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Distribution of Lipid-soluble Vitamin Intake among Exclusively Breastfeeding Mothers and its Correlation with Human Milk's Fatty Acids

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Abstract

The lipid composition such as fatty acids and fat-soluble vitamins is the second-most abundant composition of human milk providing dietary energy to infants. Micronutrient dietary intake such as vitamin A, D, E, K and C by breastfeeding mothers plays an important role in regulating the quality of human milk for optimum infant health and growth. The objective of this paper is to determine the distribution and correlation of maternal micronutrient intake of lipid-soluble vitamin and vitamin C towards fatty acids composition in human milk of exclusively breastfeeding mothers. A total of N=36 nursing women were recruited from Dengkil, Selangor, and Kuantan, Pahang, using a convenience sample method. A 24-hour dietary recall (24HR) was performed to collect thorough information on all foods and beverages ingested in the previous 24 hours by the respondent. The data on micronutrients intake per mother was tabulated using Nutritionist Pro. (NP) software. Following the diet recall, the human sample was collected in the next morning and subjected to fatty acid extraction and transesterification using the 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. The highest mean of intake occurred during the fifth to sixth months with, 1067.37±629.66 µg RE/day for vitamin A, during the first two months with, 0.89±0.84 µg RE/day of vitamin D, 5.85±2.49 mg/day while during the fifth to sixth months with, 17.28±11.74 µg /day of Vitamin E and at the first two months of lactation period with, 91.60±55.26 mg per day for vitamin C. Despite the fact that there was no significant correlation between vitamin intake and the fatty acid content of human milk, the study discovered a variety of patterns of correlation. Saturated fatty acids (SFA) in human milk were only positively correlated with vitamin D and C, while monounsaturated fatty acids (MUFA) were positively correlated with vitamin A, E, and K and negatively correlated with the rest. As a result, the fatty acid composition of human milk is less dependent on micronutrient dietary intake and more dependent on De-Novo synthesis in the mammary gland.

Keywords Fatty Acids, Human Milk, Lipid-Soluble Vitamin

INTRODUCTION

Micronutrients are vitamins and minerals that are essentials in many body functions. Since human body could not produce most of micronutrients, it is crucial to obtain them from well-balanced diet. Dietary supplement use has also risen in recent years, resulting in increased nutritional intake in the population [1]. The most of vitamins, such as vitamin C, are water-soluble; nevertheless, four fat-soluble vitamins, vitamin A, D, E, and K, do not dissolve in water and are absorbed similarly to fats.

According to WHO, human milk is composed of ideal nutritional composition, which is dynamic, and varies within a feeding, diurnally and over lactation period. It responsible to promote survival and healthy development of an infant. The fat-soluble vitamins are constituents of lipid fraction of human milk and vital for the development of infant's health especially immune system. Since human milk is the primary source of nourishment for exclusively breastfed infants, it is crucial to look at the factors that affect micronutrient content in human milk. However, there is a scarcity of information on the relationship between micronutrient dietary consumption and the macronutrient composition of human milk.

Vitamin A in human milk is derived from serum retinol and largely depends on maternal intake. According [2], dietary supplementation of vitamin A in several weeks of postpartum would increase the human milk retinol that declines over time. However, there is no evidence on health benefits gained from vitamin A supplementation for mothers' and infants' mortality and morbidity [3]. Except in cases of severe vitamin A deficiency,WHO does not suggest vitamin A supplementation for newborns, children, or postpartum women.

On the other hand, vitamin D plays an important role in developing strong and healthy bones in infants. Human milk has relatively low vitamin D, especially when the mother has very little exposure to sunlight. [4]. This had a direct impact on vitamin D levels, particularly in infants who were exclusively breastfed. Maternal supplementation is one of the ways for increasing vitamin D which could help to avoid vitamin D deficiency in infants. According to [5,] maternal vitamin D intake of 6400 IU/day elevated 25(OH)D levels in both the mother and the infant. Vitamin D supplementation for mothers and infants was also recommended by the WHO, particularly in the first few months after delivery until dietary sources of vitamin D could be found.

Vitamin E is well-known for its antioxidant capabilities, which are essential for the development of the neurological system and the prevention of cell damage caused by free radicals in infants, particularly preterm infants. The biologically active form of vitamin E, alpha-tocopherol, is found in lower amounts in human milk as lactation develops. [6] has reported a positive correlation between alpha-tocopherols level in human milk and dietary and supplementation intake of vitamin E with serum alpha-tocopherol. This suggests that when high doses of vitamin E were consumed, the vitamin E concentration in human milk was regulated by dietary and circulation maternal levels. In contrast, [7] found no association between vitamin E intake in the food and levels of this micronutrient in human milk.

Vitamin K is required in blood clotting process and lack of this vitamin will leads to vitamin K deficiency bleeding (VKDB). According to Centers for Disease Control and Prevention (CDC), VKDB occurrence rate is higher in infants from 0 to 6 months old. Hence, to prevent VKDB, vitamin K shot is given to all newborns in most developed and developing countries. Human milk contains vitamin K, but it is insufficient to meet the optimum need for infants under the age of six months. Vitamin K levels in human milk and infant blood plasma were both relatively low throughout the first six months [8]. Nonetheless, maternal vitamin K supplementation of 5 mg have been shown to boost vitamin K levels in human milk and in infants' plasma [8.9].

Ascorbic acid (AA) and dehydroascorbic acid (DHAA) are the two forms of vitamin C found in human milk. Due to its antioxidant properties, vitamin C is required to boost infants' immune system as well as reducing the risk of atopic diseases in infants. [10] found a significant result (r=0.34, P=0.047) indicating a positive association between maternal vitamin C intake and vitamin C concentration in human milk. Previous study revealed that dietary vitamin C supplementation had no influence on vitamin C concentration in human milk [10]. However, [11] reported a different observation in an earlier investigation that maternal consumption, as measured by BMI, number of pregnancies, and economic status, had no effect on vitamin C concentration in human milk.

Previous study indicated the mix outcome on the effect of lipid soluble vitamins towards human milk's nutritional composition. As stated by [12], the variation in maternal diet has no significant effect on certain macronutrient composition such as protein and fats. Even though there are certain micronutrient

deficiencies, human milk's composition still at constant level and adequate to support the infant needs at the expense on maternal body stores. The amount of antioxidant activity of vitamin E and A, as well as the content of fatty acids in humans, were found to have no correlation in a study by [13]. [14] claimed that the normative standard for baby nutrition was vitamins A in human milk, which was dependent on maternal diet and body reserves.

MATERIALS AND METHODS

This study is an observational cross-sectional study that 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. 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.

The initial round of data collection included sociodemographic and anthropometric data, as well as a nutritional intake assessment. All procedures were carried out according to procedure to improve accuracy and reduce systematic error. The maternal dietary intake was assessed using a set of diet recall and food frequency questionnaires. A 24-hour dietary recall (24HR) is a systematic questionnaire that collects thorough information on all foods and beverages consumed by the respondent in the previous 24 hours. This section demonstrates the micronutrients intake per mother which will be tabulated using Nutritionist Pro. (NP) software.

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), aluminium 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 than 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 for 15 minutes at 3000 g. Two layers of phases were formed during centrifugation. The organic layer, which was on top, was separated using a micropipette and placed in a 50ml falcon tube with a label. 5ml of chloroform was added to the remaining mixture and centrifuged for 10 minutes at 3000 g. The organic layer was separated one more before being mixed with the first extraction. An another 2.5ml of anhydrous sodium sulphate was added to the extract and allowed to sit at room temperature overnight. The extract was then filtered using Whattman No. 1 filter paper into a pre-weighted aluminium cup the next day and evaporated to dryness on a hotplate in the fume hood the next day. After evaporation, the lipid extract was weighed in an aluminium cup to determine its weight. The extract was scraped and put into another designated falcon tube using a spatula.

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 then 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).

A gas chromatography (Agilent 7890A) equipped with a flame ionisation detector (FID) and Agilent Chromatography Workstation software was used to analyse and quantify the composition of fatty acids methyl esters. In a capillary column HP-5, fatty acids methyl ester (FAME) was separated. At a flow rate of 1 ml/min, helium was used as a carrier gas. The injector and detector had temperatures of 250°C and 270°C, respectively. The oven temperature was set to rise from 50°C to 70°C at a rate of 10°C per minute, then to 170°C to 220°C at 2°C per minute. For 20 minutes, the final temperature (220°C) was maintained. The run time lasted 57 minutes in total. Within 12 hours of transesterification, the sample was analysed. 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 unemployed or works as a fulltime housewife. All the women who responded gave birth via vaginal delivery.

Table 1 Demographic Factor of Respondents

Characteristic		n	Mean ±SD	Range or Percent
Age (years)		36	30.33±5.14	19-40
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 presented the anthropometry data of the respondents. The weights and heights of the mothers ranged from 38.0 to 77.0 kg and 1.47 to 1.7 meters, respectively. Most of the mothers had a normal BMI with n=22 (61.1), although n=8 (22.2) is overweight and n=6 (16.7) is obese (BMI over 25). The latest infant weights ranged from 3.58 to 8.8kg, with birth weights ranging from 2.2 to 3.9kg. All the mothers who responded were exclusively breastfeeding.

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

Distribution of Lipid Soluble intake among lactating mothers

For vitamin A, the highest mean of intake occurred during the fifth to sixth months of lactation period with, 1067.37±629.66 µg RE/day while the lowest with 731.06±317.99 during the first two months. Overall, there were no significance difference of all means intake of vitamin A over the six months of lactation period as compared to RNI value since $p>0.005$. For Vitamin D, the highest mean of intake occurred during the first two months of lactation period with, 0.89±0.84 µg RE/day and the lowest was reported during third to fourth months with 0.27±0.39. All means value was below the daily recommended value by RNI (2017). Since $p<0.005$, there was a significant difference between the mean vitamin D consumption during the six months of breastfeeding and the RNI value. For vitamin E, the highest mean of dietary intake occurred during the first two months with, 5.85±2.49 mg/day and the lowest with 5.26±2.62 during the third to fourth months of lactation period. All means value was below the daily recommended value by RNI (2017). Since $p<0.005$, the mean dietary intake of vitamin E throughout the third to fourth months of breastfeeding and the fifth to sixth months of lactation had a significant difference when compared to the RNI value. Based on Table 4 the highest mean of intake for Vitamin K occurred during the fifth to sixth months of lactation period with, 17.28±11.74 µg /day and the lowest with 7.96±7.04 during the first two months of lactation period. All means value was below the daily recommended value by RNI (2017). Since $p<0.005$, the total dietary intake of vitamin K throughout the six months of lactation was substantially different from the RNI value. In addition, Table 4 also represented the highest mean for vitamin C dietary intake which occurred during the first two months of lactation period with, 91.60±55.26 mg per day and the lowest with 33.07±9.33 during the fifth to sixth months. All means value was below the daily recommended value by RNI (2017). Overall, all means intake of vitamin C during third to fourth months and fifth to sixth months of lactation period were significance compared to RNI value since $p<0.005$.

The data revealed a similar trend of the increases in vitamin A and K intake across the six-month postpartum period. From the third through the sixth months of lactation, the intake increased gradually. Vitamin A and K are primarily found in dark-green leafy vegetables and certain vegetable oils such as canola and olive. According to a previous study [16], proper dietary intake of other nutrients such as fat, protein, vitamin E, zinc, and dietary fiber can affect the bioavailability of Vitamin A status. It is possible that lower vitamin A and K intakes during the early postpartum period were due to cultural food restrictions. Water spinach, spinach, and pumpkins [17], which are high in both vitamins, are forbidden because they are considered "cold" and "windy" foods that can bring discomfort. Following that time, the seasonal availability of fruit and vegetable sources was related to an increase in vitamin intake. Because most working mothers return to work after two or three months of maternity leave, they can easily access or purchase vitamin A and K-rich foods. According to a recent study in China [18], lactating mothers' percentage intake of green leafy and colored vegetables (red and yellow) was lower than recommended than other plant-based meals, regardless of lactation period. Previous research among pregnant mothers in Malaysia found that they had insufficient intake of fruits and vegetables due to food taboos and a lack of nutrition knowledge [19, 20]. According to the findings, the highest mean intake of vitamin C was found during the first two months after delivery due to the highest dietary intake of vitamin C-rich foods. It was stated in NCCFN, (2017),[20] that fresh fruits such as guava, mango, papaya, citrus fruits and juices, tomato

and leafy vegetables such as cabbage, cauliflower, mustard leaves, spinach and other leafy greens and 'ulam', broccoli, turnip are rich in vitamin C. Other than that, during confinement, food always prepare with spice such as ginger and sometimes used as the main ingredient soup, or in forms of fried ginger flakes or 'serunding'. Previous study [21] determined that local herbs and spices such as ginger are also rich in vitamin C and has high antioxidant activities. However, the findings revealed a considerable decrease in vitamin C intake when compared to the RNI value, with a significant difference near the end of the breastfeeding period. Seasonal intake of specific vitamin C-rich fruit food sources in Gambia, West Africa, affects their vitamin C dietary intake, according to [22]. The blood and milk vitamin C levels were highest from April to June, when mango and orange availability and consumption were at their peak. [23] discovered that the vitamin C content of breastmilk was connected to a substantial positive association ($r = 0.61$, p.01) in an Iraqi study. As the intake of vitamin C increases, the amount of vitamin C in breast milk decreases. During less than two weeks of postpartum the mean level of vitamin C was 4.2 ± 2.08mg per 100 ml compared to at weeks 24 of lactation with 2.5 ± 0.8 mg per 100ml.

On the other hand, the same pattern was shown for Vitamin E and D which remain constant along the lactation period. According to a study conducted in the United Arab Emirates [24], vitamin D status deteriorated from pregnancy to the sixth month of lactation, with appropriate serum levels falling from 31% to 17%. Vitamin D deficiency in breastfeeding mothers relative to RNI values [25] may be linked to dietary habits and attitudes toward vitamin D [26]. In terms of dietary intake, most Malaysian women consumed a limited amount of vitamin D-fortified foods, as well as various dairy products and vitamin D-rich morning cereals [27]. The study also exhibited that, 65% to 80% of Malaysian women rarely consume egg and fatty fish such as salmon, tuna, and sardine, which are high in both vitamins. Apart from leafy vegetables, vegetable oils, nuts, and whole grains, the major dietary sources of α- and γ-tocopherols of vitamin E are leafy vegetables, vegetable oils, nuts, and whole grains. Seed and nuts were not a popular choice among Malaysians because average monthly expenditures on nuts and nut products were as low as 0.4%[20]. Almond, cashew, and peanut also consumed in small amounts as snacks or sauce ingredients [28], or completely prohibited due to allergies.

Human milk's Fatty Acids

Table 3 presented the composition of fatty acids from human milk while Table 4 presented the three fatty acids compositions from the study. As overall, the most abundance fatty acids found was SFA followed with MUFA, but PUFA was below detection limit (BDL).

As overall, SFA composition was 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 was exceed 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.

In addition, 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 composition of stearic acids presented during the last two months of lactating period with 54.168±8.57 and while the lowest presented during the first two months with 44.446±4.74. 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.

For fatty acid composition, the highest composition of SFA was determined in the study compared to another fatty acids. Previous study by [29] 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. Compared to this study data on MUFA, [29] reported much higher percentage range with 45.8 ±4.62 % to 47±4.25%. [30] reported that among 32 mothers in Poland, proportion of saturated, monounsaturated, and polyunsaturated fatty acids was $41.9 \pm 4.9\%$, $39.6 \pm 3.1\%$, and $15.1 \pm 3.4\%$, respectively which have similarity with this study. In addition, the predominant fatty

acids in human milk were oleic acid (35.4 ± 3.1%), palmitic acid (19.7 ± 2.5%), and linoleic acid (11.1 ± 2.6%). This outcome summarized that human milk of European mother today typically contain around 35 to 40% saturated fatty acids, 45–50% monounsaturated fatty acids, and approximately 15% PUFA. In addition, [31] also compared the SFA composition between Asian, Moari and Pasific Island and New Zealand European. Another study by [32] also 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%). 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. It was also supported by [4, 29, 32] that palmitic acid (C16:0) contributes approximately 25% of all milk fatty acids and represents the major composition total saturated fatty acid content. As compared the palmitic acid(C16:0) to other to the three region, Asian composition was the highest compared to Moari and Pasific 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. On the other hand, oleic acids were the major composition that represent MUFA [4, 31]. As reported by [31] the composition of oleic acid in Asian was the highest with 1.507 ± 0.344g/100ml compared to 1.225 ± 0.557 in Moari and Pasific Island and 1.294 ± 0.388 in and New Zealand European. [32] also supported the study findings that, after palmitic acids, oleic acids and linoleic acids were the main identified fatty acids in colostrum of Greece mothers.

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(%)		91.34±4.01	93.40±4.35	92.31±5.31	51.7
	81.90-97.70				
MUFA(%)		8.661±4.01	6.60±4.35	7.69±5.31	36.3
	2.3-18.1				

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 months (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

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

Correlation between human milk's SFA and MUFA concentration with macronutrient intake over the lactating period. There was no significant relationship found between human milk SFA and MUFA and dietary micronutrient intake. Only vitamin D, C, and HM SFA were positively correlated. The MUFA in human milk was found to be positively correlated with Vitamin A, E, and K and negatively correlated with the rest of the vitamins. Even though this study did not directly evaluate the concentration of vitamin A and E in human milk, no significant correlation between vitamin A and E and the composition of essential fatty acids has been observed. It was suggested that because vitamin A has a function in modifying oxidative stability in human milk, the total antioxidant activity may be impacted by other mechanisms of action that coexist to reduce the presence of free radicals directly. In another study, despite a low dietary calcium consumption, Ethiopian mothers' milk had a greater calcium concentration. This could be attributed to

increased endogenous cholecalciferol production of Vitamin K because of increased sun exposure. Earlier study also successfully proved that HM α-tocopherol of vitamin E was associated with mothers' total fat and saturated fat dietary intake but the effect of vitamin E intake on HM SFA and total fats was not documented [33].

CONCLUSION

Compares to other macronutrients, fat composition in human milk is known to have a higher fluctuation in terms of component concentrations depending on the geographical region. Diet restrictions during the confinement periods might influence the total fat compositions in human milk. Most of the time, breastfeeding mothers are adhering to traditional and conventional rules of dietary intake that can cause nutritional loss in human milk. Therefore, proper guidelines and scientifically proven dietary plan can be included in mothers' post-partum recovery plan. Apart from regional considerations, maternal nationality and age had a greater impact on the composition of HM fatty acids than other factors such as BMI and mode of delivery.

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REFERENCES

[1] Lentjes M. (2019). The balance between food and dietary supplements in the general population. *The Proceedings of the Nutrition Society*, *78*(1), 97–109. https://doi.org/10.1017/S0029665118002525

[2] Dror, D. K., & Allen, L. H. (2018). Retinol-to-Fat Ratio and Retinol Concentration in Human Milk Show Similar Time Trends and Associations with Maternal Factors at the Population Level: A Systematic Review and Meta-Analysis. *Advances in nutrition (Bethesda, Md.)*, *9*(suppl_1), 332S–346S. https://doi.org/10.1093/advances/nmy021

[3] Oliveira, J. M., Allert, R., & East, C. E. (2016). Vitamin A supplementation for postpartum women. The Cochrane database of systematic reviews, 3, CD005944. https://doi.org/10.1002/14651858.CD005944.pub3

[4] Ballard, O., & Morrow, A. L. (2013). Human milk composition: nutrients and bioactive factors. *Pediatric clinics of North America*, *60*(1), 49–74. https://doi.org/10.1016/j.pcl.2012.10.002

[5] Wagner, C. L., Hulsey, T. C., Fanning, D., Ebeling, M., & Hollis, B. W. (2006). High-dose vitamin D3 supplementation in a cohort of breastfeeding mothers and their infants: a 6-month follow-up pilot study. *Breastfeeding medicine : the official journal of the Academy of Breastfeeding Medicine*, *1*(2), 59–70. https://doi.org/10.1089/bfm.2006.1.59

[6] de Sousa Rebouças, A., Costa Lemos da Silva, A. G., Freitas de Oliveira, A., Thalia Pereira da Silva, L., de Freitas Felgueiras, V., Cruz, M. S., Silbiger, V. N., da Silva Ribeiro, K. D., & Dimenstein, R. (2019). Factors Associated with Increased Alpha-Tocopherol Content in Milk in Response to Maternal Supplementation with 800 IU of Vitamin E. *Nutrients*, *11*(4), 900. https://doi.org/10.3390/nu11040900

[7] Lima, M. S., Dimenstein, R., & Ribeiro, K. D. (2014). Vitamin E concentration in human milk and associated factors: a literature review. *Jornal de pediatria*, *90*(5), 440–448. https://doi.org/10.1016/j.jped.2014.04.006

[8] Greer F.R. (2001) Are Breast-Fed Infants Vitamin K Deficient?. In: Newburg D.S. (eds) Bioactive Components of Human Milk. Advances in Experimental Medicine and Biology, vol 501. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-1371-1_48

[9] Greer FR, Marshall SP, Foley AL, Suttie JW. Improving the vitamin K status of breastfeeding infants with maternal vitamin K supplements. Pediatrics. 1997 Jan;99(1):88-92. doi: 10.1542/peds.99.1.88. PMID: 8989344

[10] Hoppu, U., Rinne, M., Salo-Väänänen, P. *et al.* Vitamin C in breast milk may reduce the risk of atopy in the infant. *Eur J Clin Nutr* **59,** 123–128 (2005). https://doi.org/10.1038/sj.ejcn.1602048

[11] Ahmed, L, Nazrul Islam, M. N. I. Khan, S. Huque, M. Ahsan, Antioxidant Micronutrient Profile (Vitamin E, C, A, Copper, Zinc, Iron) of Colostrum: Association with Maternal Characteristics, *Journal of Tropical Pediatrics*, Volume 50, Issue 6, December 2004, Pages 357–358, https://doi.org/10.1093/tropej/50.6.357

[12] Ares Segura, S., Arena Ansótegui, J., Díaz-Gómez, N. M., & en representación del Comité de Lactancia Materna de la Asociación Española de Pediatría (2016). The importance of maternal nutrition during breastfeeding: Do breastfeeding mothers need nutritional supplements?]. Anales de pediatria (Barcelona, Spain : 2003), 84(6), 347.e1–347.e3477. https://doi.org/10.1016/j.anpedi.2015.07.024

[13] Tijerina-Sáenz A, Innis SM, Kitts DD. Antioxidant capacity of human milk and its association with vitamins A and E and fatty acid composition. Acta Paediatr. 2009 Nov;98(11):1793-8. doi: 10.1111/j.1651-2227.2009.01437.x. PMID: 19807706; PMCID: PMC2773529.

[14] Valentine CJ, Morrow G, Fernandez S, Gulati P, Bartholomew D, Long D, Welty SE, Morrow AL, Rogers LK. Docosahexaenoic Acid and Amino Acid Contents in Pasteurized Donor Milk are Low for Preterm Infants. J Pediatr. 2010 Dec;157(6):906-10. doi: 10.1016/j.jpeds.2010.06.017. Epub 2010 Sep 17. PMID: 20850762

[15] Huffman JC, DuBois CM, Mastromauro CA, Moore SV, Suarez L, Park ER. Positive psychological states and health behaviors in acute coronary syndrome patients: A qualitative study. *J Health Psychol*. 2016;21(6):1026-1036. doi:10.1177/1359105314544135

[16] Newman V. Vitamin A and Breast-Feeding: A Comparison of Data from Developed and Developing Countries. *Food and Nutrition Bulletin*. 1994;15(2):1-16. doi:10.1177/156482659401500201

[17] Fariza Fadzil, Khadijah Shamsuddin, and Sharifa Ezat Wan Puteh.The Journal of Alternative and Complementary Medicine.Jul 2016.503-508.http://doi.org/10.1089/acm.2013.0469

[18] Ding, Y., Indayati, W., Basnet, T.B. *et al.* Dietary intake in lactating mothers in China 2018: report of a survey. *Nutr J* **19,** 72 (2020). https://doi.org/10.1186/s12937-020-00589-x

[19] Mohamad, Maznorila & Ling, Chong. (2016). Food taboos of malay pregnant women attending antenatal check-up at the maternal health clinic in Kuala Lumpur. Integrative Food, Nutrition and Metabolism. 3. 10.15761/IFNM.1000140.

[20] Sawal Hamid, Z.B.; Rajikan, R.; Elias, S.M.; Jamil, N.A. Utilization of a Diet Optimization Model in Ensuring Adequate Intake among Pregnant Women in Selangor, Malaysia. *Int. J. Environ. Res. Public Health* **2019**, *16*, 4720. https://doi.org/10.3390/ijerph16234720

[21] Som, Vanita & Ashok, Purnima & Kameswari, Sri & Rajendran, Ramaswamy & Singh, Rajpreet. (2016). Comparative antioxidant and bioavailability studies of Vitamin C in Phyllanthus emblica Linn. and its combinations with Piper nigrum Linn. and Zingiber officinale Roscoe. Brazilian Journal of Pharmaceutical Sciences. 52. 35-43. 10.1590/S1984-82502016000100004

[22] C.J. Bates, A.M. Prentice, A. Prentice, A.A. Paul, R.G. Whitehead, Seasonal variations in ascorbic acid status and breast milk ascorbic acid levels in rural Gambian women in relation to dietary intake, Transactions of the Royal Society of Tropical Medicine and Hygiene, Volume 76, Issue 3,1982, 341-347, https://doi.org/10.1016/0035-9203(82)90185-7.

[23] Tawfeek HI, Muhyaddin OM, Al-Sanwi HI, Al-Baety N. Effect of Maternal Dietary Vitamin C Intake on the Level of Vitamin C in Breastmilk among Nursing Mothers in Baghdad, Iraq. *Food and Nutrition Bulletin*. 2002;23(3):244-247. doi:10.1177/156482650202300302

[24] Narchi H, Kochiyil J, Zayed R, Abdulrazzak W, Agarwal M. Maternal vitamin D status throughout and after pregnancy. J Obstet Gynaecol. 2010 Feb;30(2):137-42. doi: 10.3109/01443610903315652. PMID: 20143971

[25] Krešić, G., Dujmović, M., L. Mandić, M. i Redžić, D. (2012). Dietary intake of Croatian lactating women. *Croatian journal of food science and technology, 4* (1), 46-53. Preuzeto https://hrcak.srce.hr/84720

[26] Jamil NA, Shahudin NN, Abdul Aziz NS, et al. Knowledge, Attitude and Practice Related to Vitamin D and Its Relationship with Vitamin D Status among Malay Female Office Workers. *Int J Environ Res Public Health*. 2019;16(23):4735. Published 2019 Nov 27. doi:10.3390/ijerph16234735

[27] Nguyen Bao KL, Sandjaja S, Poh BK, Rojroongwasinkul N, Huu CN, Sumedi E, Aini JN, Senaprom S, Deurenberg P, Bragt M, Khouw I; SEANUTS Study Group. The Consumption of Dairy and Its Association with Nutritional Status in the South East Asian Nutrition Surveys (SEANUTS). Nutrients. 2018 Jun 13;10(6):759. doi: 10.3390/nu10060759. PMID: 29899251; PMCID: PMC6024724.

[28] Leong, YH., Rosma, A., Latiff, A.A. *et al.* Exposure assessment and risk characterization of aflatoxin B_1 in Malaysia. *Mycotox Res* **27,** 207–214 (2011). https://doi.org/10.1007/s12550-011-0097-4

[29] Koletzko B. Human Milk Lipids. Ann Nutr Metab. 2016;69 Suppl 2:28-40. doi: 10.1159/000452819. Epub 2017 Jan 20. PMID: 28103608

[30] Bzikowska-Jura A, Czerwonogrodzka-Senczyna A, Jasińska-Melon E, Mojska H, Olędzka G, A, Szostak-Węgierek D. The Concentration of Omega-3 Fatty Acids in Human Milk Is Related to Their Habitual but Not Current Intake. Nutrients. 2019 Jul 12;11(7):1585. doi: 10.3390/nu11071585. PMID: 31336991; PMCID: PMC6683022.

[31] Butts, C.A.; Hedderley, D.I.; Herath, T.D.; Paturi, G.; Glyn-Jones, S.; Wiens, F.; Stahl, B.; Gopal, P. Human Milk Composition and Dietary Intakes of Breastfeeding Women of Different Ethnicity from the Manawatu-Wanganui Region of New Zealand. *Nutrients* **2018**, *10*, 1231. https://doi.org/10.3390/nu10091231

[32] Sinanoglou VJ, Cavouras D, Boutsikou T, Briana DD, Lantzouraki DZ, Paliatsiou S, Volaki P, Bratakos S, Malamitsi-Puchner A, Zoumpoulakis P. Factors affecting human colostrum fatty acid profile: A case study. PLoS One. 2017 Apr 14;12(4):e0175817. doi: 10.1371/journal.pone.0175817. PMID: 28410426; PMCID: PMC5391953.

[33] Antonakou A, Chiou A, Andrikopoulos NK, Bakoula C, Matalas AL. Breast milk tocopherol content during the first six months in exclusively breastfeeding Greek women. Eur J Nutr. 2011 Apr;50(3):195-202. doi: 10.1007/s00394-010-0129-4. Epub 2010 Aug 19. PMID: 20721564.