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ORIGINAL CONTRIBUTION

Breast milk fatty acid composition differs between overweight and normal weight women: the STEPS Study

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Abstract
Objective We studied differences in breast milk fatty acid (FA) composition between overweight and normal weight women and the effect of FA composition on children's cholesterol concentrations at 13 months and growth from birth to 13 months.
Methods Samples were collected from lactating women ($n = 100$) participating in STEPS study at infant's age of 3 months, and FA composition was analyzed with gas chromatography. Diet of mother was studied with Index of Diet Quality at third trimester of pregnancy and with food frequency questionnaire on sampling day. The children's weights and heights were collected from hospital records at birth and during study visits at 13 months.
Results Overweight women's breast milk compared to normal weight women's breast milk contained higher amount of saturated FAs (46.3 vs. 43.6 %, $P = 0.012$), lower amount of n-3FAs (2.2 vs. 2.7 %, $P = 0.010$), lower ratio of unsaturated to saturated FAs (1.1 vs. 1.3, $P = 0.008$), and higher ratio of n-6 to n-3 FAs (5.7 vs. 4.9, $P = 0.031$) than those of normal weight women even after adjusting for maternal diet ($P < 0.05$ for all). Normal weight women adhered more to dietary recommendations during pregnancy, whereas no differences were found in diet at sampling 3 months postpartum. The children's weight gains correlated with saturated FAs ($R = 0.22$, $P = 0.04$) and the ratio of unsaturated to saturated FAs ($R = -0.23$, $P = 0.038$) in milk; however, effects diminished after adjusting for total duration of breastfeeding. Milk FA composition was not associated with children's cholesterol concentrations at 13 months.
Conclusions Breast milk FA composition differed between overweight and normal weight women.

Keywords Breast milk · Breast feeding · Fatty acids · Infant growth · Nutrition · Obesity

Abbreviations

BMI	Body mass index (kg/m^2)
FA	Fatty acid
GC	Gas chromatography
IDQ	Index of Diet Quality
LCPUFA	Long-chain polyunsaturated fatty acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
STEPS	Steps to healthy development study

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Introduction

Maternal health during pregnancy and early nutrition has many effects on the long-term health of the child [1] and may even affect later obesity risk of the offspring. According to a variant of Barker hypothesis, excessive maternal body weight or weight gain during pregnancy disturbs the intrauterine environment during fetal development [2].

Previously maternal overweight or obesity has been shown to associate with lower duration of breastfeeding [3]. Since breastfeeding has been shown to associate with a modest but consistent protective effect against later obesity [4–6], lower duration of breastfeeding may further increase the risk of obesity in children. Studies also indicate that breastfed infants grow differently than formula-fed infants, who, in general, reach higher body weights, lengths, and weight for length at 1 year of age than breast-fed infants, which is most likely due to higher protein and energy intake [7, 8]. Numerous mechanisms, such as the content of nutrients and bioactive components, the mode of feeding, and the mother-infant interaction, have been offered as potential reasons for the protective effect of breastfeeding against obesity in childhood [9].

The content of nutrients in breast milk influences the growth and health of infants [10]. The fatty acid (FA) composition of breast milk varies substantially between lactating mothers [11]; however, little is known about the effect of maternal overweight on the FA composition of the milk. Exclusively breastfed infants must rely on essential and long-chain polyunsaturated FAs (LCPUFA) present in the breast milk. The FA composition of breast milk reflects the long-term FA composition of the mother's diet [12, 13], although the current diet may also influence it [14, 15]. In addition to maternal diet, breast milk fatty acid composition is also dependent on mobilization of endogenous stores of fatty acids and de novo synthesis of fatty acids by liver or breast tissue. However, the optimal composition remains unknown. More importantly, the FA composition of the milk and the infant's plasma and tissues correlate with each other [16–18]. Maternal diet with low levels of essential FAs may result in a decrease of these FAs in breast milk. Fish-oil supplementation during pregnancy associates with increased n-3 LCPUFAs in breast milk and has an impact on the FA status of the erythrocytes of the infants [19]. It has also been hypothesized that the PUFA content, especially the ratio between n-6 and n-3 LCPUFA, of an infant's diet might affect early weight gain. Some studies have shown that supplementation of infant formula with n-3 LCPUFA may affect infant growth in preterm infants [20, 21] whereas others have found no such associations [22–25]. The effect of other breast milk FAs on infant growth is unknown.

Owing to partly inherited and partly environmental reasons, maternal overweight associates with children's obesity [26]. Yet, it has remained unclear whether the FA profile of breast milk differs between overweight and normal weight mothers. Therefore, we studied whether maternal overweight influences the FA composition of the breast milk or whether the maternal diet is a more important factor influencing it. Furthermore, we investigated whether the breast milk FA composition affects children's weight, growth, and cholesterol levels at the age of 13 months.

Subjects and methods

Subjects and study design

Altogether, 1797 pregnant women and their spouses were recruited from maternity clinics in South-West Finland or Turku University Hospital to participate in a prospective follow-up study STEPS (Steps to healthy development), from September 2007 to March 2010. Of those women, 144 with prepregnancy body mass index (BMI) $\geq$ 25 kg/m^2 and 108 with prepregnancy BMI <25 kg/m^2 were selected from the STEPS study based on parental BMI and recruited by sending an information letter and an invitation at 20 weeks of gestation to participate in follow-up study for early risk factors of childhood obesity. From these 144 women, 90 overweight (prepregnancy BMI $\geq$ 25 kg/m^2) women and 73 normal weight (prepregnancy BMI < 25 kg/m^2) women (mean age, 30 years; range, 17–43 years) were willing to participate in this more intensive follow-up study for early risk factors of childhood obesity. Maternal weight and height records were obtained from the maternity clinic records throughout pregnancy (weight was measured on average 13 times during pregnancy) and from study visit 13 months postpartum. Child weight and height records were obtained from the hospital records at birth and measured at the study visits at the child's age of 13 months.

Written informed consent was obtained from the participants. The study protocol was approved by the Ethics Committee of the Hospital District of South-West Finland.

Dietary evaluation

Maternal diet was studied during the third trimester of pregnancy and at the time of the sampling. At the third trimester of pregnancy, we analyzed maternal diet with Index of Diet Quality (IDQ). IDQ describes the adherence to nutrition recommendations and it has been previously validated with 7-day food records [27]. IDQ consists of 18 questions with the scoring system from 0 to 15 where the scores from 0 to 9 are defined as non-adherence and those from 10 to 15 as adherence to health-promoting diet and dietary guidelines. The health-promoting diet was defined based on current Nordic Nutrition Recommendations and scientific knowledge. More detailed information about the definition of the health-promoting diet is described by Leppälä et al. [27]. The IDQ enables assessment of the health-promoting properties of the diet taking into account possible synergistic and additive effects of foods when reflected against health outcomes.

Questions in IDQ describe the consumption and quality of dairy products, vegetables, fruits and berries, fat containing foods (i.e., spreads, oils), fish, whole grain products, sugar, and meal pattern, and the scoring system is based on current nutrition recommendations. IDQ has both qualitative (for example what kind of bread mostly used) and quantitative (for example how many slices of bread per day) questions. Quantitative questions are mainly focused on portion sizes, such as slices of bread, glasses of milk, and portions of fruit whereas the qualitative questions aim to define more specifically the quality of the different food eaten, such as the amount of fat in dairy products consumed or type of spread mostly used on bread.

At sampling time, the dietary intake of mothers was evaluated with a short food frequency questionnaire. The questionnaire focused only on foods rich in different fatty acids or in foods that constitute one of the main sources of certain fatty acids and was covering 1-week period. The questionnaire contained questions on fish, fish-oil supplements, vegetable oils, spreads, and foods rich in saturated fatty acids (SFA) such as fast food, snacks, sausages, high-fat dairy products, and chocolate. The foods included in the questionnaire represented the typical sources of different FAs in the Finnish diet [28]. The food frequency questionnaire was answered on the day of the sampling in order to define the intake of FAs during the week before sampling that could partly explain the FA profile of the breast milk samples.

Breast milk collection

Breast milk was collected at children's age of 3 months. The collection procedure was standardized by the following written instructions. The mothers collected the samples by manual expression in the morning, first milking a few drops to waste and after that collecting the actual sample of for-milk (10 ml) into a plastic container. The mothers brought the samples to the research center, or the samples were collected from their homes on the day of sampling. All samples were frozen and stored at −70 °C until analysis of the FAs.

Breast milk FA composition analyses

The total FA composition of the breast milk was analyzed at children's age of 3 months. Triheptadecanoin (Larodan Fine Chemicals, Malmö, Sweden) was used as an internal standard in breast milk samples. Internal standard was added to the sample, and total lipids were extracted with chloroform/methanol 2:1 v/v [29]. FA methyl esters were prepared from total lipids of the breast milk with boron trifluoride [30]. The samples were analyzed by gas chromatography (GC-2010 Auto Injector/Auto Sampler, Shimadzu, Japan) with a DB-23 column (60 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, DE, USA). The peaks of FAs were identified by comparing their retention times with those of known standard mixtures FAME 37 (Supelco, Bellefonte, PA) and 68D (NuChek Prep, Elysian) and then quantified in relation to the internal standard. The FA composition of the total lipids of breast milk was expressed as weight percentage of total FAs.

Statistical methods

The data were analyzed with SPSS statistical software package (version 16.0; SPSS Inc., Chicago, IL, USA). *T* test for independent samples was used for comparison of the maternal, children, and clinical characteristics except for child gender where a chi-square test was used because of the categorized variables. The *T* test for independent samples was used to investigate the effect of maternal overweight on the FA composition of breast milk. In addition, multiple regression analysis adjusted for maternal diet during sampling time was used to study the effect of maternal overweight on the FA composition of breast milk.

Pearson correlation coefficients were used in studying the effects of the total fat content of the breast milk on FA profile and in studying the correlations between breast milk FAs and foods containing high amounts of fat or foods that are significant sources of certain FAs. Pearson correlation coefficients were also used in analyzing the associations of maternal BMI with breast milk FA composition, the child's growth and the child's cholesterol concentrations. In the child's growth data, we analyzed boys and girls jointly since the numbers of boys and girls were similar in the groups of overweight and normal weight mothers, and in interaction test, no connection between maternal BMI and child gender was found. Linear regression analysis was used to investigate the association of the FA composition of breast milk on child's weight, height, BMI, weight gain, and change in BMI from birth to 13 months and the cholesterol concentrations at age of 13 months. Stepwise linear regression was also used for studying the effect of maternal socio-economic variables, age, and gestational weight gain on breast milk fatty acid composition. Statistical significance was set to a confidence level of $P < 0.05$.

Results

Basic characteristics

From a total of 163 women, 100 (61 %) donated a breast milk sample when the child was 3 months of age. The reasons for not donating breast milk were that the mother was not breastfeeding any more ($n = 36$), did not feel comfortable to donate ($n = 12$), did not know how to do manual expression ($n = 11$), had a stillborn ($n = 1$), or the reason was unknown ($n = 3$). The 163 women participating to this more intensive follow-up study did not differ in socio-economic variables from the women participating the STEPS study ($n = 1798$). The children's mean age at sample collection was 11.7 weeks (±2.2 weeks), and no difference in sampling time was found between overweight and normal weight women (11.7 vs. 11.8 weeks, respectively, $P = 0.981$). The mean duration of exclusive breastfeeding was 4 months and that of partial breastfeeding was 8 months. Fourteen percent of all women were still partly breastfeeding at child age of 13 months.

Maternal, child, and clinical characteristics are presented in Table 1. Maternal BMI before pregnancy correlated positively with child's weight ($R = 0.341$, $P = 0.001$) and BMI ($R = 0.417$, $P < 0.0001$) at 13 months and also with weight gain ($R = 0.287$, $P = 0.007$) and increase in BMI ($R = 0.239$, $P = 0.027$) from birth to 13 months. HDL cholesterol concentration and HDL to total cholesterol ratio were lower in children of overweight women than in children of normal weight women ($P < 0.05$ for both).

Breast milk fatty acid composition in overweight and normal weight women

Table 2 shows breast milk FA composition in overweight and normal weight women. Total fat content of the milk did not differ between overweight and normal weight women ($P = 0.625$). The breast milk of overweight women was significantly higher in SFA ($P = 0.012$) and lower in n-3 FAs ($P = 0.010$), and the ratio of unsaturated to saturated FAs was lower and n-6 FAs to n-3 FAs was higher ($P < 0.05$ for both) than in normal weight women. Total fat content of the milk correlated positively to PUFA in the milk ($R = 0.21$, $P = 0.032$) and, especially, n-3 FAs ($R = 0.21$, $P = 0.038$). We further expanded the analysis to investigate whether these differences between overweight and normal weight women were seen after adjusting for current maternal diet. In regression analysis, overweight women had higher amount of SFA ($\beta = 2.9$, $P = 0.008$) and lower amount of PUFA ($\beta = -1.5$, $P = 0.04$), especially n-3 FAs ($\beta = -0.5$, $P = 0.008$), in their breast milk than normal weight women after adjusting for current maternal diet. As in the unadjusted analysis, no statistically significant associations between maternal overweight and breast milk MUFA ($\beta = -1.4$, $P = 0.09$), and n-6 FAs were seen ($\beta = -0.9$, $P = 0.1$).

We also studied with stepwise linear regression analysis whether maternal age, education, household incomes, or gestational weight gain would affect breast milk fatty acid composition. Gestational weight gain was only significant predictor of breast milk SFA concentration ($R^2 = 0.24$, $P = 0.025$), and for other factors, no significant associations were found. The same pattern was found with MUFA concentration wherein gestational weight gain was significant predictor ($R^2 = 0.23$, $P = 0.031$) but other variables were not. For total PUFA, n-6 or n-3 fatty acid no significant associations were found between maternal age, education, household incomes, or gestational weight gain. Furthermore, maternal weight gain during pregnancy or changes in weight from prepregnancy to 13 months postpartum were

Table 1 Maternal, child and clinical characteristics

Maternal characteristics	Overweight women ($n = 51$)	Normal weight women ($n = 49$)	$P^{\dagger}$
Age	31.0 (5.0)	29.7 (3.6)	0.43
Prepregnancy BMI (kg/m^2)	29.7 (3.3)	20.9 (2.1)	**<0.0001**
Gestational weight gain (kg)	11.8 (5.8)	14.0 (3.9)	**0.037**
BMI at 13 months postpartum (kg/m^2)	30.6 (5.0)	21.8 (2.6)	**<0.0001**
Mean change in weight from prepregnancy to 13 months postpartum (kg)	+2.3 (6.7)	+1.5 (4.0)	0.56
Child			
Birth characteristics			
Gender (male/female)	27/24	24/25	0.31
Birth weight (g)	3641 (535)	3410 (450)	**0.024**
Birth length (cm)	51.1 (2.3)	50.4 (2.0)	0.12
Birth BMI (kg/m^2)	14.0 (1.2)	13.5 (1.3)	**0.037**
Head circumference (cm)	35.3 (1.5)	34.5 (1.3)	**0.015**
Duration of gestation (wks)	39.5 (1.9)	39.7 (1.6)	0.73
13 months characteristics			
Weight (kg)	10.7(1.1)	10.0 (1.1)	**0.003**
Length (cm)	77.8 (2.8)	77.3 (2.6)	0.39
BMI (kg/m^2)	17.7 (1.1)	16.7 (1.1)	**<0.0001**
Weight gain from 0 to 13 months (kg)	7.1 (0.9)	6.5 (1.0)	**0.010**
BMI gain from 0 to 13 months (kg/m^2)	3.8 (1.2)	3.2 (1.5)	0.052
Total cholesterol concentration (mmol/l)	4.12 (1.38)	3.77 (0.71)	0.30
HDL cholesterol concentration (mmol/l)	1.12 (0.20)	1.26 (0.20)	**0.013**
HDL to total cholesterol ratio	0.29 (0.06)	0.34 (0.06)	**0.002**

P values that are statistically significant ($P<0.05$) are in bold

Values are mean (SD) unless otherwise stated

† *P* value for comparison between overweight and normal weight women. Statistical test used: *T* test for independent samples

Table 2 Breast milk fatty acid composition in overweight and normal weight women

	Overweight women ($n = 51$)	Normal weight women ($n = 49$)	$P^{\dagger}$
Total fat (mg/ml)	31.3 (15.7)	30.0 (10.8)	0.63
Saturated fatty acids (SFA%)	46.3 (4.4)	43.6 (6.0)	**0.012**
Monounsaturated fatty acids (MUFA%)	38.8 (3.6)	40.1 (4.0)	0.080
Polyunsaturated fatty acids (PUFA%)	13.6 (2.7)	14.8 (4.0)	0.068
n-6 PUFA (%)	11.4 (2.1)	12.1 (3.4)	0.18
18:2 n-6 (%)	10.5 (2.1)	11.1 (3.2)	0.22
18:3 n-6 (%)	0.07 (0.06)	0.09 (0.06)	0.087
20:2 n-6 (%)	0.20 (0.09)	0.22 (0.08)	0.18
20:3 n-6 (%)	0.28 (0.09)	0.30 (0.10)	0.50
20:4 n-6 (%)	0.37 (0.06)	0.39 (0.09)	0.14
n-3 PUFA (%)	2.2 (0.79)	2.7 (1.1)	**0.010**
18:3 n-3 (%)	1.9 (0.7)	2.0 (0.7)	0.19
20:3 n-3 (%)	0.024 (0.044)	0.024 (0.046)	0.96
20:5 n-3 (%)	0.10 (0.13)	0.18 (0.22)	**0.028**
22:6 n-3 (%)	0.22 (0.29)	0.46 (0.54)	**0.008**
trans fatty acids (%)	0.31 (0.18)	0.32 (0.12)	0.69
Unsaturated/saturated fatty acid ratio	1.1 (0.2)	1.3 (0.4)	**0.008**
MUFA/SFA ratio	0.85 (0.15)	0.95 (0.22)	**0.011**
PUFA/SFA ratio	0.30 (0.08)	0.36 (0.16)	**0.022**
PUFA/MUFA ratio	0.35 (0.08)	0.37 (0.10)	0.31
n-6/n-3 ratio	5.7 (1.8)	4.9 (1.6)	**0.031**

P values that are statistically significant ($P<0.05$) are in bold

Values presented as percentages from all fatty acids (mean (SD)) unless otherwise stated

† Statistical test used: *T* test for independent samples

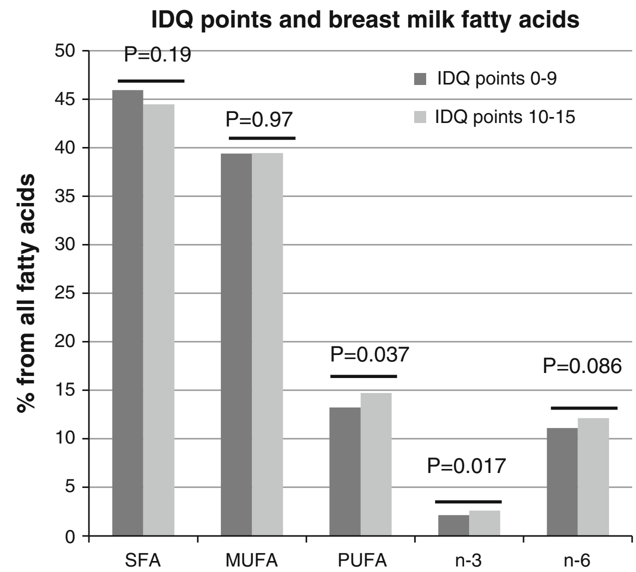

Fig. 1 Index of Diet Quality (IDQ) points during pregnancy and fatty acid composition in breast milk of lactating women ($n = 100$). Higher IDQ points (10–15) indicate better adherence to nutrition recommendations whereas lower points (0–9) indicate poorer adherence

not associated with the amount of fat in the breast milk (data not shown). Children's gender had no effect on the fat content or FA composition of the breast milk.

Maternal dietary intake during pregnancy and during sampling and their association on breast milk fatty acid profile

At third trimester of pregnancy, normal weight women followed a health-promoting diet more often than overweight women, since 75 % of normal weight women and 55 % of overweight women had IDQ points 10 or more ($P = 0.037$ between groups). Higher IDQ points during pregnancy correlated positively with PUFA levels in the breast milk ($R = 0.25$, $P = 0.012$) but not with those of SFA ($R = -0.145$, $P = 0.15$), MUFA ($R = -0.05$, $P = 0.96$), or total fat content ($R = -0.177$, $P = 0.078$). Results are illustrated in Fig. 1.

During the week of sampling, 28 % of all women had consumed fatty fish and 10 % low-fat fish during the last 2 days before sampling. Sixty-six percent of the women used a vegetable-oil-based spread, but more than a half of them used the low-fat versions (40 % or less fat). Seventy percent of the women used vegetable oils in cooking. There were no statistically significant differences between overweight and normal weight women in their dietary intakes (data not shown).

The use of fatty fish within the last 2 days before the sampling day associated with a 34 % increase in n-3 FAs in breast milk ($P = 0.007$). Breast milk PUFA was higher in women consuming vegetable-oil-based spreads than in the other women (15.1 vs. 12.8 %, respectively, $P = 0.001$), and especially, the n-6 FA contents were higher in the breast milk of vegetable-oil-based-spread users (10.5 vs. 12.5 %, $P < 0.0001$). The use of high-fat dairy products correlated with the SFA levels in breast milk ($R = 0.21$, $P = 0.04$), while no significant correlations were found for other foods. The use of vegetable oils, butter, cheese, pastries, salad dressing, sausages, and fast food did not correlate with breast milk FA distribution.

Twenty-two percent of lactating women used fish-oil or other FA supplements and 20 % of them had consumed these on the same day or previous day of the sampling. Women consuming FA supplements had higher levels of PUFA (15.2 vs. 13.7 %, $P = 0.044$) especially n-3 FAs (2.8 vs. 2.3 %, $P = 0.030$) in breast milk than the women not consuming FA supplements. No differences were seen in other FAs. Also, the use of fish, especially fatty fish, was more frequent among the women using FA supplements.

Growth and blood cholesterol concentrations of the children

The child's weight and increase in BMI from birth to 13 months correlated positively with higher breast milk SFA (Table 3). In addition, the ratio of unsaturated to saturated FAs and the ratio of MUFA to SFA correlated negatively with child's weight gain and increase in BMI from birth to 13 months. Linear regression analysis adjusted for duration of exclusive breastfeeding was used to study the association between breast milk FA composition on the child's weight, height, BMI, weight gain, and change in BMI from birth to 13 months, but no statistically significant associations were found between children's growth and breast milk FA composition (data not shown). No significant correlations or associations in linear regression analysis were found between breast milk FA composition and the child's total cholesterol and HDL cholesterol concentrations (data not shown).

Discussion

In this study, we found differences in breast milk FA composition between overweight and normal weight women. We found that overweight women had a significantly higher amount of total SFA and lower amount of n-3 FAs, especially 20:5 n-3 and 22:6 n-3, in their breast milk than normal weight women. In addition, the ratio of unsaturated to saturated FAs was significantly lower and the ratio of n-6 to n-3 was higher in overweight women than in normal weight women. These differences in FA composition between overweight and normal weight women remained even after adjusting the analysis for current maternal diet. Therefore, our results suggest that normal weight women have more essential FAs and other unsaturated FAs in their breast milk than overweight women.

Previously Marin et al. [31] reported an increase in total fat content and PUFA concentration in obese women. However, these conflicting findings may be simply due to the fact that in vulnerable population higher BMI may actually be a sign of higher accessibility to foods containing polyunsaturated fatty acids. In addition, the increase in total fat content seen in Marin's study but not in ours may also have an impact on increasing PUFA levels. In addition, the sampling was different since Marin and coworkers collected the samples at infant's age of 1–3 months whereas in our study, all samples were collected at infant's age of 3 months and sample sizes ($n = 100$ in our study, $n = 46$ in Marin's study) are different. Thus, comparisons against these two studies are highly difficult. Also a previous study has reported no association between maternal BMI and breast milk FA composition [25]; however, it was not made explicit whether the mothers were overweight or normal weight and, thus, those results cannot be directly compared to ours. The mean total fat content of the breast milk in our study was 3.1 %, which is in accordance with previous results (fat 3.22 ± 1 %) from banked donor milk analysis in the USA [32].

During the week before sampling, there were no differences between overweight and normal weight women in

Table 3 Correlations between breast milk fatty acids and children's weight, height and BMI at 13 months, and weight gain and BMI gain from birth to 13 months

Breast milk fatty acid	Weight at 13 months ($n = 88$)		Height at 13 months ($n = 88$)		BMI at 13 months ($n = 88$)		Weight gain ($n = 86$)		BMI gain ($n = 85$)	
	$R^†$	P	$R^†$	P	$R^†$	P	$R^†$	P	$R^†$	P
Total fat	0.001	0.992	−0.065	0.544	0.058	0.594	−0.047	0.666	−0.056	0.612
SFA	0.174	0.105	0.068	0.530	0.204	0.056	**0.219**	**0.043**	**0.222**	**0.041**
MUFA	−0.109	0.310	−0.005	0.963	−0.165	0.124	−0.190	0.080	−0.184	0.093
PUFA	−0.098	0.365	−0.139	0.195	−0.121	0.261	−0.124	0.257	−0.144	0.187
n-3 PUFA	−0.138	0.244	−0.126	0.200	−0.098	0.362	−0.170	0.117	−0.104	0.344
n-6 PUFA	−0.121	0.261	−0.075	0.489	−0.113	0.296	−0.090	0.410	−0.138	0.209
n-6/n-3 -ratio	0.037	0.731	0.074	0.495	−0.002	0.982	0.067	0.540	−0.020	0.858
unsaturated/saturated fatty acid ratio	−0.184	0.085	−0.079	0.463	**−0.210**	**0.050**	**−0.225**	**0.038**	**−0.242**	**0.026**
MUFA/SFA ratio	−0.175	0.103	−0.060	0.581	**−0.214**	**0.046**	**−0.239**	**0.027**	**−0.244**	**0.025**
PUFA/SFA ratio	−0.166	0.121	−0.093	0.387	−0.168	0.118	−0.166	0.126	−0.199	0.068
PUFA/MUFA ratio	−0.086	0.424	−0.091	0.398	−0.045	0.678	−0.036	0.739	−0.056	0.612

P values that are statistically significant ($P<0.05$) are in bold

† Pearson's correlation coefficient

their dietary intake of FAs measured with the food frequency questionnaire. Nevertheless, there were differences in milk FA compositions and it has, indeed, been shown that long-term diet and diet during pregnancy affect breast milk FA composition diet [12, 13]. The IDQ conducted at the third trimester of pregnancy revealed differences in adherence to dietary recommendations between overweight and normal weight women. Higher IDQ points correlated positively with breast milk PUFA, but not with SFA, MUFA, or total fat, and increased breast milk total fat was linked to increasing proportion of PUFA in the milk. Based on these results, maternal diet during pregnancy may influence PUFA concentration rather than MUFA or SFA, a phenomenon that was to some extent seen also in a previous study [31]. Some dietary factors were found to affect breast milk FA composition: PUFA concentration, especially n-6 FAs, was increased in women consuming vegetable-oil-based spreads and the use of fatty fish increased breast milk n-3 FAs. The women consuming FA supplements had higher levels of PUFA, especially n-3 FAs, in breast milk than women not consuming FA supplements. Interestingly, the use of fish was also more frequent. The difference in PUFA and n-3 concentrations may thus be related to increased use of fish, FA supplements, or both. Maternal fish-oil supplementation during pregnancy has previously been shown to be positively associated with increased PUFA in the milk and with infant erythrocytes DHA status at age of 1 year [19]. However, the differences in FA composition between overweight and normal weight women remained after adjusting regression analysis for maternal diet, thus suggesting a possible link between maternal overweight and breast milk FA composition.

Weak associations were detected between breast milk FA composition and the child's growth from birth to 13 months of age, but these effects of FA composition on growth were diminished after adjusting for total duration of breast feeding. These results are in accordance with previous research [25] and meta-analyses [22–24] in which no associations between n-3 and n-6 LCPUFA and weight and length gain in term infants were found. On the other hand, some experimental studies suggest that arachidonic acid (20:4 n-6, AA), the main n-6 LCPUFA, may promote adipose tissue development whereas n-3 LCPUFA could reduce an excessive increase in adipose tissue [33]. The content of n-6 PUFAs in breast milk of US women has increased steadily from 6–7 to 15–16 % of total FAs between 1945 and 1995 whereas the main n-3, α-linolenic acid (18:3 n-3), has remained unchanged at approximately 1 % [32]. Thus, the ratio of n-6 to n-3 seems to have progressively increased during these years whereas at the same time, childhood obesity has become an epidemic. Whether these two phenomenons are linked together remains unknown. In our study, the ratio of n-6 to n-3 in the breast milk had no association with children's growth. However, we expanded the analysis to other FAs and discovered that a higher content of SFA in milk correlated with higher weight gain in children from birth to 13 months. To our knowledge, no previous studies have reported this phenomenon. However, we do not have data on total energy intake and our sample size, and thus, the power of analysis is relatively small for analyzing early growth that depends on many factors beyond those investigated here. Therefore, further studies with larger number of subjects are needed to investigate this interesting hypothesis.

In a previous study by Powe et al. [34], infant gender was found to influence the energy content of the breast milk in a way that male gender associated with higher breast milk energy content. Infant gender had no effect on breast milk fat content or FA distribution in our current study. Instead, the association may be partially related to an increase in breast milk carbohydrate or protein content. In these exclusively breastfed children, no associations between FA composition of breast milk and children's cholesterol concentration were found even though breastfeeding may first raise the cholesterol levels of the infant and later in life be associated with lower total blood cholesterol levels [35, 36].

No association was found between maternal weight gain during pregnancy and the amount of fat in her breast milk nor did mother's weight from prepregnancy to 13 months postpartum associate with the amount of fat in the breast milk. Weight gain during pregnancy is known to be associated with prepregnancy BMI and in concordance to previous studies [37] normal weight women gained more weight (mean 14.0 kg) during pregnancy than overweight women (mean 11.8 kg). However, US Institute of Medicine (IOM) recommends higher weight gain for normal weight women, and the weight gain in both groups was on average within recommendations.

Our study has certain strengths as well as some limitations. Regarding the former, the weights and lengths of the children and mothers were measured by health care professionals, which prevented possible under-reporting or over-reporting. A limitation is that children's weight and length were collected only at birth and at 13 months of age and cholesterol concentration measured only at 13 months of age. Since we have no data on food intake of the children after the exclusive breastfeeding had ceased, the aspect of growth and cholesterol concentrations at 13 months of age requires further studies with more detailed data on food intake.

The study protocol was the same for all children irrespectively of their mothers' weight status. All breast milk samples were collected at the same age of the children, and the sampling was standardized. Sampling was designed to be as convenient as possible for the subjects. However, sample collection was done by mothers themselves, and possible differences in manual expression may result in some differences in total fat amount since the amount of fat in milk increases from for- to hind-milk. Since mean fat content was similar between overweight and normal weight women, the sample collection can be considered as valid in this aspect. FAs were analyzed with GC using both internal and external standards, which is considered an accurate method for FA composition measurements [13].

In our study, breastfeeding continued on average exclusively for 4 months, partially over 8 months, and 14 % of the subjects continued breastfeeding at 13 months of child age, which is in accordance with previously reported results in Finland [38]. In Finland, 76 % of children are breastfed at the age of 3 months [38], and in our sample, 77 % of children were breastfed at that age. Therefore, this sample can be considered as representative at least in aspect of breastfeeding duration. This study focused on children's FA intakes via breast milk alone and did not take other sources of FA intake into account. Previously, however, it has been hypothesized that other foods than breast milk make up only a small part of the dietary FAs during infancy [25]. Only one breast milk sample was collected, which is a limitation of the study. However, breast milk reflects the long-term dietary intake of FAs by the mother [12, 13] more than current diet [14], and thus, the FA composition may reflect longer time period. The recommended method for assessment of dietary intake, the 7-day food records [39], was not feasible method for this study; instead, we used IDQ that was previously validated with 7-day food records [27] and a food—frequency questionnaire.

In conclusion, the breast milk FA composition of overweight women differed from that of normal weight women by a higher level of SFA and lower level of n-3 PUFA as well as by a lower unsaturated to saturated FAs ratio, and these differences were seen even after adjusting for maternal diet. Higher SFA in breast milk associated with more rapid weight gain from birth to 13 months of age. Even though the effect on weight gain was small and diminished after adjusting for total duration of breastfeeding, it may have an impact on children's later health, especially if breast milk contained only small amounts of essential FAs. Further studies are needed to investigate whether there are long-term effects on growth or cholesterol concentrations of the children. In our study, dietary quality during pregnancy was associated with breast milk PUFA concentration, but further studies are needed to address whether maternal diet during pregnancy affects children's health through breast milk fatty acid composition.

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Conflict of interest The authors declare no conflict of interest.

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