



Effects of Maternal Exclusion Diet for Infants Suspected Food Allergy on Fatty Acid Composition in Breast Milk

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Abstract

Background: Levels of fatty acid (FA) in breast milk (BM) may vary depending on the maternal diet. This study aimed to explore FA composition in BM of lactating women following dietary restrictions due to infant allergic conditions.

Materials and Methods: Thai lactating mothers of term infants who were on exclusion diets were recruited. Mature BM was collected before and after a period (at least 2 weeks) of dietary restriction. FA in BM was analyzed by gas chromatography-mass spectrometry.

Results: Fifty lactating women 33.7 ± 3.6 years of age were enrolled. Thirty-three percent of the lactating mothers restricted more than eight food items. Most common dietary restriction were cow's milk (88%) and eggs (74%). After the period of dietary exclusion, total polyunsaturated FA showed no significant change, while saturated FA (SFA) declined, and monounsaturated FA (MUFA) increased. A decrease in fat intake was associated with an increase in arachidonic acid (ARA) and docosahexaenoic acid (DHA) content in BM ($r = -0.37$, $r = -0.36$; $p < 0.05$). However, a rise in ARA, eicosapentaenoic acid (EPA), and DHA intake was associated with an increase in linoleic acid and EPA in BM, respectively ($r = 0.38$, $r = 0.55$ and $r = 0.41$; $p < 0.05$). Infant weight-for-age z-score did not significantly change after the period of maternal dietary exclusion.

Conclusion: Maternal exclusion diet resulted in lower SFA and higher MUFA composition in BM. Further study should explore the long-term outcomes of maternal dietary restriction on infant and child health.

Keywords: maternal diet, exclusion diet, dietary restriction, fatty acid composition, breast milk, allergen

Introduction

BREAST MILK (BM) is considered the gold standard of infant feeding since it consists of nutrients and bioactive factors that support optimal infant growth and is correlated to superior health outcomes.^{1,2} Lipids in BM provide half the infant energy needs as well as essential fatty acids (FA)

for neonates. The lipid component of BM, especially long-chain polyunsaturated fatty acids (LC-PUFA), has been identified as a key nutrient for infant cognitive development. A recent systematic review revealed that BM intake and LC-PUFA supplementation may improve structural brain development and function in both preterm and term infants.³

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LC-PUFA are FA with 14 or more carbons and more than 1 double bond in their molecular structure. LC-PUFA can be classified into two groups: omega-3 and omega-6. Main omega-3 LC-PUFA components are α -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Primary components of omega-6 LC-PUFA include arachidonic acid (ARA) and linoleic acid (LA).⁴ Different LC-PUFA have their specific biological functions. LA is an essential structural component of certain dermal ceramides with a critical role in the maintenance of the epidermal water barrier. DHA is an important structural component of cell membranes and develops a marked perinatal accumulation in membrane-rich tissues such as the retina and the brain. ARA presents in large amounts in the nervous tissue and serves as a precursor to prostaglandin formation.^{5,6}

After birth, a breastfed infant receives LC-PUFA directly from BM. However, FA composition in BM may be altered by several factors such as genetic factors, gestational age, gestational weight gain, maternal nutritional status, stage of lactation, and especially maternal diet.⁷⁻⁹ Previous studies found that human milk FA varied widely depending on maternal lipid nutrition.^{10,11} Lactating women who consume a more varied diet such as fatty fish, meat, fresh seafood, and eggs had higher DHA in their BM.¹² Monounsaturated FA (MUFA) tended to be lower in sub-Saharan African samples compared to European milk samples. This may reflect a lower intake of MUFA from animal fats and olive or rapeseed oils by sub-Saharan African mothers.¹¹

Prevalence of food allergies in children has witnessed significant increases in the last few decades, both in westernized countries and developing countries including Thailand.^{13,14} Previous studies revealed that between 6% and 8% of Thai children were reported to have a food reaction experience. An elimination diet of the causative food allergen(s) is the mainstay of treatment for food allergies. However, most common food allergens such as cow milk, eggs, wheat, soy, peanut, tree nuts, fish, and shellfish are foods that comprise a major portion of essential nutrients in BM, especially in fat content. Lactating mothers who undertake dietary restrictions due to their infants' allergic conditions without appropriate substitution may experience nutritional deficiencies that could compromise their BM composition, which, in turn, may affect infant growth and neurodevelopmental outcomes.¹⁵

Currently, the data on FA composition in lactating mothers consuming exclusion diets are scarce. This study aimed to investigate the effect of maternal exclusion diets on the FA content of mature BM in a sample of Thai women. Findings can contribute to a better understanding of the effect of diet restriction on FA changes in BM and help raise clinical awareness among health care professionals about the need to provide appropriate dietary recommendations for lactating mothers.

Materials and Methods

Subjects

An observational study of healthy Thai lactating mothers was performed between November 2014 and May 2016. Lactating mothers of term infants who were on exclusion diets were recruited from the pediatric allergy clinic at King Chulalongkorn Memorial Hospital in Bangkok, Thailand.

Mothers who had any medical illness that may affect BM composition were excluded from this study. The sample size was calculated based on a previous study,¹⁶ which reported that the LA levels in the BM of mothers who consumed a low-fat diet and high-fat diet were 14.65 ± 2.32 and 13.82 ± 1.68 g%, respectively. Calculations recommended a sample size of 46 subjects to detect a similar magnitude of difference. The study protocol was ethically approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (IRB No. 027/56).

Data collection

Maternal demographic data, including age, family income, living region, details of lactation and dietary restriction, weight, and height were obtained by an interview. Body mass index (BMI) was computed by weight (kg)/height² (m) and used to classify maternal nutritional status according to the World Health Organization (WHO) recommendation for the BMI cutoff point for determining nutritional status in the Asian population.¹⁷ Information on pregnancy outcomes, gestation, infant birth weight, and weight before and after diet exclusion was also collected from baby vaccination record books. Infant weight was converted to a weight-for-age z-score according to the WHO child growth standards for birth to 5 years by using the WHO Anthro software.

Maternal dietary intake was assessed by a 24-hour dietary recall before and after a period of the elimination diet (at least 2 weeks apart). To ensure completeness and accuracy of dietary records, all participants were interviewed by the same researcher at each time of data recording. The dietary recall information was analyzed for energy and nutrients by using the INMUCAL[®] version 3 developed by the Institute of Nutrition, Mahidol University, Thailand.

Each mother was asked to express mature BM before and after the period of the exclusion diet. The breast was cleaned with cooled boiled water before BM collection. Each sample of ~60 mL was collected into a clean container and immediately placed into a household freezer until the researcher collected them. Milk samples were kept cold during transport and refrozen in a -20°C freezer until analysis.

FA analysis

The FA composition was determined by gas chromatography-mass spectrometry (GC/MS-MS) at the Halal Science Center Chulalongkorn University. A volume of 200 μL of human milk was loaded to Teflon-lined screw-cap test tubes. Next, 2 mL of methanol-hexane (4:1, v/v) was added to the milk sample and mixed in vortex vigorously. An amount of 0.2 mL of acetyl chloride was then added to the stirring tube over 1 minute. The tube was tightly closed with a Teflon-lined cap and subjected to methanolysis at 100°C for 1 hour. After the tube had been cooled at room temperature, 5 mL of 6% K_2CO_3 solution was slowly added to stop the reaction and to neutralize the mixture. The tube was then shaken vigorously with gravitational force 250 g resulting in a separate supernatant containing FA methyl esters, which were analyzed by using GC (Trace GC 2000; Thermo Finnigan).

The complete details of this method are available from a previous study.¹⁸ The emergent peaks were identified by

comparing their retention time with those of standard FA methyl esters. The relative proportion of FA was derived from the area under each peak divided by the total area of all FA that appeared in the chromatogram. The values were expressed as a percentage of total FA.¹⁹

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 22 (IBM Corp., Armonk, NY). Continuous variables, including age, weight, BMI, duration of lactation, and dietary restriction, and dietary intake, were summarized using mean $\pm$ standard deviation or median (interquartile range, IQR 1–3), as appropriate. Categorical data, including parity, family income, and number of dietary restrictions were presented as frequency and percentage. Wilcoxon signed-rank test was selected to compare the maternal dietary intake and FA composition of BM before and after the period of the exclusion diet, as well as to compare infant weight-for-age z-score before and after maternal dietary restrictions. Pearson correlation was used to assess the correlation between FA changes in BM and maternal diet composition before and after dietary restrictions. A $p < 0.05$ was considered statistically significant.

Results

Clinical characteristics and maternal dietary intake

Table 1 shows the clinical characteristics of the study participants. Fifty lactating women 33.7 ± 3.6 years of age were enrolled. Nearly all lived in the greater Bangkok area (96%) and were from intermediate to high socioeconomic status (76%). Around one-third were overweight and obese. Seventy percent of the subjects were primiparous and gestational weight gain was 14.7 ± 3.8 kg. All infants were born at term with an average birth weight of 3.24 ± 0.35 kg.

The mean duration of lactation was 4.2 ± 3.7 months (range from 0.5 to 17 months). The average duration of dietary restriction was 2.2 ± 1.9 months. Most of them restricted their dietary intake of cow's milk (88%) and eggs (74%) and 16 mothers (32%) restricted more than 8 food items.

The comparison of maternal energy and nutrient intake before and after diet exclusion is presented in Table 2. Before the dietary exclusion, energy intake ranged from 1,179 to 2,987 kcal/day with a median of 1,889 (IQR 1,674–2,260) kcal/day. The caloric distribution of carbohydrates, protein, and fat was 38%, 17%, and 45% of total calorie intake, respectively. After the exclusion diet period, the median energy intake was 1,786 (1,758–2,108) kcal/day with no significant change in terms of caloric distribution. The median energy and macronutrient intake were not significantly different between both periods. There was no statistically significant difference in the maternal FA intake before and after dietary restrictions.

FA composition in BM

The comparison of FA in BM before and after the dietary restriction is shown in Figure 1. After the restriction period, total saturated FA (SFA) was significantly lower than before the dietary restriction (34.82 [IQR 32.27–36.83] versus 36.98 [IQR 34.40–40.47] g/100 g of total FA, $p = 0.001$). Myristic acid (C14:0) and palmitic acid (C16:0), which are parts of SFA, also significantly decreased (Fig. 1c, d). In contrast,

TABLE 1. CLINICAL CHARACTERISTICS OF LACTATING MOTHERS WHO WERE ON EXCLUSION DIET (N=50)

Variables	Mean (SD)/n (%)
Maternal variables	
Age (years)	33.7 (3.6)
Pregestational BMI (kg/m ²)	20.8 (4.2)
Postpartum BMI (kg/m ²)	26.6 (4.0)
Gestational weight gain (kg)	14.7 (3.8)
Parity	
Primipara	35 (70)
Multipara	15 (30)
Nutritional status	
Underweight	2 (4)
Normal weight	32 (64)
Overweight	13 (26)
Obesity	3 (6)
Duration of lactation (months)	4.2 (3.7)
Duration of dietary restriction (months)	2.2 (1.9)
Living region	
Bangkok and surrounding areas	48 (96)
East	1 (2)
Northeast	1 (2)
Family income	
15,000–50,000 bath/month	11 (22)
50,001–100,000 bath/month	16 (32)
>100,000 bath/month	23 (46)
No. of dietary restrictions	
1 Item	13 (26)
2–4 Items	10 (20)
5–7 Items	11 (22)
≥8 Items	16 (32)
Dietary restriction	
Cow's milk and dairy products	44 (88)
Egg	37 (74)
Wheat	32 (64)
Soy	29 (58)
Seafood	27 (54)
Peanut	25 (50)
Marine fish	22 (44)
Freshwater fish	16 (32)
Others	13 (26)

BMI, body mass index; SD, standard deviation.

total MUFA and myristoleic acid (C14:1) notably increased (MUFA: 45.41 [43.11–46.97] versus 42.74 [39.95–45.00] and myristoleic acid: 0.07 [0.04–2.92] versus 0.04 [0.00–0.06] g/100 g of total FA, $p = 0.001$) (Fig. 1a, c). However, no significant difference in the total amount of polyunsaturated fatty acid (PUFA), LA, ALA, ARA, EPA, DHA, and other types of FA in BM was found between these two periods (Fig. 1a, b, d).

The correlations between the changes of BM FA composition and maternal diet before and after diet restriction are presented in Table 3. The decrease in total fat intake was correlated with the increase in ARA ($r = -0.37$, $p = 0.04$). Similarly, the reduction in the fat-to-energy ratio was correlated with the increase in DHA content in BM ($r = -0.36$; $p = 0.03$). In contrast, the decrease in ARA intake was associated with the decline of LA in BM ($r = 0.38$, $p = 0.03$). In addition, the reduction in EPA and DHA intake was associated with the decline of EPA in BM ($r = 0.55$ and $r = 0.41$; $p = 0.04$, respectively). Also, subgroup analysis showed that

TABLE 2. COMPARISON OF MATERNAL DIETARY INTAKE BEFORE AND AFTER DIET RESTRICTION (N=50)

	Before restriction (n=50) median (IQR 1–3)	After restriction (n=50) median (IQR 1–3)	p ^a
Dietary intake			
Total energy intake (kcal/day)	1,889 (1,674–2,260)	1,729 (1,389–2,168)	0.12
Carbohydrate (g/day)	178.1 (133.6–212.8)	161.5 (115.9–188.1)	0.13
Protein (g/day)	83.6 (59.4–103.6)	79.7 (63.5–105.5)	0.91
Fat (g/day)	90.2 (69.9–106.2)	86.1 (69.6–100.8)	0.49
% Fat (% of total energy)	44.9 (37.2–49.6)	46.1 (38.7–50.9)	0.93
Fatty acid intake (g/day)			
Total SFA	34.3 (22.6–41.0)	28.0 (20.7–35.2)	0.15
Total MUFA	35.7 (23.8–45.3)	35.8 (28.0–39.5)	0.28
Total PUFA	19.0 (11.6–32.5)	15.6 (11.9–25.2)	0.37
LA	17.2 (10.7–30.2)	14.2 (10.4–23.4)	0.37
ALA	0.8 (0.5–1.3)	0.7 (0.5–1.1)	0.31
ARA	0.29 (0.16–0.38)	0.20 (0.15–0.32)	0.52
EPA	0.06 (0.02–0.12)	0.09 (0.03–0.16)	0.20
DHA	0.27 (0.15–0.40)	0.18 (0.07–0.54)	0.39

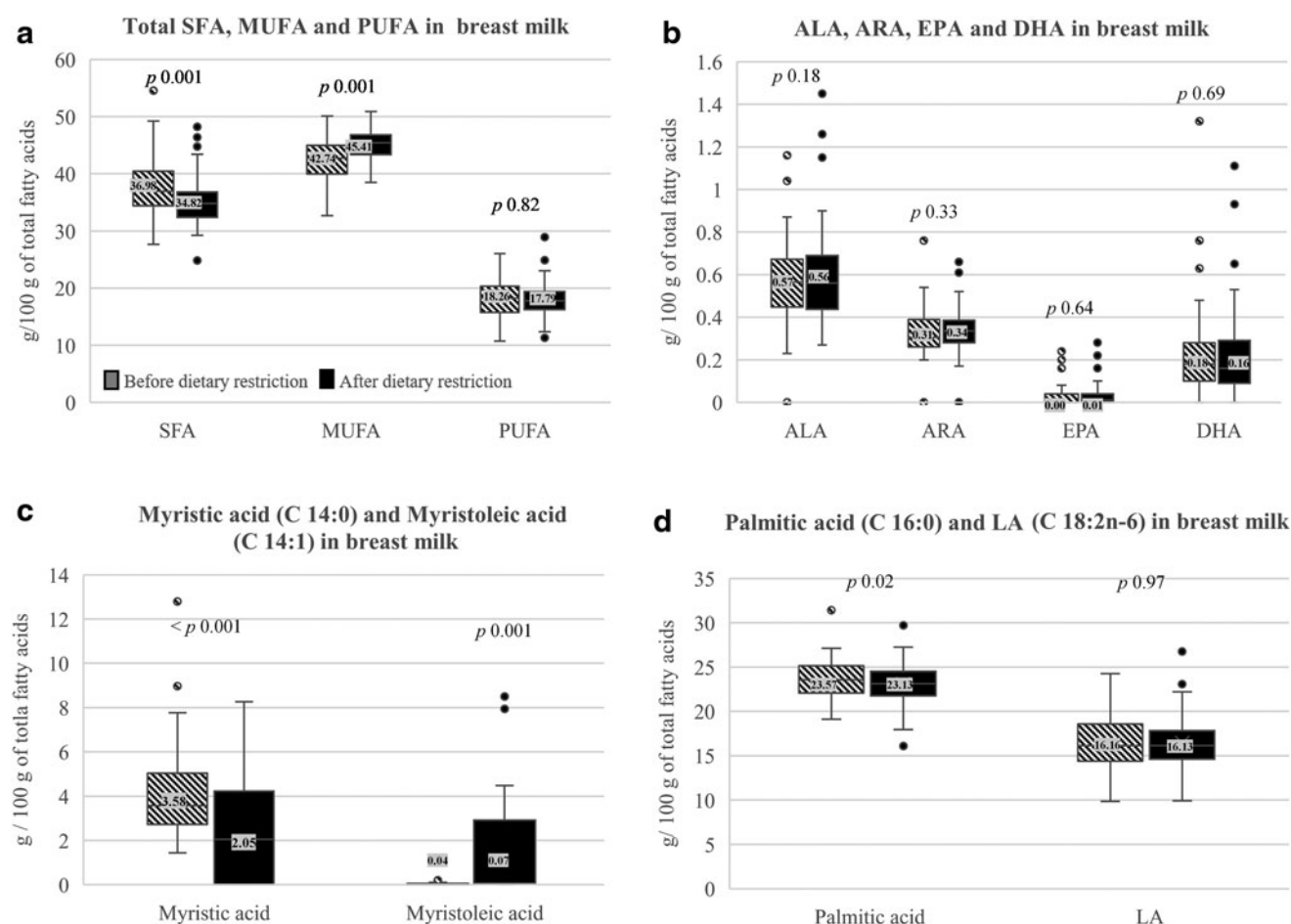
^aWilcoxon signed-rank test.ALA, α -linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IQR, interquartile range; LA, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

FIG. 1. Comparison of FA in breast milk before and after dietary restriction ($n=50$). **a, b, c, d** represent the comparison between various components of FA in breast milk before and after dietary restriction: **(a)** comparison of total SFA, MUFA, and PUFA; **(b)** comparison of ALA, ARA, EPA, and DHA; **(c)** comparison of myristic acid and myristoleic acid; **(d)** comparison of palmitic acid and LA. Data are presented as box plots. The median is presented by the line and number in the middle of the box, whereas the error bars demonstrate the interquartile range 1–3. Wilcoxon signed-rank test indicates that SFA and myristic acid decrease, but MUFA and myristoleic acid increase after the restriction period. ALA, α -linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

TABLE 3. CORRELATION COEFFICIENT BETWEEN THE CHANGES OF MATERNAL DIET AND FATTY ACID COMPOSITION IN BREAST MILK (N=50)

	<i>Changes of fatty acid composition in breast milk</i>				
	LA	ALA	ARA	EPA	DHA
Changes of macronutrient intake					
Energy (kcal/day)	0.11	0.23	-0.19	0.16	-0.22
Fat (g/day)	0.18	0.17	-0.37 ^a	-0.02	-0.26
Protein (g/day)	-0.10	-0.02	-0.01	0.27	0.16
% Fat (% of total energy)	0.07	-0.06	-0.30	-0.22	-0.36 ^a
Changes of fatty acid intake					
LA	0.24	0.25	-0.22	-0.10	-0.24
ALA	0.23	0.30	-0.16	-0.04	-0.28
ARA	0.38 ^a	0.06	-0.34	-0.23	-0.28
EPA	0.06	0.19	-0.25	0.55 ^b	0.17
DHA	0.22	0.20	-0.25	0.41 ^a	0.08

^aPearson correlation, *p*-value <0.05.

^bPearson correlation, *p*-value <0.01.

ALA, α -linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid.

the directions of the associations were similar in mothers who reported an increase or decrease in the proportion of dietary fat intake (data not shown).

Moreover, there was no difference found between the category of food restriction and FA composition. No difference in the FA contents of BM among mothers who restricted 1, 2–4, 5–7, and ≥ 8 food items were found.

Infant outcomes after dietary restriction

All infants were born full term with average birth weight 3.14 ± 0.35 kg. The mean weight-for-age z-score of infants before exclusion diet was -0.9 ± 1.5 . In addition, around one-fifth of the infants were underweight according to the WHO classification of nutritional status. After the restriction period, the weight-for-age z-score of the infant was -0.5 ± 1.2 . There was no significant difference in the weight-for-age z-score of infants after maternal dietary restrictions (mean difference 0.46; 95% confidence interval -0.10 to 0.43 , $p=0.06$). In addition, no correlation between the change in infant weight for age z-score and the change in the amount of FA intake, the change in FA in BM, or the duration of dietary restrictions was found in this study (data not shown).

Discussion

Our study demonstrated the effect of maternal exclusion diets on FA composition in the BM of Thai lactating women undergoing an elimination diet due to their infants' food allergic conditions. After the restriction period, the total PUFA in BM showed no significant change, while total SFA declined and total MUFA raised. A decrease in fat intake was associated with an increase in ARA and DHA content in BM. In contrast, a decrease in ARA intake correlated with a decrease in LA, while a reduction in EPA and DHA intake was associated with a decline of EPA in BM.

In our study, despite no significant differences in maternal FA intake after dietary restriction, SFA and PUFA intake showed a decline. Total SFA in BM decreased significantly responding to the trend of the decrease in SFA intake, while total MUFA increased after the restriction period. However, no significant change in total PUFA in BM was found. This result is similar to evidence from previous studies that reported on an association between dietary intake and each type of FA in human milk.^{11,20,21} Although many studies report a positive association between maternal diet and FA in BM, some research has shown no association.²² A previous interventional study found no significant change in total SFA, MUFA, and PUFA concentrations in BM after mothers were put on either a low- or high-fat diet (17.6% and 40.3% fat of total energy).¹⁶

A study in five regions of China showed that maternal dietary FA was positively associated with SFA and PUFA, but negatively associated with MUFA in BM. In addition, a study from South Korea reported that the dietary intake of SFA and PUFA was positively correlated with FA in BM.²³ This divergence in study results might be due to the use of different techniques in the extraction of lipids and FA composition in BM and different assessment methods for maternal diet analysis. The other possible explanation might be a compensatory physiological mechanism that may buffer against variation in maternal dietary intake.

Physiologic upregulations and downregulations, including the transport of FA from plasma to mammary glands, the release of FA from lipoprotein, and milk triglyceride synthesis, were proposed as being responsible for controlling FA levels in BM.²⁴ The alternative explanation could be that the magnitude of changes in maternal dietary fat intake in this clinical scenario was not enough to override normal physiologic regulations. Moreover, we hypothesized that there may be some changes in the maternal diet, but the sample size is too small to show any statistical significance. However, it may be enough to cause the change in BM. Another explanation could be that the 24-hour dietary recall may not fully reflect the change in maternal FA intake after dietary exclusion.

LC-PUFA (LA, ALA, ARA, EPA, and DHA) intake and LC-PUFA presence in BM did not significantly differ before and after maternal dietary restrictions. These findings could be explained by the subtle change in FA composition in diet. Another possible explanation for the results may be the short duration of the exclusion diet. It is well established that both current diet and long-term dietary intake and maternal nutritional status can affect BM FA composition, especially DHA.²⁵ The previous randomized control trial was conducted among mothers who received fish oil supplementation, comparing with primrose oil supplementation for 15 days at gestational age 30 weeks.

The result found that lactating mothers who received fish oil supplementation during pregnancy had higher DHA and EPA level of BM than the group receiving primrose oil supplementation. Similarly, a recent systematic review found BM of women with excessive body weight before pregnancy had a higher omega-6/omega-3 ratio compared to normal-weight women.²⁶ We postulate that dietary intake during lactation may not be the sole determinant for the size of fat mass during lactation, which, in turn, affects FA composition in BM. Dietary

intake during pregnancy may be a better determinant of maternal fat mass during lactation, due to intense fat deposition during this period.²⁷

This study shows that the decrease in fat content and proportion in a maternal diet correlated with the increase in ARA and DHA, respectively, whereas the reduction of ARA, EPA, and DHA intake correlated with the decline of LA and EPA in BM. A previous study⁹ also showed a positive correlation between changes in EPA intake and EPA in BM ($r=0.55$, $p=0.002$), but no such correlation found for DHA. Jirapinyo et al.²⁸ also found no correlation between DHA intake and DHA in BM. No change in DHA may be explained by the compensatory physiologic mechanisms of lactation mentioned earlier. However, many studies have shown that BM DHA increased after consumption of fatty sea fish or fish oil supplementation.^{6,29} In such interventional studies, the magnitude of alteration of dietary DHA was much larger than in our study.

Although Thai lactating mothers in our study were on habitual diets before the dietary restriction period, the median energy intake was lower than the Thai dietary reference intake for lactating women.³⁰ The LC-PUFA, LA, ALA, and EPA intake were lower than the Thai dietary reference intake during both periods.^{31,32} The data demonstrated that energy intake, carbohydrate, protein, fat, and FA intakes of the mother's diet were not significantly different before and after the dietary restriction period. This might be explained by the intermediate to high socioeconomic status of these mothers, resulting in a more diverse substitution diet.

In our study, infant growth after the dietary restriction period did not seem to be affected, which could be due to the short follow-up period and the small number of participants. Currently, few studies have investigated the impact of maternal dietary avoidance during the lactating period on infant growth. Previous meta-analysis showed that maternal dietary antigen avoidance during pregnancy may be associated with lower infant birth weight.³³ Therefore, further large-scale longitudinal studies should be performed to explore the impact of a maternal exclusion diet during the lactating period on maternal nutritional status and infant growth.

This is the first study on the effect of a maternal exclusion diet on BM FA in Thailand. Since our matched sample methodology ensured that the BM samples were collected from the same mothers before and after dietary restriction, we can limit any confounding influence on the milk FA such as maternal genetics, parity, gestational age, stage of lactation, and pregnancy weight gain. Our study does have several limitations. First, BM samples were not collected at the same time of the day; thus, the FA results might be influenced by the diurnal variation. Second, 24-hour dietary recall by mothers might not be an adequate method to assess dietary FA intake.

Finally, due to the overrepresentation of intermediate to high socioeconomic status of the mothers, the results do not represent the overall population of lactating women in Thailand. Future studies should be performed using a food-frequency questionnaire to estimate more accurately FA intake and by collecting BM samples at the same time of the day.

Conclusions

This study demonstrated that a maternal exclusion diet affects the FA content in BM. The BM SFA was lower

and MUFA was higher after the maternal dietary restriction period. Results suggest that diagnostic testing for infant food allergy diseases should be performed to avoid unnecessary maternal exclusion diet. The average LC-PUFA contents of a majority of the mothers' diet were found to be lower than recommendations from the Thailand national dietary reference intake guidelines. Consumption of foods rich in LC-PUFA such as oily fish, nuts, seeds, and eggs should be encouraged for lactating women to address this deficiency.

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Authors' Contributions

T.F., O.S., and S.C. conceived and designed the study. T.F., P.H., P.C., and N.S. were involved in data collection. S.S., V.T., and W.D. assessed and analyzed the fatty acid composition in breast milk. T.F., S.S., V.T., and O.S. analyzed the data. T.F., S.C., and O.S. interpreted the data, revised the article, and approved the final draft.

Disclosure Statement

The authors declare that they have no conflicts of interest.

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