



## Original Article

## Comparisons of Breast Milk Fatty Acid Profiles in Overweight and Obese Women

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## ABSTRACT

**Background and Objectives:** Breast milk composition is affected by several factors such as maternal diet and body mass index. Milk fats include a major calorie content of the milk, which changes dramatically throughout the lactation. The aim of the present study was to assess breast milk fatty acid profile in overweight and obese mothers and its correlations with maternal body mass index.

**Materials and Methods:** Milk samples were collected from 73 healthy lactating women referring to various health centers in Mashhad. Data were collected using food frequency questionnaire to assess the maternal dietary intakes. Breast milk fatty acid profile was assessed using gas chromatography.

**Results:** Breast milk content of saturated fatty acids was higher in obese mothers than overweight mothers ( $p = 0.03$ ) with positive correlations with maternal body mass index ( $r = 0.256$ ;  $p = 0.037$ ). Furthermore, breast milk monounsaturated fatty acids were negatively correlated with pre-pregnancy body mass index ( $r = -0.385$ ;  $p = 0.003$ ) and pre-pregnancy body weight ( $r = -0.276$ ;  $p = 0.026$ ). However, no significant correlations were observed between the maternal body fat mass and fat proportion with the breast milk fatty acids ( $p > 0.05$ ). Moreover, no significant associations were seen between dietary saturated fatty acids, milk monounsaturated fatty acids, polyunsaturated fatty acids, n-3 polyunsaturated fatty acids and n-6 polyunsaturated fatty acids with the corresponding fatty acids in breast milk ( $p > 0.05$ ).

**Conclusions:** Based on the results, breast milk of the obese mothers included higher proportion of saturated fatty acids, while no significant differences were observed in the contents of milk monounsaturated fatty acids and polyunsaturated fatty acids between the overweight and obese women. Furthermore, maternal pre-pregnancy body mass index was negatively correlated with the contents of milk monounsaturated fatty acids, while it was positively correlated with saturated fatty acids contents.

**Keywords:** Human milk, Fatty acids, Body mass index, Obesity, Gas chromatography

## Introduction

Breast milk is an optimal food for infants during the first six months of their lives (1). With its unique composition of essential nutrients and various bioactive factors, breast milk provides ideal nourishments to the infants. Nevertheless, the human breast milk composition may vary largely, particularly in its fat contents, which play key roles in development of the central nervous system (CNS) in infants, providing approximately 50% of the milk energy (2, 3). Previous studies have indicated that human milk composition is affected by the environmental factors, such as infant maturity (4), maternal nutritional status (5) and breastfeeding stage (6). Furthermore, maternal body mass

index (BMI) has been reported to positively correlate with breast milk energy (7) and lipid contents (8, 9). Maternal diets can affect breast milk lipid components with no effects on total fats. Diets with high levels of polyunsaturated fatty acids (PUFAs) can increase the levels of PUFA in breast milks with no changes in total fat contents (10).

The suggested fatty acid (FA) ratio of human breast milks is nearly 30% of the maternal FA intakes, 70% of the FA reservoirs and the minimal quantities derived from *de novo* synthesis (10, 11). Panagos et al. reported that breast milk of mothers with overweight and obesity included

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lower n-3 PUFA (12). Similarly, other studies have reported that maternal obesity is associated with higher n-6 PUFA/n-3 PUFA ratios of the breast milks (12–14). General changes in dietary habits with increased intakes of n-6 PUFA compared to n-3 PUFA are reflected in breast milks (15). It is suggested that increased n-6 PUFA intakes might contribute to obesity epidemics (16). Moreover, studies have demonstrated that neonates supplemented with n-3 FAs have lower fat mass than those receiving no supplementations (17). Being overweight or obese are factors for having extra body fats. Although evidence suggest that breast milks of mothers with normal weights vary from those of overweight and obese for saturated fatty acids (SFA) (18), n-3 PUFA (12, 18, 19) and n-6 PUFA/n-3 PUFA ratios (13, 14, 18), a few studies have addressed these variations in breast milk FA profiles in obese and overweight mothers. However, these studies did not explain distinct characteristics of the categories (20). Since FA reservoirs in the body are responsible for most breast milk FA variations (10), the aim of the present study was to investigate if FA compositions of the breast milks are similar in overweight and obese mothers.

## Materials and Methods

### Participants

This observational study was carried out on breastfeeding volunteers referred to health centers in Mashhad, Iran, September 2018 to March 2019. The inclusion criteria of the study were as follows: 1) ages of 18–55 years; 2) current BMI of  $\geq 25$  kg/m<sup>2</sup>; 3) mothers with apparently healthy infants; and 4) neonatal ages of  $> 10$  to  $< 90$  days. The exclusion criteria were as follows: 1) twin delivery/premature births (before 37 weeks of gestation); 2) history of gestational diabetes; 3) use of probiotics or n-3 supplements; and 4) alcohol/tobacco consumption. After receiving written informed consents from the participants, demographic data were collected, including mother age at delivery, mode of delivery, mode of breastfeeding, self-reported pre-pregnancy weight and height and weight gain during pregnancy as well as infant gender and birth weight. Obese women (BMI  $\geq 30$  kg/m<sup>2</sup>) and a matched overweight group with BMI of 25–29.9 kg/m<sup>2</sup> were included in the study based on the World Health Organization (WHO) criteria. Matching was carried out based on maternal age and weeks postpartum between the overweight mothers from the same health centers. The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (code: IR.MUMS.MEDICAL.REC.1397.114).

### Sample size

Sample size was calculated ( $n = 36$ ) based on the study of Rudolph et al. (21) and the following formula. Where,  $n$  was the predicted sample size,  $\alpha$  was the

probability of a type 1 error (0.05),  $\beta$  was the probability of a type 2 error (0.1),  $S$  was S.D and  $\bar{X}$  was the mean.

$$n_1 = n_2 = \frac{(S_1^2 + S_2^2)(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2}{(\bar{X}_1 - \bar{X}_2)^2}$$

### Data collection

Anthropometric measurements included maternal body weight, BMI and body fat proportion, using body composition analyzer (Tanita BC 418MA, Tokyo, Japan) at the inclusion. Height was measured using SECA Portable Stadiometer (SECA, Hamburg, Germany). The BMI classification by the World Health Organization (WHO) was used as follows: overweight, 25.0–29.9 kg/m<sup>2</sup>; and obese,  $> 30$  kg/m<sup>2</sup> (22). Selected participants were asked to collect their breast milk samples manually. First, they let a few drops of their breast milks to remove and then collected breast milks as much as possible (at least 20 ml), storing samples in plastic containers (18). Breast milk samples were collected from the fasting mothers (8:00–11:00 AM) by a trained expert and stored at  $-20$  °C until use. Maternal dietary intakes within the past 30 days were assessed using semi-quantitative food frequency questionnaire (FFQ) with 160 Iranian food items, validated by Nematy et al. (23). The completed FFQ were analyzed using United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference Legacy Release (April 2018).

### Milk fatty acid analysis

Breast milk FAs were extracted from the samples using modified Folch method (24, 25). In brief, 500  $\mu$ l of homogenized breast milk samples were added to a mixture of chloroform/methanol (v/v, 2:1) and 600  $\mu$ l of normal saline and vortexed for 1 min. After centrifugation at 4,000 rpm for 4 min, the lower phase was collected, and the solvent was evaporated using a rotary evaporator (Model Laborota 4003, Heidolph WB, Germany). Then, fatty acid methyl esters (FAME) were assessed through dissolving dried lipid extracts in n-heptane and methanolic KOH (2 N). The FAs were analyzed using gas chromatograph (Varian 450-GC, Varian Associates, Walnut Creek, CA, USA) equipped with a fused silica capillary column (length, 100 m; film thickness, 0.25 mm  $\times$  0.2  $\mu$ m), which coated with silicon-based polymers (polysiloxane), polyethylene glycols and solid adsorbent (EU). Then, standard FAME mix (Supelco 37 Component FAME Mix, Sigma-Aldrich, USA) was injected prior to sample analysis to characterize FAs. Quantification was accomplished using peak areas, which were retrieved from the integrator coupled to the chromatograph. The FA data were reported as proportions of the total detected FAs.

## Statistical analysis

Data analysis was carried out using SPSS Software v.11.0 (SPSS, Chicago, IL, USA). Variable distributions were evaluated using Kolmogorov-Smirnov test. In addition, descriptive statistics (mean, standard deviation [SD], median and interquartile range) were used. Student's t-test and Mann-Whitney U test were used to compare demographic data, anthropometric data and nutritional values of the maternal diets between the study groups. Furthermore, Pearson's or Spearman's correlation-coefficients were used to assess correlations between the breast milk compositions, maternal diets and body compositions based on data normality. Linear regression analysis was carried out to show if maternal BMI significantly predicted breast milk FA profiles. Maternal age, days postpartum at the time of sampling, mode of delivery, mode of breastfeeding, energy intake, and dietary FA intake were considered as confounders. In all the statistical analyses, *p*-values of less than 0.05 were considered as significant.

## Results

In total, 73 eligible participants (37 overweight and 36 obese participants) enrolled in the study. Table 1 shows baseline demographic characteristics of the participants. No significant differences were seen between the groups in time points after birth when breast milk samples were collected (75.31 and 69.08 days for overweight and obese participants, respectively). In addition, no significant differences were observed between the study groups in mean maternal age, pregnancy weight gain and infant birth weight. No significant differences were reported in exclusive breastfeeding rate, mode of delivery and infant gender between the study groups. Table 2 shows data on

breast milk FA compositions in overweight and obese women. The SFA (43%) was the major constituent of breast milk FAs, whereas PUFA (23%) included the lowest proportion. In addition, SFA contents of the breast milk samples were significantly higher in obese mothers, compared to overweight mothers (*p* = 0.03). Ratios of unsaturated to saturated fatty acids were lower in breast milk samples of the obese groups (*p* = 0.04). However, no significant differences were seen in ratios of other FAs, including MUFA, PUFA, n-6 PUFA, n-3 PUFA and n-6 PUFA/n-3 PUFA between the study groups (*p* > 0.05).

Based on the information in Table 3, total rates of the simple sugars in maternal diets of the participants were negatively correlated with the breast milk contents of PUFA (*r* = -0.369, *p* = 0.035), n-6 PUFA (*r* = -0.370, *p* = 0.034) and PUFA/SFA ratio (*r* = -0.407, *p* = 0.019). However, no significant correlations were reported between the dietary intakes of SFA, MUFA, PUFA, n-3 PUFA and n-6 PUFA in breast milk samples and their corresponding FAs (*p* > 0.05). In addition, energy, protein, carbohydrate, total lipids and dietary fiber intakes were not associated with the breast milk FA profiles (*p* > 0.05). Based on the information from Table 4, maternal age was positively correlated with SFA (*r* = 0.250, *p* = 0.042) and negatively with MUFA (*r* = -0.25, *p* = 0.036), MUFA/SFA ratio (*r* = -0.255, *p* = 0.038) and MUFA/SFA ratio (*r* = -0.304, *p* = 0.012) in breast milk samples. It is noteworthy that associations between these parameters were significant after adjustment for maternal BMI. Moreover, maternal weight was negatively associated with MUFA/SFA ratios (*r* = -0.251, *p* = 0.040) in breast milk samples. Pre-pregnancy maternal weight was inversely correlated with MUFA (*r* = -0.276, *p* = 0.026) and MUFA/SFA ratios (*r* = -0.285, *p* = 0.022).

**Table 1.** Demographic and anthropometric characteristics of the study groups

Characteristics		Overweight n=37	Obese n=36	p-value
Mother's age (years)		28.73 ±5.19	30.83 ±4.65	0.195
Prepregnancy weight (Kg)		66.24 ±7.87	78.72 ±9.38	0.001
Weight gain during pregnancy (Kg)		11.85 ±4.56	13.3 ±5.31	0.228
BMI (kg/m <sup>2</sup> )		27.48 ±1.26	33.51 ±2.74	0.001
Body fat mass (Kg)		24.86 ±3.66	35.31 ±5.09	< 0.001
Body fat proportion (%)		35.17 ±2.50	42.04 ±2.87	< 0.001
Infant age (day)		75.31 ±26.04	69.08 ±26.35	0.320
Birthweight (gr)		3344 ±334	3278 ±564	0.562
Exclusive breastfeeding (n)%		26 (70.2%)	29 (80.6%)	0.580
Infant gender (n)%	Female	20 (54%)	14 (39%)	0.243
	Male	17 (46%)	22 (61%)	
Delivery type (n)%	Vaginal	15 (41%)	16 (44%)	0.815
	Cesarean	22 (59%)	20 (56%)	

(Quantitative variables are expressed as mean ±SD)

**Table 2.** Breast milk fatty acid compositions in overweight and obese women

	Overweight	Obese	p-value		Overweight	Obese	p-value
SFA (%)	41.75 ±5.42	44.52 ±4.96	0.03 <sup>‡</sup>	C20:1 (%)	1.24 (1.07, 1.49)	1.18 (0.91, 1.45)	0.35 <sup>±</sup>
C4:0 (%)	0.12 (0.06, 0.21)	0.12 (0.08, 0.17)	0.54 <sup>±</sup>	C22:1n9 (%)	0.16 (0.06, 0.44)	0.08 (0.05, 0.32)	0.43 <sup>±</sup>
C8:0 (%)	0.05 (0.03, 0.08)	0.04 (0.02, 0.08)	0.15 <sup>±</sup>	C24:1 (%)	0.10 (0.07, 0.11)	0.09 (0.08, 0.10)	0.71 <sup>±</sup>
C10:0 (%)	0.90 (0.67, 1.18)	0.90 (0.75, 1.28)	0.57 <sup>±</sup>	PUFA (%)	23.23 (19.44, 24.11)	21.15 (17.43, 25.50)	0.17 <sup>±</sup>
C12:0 (%)	4.96 ±1.79	5.35 ±2.08	0.39 <sup>‡</sup>	C18:2n6c (%)	21.90 ±4.33	20.82 ±4.75	0.31 <sup>‡</sup>
C14:0 (%)	5.64 ±2.00	6.50 ±2.12	0.08 <sup>‡</sup>	C18:3n6 (%)	0.19 (0.15, 0.22)	0.22 (0.19, 0.24)	0.18 <sup>±</sup>
C15:0 (%)	0.26 ±0.09	0.27 ±0.09	0.43 <sup>‡</sup>	C18:3n3 (%)	0.22 (0.15, 0.40)	0.23 (0.18, 0.28)	0.83 <sup>±</sup>
C16:0 (%)	23.24 ±3.31	23.86 ±3.35	0.43 <sup>‡</sup>	C20:2 (%)	0.32 ±0.11	0.35 ±0.07	0.14 <sup>‡</sup>
C17:0 (%)	0.31 (0.23, 0.47)	0.33 (0.27, 0.41)	0.28 <sup>±</sup>	C20:3n3 (%)	0.43 ±0.13	0.40 ±0.11	0.34 <sup>‡</sup>
C18:0 (%)	5.91 ±1.24	6.09 ±0.81	0.47 <sup>‡</sup>	C20:4n6 (%)	0.04 ±0.02	0.05 ±0.02	0.40 <sup>‡</sup>
C20:0 (%)	0.25 ±0.11	0.22 ±0.10	0.36 <sup>‡</sup>	C20:5n3 (%)	0.03 (0.02, .03)	0.03 (0.02, 0.03)	0.39 <sup>±</sup>
C21:0 (%)	0.03 ±0.02	0.02 ±0.01	0.09 <sup>‡</sup>	C22:2 (%)	0.02 (0.01, 0.03)	0.03 (0.02, 0.03)	0.15 <sup>±</sup>
C22:0 (%)	0.48 (0.35, 0.57)	0.48 (0.40, 0.64)	0.30 <sup>±</sup>	C22:6n3 (%)	0.04 (0.02, 0.07)	0.03 (0.02, 0.05)	0.11 <sup>±</sup>
C23:0 (%)	0.05 (0.03, 0.14)	0.06 (0.04, 0.10)	0.67 <sup>±</sup>	USFA/SFA (ratio)	1.35 (1.19, 1.65)	1.22 (1.06, 1.48)	0.04 <sup>±</sup>
C24:0 (%)	0.05 ±0.02	0.06 ±0.02	0.32 <sup>‡</sup>	MUFA/SFA (ratio)	0.82 (0.74, 1.01)	0.74 (0.66, 0.86)	0.05 <sup>±</sup>
MUFA (%)	34.34 (32.36, 36.21)	32.89 (31.30, 35.24)	0.15 <sup>±</sup>	PUFA/SFA (ratio)	0.55 (0.43, 0.62)	0.47 (0.39, 0.63)	0.08 <sup>±</sup>
C14:1 (%)	0.11 ±0.06	0.12 ±0.07	0.55 <sup>‡</sup>	PUFA/MUFA (ratio)	0.67 ±0.17	0.66 ±0.20	0.74 <sup>‡</sup>
C16:1 (%)	1.22 (0.98, 1.86)	1.28 (1.01, 1.52)	0.84 <sup>±</sup>	n-3 PUFA (%)	0.68 ±0.29	0.61 ±0.23	0.23 <sup>‡</sup>
C17:1 (%)	0.26 (0.20, 0.33)	0.26 (0.21, 0.39)	0.64 <sup>±</sup>	n-6 PUFA (%)	22.33 (18.63, 23.61)	20.63 (16.99, 24.88)	0.20 <sup>±</sup>
C18:1n9c (%)	30.85 (28.95, 32.87)	30.21 (28.3, 32.5)	0.33 <sup>±</sup>				

Values presented as proportions from all fatty acids (mean ±SD or Median (IQR)). p<0.05 were considered statistically significant. Statistical tests: Student's t-test <sup>‡</sup>, Mann-Whitney U test <sup>±</sup>

**Table 3.** Correlations between the maternal dietary intakes and the breast milk fatty acids

Dietary intake		Breast milk fatty acid composition									
		SFA (%)	MUFA (%)	PUFA (%)	USFA/SFA ratio	MUFA/SF A ratio	PUFA/SFA ratio	PUFA/MU FA ratio	n-3 PUFA (%)	n-6 PUFA (%)	n-6 PUFA/n-3 PUFA ratio
Energy (Kcal)	r	0.216	0.011	-0.262	-0.249	-0.137	-0.302	-0.211	-0.056	-0.258	-0.173
Protein (EI%)	r	-0.027	-0.095	0.139	0.029	-0.052	0.126	0.131	-0.193	0.150	0.303
Total lipid (EI%)	r	0.086	0.303	0.279	-0.069	-0.230	0.165	0.307	0.046	0.276	0.156
Carbohydrate (EI%)	r	-0.107	0.306	-0.219	0.093	0.220	-0.113	-0.319	0.023	-0.220	-0.115
Fiber (g/1000Kcal)	r	-0.013	0.002	0.014	-0.017	-0.013	-0.016	0.028	0.224	0.001	-0.158
Starch (EI%)	r	-0.162	-0.063	0.262	0.145	0.033	0.240	0.249	-0.169	0.272	0.289
Sugars, total (EI%)	r	0.080	0.263	-0.369*	-0.087	0.097	-0.287	-0.407*	0.049	-0.370*	-0.023
Cholesterol (mg)	r	0.230	-0.066	-0.195	-0.259	-0.187	-0.254	-0.136	-0.081	-0.190	-0.056
Trans fatty acids (EI%)	r	-0.015	-0.054	0.076	0.039	-0.003	0.078	0.083	0.122	0.068	-0.181
SFA (EI%)	r	0.073	-0.101	0.034	-0.075	-0.100	-0.014	0.081	-0.022	0.035	0.123
MUFA (EI%)	r	-0.184	-0.097	0.311	0.186	0.050	0.291	0.286	0.283	0.293	-0.127
PUFA (EI%)	r	-0.023	-0.059	0.095	0.048	0.018	0.072	0.152	-0.006	0.095	-0.015
USFA/SFA ratio	r	-0.004	0.029	-0.031	0.012	0.027	-0.012	-0.027	-0.019	-0.030	-0.118
MUFA/SFA ratio	r	-0.016	0.012	0.001	0.032	0.031	0.022	0.001	0.023	0.000	-0.180
PUFA/SFA ratio	r	-0.029	0.003	0.025	0.037	0.027	0.035	0.036	0.022	0.024	-0.116
PUFA/MUFA ratio	r	-0.119	0.025	0.112	0.112	0.081	0.111	0.120	0.110	0.105	-0.061
n-3 PUFA (EI%)	r	-0.059	-0.098	0.183	0.087	0.011	0.155	0.208	0.171	0.172	-0.215
n-6 PUFA (EI%)	r	-0.015	-0.077	0.105	0.033	-0.012	0.082	0.148	-0.031	0.107	0.018
n-6 PUFA/n-3 PUFA ratio	r	0.050	-0.105	0.046	-0.070	-0.102	-0.001	0.091	-0.135	0.055	0.187

The results are shown with Pearson's correlation coefficients. The statistically significant correlations are shown as highlights (\*p < 0.05, \*\*p < 0.01).

**Table 4.** Correlations between the maternal anthropometric characteristics and the breast milk fatty acids

Breast milk fatty acid composition		Mother's age (years)	Prepregnancy weight (Kg)	Weight gain during pregnancy (Kg)	Maternal weight (Kg)	Prepregnancy BMI (kg/m2)	BMI (kg/m <sup>2</sup> )	Body fat mass (Kg)	Body fat proportion (%)
SFA	r	0.250*	0.211	0.137	0.212	0.285*	0.256*	0.226	0.214
MUFA	r	-0.256*	-0.276*	0.132	-0.175	-0.358**	-0.224	-0.151	-0.096
PUFA	r	-0.142	-0.081	-0.189	-0.116	-0.125	-0.167	-0.165	-0.209
USFA/SFA	r	-0.255*	-0.211	-0.135	-0.212	-0.287*	-0.256*	-0.226	-0.212
MUFA/SFA	r	-0.304*	-0.285*	-0.066	-0.251*	-0.351**	-0.277*	-0.235	-0.183
PUFA/SFA	r	-0.177	-0.139	-0.180	-0.169	-0.205	-0.221	-0.208	-0.232
PUFA/MUFA	r	-0.013	0.009	-0.231	-0.069	0.029	-0.077	-0.114	-0.169
n-3 PUFA	r	0.106	-0.053	0.033	-0.080	0.037	-0.044	-0.011	0.077
n-6 PUFA	r	-0.152	-0.072	-0.189	-0.103	-0.117	-0.157	-0.155	-0.205
n-6 PUFA/n-3 PUFA	r	-0.174	-0.052	-0.162	0.032	-0.116	-0.009	-0.025	-0.101

The results are shown with Spearman correlation coefficients. The statistically significant correlations are shown as highlights (\*p < 0.05, \*\*p < 0.01).



In this study, maternal pre-pregnancy BMI was positively correlated with SFA ( $r = 0.285$ ,  $p = 0.019$ ) and negatively with MUFA ( $r = -0.385$ ,  $p = 0.003$ ), USFA/SFA ratios ( $r = -0.287$ ,  $p = 0.019$ ) and MUFA/SFA ratios ( $r = -0.351$ ,  $p = 0.004$ ) in breast milks. During sample collection, the current maternal BMI was positively correlated with SFA ( $r = 0.256$ ,  $p = 0.037$ ) and negatively with USFA/SFA ( $r = -0.266$ ,  $p = 0.036$ ) and MUFA/SFA ( $r = -0.277$ ,  $p = 0.023$ ) ratios. However, no significant associations were seen between the body fat mass, fat proportion, weight gain during pregnancy and breast milk FA profile ( $p > 0.05$ ). Results of linear regression (Table 5) revealed that maternal BMI significantly contributed to the model that predicted SFA ( $\beta = 0.392$ ,  $p = 0.023$ ) and USFA/SFA ratios ( $\beta = -0.022$ ,  $p = 0.027$ ) in breast milks. The current maternal BMI was not a significant predictor of breast milk FA profiles after adjustment of the confounding factors (maternal age, days postpartum at the time of sampling, mode of delivery, mode of breastfeeding, energy intake and dietary intake of each FA).

## Discussion

Based on the present results, SFA was the major fraction of breast milks (43%) and included significant differences between obese and overweight mothers as well as the USFA/SFA ratios. Although PUFA included the smallest proportion of breast milk FAs, the averages of total PUFA and linoleic acid proportions were higher (22.3 and 21.3%, respectively) than those reported in the previous studies (19, 26). However, no significant differences were seen in breast milk FAs between the groups. Adiposity measurements and higher BMI have been associated with the higher total fat contents of breast milks (27). However, a few studies have compared the breast milk FA profiles between the obese and overweight mothers, suggesting inconsistent results. Although the breast milk ratios of n-6 PUFA/n-3 PUFA have been higher in obese and overweight mothers than normal-weight mothers (12–14, 18), no significant differences were observed in n-6 PUFA/n-3 PUFA ratios of breast milks between the overweight and obese mothers. The current maternal BMI

was positively correlated with SFA and negatively with USFA/SFA ratios. Maternal BMI contributed to the linear regression model that predicted SFA and USFA/SFA ratios. However, the BMI effects were not significant after adjusting for confounding factors. In a similar study, Makela et al. reported that breast milk SFA contents were significantly higher ( $p = 0.012$ ) and n-3 PUFA FAs ( $p = 0.010$ ) were significantly lower in overweight mothers than normal-weight mothers. However, no significant associations were reported between MUFA and n-6 PUFA (18).

In the present study, maternal age and pre-pregnancy BMI were positively correlated with SFA and negatively with MUFA contents of the human breast milks. However, no significant correlations were seen between the PUFA, n-3 PUFA, n-6 PUFA contents as well as the n-6 PUFA/n-3 PUFA ratios in breast milk samples with maternal anthropometric indices. Garcia-Ravelo et al. reported low concentrations of n-3 long-chain polyunsaturated fatty acids (LCPUFA), including eicosapentaenoic acid (EPA) ( $p = 0.005$ ) and docosahexaenoic acid ( $p = 0.015$ ), in breast milks of overweight women (28). In contrast, breast milk contents of SFA, MUFA, total n-6 PUFA, total n-3 PUFA, and free FAs included no significant differences between the normal and overweight mothers (13). In contrast to maternal weight and BMI, no significant associations were reported between the maternal body fat masses, fat proportions and breast milk FA profiles. Therefore, it could be concluded that maternal body fat masses and visceral fats increased regardless of postpartum weight losses (29). Human milk FAs are either synthesized by mammary alveolar epithelia or originate from the blood plasma (10). Availability of the circulating FAs in mammary glands, which is affected by the maternal fat reservoirs and dietary habits, determines FA profiles of the breast milks (30–32). In a study by Ahmadi et al., obesity was negatively associated with plasma n-3 PUFA (33). However, the SFA content of breast milks increased as lipids were synthesized from the tissues when breastfeeding mothers consumed excessive non-fat calories (10).

**Table 5.** Linear regression models for assessing associations between the maternal BMI and the breast milk fatty acids

Variables	Unadjusted model $\beta$ [95% CI]	p-Value	Adjusted model $\beta$ [95% CI]	p-Value
SFA	0.392 [0.056 _ 0.728]	0.023*	0.449 [-0.206 _ 1.104]	0.170
MUFA	-0.146 [-0.399 _ 0.108]	0.255	-0.043 [-0.651 _ 0.565]	0.885
PUFA	-0.156 [-0.454 _ 0.152]	0.316	-0.383 [-1.053 _ 0.288]	0.250
USFA/SFA	-0.022 [-0.041 _ -0.003]	0.027*	-0.022 [-0.063 _ 0.018]	0.260
MUFA/SFA	-0.012 [-0.024 _ 0]	0.054	-0.001 [-0.032 _ 0.031]	0.965
PUFA/SFA	-0.008 [-0.019 _ 0.003]	0.158	-0.014 [-0.040 _ 0.012]	0.276
PUFA/MUFA	-0.001 [-0.013 _ 0.011]	0.868	-0.018 [-0.048 _ 0.011]	0.213
n-3 PUFA	0 [-0.13 _ 0.018]	0.961	0.003 [-0.038 _ 0.043]	0.900
n-6 PUFA	-0.155 [-0.464 _ 0.153]	0.318	-0.365 [-1.041 _ 0.311]	0.276
n-6 PUFA/n-3 PUFA	0.229 [-0.606 _ 1.065]	0.585	0.596 [-1.584 _ 2.776]	0.573

The statistically significant correlations are shown as highlights (\* $p < 0.05$ ).

Although SFA is suggested to increase low-density lipoprotein cholesterol (LDL-C) and increase cardiovascular disease (CVD) risks (34), evidence have shown no associations between higher intakes of total fats or SFAs with growth, adiposity and cardiometabolic health in children up to 6 years of age (35). However, higher plasma levels of long-chain saturated fatty acids (LCSFA) and high n-3 and low n-6 LCPUFA patterns were positively associated with psychomotor development scores in infants up to six months of age (36). Laiho et al. suggested that the proportion of total SFA might be an important regulator of breast milk immune milieu as it was negatively associated with TGF- $\beta$ 2 levels (37). Naturally, maternal diets affect constituents of the breast milk lipids (10). Although breast milk FAs are predominantly composed of the FAs deposited in fat tissues, maternal diets might affect breast milk FA contents, especially its PUFA contents. Diets with fish and seafood increase the intake of n-3 PUFA FAs, EPA and DHA as well as concentration of the corresponding FAs expressed in breast milks (20, 38). Based on the results of the present study, dietary intakes included no significant differences between the overweight and obese mothers, except for the total energy intakes ( $p = 0.014$ ). In addition, no significant correlations were observed between the breast milk FAs and energy intakes, carbohydrates, proteins, SFA, MUFA, PUFA, n-3 PUFA and n-6 PUFA in maternal diets. However, the breast milk levels of PUFA and n-6 PUFA were negatively correlated with the total sugar intakes of the mothers. These differences could be attributed to various breast milk FA extraction methods and maternal diet analyses. Therefore, genetic differences in FA desaturases should be considered important, affecting breast milk fat contents (39, 40). Studies have reported that maternal age is positively correlated with breast milk fat contents, especially colostrum and transitional milk (41). Antonakou et al. reported that MUFA levels in breast milks were negatively correlated with maternal ages (20). In the present study, increased maternal age was similarly associated with decreased MUFA and increased SFA breast milk contents. Although the exact mechanisms are still unclear, dietary habits and gut microbiome differences may be involved in such changes (20). One of the limitations of the present study included lack of local food composition databases for the maternal diet nutritional assessments.

## Conclusion

The current findings supported the hypothesis that maternal weight and BMI could affect breast milk FA compositions. Although no significant differences were observed in MUFA and PUFA contents of the breast milks, breast milks of the obese mothers included higher proportions of SFA and lower ratios of unsaturated to saturated fatty acids, compared to that the breast milks of

the overweight mothers did. This could be linked to differences in the metabolic functions between the obese and overweight mothers. Considering similar dietary habits in the two study groups, maternal diets were not significant affecting factors in FA contents of the breast milks.

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