unsaturated fatty acids (especially LA) and a low intake of food rich in *n*−3 LC PUFAs is a trend seen in the typical Western diet, with a marked shift in breast milk composition witnessed in parallel with the changes in fatty acid dietary profile (Gibson et al. 2011).

Since LA intake recommended during lactation is set at 13 g.day^{-1} (Institute of Medicine 2002), Croatian breastfeeding women having a daily dietary LA intake of 13.81 g.day^{-1} on the average, meet and even beat these recommendations by 105%. Although the daily intake of the predominating *n*−3 unsaturated fatty acid ALA (1.27 g.day^{-1}) was close to recommended (1.3 g.day^{-1}), the intake of other two important *n*−3 fatty acids—eicosapentaenoic (EPA, C20:5*n*−3) and DHA—was low because of an inadequate fish consumption. Foodstuffs which contributed to the daily intake of ALA were nuts and dark green leafy vegetables. Although ALA can be converted into DHA, the efficiency of this conversion is low. Hence, an increased daily intake of DHA is recommended. A daily intake of DHA of 0.12 g poses as the grounds for concern, although even lower LC PUFA intakes (of about 0.05 g of DHA and 0.03 g of EPA *per* day) have been recorded among the lactating women in the USA (Brenna and Lapillonne 2009), Sweden and China (Xiang et al. 2005), and Canada and Australia (Innis 2004). However, the intake of this fatty acid was high in the countries having a tradition of abundant fish consumption (Innis 2004). In order to obtain the sufficient amounts of DHA, breastfeeding women should aim to achieve an average daily intake of at least 200 mg of DHA (Koletzko et al. 2008).

Fatty acid composition of the breast milk mirrors maternal diet. SFA share present in the breast milk is related to the content of carbohydrates and fats in a daily diet, as well as to the total energy intake and the mobilisation of adipose tissue (in a caloric deficit situation) during the postpartum period. The average concentration of SFA in the breast milk of Croatian women ranged from 26% to 51% of total fatty acids, which is similar to the results obtained in Turkey (Samur et al. 2009), Brazil (Cunha et al. 2005), China and Sweden (Xiang et al. 2005), Iran (Bahrami and Rahimi 2005) and Italy (Scopesi et al. 2001). The level of PA in the predominant breast milk SFA, is a significant indicator of the maternal daily intake (Nasser et al. 2010), the fact that was confirmed by our study as well (r=0.18, P=0.021). Dietary sources of PA were red meat, poultry and dairy. Generally speaking, SFAs constituted of more than 14 C atoms originate from maternal diet or from the adipose tissue mobilisation, while fatty acids having a length of less than 14 C atoms are synthesised de novo in the mammary glands (Innis 2007).

OA level found in the breast milk of Croatian women is higher as compared to the usual 25–30% level obtained in other studies (Samur et al. 2009; Silva et al. 2005; Xiang et al. 2005), probably due to the regular consumption of olive oil. Similar to our results, the breast milk of Italian and Spanish women whose diet includes the elements of the Mediterranean diet featured, among other, by a habitual olive oil consumption, contains about 40% of MUFAs, or even less when it comes to Spanish women (Antonakou et al. 2012; Sala-Vila et al. 2005; Scopesi et al. 2001).

The concentration of *trans* fatty acids found in the breast milk of our examinees is quite similar to that obtained for other European countries, i.e. Poland (2.7%), Italy (2.7%), the Czech Republic (2.1%), France (2%), Germany (2.4%) and Turkey (2.13%) (Samur et al. 2009). Due to their capacity to disturb the mechanism of conversion of essential fatty acids into long-chain metabolites, the intake of *trans* fatty acids via breast milk has been a continuous topic of scientific interest in the last decade.

Our results confirmed the relationship between maternal dietary LA intake and the content of this fatty acid in the breast milk (r=0.24, P=0.032), observed previously by other authors as well (Samur et al. 2009). The average share of LA in the breast milk of our study participants is higher than that found in the breast milk of the European female population, which contains 10–16% of LA on the average (Samur et al. 2009; Silva et al. 2005; Xiang et al. 2005). The level of another essential fatty acid, ALA, found in the breast milk of Croatian women, is higher than the worldwide average of 0.1–1%, but is quite similar to the levels obtained in Turkey, Brazil and Sweden (Samur et al. 2009; Silva et al. 2005; Xiang et al. 2005). The recommended LA/ALA ratio spans from 5 to 10 (Xiang et al. 2005). The higher ratio of these two fatty acids obtained in this research (12.24) can be attributed to the high consumption of vegetable oils resulting in an increased LA concentration. The ratio of total n−6 over n−3 PUFAs established within this study (11.00), ranging from 7.01 to 13.38, conforms to the recommended ratio of 5–15 (Xiang et al. 2005).

The concentration of AA (0.42%) in the breast milk of Croatian women was similar to that obtained by meta-analysis of global data (0.47±0.13%) (Brenna et al. 2007). However, this concentration is significantly higher as compared to the breast milk of women populating other Mediterranean countries (Antonakou et al. 2012; Sala-Vila et al. 2005; Scopesi et al. 2001). According to Del Prado et al. (2001), AA in the breast milk originates from maternal adipose tissue rather than from dietary

intake. On the opposite, the results of the interventional study conducted by Weseler et al. (2008) confirmed that AA consumed by breastfeeding women in addition to an extra *n*−3 LC PUFA dose, increases the breast milk AA concentration and its share in total breast milk lipids.

Among *n*−3 LC PUFAs, the main fatty acid identified in our breast milk samples was DHA (0.21%). According to the previously mentioned meta-analysis, the average worldwide DHA breast milk share equals to 0.32±0.22%, ranging from 0.06% to 1.4% (Brenna et al. 2007). Global data analysis showed that the breast milk secreted by women originating from the cultures having a tradition of high fish consumption (Arctic part of Canada, Japan, Philippines and Dominican Republic) contains the highest DHA concentrations. Contrary to that, the lowest level of DHA was detected in the milk of lactating women of Pakistan, the Netherlands, Canada and France (0.06–0.14%). Of concern is the fact that the concentration of DHA found in the breast milk of Croatian women was the lowest in the Mediterranean (Antonakou et al. 2012; Sala-Vila et al. 2005; Scopesi et al. 2001). The significant relationship between breast milk DHA and AA concentration seen in our research is lower than the value of r=0.250, P=0.02 which was recognised globally by Brenna et al. (2007). These authors confirmed that the mean DHA over AA in breast milk ratio widely varies across different regions.

The recommended range of breast milk AA/DHA ratio is 0.5–1 (FAO/WHO 1994), i.e. lower than that found in our study sample (2.37). On the global scale, AA/DHA ratio substantially varies from 0.5:1 in Japan to 3.16:1 in the USA; this comes as a result of a relative steadiness of AA levels as compared to highly variable DHA levels. Such variability in both DHA levels and AA/DHA ratios also implies the non-existence of a unique, generally recognised standard applicable to breast milk PUFAs composition.

Our study has several strengths; one of the most important among them is the fact that data on fatty acid intake and their concentrations in breast milk of Croatian women were obtained for the first time ever. These results should also fill the gap in knowledge and reduce the scarcity of data on countries observing the Mediterranean dietary pattern. Furthermore, we have confirmed that diet represents an important factor which may influence the breast milk composition. Within the frame of our study, dietary intakes were recorded and the breast milk samples were collected without interfering with the lactating women's lives. A possible study limitation lies within the fact that the breast milk fatty acids analysis embraced a single milk sample since we lacked capacities to collect the milk throughout the day. Although the total breast milk fat content could change during the day, the shares of individual fatty acids in the total milk fat remain constant. At the end of the breastfeeding session, when the milk samples were taken, the amount of total fat was the highest, which is the fact that contributes to the representativeness of our results.

5 Conclusion

Dietary pattern of Croatian breastfeeding women was proven to be similar to that of women from other countries adhering to the Mediterranean diet. Fatty acid dietary intake pattern was seen to be characterised by a high share of total fat, a low share of

SFAs, as well as by high MUFA and PUFA contents. However, of concern is the high ratio of *n*−6 over *n*−3 fatty acids in the consumed food and the predominance of *n*−6 fatty acids in it. In the breast milk secreted by the women included into this study, MUFAs, SFAs and PUFAs were shown to account for 42.26%, 34.95% and 20.01% of total fatty acids, respectively. The predominant fatty acid was OA (39.63%), whose share was amongst the highest in the Mediterranean. The share of *trans* fatty acids was 2.46%. Although total *n*−6:*n*−3 fatty acids ratio found in the breast milk falls within the recommended boundaries and indicates the capability of human body to alleviate the effect of an inadequate maternal dietary intake, the content of *n*−3 LC PUFAs in the breast milk, especially that of DHA, revealed by our study, should be deemed inadequate. DHA breast milk concentration found in Croatian women turned out to be the lowest in the entire Mediterranean region. The obtained data open up new areas of future research in cognitive and visual functions and the incidence of obesity among the infants of the involved mothers.

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