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Effects on Maternal Macronutrient Intake Towards Human Milk's Fatty Acids Composition

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Abstract

While fatty acids found in human milk account for half of the energy consumed by exclusively breastfed infants, fatty acids such as monounsaturated fatty acids (MUFA) and long chain polyunsaturated fatty acids (LC-PUFA) plays critical roles in infant growth. Fatty acids components in human milk are vary widely accordance to the maternal diet during lactation but has not been sufficiently studied. The objective of this paper was to determine the correlation between maternal macronutrient intake with human milk's fatty acids composition among exclusively breastfeeding mothers. A total of N=36 lactating mothers were recruited based on convenience sampling basis from Dengkil, Selangor and Kuantan, Pahang. A 24-hour dietary recall (24HR) was used to capture mother's dietary intake in the past 24 hours. The human milk sample was collected in the next day morning after the diet recall and stored before proceeded to another fatty acids extraction and transesterification process namely Blight and Dyer method. The composition of fatty acids methyl esters was analyzed and quantified by a gas chromatography (Agilent 7890A), equipped with a flame ionization detector (FID) and Agilent Chromatography Workstation software. As overall, the most abundance fatty acids found was SFA ranged (81.90 to 97.7 %) followed with MUFA (2.3 to 18.1%), but PUFA was below detection limit (BDL). Result also indicated that palmitic, stearic, and oleic acids were the three major types of fatty acids determined from human milk. Correlational study also determined that, there was no significant correlation between the human milk's SFA and MUFA with the same dietary intake and another macronutrient like carbohydrate and protein. Even though there was no significant correlation determined for the most composition, various pattern of correlation was found in the study. Human milk's SFA only had a positive correlation with dietary carbohydrate but negative with the rest. Different pattern also showed for human milk's MUFA which only negatively correlate with carbohydrate and fats while positive for the rest. Thus, overall, this fat composition is known to have higher variation in terms of concentration of its components compared to another macronutrient even within the same population. Aside from geographical considerations, maternal nationality and age have a substantial impact on the fatty acid composition of human milk.

Keywords Fatty Acids; Human Milk; Mono-Unsaturated fatty acids (MUFA); saturated fatty Acids (SFA)

INTRODUCTION

Human milk is invariably known as the optimal food for infants up to 6 months of age. It contains adequate amount of macronutrient, micronutrient, bioactive component, and growth factors. Human milk is mostly composed of macronutrients such as fat, protein, and lactose, with micronutrients such as vitamin A, D, B1, B2, B6, B12, and iodine which present in small proportions. In general, human milk's macronutrient can be determined by a few maternal factors such as BMI, dietary intake, menstruation, and nursing frequency [1]. It is responsible to the changes of the human milk quality and quantity [2]. Previous study suggested that human milk composition such as fat, carbohydrate, protein can be affected selectively by maternal diet [18,19]. Considering maternal diet in the context of exclusively breastfeeding infant, the changes of macronutrient and micronutrient content will subsequently affect nutritive values that receive by them. MUFA, LC-PUFA and saturated fatty acid (SFA) which cannot be synthesized by an infant have an important role for healthy growth of infants specifically in the development of central nervous system [3]. Several human milks' fatty acid such as arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) have already been sufficiently studied and been thought key factors for foetal growth and vital for tissue, organ development of infants. [4,5]

MATERIALS AND METHODS

An observational cross-sectional study was conducted in Kuantan, Pahang and Dengkil, Selangor. Ethical approval was obtained from the Kulliyah of Allied Health Science (KAHS42/18) and IIUM Research Ethics committee (IREC-2019/011). Recruited respondents were chosen based on several inclusion factors such as age that ranged between 18 to 39, singleton pregnancy, full term delivery and six months of exclusively breastfeeding period. The sample size calculation of 152 respondents was determined using single mean formula since this study involved only a single population with specified absolute precision of anticipated population (P) of 30% or 0.3, 95% confidence interval (at a Z-score of 1.96) and absolute precision of 5%, and a 10% non-response rate. However, based on convenience and snowball sampling method only 36 respondents who attending government health clinic for post-partum regular check-up and follow up were successfully recruited due to the low response rate.

Firstly, the data collection was done with the collection of sociodemographic and anthropometry data. The sociodemographic data included information on the mother's ethnicity, age, and delivery method, as well as chronic illness. Besides, the anthropometry factor listed the respondent's and infants' weight, height, and Body Mass Index (BMI) and lactation stage as the required data. All procedures were done with properly standard of procedures to improve accuracy and minimized the systematic error. BMI was then calculated as weight (kg) divided by height (m^2). The recommended BMI categories in Asian populations were as follows: <18.5, 18.5-23, 23-27.5, and ≥27.5 for underweight, normal weight, overweight and obese, respectively [6].

Fatty acids were determined by two phases; first is the lipid extraction by Blight and Dyer method and second phase, the transesterification of lipids. Chemical such as methanol (Sigma-Aldrich), chloroform (Sigma-Aldrich), 2% anhydrous sodium sulphate (Sigma-Aldrich), sodium hydroxide (Sigma-Aldrich), boron trifluoride (Sigma-Aldrich), ammonium chloride, Butylated hydroxytoluene (BHT) (Sigma-Aldrich) and hexane (Sigma-Aldrich) were used in the study. The equipment used were Pasteur pipette, 50ml falcon tube, Micropipette (Eppendorf) and 1000 µl and 500 µl sterile tip, Schott bottle (250 ml/ 100 ml / 50 ml) Schott Duran, beaker (250 ml / 100 ml / 50 ml) (Pyrex®), volumetric flask (100 ml) (Pyrex®), disposable tips (200 µl / 100 µl) (Fisher Scientific), aluminum cup, filter paper (Whattman No.1), test tube, metal spatula, 2ml HPLC vial with cap, syringe filter (PTFE, hydrophilic, 0.45µm), vortex mixer Genie 2 (Scientific Industry Inc,), Shaker Cleaver (Scientific Industry Inc,), Analytical Balance FX-3000i (AND), Refrigerated Universal Centrifuge 320R (Hettich), Hot Plate (V-go), Stainless steel Forced-Air Convention Oven/Incubator (PROTECH), Fume Hood and gas chromatography (Agilent 7890A).

During lipids extraction by fatty acid methyl Ester (FAME), 1.5ml of sample was replaced into labelled 50ml falcon tube for further reaction. Sample then was mixed with 9.5ml solvent mixture (with the ratio of methanol: chloroform: water at 2: 1: 0.8 ratio. 2.5ml of chloroform and 2.5ml of 2% anhydrous sodium sulphate solution were then added to the mixture. It was then agitated for another 2 minutes. After

that, the mixture was centrifuged at 3000 × g for 15 minutes. After centrifugation, two layers of phases were formed. The organic layer which the upper one was separated using micropipette into another labelled 50ml falcon tube. 5ml of chloroform was added again into the remaining mixture and centrifuged again at 3000 × g for 10 minutes. The organic layer was separated again and combined to the first extraction. Another 2.5ml of anhydrous sodium sulphate than was added to the extract and left overnight at room temperature. In the next day, the extract was then filtered using filter paper (Whattman No.1) into pre-weighted aluminum cup and evaporated to dryness on a hotplate in the fume hood. After evaporation, aluminum cup was weighed to record the weight of the lipid extract. The extract was then scrapped using a spatula and transferred into another labelled falcon tube.

During transesterification of lipids, 1 ml of sodium hydroxide solution in methanol with the concentration of 1 mol/L was added to the extract in the falcon tube. The mixture was vortexed and then incubated at 70°C for 30 minutes. 2 ml of boron trifluoride solution was added, and the extract was then incubated again at 70°C for 10 minutes and left to cool at room temperature. 2ml of hexane containing 0.2% BHT and 1 ml of 20% ammonium chloride solution were added to the mixture which resulted in suspension. The mixture than was centrifuged for 15 minutes at 3000 rpm. The clear organic phase which was the upper layer was extracted using Pasteur pipette and filtered into a clean vial using a syringe filter (PTFE, hydrophilic, 0.45µm).

The composition of fatty acids methyl esters was analyzed and quantified by a gas chromatography (Agilent 7890A), equipped with a flame ionization detector (FID) and Agilent Chromatography Workstation software. The fatty acids methyl ester (FAME) was separated in a capillary column HP-5 (30m × 320µm and a film diameter of 0.25µm). Helium was used as a carrier gas at a flow rate of 1 ml/min. The temperatures of the injector and detector were 250°C and 270°C, respectively. The oven temperature was programmed from 50°C to 70°C at 10°C/min, followed by 170°C to 220°C at 2°C/min. The final temperature (220°C) was maintained for 20 minutes. The total run time was 57 minutes. Sample was analyzed within 12 hours of transesterification. The FAMEs were identified by comparing the retention times of the peaks generated by the samples with those of the peaks obtained from gas chromatography-mass spectrometry (GC-MS) analysis. Peaks obtained from GC-MS were identified based on its database library.

RESULTS AND DISCUSSION

Sociodemographic and anthropometry factor

Table 1 showed the demographic factor of respondents. Respondents' age ranged from 19 to 39 years old with n=35(97.2) were Malay and n=1(2.8%) was Chinese. Most of them with n=13(36.1) was government servant and an unemployed housewife. All the respondents gave birth through normal vaginal delivery. **Table 2** presented the anthropometry data of the respondents. Maternal weights and heights ranged from 38.0 to 77.0kg and 1.47 to 1.7m, respectively. The Body Mass Index (BMI) showed that majority of the mothers have normal BMI with n=22(61.1) but n=8(22.2) was overweight and n=6(16.7) was obese with BMI over 25. Infant birth weights ranged from 2.2 to 3.9kg while the latest infant weights ranged from 3.58 to 8.8kg. All the respondents were an exclusively breastfeeding mother with 0 to 180 days of lactating stage.

Table 1 Demographic Factor of Respondents

Characteristic		**n**	**Mean ±SD**	**Range or Percent**
Age (years)		36	30.33±5.14	19-39
Races	Malay	35		97.2
	Chinese	1		2.8
Employment	Government	13		36.1
	Private	8		22.2

	Free-Lance	2	5.6
	Unemployed/Housewife	13	36.1
Mode of Delivery	Normal Vaginal Delivery	36	100

Table 2 Anthropometry Data of Respondents

Characteristic	n	Mean ±SD	Percentage or range
Latest Weight (kg)	36	59.47±9.74	38.0-77.0
Height(m)	36	1.56±0.056	1.47-1.7
Body Mass Index (BMI)	36	24.38±4.39	17.59-34.22
Normal	22		61.1
Overweight	8		22.2
Obese	6		16.7
Infant Birth Weight (kg)	36	3.05±0.39	2.2-3.9
Infant Latest Weight (kg)	36	5.70±1.75	3.58-8.8
Infant's Age (day)	36	114.17±50.10	30-180
30	4		11.1
60	6		16.7
90	5		13.9
120	5		13.9
150	10		27.8
180	6		16.7

Human milk's Fatty Acids

Table 3 presented the composition of fatty acids from human milk. As overall, the most abundance fatty acids found was SFA followed with MUFA, but PUFA was below detection limit (BDL).

Table 3 Fat's concentration in human milk over the lactating period

Nutrient	**(0-6 months) (n=36)**	**0-2 month(n=10)**	**3-4 month(n=10)**	**5-6 month(n=16)**	**Reference range**
	Range		Mean±		
SFA (%)	81.90-97.70	91.34±4.01	93.40±4.35	92.31±5.31	51.7
MUFA (%)	2.3-18.1	8.661±4.01	6.60±4.35	7.69±5.31	36.3

SFA composition was predominantly, ranged from 81.90 to 97.7 % over the lactation period. The highest SFA composition presented during three to four months of lactating period with 93.40±4.35% while the lowest presented during the first two months with 91.34±4.01%. The percentage composition of SFA exceeded the reference range through the lactating period. For MUFA which represented by only oleic acid from the study, the composition was ranged from 2.3 to 18.1% for zero to sixth months of lactation period which was lower as compared to reference range. The highest MUFA composition presented during the first two months of lactation period with the means of 8.661±4.01 and the lowest during three to four months with 6.60±4.35. This outcome also parallel to the oleic acid composition that ranged from 7.6572±4.655.

Table 4 Fatty acids composition in human milk over the lactating period

Nutrient	0-6 month(n=36)	0-2 month(n=10)	3-4 month(n=10)	5-6 month(n=16)
	Area Percentage (Mean±)			
Palmitic Acid	40.693±5.37	44.892±4.66	40.581±5.25	38.139±4.37
Stearic Acid	51.649±8.13	44.446±4.74	52.822±8.36	54.168±8.57
Oleic Acid	7.6572±4.655	8.661±4.01	6.60±4.35	7.69±5.31

Table 4 presented the three fatty acids compositions from the study. Palmitic, stearic, and oleic acids were the three types of fatty acids determined from the study. The presence of palmitic and stearic acids composition in human milk were abundance as compared to oleic acid. The highest stearic acid composition was found in the last two months of lactating, at 54.168±8.57, while the lowest was discovered in the first two months, at 44.446±474. However, different pattern showed for palmitic acids where the highest composition occurs during the first two months with 44.892±4.66 while the lowest presented during the last two months of lactation period with 38.139±4.37.

As overall, the highest composition of SFA has been determined in the study compared to other fatty acids. Previous study in USA indicated that the range percentage of SFA to the overall fat composition during zero to sixth months of lactation period was from 36.8±4.64 % to 39±5.62% which much lower compared to the study data [7]. Compared to this study data on MUFA, they reported much higher percentage range with 45.8±4.62 % to 47±4.25% [7]. It was reported that among 32 mothers in Poland, proportion of saturated, monounsaturated, and polyunsaturated fatty acids was 41.9±4.9%, 39.6±3.1%, and 15.1±3.4%, respectively which have similarity with this study [8]. In contrast, the predominant fatty acids in human milk were oleic acid (35.4±3.1%) not palmitic acid as stated in the study, followed with palmitic (19.7±2.5%), and linoleic acid (11.1±2.6%). This outcome summarized that human milk of European mother typically contain around 35 to 40% saturated fatty acids, 45–50% monounsaturated fatty acids, and approximately 15% PUFA. Another study that compared the SFA composition between Asian, Māori, Pacific Island and New Zealand reported the similar outcome which among fatty acids, saturated fatty acids predominated (47.61%), followed by monounsaturated fatty acids (39.26%), while polyunsaturated fatty acids had the lowest proportion (13.13%) [10,9]. The palmitic acid (C16:0) composition was the highest in Asian as compared to stearic acid (C18:0) with (0.929±0.319g/100ml vs 0.258±0.094 g/100ml) respectively [9]. It was also supported by many studies that palmitic acid (C16:0) contributes approximately 25% of all milk fatty acids and represents the major composition to total saturated fatty acid content [1,7,10]. As compared to other of the three regions, the palmitic acid (C16:0) composition of Asian was the highest compared to Māori, Pacific Island and New Zealand European with 0.801±0.339 and 0.780±0.232g/100ml respectively while stearic acid (C18:0) composition was eventually similar across the region within the small range of 0.243±0.103 to 0.258±0.094. Supporting this study, oleic acids was the major composition that represent MUFA [1,9]. As reported by [9], the composition of oleic acid in Asian was the highest with 1.507±0.344g/100ml compared to 1.225±0.557 in Māori and Pacific Island and 1.294±0.388 in New Zealand and European. Findings by [10] reported that, after palmitic acids, oleic acids and linoleic acids were the most found fatty acids in colostrum from Greece mothers.

Correlation between Maternal Macronutrient intake with Human milk's fatty acids

Table 5.0 presented the correlation between human milk's SFA and MUFA concentration with macronutrient intake over the lactating period.

Table 5 Correlation between SFA and MUFA concentration in Human Milk with macronutrient of dietary intake over the six months of lactating period

Nutrient		**CHO**		**Protein**		**Fats**		**SFA**		**PUFA**		**MUFA**	
	n	**r**	***P*-value**	**r**	***P*-value**	**r**	***P*-value**	**r**	***P*-value**	**r**	***P*-value**	**r**	***P*-value**
SFA	36	0.317	0.059	-0.009	0.957	0.067	0.696	-0.29	0.868	-0.177	0.3	-0.109	0.529
MUFA	36	-0.317	0.059	0.009	0.957	-0.067	0.696	0.029	0.869	0.267	0.116	0.193	0.239

As illustrated in Figure 1 (a and b), there was no significant correlation between the human milk's SFA and MUFA with the same dietary intake. Even though no significant association was identified for the most composition, this study found a pattern of correlation. Human milk's SFA only had a positive correlation with dietary carbohydrate and fats but negative with the rest. Different pattern also showed for human milk's MUFA which only negatively correlate with carbohydrate and fats while positive for the rest.

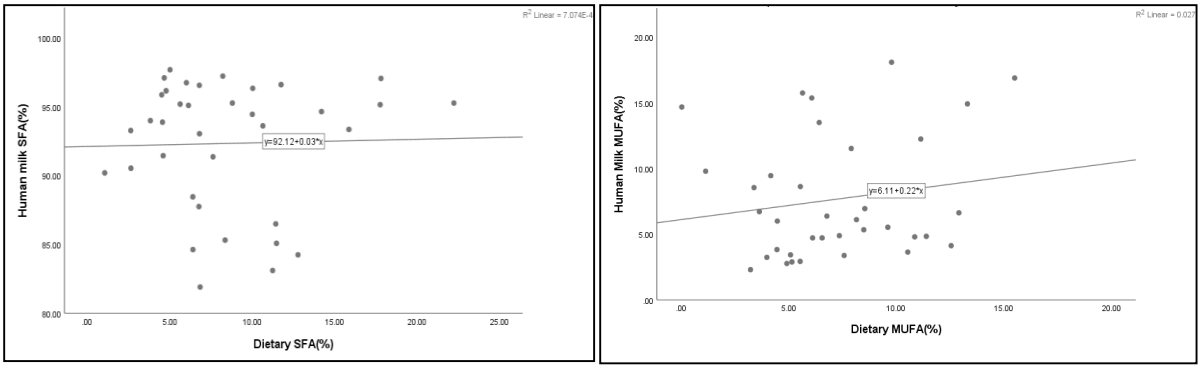

Figure 1 (a) and (b) Correlation line graph of SFA and MUFA from dietary intake against the same composition in human milk over the lactation period

Earlier study revealed that human milk's composition such as SFA and MUFA did not significantly associate with the corresponding dietary intake [8,11,12,13,17]. As mentioned by [15], the buffering effect within human milk could be one of the reasons against the variation of human milk based on nutritional intake. Body has an ability to limit the dynamic changes of human milk via physiological compensation mechanism. One of it is through de novo synthesis of mammary gland. Apart from plasma serum nutrient composition, de novo synthesis of mammary glands theoretically discussed in earlier study to cause changes in HM nutrient composition. De novo lipogenesis is one of the well-known mechanisms in mammary gland to affect fatty acids composition in milk. Mimics the study by [20], dietary pattern of high carbohydrate or high fats intake, the outcome indicated that there was assimilation of 13C2 from glucose into milk fatty acids as it increased in the length of chain from C2:0 to C12:0 but progressively declined in C14:0 and C16:0. The outcome also reported that, regardless of diets treatment, the incorporation of 13C2 in milk fatty acids and 13C3 in milk glycerol were three and seven fold higher compared with plasma FA and glycerol, respectively. Even in the scarce of carbohydrate and fat intake during fasting, medium chain fatty acids (C6-C12) increased significantly from 25% to 75% for carbohydrates and 6% to 25% for fats. This outcome supports the findings that the human mammary gland is capable of de novo lipogenesis which is influenced by the macronutrient composition of the maternal diet. It was also reported among Filipino mothers, dietary macronutrient such as protein, carbohydrate, and fatty acids such as PUFA and MUFA did not associated to the total HM fats composition [17]. However, another study by [14] of three to four months postpartum Canadian mother reported that, there was a significant negative weak correlation between fatty

acids and diary HEI with total SFA including palmitic, stearic and myristic acid but had a significant positive weak correlation with total MUFA and oleic acids. A review by [15] reported that most observational studies of the relationship of FA dietary intake and its presentation in HM showed a positive correlation. However, while focusing on specific study such as PUFA, no relationship has been reported. It is well known that levels of ω-6 and ω-3 PUFA vary widely among individual women, a difference which is explained largely by the types of ω-6 and ω-3 PUFA in the breastfeeding mother's diet. According to [11], only selected human milk fatty acid profile such as linoleic, α-linolenic, docosahexaenoic, intake may be affected by the immediate and habitual diet consumed by the mother. For essential fatty acids like MUFA, mixed outcome has been reported. Study by [13] among Malaysian mothers indicated the same result with this study but another study by [16] among Dutch mothers, there was a positive correlation between HM MUFAs within a week of postpartum total dietary MUFAs with ($r=0.63$, $P=0.01$). However, there was no significant relations between HM MUFAs with dietary intake of fat, PUFA, total SFA, carbohydrate, or protein intakes.

CONCLUSION

In comparison to MUFA, SFA is the most prevalent fatty acid in human milk. The findings also discussed the differences of fatty acids in human milk during lactating period. The BDL status of PUFA, which is an important element of human milk's fatty acids, determines that exceeded presentation of certain fatty acids may affect the presentation of another fatty acid's element in human milk. Although it is known in the literature that fatty acid composition varies more than other human milk macronutrients, the results are not significant when it comes to food consumption, showing that dietary intake is not the only factor that can regulate these compositions. Even though dietary intake dictates some changes over the lactating period, hypothetically, physiological factors such as De-Novo fatty acids synthesis in the mammary gland, can be a reason for the particular outcome. Other factors impacting the fatty acid content of human milk, such as maternal nationality, genetic factors, age, lactation stage, and BMI of breastfeeding women, should be studied more in the future.

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