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Research Article

Fatty Acid Composition of Mature Breast Milk in Malay Women from Kuala Lumpur, Malaysia

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ABSTRACT

This study aimed to determine the Fatty Acid (FA) composition in human mature milk and the relationship between essential FA levels in breast milk and maternal dietary intake during postpartum period. This study enrolled seventeen exclusively breastfeeding women with full-term, disease-free babies (26–64 days). Milk samples were collected using Absorbent Spot Cards. Subsequently, gas chromatography was performed to analyze FA composition. The total FA constituted saturated fatty acids (40.56±2.62%), monounsaturated fatty acids (43.54±2.60%), and polyunsaturated fatty acids (14.01±1.34%). No relationship was observed between maternal dietary omega-3 FA intake and human milk omega-3 content. These findings suggest that humans have a reservoir to store FA for babies during pregnancy and that omega-3 content in human milk was not influenced by postpartum maternal diet. It highlights the importance of maternal diet during pregnancy for optimal fatty acid levels in breast milk.

INTRODUCTION

Human breast milk contains macronutrients, micronutrients, and fatty acids that are essential in the healthy development of newborns (Kim & Yi 2020). Human milk can be categorized into colostrum, transitional milk, and mature milk (Mosca & Gianni 2017), each differing in lipids and protein composition. Colostrum, produced immediately after the mother gives birth, has higher protein, but less fat, lactose, and total energy (Gidrewicz & Fenton 2014). Following the production of colostrum, the milk contents undergo transition and it becomes stable after 21 days postpartum. At this stage, the milk secreted is known as mature milk (Gidrewicz & Fenton 2014). Mature milk comprises 87% water, 3.8% fat, 1.0% protein, and 7% lactose (Guo 2014), with total energy 62–80 kcal/100 mL (Martin *et al.* 2016).

Essential Fatty Acids (EFAs), particularly omega-3 (Alpha-Linolenic Acid or ALA, Eicosapetaenoic Acid or PA, Docosahexaenoic Acid or DHA), and omega-6 (Linoleic Acid or LA) are vital components of human breast milk. Among these, DHA representing the major structural phospholipid in the brain grey matter is of paramount importance to ensure healthy brain and visual development (Echeverria *et al.* 2017). In recent years, DHA has been recognized as a crucial nutrient during pregnancy and lactation as it is actively involved in the nervous system development at an early age of life (Echeverria *et al.* 2017). Imbalance and insufficiency of DHA

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might impair normal visual development and lead to behavioral abnormality (Echeverria *et al.* 2017; Lauritzen *et al.* 2016; Schuchardt *et al.* 2010).

Human milk composition of total Polyunsaturated Fatty Acids (PUFAs), including omega-3 and omega-6 FAs exhibit significant variation across countries (Butts *et al.* 2018). DHA content in breast milk influenced by maternal diet. Lactating women who frequently eat fish have higher DHA in their breast milk compared to those who consume little or no fish (Aumeistere *et al.* 2018). In general, the average fish consumption among Malaysia's population is 168 g/day, but it varies by ethnicity. Malay population eats more fish than the Chinese and Indian community. Commonly eaten fish are Indian Mackerel, anchovy, tuna, and sardine (Ahmad *et al.* 2016). The average fish consumption among Malaysia's population is considered high as it is higher than the fish consumption of most of the countries and but only lower than Japanese and Korean. An earlier study by Kneebone *et al.* (1985) compared the fatty acid composition of fatty acids in breast milk across Malay, Chinese and Indian in Penang state. Shift in the dietary pattern in the community over the decades, reassessment of the dietary intake pattern among lactating mothers is necessary. Due to the uncertainty of the omega-3 food consumption pattern among lactating women, this study aimed to determine the relationship between fatty acid level in breast milk and maternal dietary intake of Malay women who exclusively breastfed babies aged 26 to 64 days in the capital of Malaysia, Kuala Lumpur.

METHODS

Design, location, and time

This cross-sectional study complied with the World Medical Association (Declaration of Helsinki) code of ethics and obtained research ethics approval from the Research and Ethics Committee, UKM (JEP-2019-839). Federal Territory of Kuala Lumpur was chosen as the study location. The sampling frame was breastfeeding mothers who live in Kuala Lumpur and Selangor. Researchers conducted home visits to administer interviews and collect milk samples from participating lactating women. The study was carried out from January 2020 to September 2020.

Sampling

This study used purposive sampling to select a specific population group with similar sociodemographic characteristics (ethnicity, household income) representative of Malaysia. The sample size was set at 25 subjects, based on Brenna *et al.* (2007) and aligned with common practices in human breast milk studies, which often use small sample sizes (<25). Additionally, due to financial constraints, the collaborating international researchers specified this sample size to optimize available resources. Eighteen Malay women who exclusively breastfeed and have full-term, disease-free babies aged 26 – 64 days were recruited. The age range was chosen based on the stability of breastmilk after 21 days postpartum (Martin *et al.* 2016). The study population was Malay ethnicity as this is the main ethnic group in Malaysia (MyGoverment 2016). Women who took essential fatty acid supplements when the research was conducted were excluded from this study. Informed consent from subjects was collected before the commencement of the research project.

Data collection

Sociodemographic status and omega-3 consumption data. Subjects' sociodemographic data comprising subjects' age, educational level, occupation, and household income were captured. Health status of lactating women and their breastfeeding practices were recorded. A Dietary History Questionnaire (DHQ) validated by Shahar *et al.* (2000) was used to capture maternal dietary patterns. To minimize recall bias, DHQ was administered as a structured and interviewer-guided questionnaire using non-leading and open-ended questions. Subjects required to recall all the food and beverages they had consumed in the past week. Subsequently, the interviewer probed further details including food type, portion size, cooking methods and home recipes. Household measurements such as spoon, cup, and glass were used to assist subjects in estimating the portion size correctly.

Frequency consumption of omega-3 polyunsaturated fatty acid by the subject was captured using omega-3 fatty acid Food Frequency Questionnaire (FFQ) which was developed and validated by Lee *et al.* (2013). Subjects were required to recall the frequency of the consumption of all food items that contained omega-3 fatty acids in the previous month. The average daily consumption of omega-3 fatty acids was analyzed based on the United States Department of Agriculture Nutrient Database (2007). The average daily Alpha-Linolenic Acid (ALA), Eicosapentaenoic Acid (EPA), and Docosahexaenoic Acid (DHA) were calculated using formulas below (Wu *et al.* 2017):

Mean ALA intake: ALA content x Frequency of consumption

Omega-3 PUFAs intake: ALA content + EPA content + DHA content

Total omega-3 PUFAs intake: Total content of ALA + Total content of EPA + Total content of DHA

Breast milk collection and fatty acid analysis. Less than 1 ml of maternal milk sample was needed in this study. Prior to milk collection, researchers verified key breastfeeding parameters, including the timing of last breastfeeding and side of the breast last used to feed the baby. Next, subject manually expresses the milk sample from the opposite breast that had not been used to feed the baby. The breast milk was first collected in a plastic cup, then transferred onto an adsorbent spot card via disposable pipette.

Absorbent Spot Cards (PerkinElmer 266 Bioanalysis RUO Collection Cards) were used to analyze the fatty acid profile in breast milk. The absorbent spot card was left and dry at room temperature for 45 to 60 minutes. It was placed into a zipper bag and stored in a freezer (-18°C) before being shipped to the laboratory Lipid Technologies LLC in Austin, Minnesota in the US for further analysis. The samples were analyzed using Gas Chromatography (PerkinElmer, US) (López-López *et al.* 2001).

Data analysis

Statistical Package for the Social Sciences Version 22.0 (SPSS version 22.0) was used to analyze the data. Shapiro-Wilk test was used to test for the normality of the data. In this study, 95% confidence interval was used, with a significant level of $p<0.05$. A descriptive test was used to analyze sociodemographic data (mean±SD, %), macronutrient and micronutrient intake, frequency of the consumption of foods containing omega-3 fatty acids as well as breast milk fatty acids profile. Maternal dietary intake was compared with Recommended Nutrients Intakes for Malaysia, RNI (NCCFN 2017). Pearson's Correlation was used to determine the relationship between maternal dietary intake and essential fatty acids profile in breast milk.

RESULTS AND DISCUSSION

Subjects' sociodemographic data

A total of 17 exclusively breastfeeding women participated in the study. Due to the COVID-19 pandemic, the researcher was unable to recruit the ideal number of subjects as planned (25 subjects). All participants recruited in this study were Malay women, with the majority aged between 30 to 39 years old (72.2%), 22.2% aged between 20 to 29 years old, and 5.6% aged 40 to 49 years old. All participants were in good health, without taking any medication. The details of the subjects' sociodemographic profiles are shown in Table 1.

Table 1. Subjects' socio-demographic characteristics

Characteristics	n (%) (n=17)
Age group	
20–29	4 (23.5)
30–39	12 (70.6)
40–49	1 (5.9)
Educational level	
Diploma	3 (17.6)
Bachelor's degree	13 (76.5)
Masters/PhD	1 (5.9)
Employment status	
Civil service	7 (41.1)
Private service	6 (35.3)
Self-employed	1(5.9)
Student	2 (11.8)
Unemployed	1 (5.9)
Household income	
<RM4,850	2 (11.8)
RM4,851–RM10,970	11 (64.7)
>RM10,970	4 (23.5)
Health status	
With medication	0 (0.0)
Without medication	17 (100)

RM: Ringgit Malaysia

Macronutrient and omega-3 fatty acids intake

The validity of the subjects' energy intake was determined based on the calculation of energy intake over basal metabolic rate (EI:BMR) ratio (Black 2000; Goldberg *et al.* 1991). Following the exclusion of over-reported data, 17 normal reported data were used to analyse the maternal macronutrient intake and omega-3 food intake. Overall, total energy intake of the subjects was 1,819±170.6 kcal, comprised of 50.5±6.7% carbohydrate, 30.1±5.7% fat and 19.5±3.9% protein, aligning with the Malaysian Recommended Nutrient Intake (RNI) guidelines (NCCFN 2017).

Meanwhile, the average omega-3 intake among breastfeeding women was 1.50±0.74g, equivalent to 0.74% Total Energy Intake (TEI) and it complied with the RNI (NCCFN 2017).

Based on the research interview conducted, all women followed traditional Malay confinement practices after delivery. In Malay culture, the confinement period is considered a vulnerable period, requiring adherence to specific dietary taboos or restrictions. The confinement period typically lasts for 40 to 44 days or 6 weeks (Fadzil *et al.* 2015). During the confinement period, women were not allowed to eat foods that were culturally known as cold, gas-inducing, acidic, and poisonous (Jamaludin 2014). Despite the food restriction during the confinement period, postpartum women selected food rich in omega fatty acids together with meat can help in postpartum recovery (Jamaludin 2014). Commonly consumed Omega-3 fatty acids food among Malay postpartum women are anchovy, Spanish mackerel, salmon, and sea bass. Table 2 shows the details of maternal omega-3 fatty acids intake.

Fatty acids profile in breast milk

Table 3 shows the saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids found in the lipid fraction of breast milk. The average percentage of Saturated Fatty Acids (SFA), Monounsaturated Fatty Acids (MUFA), Highly Unsaturated Fatty Acids (HUFA), and Polyunsaturated Fatty Acids (PUFA) content in breast milk were 40.5±2.62%, 43.54±2.60%, 1.53±0.47%, and 14.01±1.34% respectively. In this study, SFA palmitic acid (C16:0) accounts for approximately 30% of total milk fatty acids, followed by stearic acid (C18:0), myristic acid (C14:0), and lauric acid (C12:0). Maternal carbohydrate intake was found to increase the SFA in breast milk (Kim *et al.* 2017). However, in this study, the significant relationship was not found between maternal carbohydrate intake and SFA in breast milk, p=0.275. While SFA provides energy and nutrients for infant growth and development (Freitas *et al.* 2019), their consumption requires careful consideration (Pirillo & Catapano 2024). Upon absorption, lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0) were directed to triglyceride formation and

Table 2. Maternal omega-3 fatty acids intake from each food group

Food group	mean±SD (n=17)
Fish and seafood (g/day)	1.30±0.68
Anchovy	0.22±0.18
African bream	0.01±0.02
Sea bass	0.15±0.22
Sardine	0.01±0.02
Tuna	0.03±0.05
Salmon	0.01±0.03
Spanish mackerel	0.70±0.66
Black pomfret	0.06±0.11
Silver pomfret	0.09±0.19
Shrimp	0.01±0.01
Stingray	0.01±0.02
Plant-based oil (g/day)	0.09±0.16
Palm olein oil	0.01±0.01
Canola oil	0.04±0.16
Margarine	0.04±0.07
Vegetables (g/day)	0.05±0.06
Soya bean milk	0.01±0.03
Soya bean curd	0.01±0.01
Bean sprout	0.01±0.03
Green leavy vegetables	0.01±0.01
Fermented soy bean	0.01±0.01
Meat, poultry, and egg (g/day)	0.03±0.01
Hen egg	0.01±0.01
Chicken	0.02±0.01
Milk and dairy products (g/day)	0.02±0.02
Full cream milk	0.02±0.02

SD: Standard Deviation

Table 3. Fatty acids profile in the lipid fraction of breast milk

Fatty Acid (FA)	Common name	mean±SD (n=17)
Saturated FA		
12:0	Lauric Acid	0.62±0.31
14:0	Myristic Acid	4.58±1.55
15:0	Pentadecylic Acid	0.22±0.06
16:0	Palmitic Acid	29.69±1.38
18:0	Stearic Acid	5.20±1.03
20:0	Arachidic Acid	0.18±0.05
22:0	Behenic Acid	0.06±0.03
Monounsaturated FA		
14:1	Myristoleic Acid	0.30±0.35
15:1	10-Pentadecenoic	0.03±0.02
16:1ω7	Palmitoleic Acid	3.50±0.87
17:1	10-heptadecanoate	0.16±0.04
18:1ω9	Oleic Acid	39.08±2.15
20:1ω9	11-Eicosenoic Acid (Gondoic Acid)	0.37±0.06
22:1ω9	Euricic Acid	0.05±0.02
24:1	15-Tetracosenoic (Nervonic Acid)	0.05±0.02
Polyunsaturated FA		
18:2ω6	Linoleic Acid	11.47±1.24
18:3ω6	Gamma-Linolenic Acid	0.13±0.04
20:2ω6	Eicosadienoic Acid	0.22±0.04
20:3ω6	Dihomo-Gama-Linolenic Acid	0.28±0.08
20:4ω6	Arachidonic Acid	0.36±0.08
22:4ω6	Adrenic Acid	0.07±0.03
22:5ω6	Docosapentaenoic Acid	0.06±0.04
18:3ω3	Alpha-linolenic Acid	0.55±0.19
18:4ω3	Stearidonic Acid	0.12±0.07
20:3ω3	Eicosatrienoic Acid	0.03±0.01
20:4ω3	Eicosatetraenoic Acid	0.04±0.02
20:5ω3	Eicosapentaenoic Acid	0.08±0.05
22:5ω3	Docosapentaenoic Acid	0.14±0.07
22:6ω3	Docosahexaenoic Acid	0.48±0.24
Summary		
Total SFA		40.56±2.62
Total MUFA		43.54±2.60
MUFA n-9		39.85±2.03
Total PUFA		14.01±1.34
PUFA n-6		12.57±1.20
PUFA n-3		1.44±0.44
n-6:n-3		9.57±3.23

Results were expressed in weight % of human milk fat; The numbers to the left of the colon indicate the number of carbons in the chain, and the number to the right of the colon indicates the number of double bonds; Specific unsaturated fatty acids were designated by ω notation, indicating the position of the first double bond relative to the terminal methyl end group FA: Fatty Acid; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA:Polyunsaturated Fatty Acid; SD: Standard Deviation elevating total cholesterol level in human plasma (Silberstein *et al.* 2013). Apart from SFA, the predominant components of Monounsaturated Fatty Acids (MUFA) were oleic acid (C18:1) and palmitoleic acid (C16:1).

Essential fatty acids with a chain length of C18 and the Long-Chain Polyunsaturated Fatty Acids (LC-PUFA) with 20 and 22 carbon atoms are important structural and functional components of cell membranes (Schuchardt *et al.* 2010). Among the LC-PUFA, Arachidonic Acid (AA) (20:4ω6), Docosahexaenoic Acid (DHA) (22:6ω3), and Eicosapentaenoic Acid (EPA) (20:5ω3) were the primary fatty acids of interest in this study. The mean AA, DHA, and EPA content in the breast milk were 0.36±0.08%, 0.48±0.24%, and 0.08±0.05% respectively. The mean AA and EPA contents in the breast milk in this study were lower than those reported in previous meta-analysis (0.50±0.25% and 0.12±0.67% respectively), whereas the DHA content was higher than the result of the previous meta-analysis (0.37±0.31%) (Zhang *et al.* 2022). None of the women consumed fatty acids supplements during the study period. This was to ensure the fatty acids content in breast milk reflected the maternal dietary intake and without confounding effect from supplementation.

Compared to the previous studies conducted in Kuantan, Pahang (Basir *et al.* 2019). mothers in Kuala Lumpur in this study had lower SFA levels and higher levels of MUFA and PUFA in human milk The difference in fatty acid composition found in human milk may be attributed to variations in urbanization and food availability between both cities. Kuala Lumpur, the most urbanized city in Malaysia, contrasts with the less urbanized Kuantan. Therefore, people in Kuala Lumpur may eat out frequently and thus lead to the differences in their dietary fat quality and variety, which in turn may be reflected in breast milk fatty acid profiles (Poulain *et al.* 2020). Besides, people who live in urban area may have greater access to variety animal products such as fish that is high in MUFA and PUFA compared to less urban areas which usually rely more on staple carbohydrates and saturated fats (Ja'afar *et al.* 2024). Similar results were observed when compared to the data reported by Kneebone *et al.* (1985) on women in Penang. Women in Penang had higher human milk SFA (51.72%) Kneebone *et al.* (1985) than the women in Kuala Lumpur in this study (40.56%). The reduction of SFA and increase in MUFA and PUFA content in breast milk among Malaysian women might be due to the change in the maternal dietary pattern after a few decades.

Besides, this study examined the n-6:n-3 FA ratio in human milk, with a mean value of 9.57±3.23:1. Previous research suggested that an increased n-6:n-3 FA ratio in human milk may increase childhood obesity risk (Simopoulos 2016). To mitigate the risk, mothers were encouraged to consume more DHA through diet or supplements to lower the n-6:n-3 ratio in human milk (Rudolph *et al.* 2017). In the present study, Malaysian mothers show a lower n-6:n-3 ratio (9.57±3.23) compared to Singaporean mothers (10.65±4.11) (Thakkar *et al.* 2013). This is because Singapore is a high-income country which uses less than 1% of its land for agriculture (Diehl *et al.* 2020). Therefore, most of its food is imported and processed, and urbanization also led to dietary shifts to processed food (Diehl *et al.* 2020; Sukereman *et al.* 2021; Satterthwaite *et al.* 2010). Besides, women in Singapore also favour processed food due to the affordability and the convenience. In contrast, women in Malaysian tends to spend a consistent amount of money on foods associated with the local traditional diet for example fish (Gaupholm *et al.* 2022). Their preferences of different diet may influence the fatty acid composition of human milk.

According to the Malaysian RNI (NCCFN 2017), the recommendation of n-6 FA is 3–7% of Total Energy Intake (TEI) and n-3 FA is 0.3–1.2% TEI. Dietary substitution of SFA with n-6 PUFA decreased the risk of coronary heart disease (Wang 2018). Primary dietary sources of n-6 and n-3 PUFAs include meat, poultry, milk and dairy products, fats and oils, fish and seafood products, and eggs (Meyer *et al.* 2003).

The relationship between maternal dietary intake and essential fatty acids profile was investigated using Pearson's Correlation. No relationship was found between maternal dietary omega-3 fatty acids intake and breast milk PUFA and omega, p=0.992. This result was similar to Bzikowska-Jura *et al.* (2019) who demonstrated the omega-3 FA content in human milk was associated with maternal habitual intake but not the current intake. Maternal dietary intake of EPA and DHA during the third trimester of pregnancy directly influenced the fatty acids content in mature human milk (Nishimura *et al.* 2014). A possible explanation would be the increased weight gain and body fat storage in mothers during late pregnancy (Kominiarek & Peaceman 2017; Gilmore *et al.* 2016). These results suggested the fatty acids stored in the maternal body had a significant impact on the human milk fatty acids composition. Previous study by Giuffrida *et al.* (2022) found positive correlations were between plamitic, stearic, oleic, and PUFA in breast milk with adipose tissue. Additionally, previous study also found that pre-pregnancy BMI had significant positive correlation with fatty acid profile of human milk specifically six SFAs, five MUFAs and six PUFAs (Meng *et al.* 2023).

A limitation of this study is the inadequate sample size as planned due to the COVID-19 pandemic. This limitation reduced the statistical power of the study and at the same time restrict the ability of the findings to be generalized. Therefore, the results should be interpreted with caution and considered preliminary. Despite this limitation, the data can provide valuable insights and serve as pilot data to guide future research in similar populations.

CONCLUSION

In conclusion, this study showed that the lactating women had sufficient Omega-3 intake, averaging 1.50 g or 0.74% TEI, within the RNI recommended range. Omega-3 food source primarily from fish and seafood, plant-based oil, vegetables, meat, poultry, eggs, milk, and dairy products. However, no significant correlation was observed between maternal dietary Omega-3 fatty acids intake and the Omega-3 content in breast milk. The limitation of this study is its small sample size and cross-sectional design, which should be interpreted carefully and did not account for maternal dietary intake during pregnancy.

Future study should involve larger longitudinal studies to better understand how maternal dietary patterns, particularly during and after the confinement period, influence breast milk fatty acids composition. It will give deeper insight into the impact of the maternal diet on breast milk fatty acids profile.

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DECLARATION OF CONFLICT OF INTEREST

The authors have no conflict of interest.

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