Eur J Nutr (2013) 52:963–973
DOI 10.1007/s00394-012-0403-8

ORIGINAL CONTRIBUTION

Breast milk fat concentration and fatty acid pattern during the first six months in exclusively breastfeeding Greek women

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Received: 20 October 2011 / Accepted: 12 June 2012 / Published online: 30 June 2012

Abstract
Purpose To determine fat and fatty acid (FA) profile of Greek mother's milk during the first 6 months of exclusive breastfeeding and to examine their correlation with dietary and other maternal characteristics.
Methods Milk samples and dietary records were obtained by mothers at 1st ($n = 64$), 3rd ($n = 39$), and 6th ($n = 24$) month postpartum. Fatty acid methylesters were separated and quantified by gas chromatography (GC/FID) and fat concentration by the creamatocrit method.
Results At the 3 time points, milk fat concentration ranged between 26.3 and 30.2 g/l ($p > 0.05$). Milk's FA composition was expressed as weight percentage (% wt/wt of all FAs detected with a C6 to C22 chain length). Maternal macronutrient and FA dietary intake, as well as the FAs' profile in maternal milk, remained constant over the 6 months. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) represented approx. 46, 35, and 18 % of all FAs, while ω6 and ω3 PUFA were 17.4 and 0.8 %, respectively. Body weight gain during pregnancy was positively related to breast milk's concentration in SFA ($p < 0.01$) and negatively to milk's concentration in MUFA ($p < 0.01$). Age and parity were also independent factors affecting the FA profile in maternal milk. A strong positive effect was found during the first month postpartum, between mother's PUFA intake and the concentration of PUFA, ω3 fatty acids, docosahexaenoic and linoleic acid (LA) in the milk, while MUFA intake was strongly correlated with the concentration of PUFA, ω6 fatty acids, and LA.
Conclusion This study is among few in literature to determine FA profile of breast milk in European populations and verified certain dietary factors that influence this profile. Maternal PUFA and MUFA intake were found to be important factors affecting breast milk's FA profile.

Keywords Fatty acid content · Breast milk · DHA · AA · Fat · Maternal diet

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Introduction

Breastfeeding is the natural next step in the continuum of pregnancy. Breast milk is universally recognized as the optimal food for infants up to the first 6 months of age [1]. Over the past two decades, fat was proven to be a critical component of breast milk, providing energy and important nutrients, which are key to the development of the central nervous system and cannot be synthesized de novo by the infant [2]. Polyunsaturated fatty acids (PUFA) are structural components of cellular membranes and the precursors of thromboxanes, prostaglandins, and leukotrienes. Principal among these are the long chain polyunsaturated fatty acids (LC PUFA), in particular arachidonic (AA; 20:4ω6) and docosahexaenoic (DHA; 22:6ω3) acid. Healthy brain tissue consists of about 60 % structural fat; of this, about 25 % is DHA and 15 % AA [3]. Sufficient PUFA supply ensures optimum growth and development, particularly

neurodevelopment [4, 5] and visual acuity [6] for newborns and infants. In neonates, AA and DHA can be synthesized by chain elongation and desaturation of essential fatty acids, such as linoleic acid (LA; 18:2ω6) and linolenic acid (LNA; 18:3ω3), respectively. This process, however, changes with development because the enzyme activities decrease with growth and maturation [7]. Most infants are incapable of synthesizing these compounds from precursor fatty acids [8, 9]. Thus, it is extremely important to provide adequate AA and DHA in the diet from early infancy. It is generally accepted that several factors such as mammary gland endogenous production, adipose tissue release, and maternal diet intake are involved in human milk profile regulation [10–12]. Numerous studies [13–16] indicate the influence of maternal diet on fatty acid composition of the milk lipid fraction. Clinical trials have demonstrated that PUFA profile in the infants' blood and tissues depends on their diet fatty acid pattern and that breast-milk PUFA profile can partly reflect the dietary habits of the mother [17]. Fish-eating populations have been shown to have higher breast-milk DHA concentrations than do populations that do not consume marine foods [18, 19]. Brenna et al. [20] in a meta-analysis of 65 studies worldwide found that DHA is the component of human milk that varies greatly among populations, that is, mean concentration ($\pm$SD) 0.32 ± 0.22 % with a range of 0.06–1.4 %. In particular, it was found that the 4 out of 5 locales with the greatest breast-milk DHA concentration (0.6–1.4 %) were coastal or islands with high marine food intake. In contrast, the lowest breast-milk DHA values (0.06–0.14 %) were found in inland or developed countries, both of which are usually associated with low marine food consumption.

Nevertheless, the actual effect of maternal diet to the breast milk lipid content during long periods of lactation has not still been fully indentified. There are numerous studies referring to the early stages of lactation [21–23] but very few that examined latter stages [16, 24, 25]. In general, however, it has been proven that maternal status and essential fatty acid stores developed from habitual diet are the main influence of human milk lipid content. Specifically, the maternal circulating fatty acids are the major direct sources of PUFA present in human milk [26, 27]. Research conducted among lactating mothers in the Mediterranean region is fragmentary and mainly concerns Spain and Italy [28–30]. Given that Mediterranean women and Greek women in particular are known to have distinct dietary patterns, that is, high total, low saturated, and high monounsaturated fat intake [30–32], the composition of breast milk in these populations remains an issue that has been under-investigated. The present study aimed to address the hypothesis that the fatty acid profile of Greek women's human milk during the first 6 months postpartum has distinct characteristics which may be attributed to the particular diet of this population.

Methods

In this prospective study, mothers who delivered healthy term newborns (>37 weeks of gestation) weighing >2.5 kg at obstetric clinics in the area of Athens and were planning to exclusively breastfeed their infants for at least 6 months were recruited. Mothers were approached by a member of the research group during the last trimester of their pregnancy and were thoroughly informed about the study protocols and aims. They were asked to sign an informed consent form. Ethical approval was obtained by the Harokopio University Ethics Committee and permission to approach the mothers was secured from the clinic's executive board.

Mothers participating in the study had no medical history, no history of alcohol or substance abuse, no obstetric pathology during pregnancy, that is, preeclampsia, gestational diabetes, cardiac or renal disease and were not receiving any dietary oil or vitamin supplements, apart from folic acid and iron supplements, during their pregnancy or postpartum. Samples of milk were obtained at three different time points. First sampling was performed at 20–30 days postpartum (1st month, $n = 64$). The second milk sampling was performed at the beginning of the 3rd month postpartum among the 39 women who continued to exclusively breastfeed. Third sampling was done at the beginning of the 6th month postpartum and included 24 women who still continued to exclusively breastfeed their infants.

Data collection

Anthropometric data

Data were collected during early morning hours. Weight and height were measured with the subjects wearing only underwear and with the use of a digital electronic balance (range 0.1–150 kg) and a tape measure (range 0–200 cm). Body mass index (BMI) (kg/m^2) was thus calculated. Prepregnancy weight was derived from the subjects' medical record kept at the antenatal clinic.

Dietary records analysis

Dietary intake was assessed using a 3-day dietary record at 1st, 3rd, and 6th month postpartum. The process has been described elsewhere [33]. In summary, participants were advised not to change their habitual diet during the 3 days of recording and then they were thoroughly explained how to fill in the dietary records. Consequently, mothers recorded the type and amount of food and beverages consumed for two consecutive weekdays and one weekend day, using standard household measures (cups, tablespoons, etc.).

The 3-day dietary records were analyzed using the Nutritionist Pro diet analysis software (FirstDataBank Inc, San Bruno, California, US) to estimate intakes of energy, carbohydrate, protein, fat and its proportions of Polyunsaturated fatty acids (PUFA), Monounsaturated fatty acids (MUFA) and Saturated fatty acids (SFA). Traditional Greek foods were also included in the food database.

Milk sampling

After completion of dietary records, a member of the research team visited mothers at home and a total of 30 ml of foremilk was collected from one breast by an electric breast pump (mini electric breast pump, MEDELA Inc, USA). Milk was placed in three dark sterile plastic tubes without preservatives and was immediately transferred in a cool-box to Harokopio University, where it was stored at −80 °C until analyzed. Home visits were made during morning hours and mothers were instructed not to have breastfed their infants for at least 2 h prior to using the breast pump, in order to obtain a significant amount of milk fat [34, 35].

Other data

The date of each subject's last menstruation plus data from their first ultrasound were used to establish gestational age at recruitment. Any pathology during pregnancy was also recorded. Mothers' age, educational level, number of children previously born, and use of tobacco were recorded during the first interview.

Laboratory methods

Materials

Methanol, hexane, and chloroform of analytical grade, sodium chloride and boron-trifluoride (BF_3 7 % in methanol) were purchased from Merck (Darmstadt—Germany).

Lipid extraction and fatty acid methyl esters analysis

A modified Folch extraction [36] was used in order to extract total lipids from milk samples [37]. Briefly, samples were allowed to thaw and reach room temperature on a vortex-shaker to prevent separation of the fat and aqueous phases [38]. In 300 µl of human milk, chloroform/methanol (2:1, v/v, 6 ml) was added and the mixture was thoroughly vortexed for 5 min. Subsequently, NaCl 0.05 M (1 ml) was added and the mixture was centrifuged at 4,000 rpm for 15 min at 22 °C. The lower (chloroform) layer was collected and solvent was evaporated using speedvac (AES 1010 SpeedVac System, Thermo Savant, Holbrook, NY, USA). Fatty acid methyl esters (FAMEs) were obtained after trans-methylation of total lipids by using 7 % BF_3 in methanol (1 ml) for 10 min at 90 °C [16]. FAMEs were extracted twice with hexane (1 ml), the solutions being centrifuged for 10 min at 4,000 rpm at 22 °C after each extraction step. The upper phases were collected and dried using speedvac; complete dryness of samples was prohibited in order to limit losses of volatile short-chain FAs which are normally present in breast milk. The residue obtained was re-suspended in hexane (0.5 ml final volume). FAMEs were separated and quantified by gas chromatography (GC System, 6890A Series, G1540A, Agilent Technologies, Wilmington, Denver, USA); flame ionization detection (FID) was applied. A 60-m capillary column was used (DB-23; Agilent Technologies, Wilmington, Denver, USA), calibrated against a standard containing 37 FAMEs, ranging in chain length from 4 to 24 carbon atoms (Supelco 37 Component Fame Mix; Supelco, Bellefonte—Pennsylvania, USA). This column provided a good separation of trans 16:1, 18:1, and 18:2 FAMEs isomers as well as of all the polyunsaturated FAMEs in human milk. The following chromatographic conditions were used: oven temperature was programmed from 130 to 230 °C at a rate of 5 °C/min with a total run time of 30 min. Helium was used as carrier gas at a flow rate of 1 ml/min. Injector and flame ionization detector temperatures were set at 280 °C. Air and hydrogen flows were adjusted to give maximal detector response. Aliquots of 1 µl were injected into the gas chromatograph at a split ratio 1:20. Quantification was performed based on a series of six standard mixtures.

Milk fat determination

Fat in milk samples was analyzed according to creamatocrit method [39]. The same researcher read all the creamatocrits, and the numerical formula defined by Lucas et al. [39] was used to estimate the fat content of whole milk.

Statistical analysis

Dependent variables were the total fat, PUFA, MUFA, SFA, DHA, AA, LA, LNA, $\omega 6$ and $\omega 3$ proportion of mature milk. Equality of means of all the above measurements within the three time points (1st, 3rd, and 6th month) was tested with repeated measures analysis of variance (ANOVA). The effect of food and energy intake on the lipid profile of mature milk was tested with the general linear model (univariate analysis of variance) using the intake values as covariates. Values are reported as mean ± SD. The level at which differences were considered significant was $p = 0.05$. All analyses were performed

with the aid of the SPSS version 17.0 software (SPSS Inc, Chicago, Illinois, USA).

Results

Maternal characteristics

Mothers' mean age was 32.5 ± 3.1 years. All participants were married, 78 % were first time mothers, the great majority (94.3 %) was employed, two-thirds (64 %) of them had university degrees. The study population is hence more likely to reflect women with good nutritional care. Anthropometric characteristics of the initial sample ($n = 64$) are presented in Table 1.

Dietary parameters

Mothers' macronutrient intake, as estimated from the 3-day food diaries, are presented in Table 2. These results are described in more detail in previous publication [33]. In summary, dietary parameters examined were energy intake, energy contribution of carbohydrate, protein, fat intake (SFA, MUFA, PUFA), and fiber intake. Food intake was recorded in 3-day diaries at the 1st, 3rd, and 6th month of lactation. However, repeated ANOVA measures revealed that dietary parameters did not differ significantly among the three time points; hence, in Table 2, only data obtained during the 1st month are presented. More specifically during the 6th month measurements, an increase in the intake of fiber (i.e., 19.4 ± 8.8 g at the 6th month–16.2 ± 7.1 g at 1st month) was noted; this increase, however, did not reach statistical significance.

Milk fat and fatty acid profile

Repeated ANOVA measures revealed that milk total fat concentration did not differ among the three time periods, being 31.7 ± 16.4 g/l at the first month, 28.2 ± 17 g/l at the third month, and 26.3 ± 11.1 g/l at the sixth month of lactation ($p = 0.716$). Although fat concentration did show a decrease during the six-month study period, this did not reach statistical significance probably due to the great inter-subject variability that was observed and the small sample size at the sixth-month measurement ($n = 24$). The main fatty acids proportions of breast milk are presented in Table 3. The three fatty acid classes accounted for approximately 46 % of total FAs in the case of SFA, and for approximately 36 and 18 % in the case of MUFA and PUFA, respectively; this profile remained remarkably constant throughout the 6-month study period. DHA represented 0.50 % of total FAs and remained practically constant throughout the study period, while AA accounted for 1.1–0.7 % of total FAs and its contribution declined significantly only in the 6th month ($p = 0.036$). LA and LNA represented 14.5 and 0.15 % of total FAs, respectively, and remained virtually constant during all three time points. The rest of the fatty acids identified did not exhibit any significant change throughout the study period.

Table 1 Anthropometric characteristics of subjects

	Mean ± SD	Range
Height (m)	1.67 ± 0.06	1.52–1.80
Pre-pregnancy body weight (kg)[a]	62.3 ± 11.5	45–106
Pre-pregnancy BMI (kg/m^2)[a]	22.2 ± 4.1	17.4–36.6
Underweight (<18.5)	6.3 % ($n = 4$)	
Normal (18.5–24.9)	78.1 % ($n = 50$)	
Overweight (25.0–29.9)	10.9 % ($n = 7$)	
Obese (≥30)	4.7 % ($n = 3$)	
Weight gain during pregnancy (kg)	15.9 ± 6	4–30

[a] Weight measurements were recorded during their first visit to the antenatal clinic at 4th–5th gestational week. Weight was also recorded before labor upon admission in order to calculate weight increase during pregnancy

Table 2 Mothers' macronutrient intake, as estimated from the 3-day food diaries at the 1st month of lactation

Energy and macronutrients	Energy and macronutrients intake ($n = 64$)
EI (kcal)	1,999.8 ± 452.3
EI/body weight (kcal/kg)	29.2 ± 8.7
Carbohydrates (%EI)	44.7 ± 6.4
Proteins (%EI)	16.2 ± 3.4
Total fat (%EI)	38.5 ± 6.4
SFA (%EI)	13.4 ± 2.8
MUFA (%EI)	16.0 ± 2.8
PUFA (%EI)	5.6 ± 1.6
Fiber intake (g)	16.2 ± 7.1

Data are given as mean ± SD

EI Energy intake, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

Relationship between maternal characteristics and lipid content

The associations between the breast-milk lipid content and certain maternal characteristics, that is, age, BMI, body weight gain during pregnancy, and parity, were tested by applying the *general linear model* for fixed effects after adjustment for various potential confounders. Differences in lipid content between particular subgroups, that is, parity, were tested by using post hoc analysis after Bonferroni correction of the probability for multiple comparisons. Results demonstrated a significant positive association

Table 3 Milk fat concentration and fatty acid pattern at 1st, 3rd, 6th month of lactation of Greek solely breast-feeding women

	Fatty acids	1st month $n = 64$	3rd month $n = 39$	6th month $n = 24$
%wt/wt of all FAs				
C6:0	Caproic	0.18 ± 0.53	0.53 ± 2.14	0.32 ± 0.83
C8:0	Caprylic	0.60 ± 0.45	0.51 ± 0.43	0.45 ± 0.45
C10:0	Capric	3.10 ± 0.85	2.93 ± 0.65	2.83 ± 0.72
C12:0	Lauric	8.42 ± 3.10	8.81 ± 2.56	8.91 ± 2.98
C14:0	Myristic	6.87 ± 2.11	7.27 ± 2.01	8.22 ± 2.07
C15:0	Pentadecanoic	0.28 ± 0.26	0.22 ± 0.23	0.13 ± 0.19
C16:0	Palmitic	19.82 ± 3.65	18.96 ± 3.91	18.68 ± 3.84
C17:0	Heptadecanoic	0.19 ± 0.20	0.15 ± 0.19	0.05 ± 0.10
C18:0	Stearic	6.18 ± 1.62	5.79 ± 1.49	6.07 ± 1.63
C20:0	Arachidic	0.04 ± 0.10	0.04 ± 0.09	0.18 ± 0.31
C21:0	*cis*-11-Eicosenoic	0.01 ± 0.05	0.07 ± 0.24	0.04 ± 0.12
	Total SFA	45.67 ± 8.71	45.29 ± 7.97	45.62 ± 8.98
C14:1ω5	Myristoleic	0.26 ± 0.25	0.25 ± 0.25	0.32 ± 0.94
C15:1	*cis*-10-Pentadecenoic	0.02 ± 0.12	nd	0.02 ± 0.08
C16:1ω7	Palmitoleic	2.63 ± 0.84	2.38 ± 0.63	2.24 ± 0.49
C17:1	*cis*-10-Heptadecenoic	0.10 ± 0.16	0.09 ± 0.14	0.11 ± 0.29
C18:1ω9c	Oleic	31.65 ± 9.99	32.79 ± 10.63	33.87 ± 9.09
C20:1ω9	*cis*-11-Eicosenoic	0.28 ± 0.28	0.31 ± 0.42	nd
C22:1ω9	*cis*-13,16-Docosadienoic	0.004 ± 0.02	nd	0.0013 ± 0.061
	Total MUFA	34.95 ± 9.51	35.77 ± 10.18	36.73 ± 9.09
C18:2ω6c	Linoleic	15.02 ± 3.59	15.15 ± 5.01	14.68 ± 4.09
C18:3ω6	Y- Linolenic	0.90 ± 0.44	0.86 ± 0.46	0.74 ± 0.49
C20:2 ω6	*cis*-11,14-Eicosadienoic	0.51 ± 0.59	0.34 ± 0.30	0.21 ± 0.30
C20:3ω6	Dihomo-γ-linolenic acid	0.36 ± 0.62	0.22 ± 0.25	0.15 ± 0.21
C20:4ω6	Arachidonic	1.08 ± 0.41	0.89 ± 0.35	**0.67 ± 0.40***
	ω6 PUFA	17.86 ± 4.42	17.42 ± 5.66	16.46 ± 4.43
C18:3ω3	Linolenic	0.14 ± 0.21	0.16 ± 0.21	0.06 ± 0.11
C20:3ω3	cis-8,11,14-Eicosatrienoic	0.16 ± 0.65	0.13 ± 0.55	0.12 ± 0.59
C20:5ω3	EPA	0.05 ± 0.13	0.15 ± 0.25	0.11 ± 0.32
C22:6ω3	DHA	0.55 ± 0.14	0.45 ± 0.15	0.52 ± 0.97
	ω3 PUFA	0.76 ± 0.70	0.76 ± 0.72	0.68 ± 0.92
	Total PUFA	18.59 ± 4.64	18.19 ± 5.72	17.15 ± 4.30
C18:1ω9t	Elaidic	0.62 ± 0.40	0.64 ± 0.35	0.46 ± 0.38
C18:2ω6t	Linolelaidic	0.16 ± 0.22	0.14 ± 0.18	0.04 ± 0.10
	TFA	0.78 ± 0.47	0.48 ± 0.50	0.19 ± 0.34
	Ratios			
	ω6/ω3	25.85 ± 12.41	25.94 ± 13.31	26.51 ± 12.41
	C18:2ω6c/C18:3ω3	46.22 ± 10.42	45.41 ± 17.72	69.41 ± 28.12
	Total fat (g/l)	31.73 ± 16.42	28.23 ± 17.13	26.32 ± 11.13

Results are presented as mean ± SD, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *TFA* trans fatty acids, *nd* not detected

Bold value is statistically significant

* Significant decline at the 6th month measurement ($p = 0.036$); ** mean C18:2ω6c/C18:3ω3 ratio was calculated as the mean value of each individual milk sample C18:2ω6c/C18:3ω3 ratio; samples for which C18:3ω3 was not detected were unavoidably excluded from the calculation

between the body weight gain during pregnancy and the SFA proportion in human milk. This association remained strong throughout the 6-month study period ($p < 0.01$). Respectively, a strong negative association between the weight gain during pregnancy and the MUFA proportion in breast milk was also found. This finding remained strong

during the first 3 months of lactation ($p < 0.01$) but during the sixth-month measurement, although still negative, it did not reach the level of significance ($p = 0.09$). This might be due to the small sample size at the sixth-month measurement. A positive correlation ($p < 0.05$) between maternal BMI and the percentage of LNA in breast milk during the first month was found. A strong negative correlation ($p < 0.05$) between maternal age and MUFA and oleic acid proportion during the first month was also found. Finally, a significant positive correlation ($p < 0.05$) was found between the number of previously born children and the total milk fat concentration, at the first month postpartum. That is, milk from multiparous mothers had higher total fat concentration compared with the milk of primiparous (Table 4). However, none of these correlations remained significant during the whole 6-month study period. No other significant correlation between maternal characteristics and milk's fatty acid profile was noted.

Influence of maternal diet to lipid concentration and fatty acid composition

The impact of macronutrient intake on the lipid profile of breast milk was assessed by applying the *general linear model*. The following macronutrients were examined: Energy Intake (EI, kcal), total fat (%EI), MUFA (%EI), PUFA (%EI), SFA (%EI), protein (%EI), and carbohydrate (%EI). Results revealed positive relationships between some of these dietary parameters and milk fatty acid profile during the first month postpartum. More specifically, both total fat dietary intake and PUFA intake were strongly correlated with the human milk DHA value at the 1st month of lactation ($p = 0.03$). A strong positive effect was also found between PUFA intake and milk's PUFA, $\omega 3$ fatty acid and LA percentage. Dietary MUFA intake was strongly correlated with milk's PUFA, $\omega 6$ fatty acid and LA values. Finally, carbohydrate dietary intake was negatively correlated with $\omega 3$ fatty acids and DHA percentages in milk (Table 5). No significant correlation was found between breast milk lipids and fiber intake ($p = 0.275$). Finally, no correlation was found to last during the whole six-month period, between the dietary macronutrients and either the total milk fat or any of the individual fatty acid examined, namely DHA, AA, eicosapentaenoic acid (EPA), LA, and LNA. However, it is worth mentioning that a significant positive correlation ($p < 0.05$) was found between breast milk's total fat concentration and energy intake, albeit only for the sixth-month measurement.

Discussion

This is one of a few studies to report on the lipid profile of breast milk among solely breastfeeding Mediterranean women for up to 6 months postpartum. Data regarding milk fat concentration indicate that typical values range from 30 to 44 g/l [25, 40–42]. A recent study has shown an increase of total fat during the first 6 months of lactation

Table 4 Correlation of maternal characteristics with the lipid content in mature milk at 1st month of lactation ($n = 64$, Pearson's Correlation coefficient (r) is presented)

Milk fatty acids (% wt/wt of all FAs)	BW pre-pregnancy	BW (1st mo)	Weight gain	Age	Parity	Gestational age	Smoking[a]
DHA	0.004	−0.06	−0.09	0.13	−0.06	−0.13	0.09
AA	−0.09	−0.07	−0.03	0.15	0.01	0.12	0.08
EPA	−0.15	−0.08	0.03	0.09	0.18	−0.05	−0.92
LA	0.20	0.19	−0.07	0.16	−0.63	0.11	−0.16
LNA	**0.37***	**0.32***	−0.13	−0.01	0.03	−0.06	0.53
γ-LNA	−0.002	0.03	0.07	0.24	0.34	−0.07	0.15
Oleic	0.06	−0.13	**−0.26***	**−0.26***	−0.19	0.26	0.15
Palmitic	−0.12	−0.05	0.04	0.01	0.16	0.03	−0.16
$\omega 3$	0.05	−0.04	−0.13	0.13	0.06	−0.1	0.06
$\omega 6$	0.176	0.19	−0.01	0.17	−0.03	0.07	0.04
SFA	−0.18	0.04	**0.32***	0.22	0.02	−0.04	0.01
MUFA	0.08	−0.11	**−0.28***	**−0.29***	−0.02	0.02	−0.01
PUFA	0.18	0.18	−0.03	0.19	0.03	0.04	0.16
Total fat (g/l)	0.141	0.07	0.01	0.16	**0.32***	0.025	0.07

n number of samples, *LA* Linoleic acid, *LNA* linolenic acid, *AA* arachidonic acid, *DHA* docosahexaenoic acid, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *BW* Body weight

Bold values are statistically significant

* Significant at the level of $p < 0.05$

[a] The correlations concern the number of cigarettes smoked by mothers per day

Table 5 Correlation of dietary intake parameters with the lipid content in mature milk at 1st month of lactation (Pearson's Correlation coefficient (r) is presented)

Milk fatty acids (% wt/wt of all FAs)	Dietary intake						
	EI (kcal)	Fat (%EI)	MUFA (%EI)	PUFA (%EI)	SFA (%EI)	CHO (%EI)	PRO (%EI)
DHA	−0.08	**0.25***	0.18	**0.27***	0.16	**−0.028***	0.10
AA	0.10	0.23	0.24	0.16	0.09	−0.19	0.04
LA	−0.09	0.15	**0.26***	**0.26***	−0.15	−0.01	−0.18
ω3	−0.08	0.24	0.18	**0.26***	0.15	**−0.29***	0.14
ω6	−0.01	0.17	**0.27***	0.22	−0.08	−0.05	−0.13
SFA	−0.01	−0.05	−0.16	−0.02	0.01	0.12	−0.15
MUFA	0.02	−0.05	0.01	−0.09	0.01	−0.07	0.19
PUFA	−0.03	0.20	**0.29***	**0.25***	−0.05	−0.10	−0.10
AA/DHA	0.15	0.08	0.16	0.01	−0.05	0.00	−0.19
ω6/ω3	0.12	0.05	0.10	0.03	−0.09	0.02	−0.10
Total fat (g/l)	−0.04	0.12	−0.01	0.08	0.10	−0.10	−0.02

DHA Docosahexaenoic acid, *AA* arachidonic acid, *LA* linoleic acid, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *EI* Energy intake, *Fat* total fat, *CHO* carbohydrates, *PRO* proteins

Bold values are statistically significant

* Significant at the level of $p < 0.05$

[43]; however, earlier longitudinal studies did not reveal significant variations [25, 40]. Our results concerning the values of total milk fat (approx. 30 g/l) are in agreement with previously reported values; moreover, we did not observe any significant variations during the 6-month study period.

As far as the ratio of the fatty acids classes present, our results are in accordance with previous studies performed in southern Europe. SFA were found to represent about 46 % of total FAs, MUFA about 36 %, and PUFA about 18 %. Milk fatty acid classes values, as well as the essential fatty acids values found in the present study (first month), can be compared with the respective values obtained from five published studies, as shown in Table 6. Milk's proportion of (%) total PUFA, ω6 FAs, DHA, and AA was higher (18.6, 17.9, 0.55, and 1.1 %, respectively, during the first month) than that reported for mothers' milk in other European countries including Spain and Italy [21, 43–45] (Table 6). For AA in particular, values found in the present study (approx. 1.1 %) were at least two-fold higher than those previously reported. Expert panels worldwide recommend that the ideal AA's proportion in breast-milk lipids should be between 0.35 and 1.00 % [46], considering its importance for growth and development of the brain during the perinatal period.

Another important fact that came up in the present study was the lower values of total ω3 FAs, as compared to those reported for other European countries, mainly due to the small amounts of EPA and LNA found in our samples. At the same time, DHA's values were found to be among the highest. It is of interest that previous similar studies that found high DHA value in breast milk, also found low EPA's value [45, 47]. EPA is a precursor metabolite to DHA and may be converted to DHA through elongation and desaturation, thus contributing further to the level of DHA in milk. Additionally, LA levels in our study were similar to other reported data for European populations while LNA levels were lower [44, 45]. The high ratio of LA/LNA in breast milk is found in other recent studies as well, and it raises concerns about linoleic acid—rich plant oils consumption increase and, consequently, linoleic acid value increase, which may affect ω3 PUFA biosynthesis through the elongation–desaturation mechanism [48]. This work further supports the findings that high levels of LA found in the milk lipid fraction may interfere with the biosynthesis of ω3 FA group [45, 49].

With regard to the proportion of DHA in human milk fat, it has been found that it is highly variable both within and among cultures, ranging from 0.05 to 0.10 % in women whose diets do not habitually include foods or supplements that provide relatively large amounts of DHA [14, 20, 24, 45], to proportions greater than 1.00 % in women who consume salmon or other ocean fish at least once a week or receive fish oil supplements [47, 50]. Meanwhile, DHA proportion in the samples of the present study was found to be relatively high, representing 0.50 % of total FAs and remained practically constant throughout the study period. This finding suggests that Greek women, although they do not receive fatty acid supplements, they follow a diet that supplies them with significant amounts of DHA. In Greece, the consumption of wild food, which began in the earliest period, retained its importance in

Table 6 Human milk lipid content: comparison of fatty acid classes and essential fatty acids values at 1st month postpartum between the present study and other published studies

	Greece Athens $n = 64$	Spain Navarre and Catalonia $n = 40$	Italy Genoa, Liguria and Trento, Trentino $n = 34$	Sweden Stockholm $n = 19$	China Nothern China $n = 52$	Germany Ulm, Baden-Wurttemberg and Bavaria $n = 462$
(*% wt/wt of all FAs*)						
DHA	0.55 ± 0.14	**0.34 ± 0.20***	**0.18 ± 0.04***	**0.25 ± 0.01***	**0.19 ± 0.08***	**0.17 ± 0.23***
AA	1.08 ± 0.41	0.5 ± 0.32	**0.15 ± 0.09***	**0.38 ± 0.02***	**0.30 ± 0.15***	**0.46 ± 0.32***
EPA	0.05 ± 0.13	0.14 ± 0.20	**0.30 ± 0.06***	0.06 ± 0.01	–	0.04 ± 0.07
$\omega 3$	0.76 ± 0.70	**1.39 ± 0.78***	0.95 ± 0.45	**1.95 ± 0.14***	**1.20 ± 0.30***	**1.15 ± 0.92***
$\omega 6$	17.86 ± 4.42	**13.73 ± 7.60***	**11.31 ± 1.21***	**12.19 ± 0.47***	18.70 ± 4.60	**11.48 ± 4.76***
SFA	45.67 ± 8.71	41.09 ± 9.40	45.49 ± 1.57	**40.72 ± 1.04***	**35.90 ± 7.30***	47.12 ± 9.12
MUFA	34.95 ± 9.51	**41.97 ± 15.00***	**42.25 ± 1.58***	**45.15 ± 0.74***	32.60 ± 7.20	**39.33 ± 6.91***
PUFA	18.59 ± 4.64	15.23 ± 10.70	**12.26 ± 1.24***	**14.14 ± 0.57***	19.90 ± 4.90	**13.57 ± 5.97***
	Present study	de la Presa-Owens et al. [44]	Scopesi et al. [21]	Xiang et al. [45]	Wan et al. [59]	Szabo et al. [43]

Data are given as mean ± SD

n sample size

Bold values are statistically significant

* After applying the $t = \frac{m1-m2}{\sqrt{sd1^2/n1+sd2^2/n2}}$, statistical significance was met at level of $p < 0.01$

where

*m*1, sd1, and *n*1 are the mean value, standard deviation, and size of our sample

*m*2, sd2, and *n*2 are the mean value, standard deviation, and size of the second sample

native peoples' diet well after domestication and the spread of cultivation, through the beginning of the twentieth century [51]. It has actually been argued that the health-promoting properties of the Mediterranean diet basically stem from its relation to preagricultural diets, when people relied on nature's "super-market" for nourishment [52]. Wild greens and herbs, snails, and invertebrate seafood in particular, are wild food products that find widespread use in Greece and other areas of the Mediterranean region even in our days, contributing to $\omega 3$ fatty acid intake. Similarly, a portion of the animal production in contemporary Greece has preserved its age-old character, making free-range kids and chicken available to the consumer. It was found that Greek chickens that roam freely, eating a variety of plants and insects, produce eggs six times higher in DHA than USA supermarket eggs [53]. Consumption of organ meats, food items that are still popular among Greeks, may also be related to mothers' DHA intake. It might be hence assumed that the food supply in Greece, though moderate in fish and seafood [54, 55], may contain considerable amounts of DHA. A recent study from Iran concerning DHA values in colostrum also came to give interesting new data. It was found that DHA in colostrum was increased in population where they consumed large portions of olives and olive oil as well as fish products. While no direct associations were made for olive oil consumption and DHA values in breast milk, this seems to be an area for further research [56].

Data regarding the impact that dietary intake has on lipid profile of human milk are controversial. An early study conducted by WHO [57] reported that the lipid profile of mature milk is little influenced by variations in mothers' current dietary intake. Similar are recent published results with regard to dietary intake and vitamin E content of mature human milk [33]. However, other recent studies showed that specific elements of mature human milk are affected by maternal nutrition [16, 18, 20]. Studies have shown that DHA's concentration in human milk is affected by maternal dietary DHA intakes [25]. It has also been found that low soybean oil consumption by mothers can lead to low LNA milk's concentration [47]. In the present study, we have shown that the ratio of total fat, PUFA, MUFA dietary intake during lactation may play an important role on the corresponding ratio of these elements in breast milk. Specifically, it was found that PUFA intake was strongly correlated with milk's PUFA, $\omega 3$ fatty acid, DHA and LA values and that dietary MUFA' intake was strongly correlated with milk's PUFA, $\omega 6$ fatty acid and LA values. At the same time, DHA and $\omega 3$ values in breast milk during the first month were negatively associated with carbohydrate intake. We do not have a clear explanation to this, but the fact that populations with increased carbohydrate intake tend to have lower total fat intake, and therefore lower total $\omega 3$ intake, might provide us with a probable etiology.

Our results demonstrate an effect of weight gain during pregnancy to the specific lipid profile of the breast milk. Specifically, weight gain during pregnancy was found to have a significant positive association with SFA value in human milk throughout the study period and a negative association with MUFA value for the first 3 months of observation. This implies that the more weight women gained during pregnancy, the higher the proportion of saturated fat, and the lower the proportion of monounsaturated fat in their milk were. Additionally, high maternal BMI (>25 kg/m^2) status during lactation was found to have a positive correlation only with LNA's value in breast milk during the first month. Butte et al. [58] has also reported that among well-nourished women, with adequate pregnancy weight gain – such as our subjects were – the physiological effects of rapid fat mobilization may overwhelm any relationship of maternal BMI to milk lipid concentration, at least in the first 4 months of lactation. Similarly, Nommsen et al. [25] have found that women with higher BMIs had significantly higher milk lipid concentrations only after the 6th month postpartum. We also found a strong negative correlation of maternal age and monounsaturated fat and oleic acid values during the first month; however, there are no similar data to compare these findings. Finally, a significant positive correlation ($p < 0.05$) was observed between the number of previously born children and the total milk fat concentration, at the first month postpartum. That is, milk from multiparous mothers had higher total fat concentration compared with the milk of primiparous. Nommsen et al. [25], however, found that milk fat was significantly higher among primiparous women by the twelfth month of lactation, with no difference being noted in earlier stages of lactation.

Lastly, it should be mentioned that the Nutritionist Pro diet analysis software (FirstDataBank Inc, San Bruno, California, US) that was used for the determination of energy, carbohydrate, protein, fat content of foods, and its proportions of MUFA, SFA, and PUFA does not provide individual values for the ω3 and ω6 content of foods. Therefore, we were not capable to calculate correlations between ω6/ω3 ratios in maternal diet to ω6/ω3 ratio in breast milk. Most of the diet analysis software faces similar limitations. On the other hand, the particular software is enriched with traditional Greek foods, which was an important positive element in order to estimate accurately the ratio of MUFA, PUFA, and SFA of often mentioned foods.

Conclusion

This is the first study in Greece, and one of a few in Europe, to examine mature milk's lipid profile in exclusively breastfeeding women. Breast milk of Greek mothers was found to be richer in DHA, AA, and total ω6 fatty acids compared with the milk from mothers in other European countries. The proportion of total ω3 fatty acids, on the other hand, was found to be lower than in other European populations. A significant positive association was found during the first month postpartum, between mother's PUFA intake and the proportion of PUFA, ω3 fatty acids, DHA, and LA in the milk, while MUFA intake was strongly correlated with PUFA, ω6 fatty acids, and LA values. These findings showed that Greek women distinct diet characteristics, that is, high total, low saturated and high monounsaturated fat intake, affect their fatty acid milk profile during the first important 6 months of exclusive lactation. The verification of these results in greater study populations may lead to specific dietary advice in lactating women in order to increase the DHA and AA values in breast milk.

Acknowledgments Dr. N. Kalogeropoulos is gratefully acknowledged for his valuable contribution in verifying the validity of our methodology.

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