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Correlation Between Fatty Acid Profile in Plasma and Mature Breast Milk

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Abstract

Long-chain polyunsaturated fatty acids are crucial for infants as they reach mature breast milk through the bloodstream, originating from the digestion and absorption of ingested fat and/or mobilization of maternal fat reserves. Research indicates that blood plasma fatty acids are of dietary origin, suggesting that their content in mature breast milk is dependent on their circulating quantity in the plasma.

Objective: To evaluate the serum fatty acid profile and its influence on the fatty acid content in mature breast milk.

Materials and Methods: Fifty women breastfeeding for a minimum of 30 days participated. Thirty milliliters of mature breast milk were collected from both breasts between 7 to 9 AM using manual extraction, along with 2 milliliters of blood. Fatty acid profiling for mature breast milk and plasma was conducted using gas chromatography, employing an internal standard and a reference pattern (FAME Mix of 37 components) for fatty acid identification.

Results: Minimal correlation was found, such as in linoleic acid, cis-docosahexaenoic acid, and cis-eicosapentaenoic acid; low correlation was observed with palmitoleic acid, while there was no correlation with the rest of the fatty acids. ***Conclusions:*** There was no significant correlation between plasma fatty acid content and mature breast milk. Its content appears to be primarily driven by the baby's nutritional needs and is directly related to a physiological process of the mammary gland.

Keywords: Human milk; fatty acids; blood plasma; data correlation

Introduction

In recent years, there has been increasing recognition of the importance of promoting and supporting breastfeeding from birth to at least 2 years of age to optimize growth and development, including neurological development [1]. Human milk (HM), due to its nutritional, immunological, and bacteriological advantages, is considered the ideal food for infants [1]. Its production involves complex biological processes within the mammary gland, comprising various nutrients and substances [1]. The components integrated into HM undergo diverse changes according to lactation stages, and some authors suggest that these components might also vary based on the mother's nutritional status [2]. The production of mature human milk (MHM) begins approximately 15 days after delivery, characterized by a stable nutritional composition (87% liquid, with varying amounts of other macronutrients) [1].

Among the primary macronutrients in MHM, lipids are fundamental and present as emulsified globules in the aqueous phase. Their content can range from 3.5 to 4.5%, comprising at least 7 forms of fatty acids, influenced by the quantities of dietary fatty acids ingested by the mother (clarifying that this affects the final fatty acid composition rather than the total fat content) [3].

HM provides essential long-chain fatty acids, which are not synthesized de novo via endogenous biochemical processes in the human body. This implies that these fatty acids must be obtained through the maternal diet [1] to be passed on to the baby through HM. Therefore, some authors emphasize the importance of maternal nutrition to ensure the provision of these fatty acids [4].

Long-chain polyunsaturated fatty acids' (LC PUFAs) function is believed to facilitate the movement of proteins on and within the lipid bilayer, promoting greater fluidity in cellular membrane activity [4]. Fatty acids in HM derive from endogenous synthesis in the mammary gland and absorption from maternal plasma. Both mammary gland fatty acid synthesis and fatty acids available in maternal plasma are influenced by maternal nutrition [4]. They are formed from smaller chain precursor molecules through elongation and desaturation processes. Arachidonic acid (AA) and docosahexaenoic acid (DHA) are synthesized from 18-carbon fatty acids obtained from the diet: linoleic acid (LA) and α-linolenic acid (ALA), respectively, with LA being the most abundant source of LC PUFAs in the diet [5]. As essential fatty acids (EFAs), their synthesis directly depends on the concentration of their respective precursors, necessitating a properly balanced dietary intake, as mentioned earlier [5]. EFAs are necessary for normal growth, development, and various physiological functions [5]. Disruptions in visual and nervous tissue functionality have been observed in children who did not receive adequate PUFA intake during gestation and early infancy [6].

DHA, along with other PUFAs, accounts for one-third of all lipids in the gray matter of the brain, considered crucial for nervous tissue and synaptic membrane development and playing indispensable roles in normal neuronal function. These fatty acids form part of the structure of the retina's cones and rods, facilitating proper visual tissue formation [6].

Given the significance of PUFAs for the proper development of breastfeeding infants, understanding how various variables can affect the HM fatty acid content is essential. One such variable is the maternal lipid profile. The concentrations of PUFAs in maternal serum can influence their synthesis and conversion, thereby impacting their availability and content in HM [6].

The sources of HM fatty acids are lipids circulating in the blood, derived from the digestion and absorption of ingested fat and the mobilization of adipose tissue fatty acids [7]. It is asserted that the majority of plasma-derived fatty acids originate from the diet (> 80%) [7]. This circulation of fatty acids in plasma similarly influences their content in HM. Researchers suggest that this quantity may differ based on lactation stage, milk production, and diet type, indicating that more than half of the milk's fatty acids are derived from blood plasma lipids [7]. To date, it has been considered that the balance or increase of HM fatty acids is induced by maternal diet [7]. Numerous studies have described differences in human milk fatty acids and dietary interventions, strongly suggesting that maternal lipid consumption is the most significant factor contributing to the variability of human milk fatty acids [7-11].

This study aimed to evaluate how the serum fatty acid profile may alter the fatty acid content in HM, offering insights into nutritional strategies to promote the consumption of foods rich in these nutrients and stressing the importance of monitoring the nutritional status of lactating mothers in terms of plasma lipid profile measurements.

Materials and Methods

This is a descriptive, cross-sectional, and quantitative study conducted at San Vicente Foundation Hospital (HSVPF) in Medellín, Antioquia, Colombia, between 2017 and 2018. A total of 50 women aged between 19 and 45 participated, predominantly between 19 and 35 years old, with a minimum breastfeeding duration of 30 days and a maximum period of 21 months. The majority of women (44 participants) had been breastfeeding for less than 12 months. Among the total participating mothers, 46% were employed, 44% were housewives, and 12% were self-employed.

Regarding the mothers' education levels, 26% had a high school degree, another 26% had some technology education, 28% had a

university level of education, and 20% had a postgraduate degree. In terms of socioeconomic status, 34% belonged to the low strata, 46% to the middle stratum, and the remaining 20% to the upper strata.

The study received approval from the Bioethics Committee of the University Research Headquarters at the University of Antioquia, as well as from the San Vicente de Paul University Hospital Foundation. Ethical considerations encompassed adherence to the Declaration of Helsinki and Colombian national regulations [12, 13], always ensuring the informed consent of the participating mothers.

Inclusion criteria

The study included breastfeeding mothers, irrespective of smoking or alcohol consumption, dietary patterns, mode of delivery (normal or cesarean section), term or preterm childbirth, and socioeconomic stratum.

Exclusion criteria

Mothers with pathologies affecting the fatty acid profile (diabetes mellitus, thyroid disease, hypertension, metabolic syndrome, liver or kidney disorders, or under therapeutic control, HIV), those using contraindicated medications during breastfeeding, pregnant women, consumers of psychoactive substances, and those engaged in high-performance sports were excluded from the study.

Breast milk samples

Mothers donated 30 mL of low-fat mature milk (LMM) per breast through manual expression, resulting in a total of 60 mL per subject, collected between 7 and 9 AM. The samples were placed in tubes with screw caps, identified, and transported to the laboratory in a refrigerator at room temperature. Subsequently, they were frozen at -22 °C until the time of analysis.

Extraction of Maternal Blood Samples

The extraction was performed after fasting via peripheral puncture, extracting 8 to 10 cc of blood into adult vials per lactating woman while adhering to the required biosafety standards for this procedure. These samples were labeled, refrigerated at 4°C, and transported to the laboratory in a dedicated container for biological risk samples.

Both blood and mature human milk (MHM) samples were analyzed using gas chromatography. An Agilent 6890N gas chromatograph with a flame ionization detector (FID) was employed, using a TR-CN100 capillary column (60 m x 250 um x 0.20 um ID), split/splitless injector at a 100:1 ratio, 1.0 uL injection volume, injector temperature of 260 ºC, and a programmed temperature: 90 ºC for 7 min, increasing by 5ºC per minute until reaching 240 ºC, maintained for 15 min, with a detector temperature of 300ºC, and helium carrier gas at a flow rate of 1.1 mL/min. Fatty acid identification was conducted by comparing sample retention times with those of a reference standard (FAME Mix of 37 components: C4-C24, Supelco). Quantification of methyl esters for each fraction was performed by area normalization and carried out in triplicate [15].

Lipid extraction from both samples followed the Folch method, suitable for biological samples. This method involves adding 40 µL of internal standard (tridecanoic acid, 50 mg/mL) to 100 µL of the sample in a Pyrex tube with a screw cap. Subsequently, 2 mL of chloroform/methanol (2:1) was added for lipid extraction and protein precipitation, vortexed for 1 minute, followed by the addition of 1 mL of saturated sodium chloride, vortexed again for 1 minute. After centrifugation at 3400 rpm for 7 minutes, the organic phase was carefully aspirated using a Pasteur pipette and transferred to another Pyrex tube, while the aqueous phase underwent two more extraction cycles, each with the addition of 2 mL of chloroform. The organic phases were combined and dried in a dry bath at 90ºC [15].

To solubilize the present fatty acids, 1 mL of hexane was added to the dry sample, followed by the addition of 1 mL of 20% BF3 (Boron trifluoride) in methanol for methylation. The mixture was placed in a water bath at 80 - 90 ºC for 1 hour, then cooled to room temperature. Subsequently, 5 mL of saturated sodium chloride solution was added, allowing the phases to separate. The upper phase (organic phase) was collected, transferred to an Eppendorf tube containing a pinch of anhydrous sodium sulfate, 200 µL were with-

drawn and placed into a vial for gas chromatography analysis [15]. Later, methyl esters from each fraction were quantified using the chromatograph.

Classification of Fatty Acids in Plasma

After quantifying the different fatty acids, a search was conducted across various bibliographic databases (MEDLINE (2018 - 2020), Embase (2018 - 2020), EBSCO (2018 - 2020), Web of Science (2018 - 2020), Servier (2018 - 2020), Medes (2018 - 2020), and Dialnet (2018 - 2020)) and the World Health Organization's International Clinical Trials Registry Platform (WHO ICTRP) to retrieve scientific bases for classifying the identified fatty acids as normal, high, or low. Keywords used included: Serum fatty acids, comparison of blood serum, blood serum in humans, blood fatty acids.

No scientific information referencing a classification for these fatty acids was found. Therefore, it was considered to establish a classification based on the data reported in this study summarized in Table 1, presenting the minimum and maximum values of the studied fatty acids in the blood serum of the 50 lactating mothers.

Fatty acid	*Minimal*	*Normal*	*Maximum*
Lauric acid (C12:0)	< 0.0020	0,0021 - 0,0029	>0.0030
Myristic Acid (C14:0)	<0.0030	0,0031 - 0,0034	>0.0035
Palmitic acid (C16:0)	<0.09	0,10 - 0,09	>0.1
Palmitoleic acid (C16:1)	<0.03	0,02 - 0,05	>0.06
Heptadecanoic acid (C17:0)	<0.001	0,0001 - 0,002	>0.003
Stearic acid (C18:0)	<0.09	0,091 - 0,99	>0.1
Oleic acid (C18:1n9c)	<0.1	0,11 - 0,19	>0.2
Linoleic acid (C18:2n6c)	<0.05	0,06 - 0,09	>0.1
Cis-8,11,14-eicosatrienoic acid (S20:3n6)	<0.010	0,011 - 0,14	>0.015
Arachidonic acid (C20:4n6)	<0.020	0,021 - 0,029	>0.030
Cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n3)	<0.001	0,011 - 0,029	>0.003
CIS-4,7,10,13,16,19-Acid docosahexaenoic (C22:6n3)	<0.020	0,021 - 0,029	>0.030
Total saturated (%)	<0.09	0,091 - 0,19	>0.2
Total monounsaturated (%)	<0.1	0,11 - 0,10	>0.2
Total polyunsaturated (%)	<0.09	0,091 - 0,99	>0.1
Total Fat (%)	<0.5	0,49 - 0,59	>0.6

Table 1: Classification of whey fatty acids according to the results of laboratory.

Statistical Analysis

The collected data were analyzed using SPSS version 25. Descriptive statistics were applied to the fatty acid content reported in plasma and mature human milk (means and deviations). Correlation analysis assessed the normality of continuous variables using the Kolmogorov-Smirnov test. Subsequently, the correlation between the fatty acid content in mature human milk and plasma was evaluated. Given that the correlation data for serum and mature human milk fatty acids did not follow a normal distribution curve, Spearman's correlation was employed to establish a linear relationship between the two variables of interest, as tabulated in Table 4.

Results

In Table 2, the fatty acid content in grams in the mothers' serum is presented, revealing that the highest content is for oleic acid (0.1669), followed by palmitic (0.1267), stearic (0.0923), linoleic (0.0680), docosahexaenoic (0.0292), and lower contents are found

for eicosatrienoic (0.0075), palmitoleic (0.0031), myristic (0.0027), lauric (0.0021), heptadecanoic (0.0018), with the lowest content of all being eicosapentaenoic (0.0005).

Fatty acid	*Minimal*	*Normal*	*Maximum*
Lauric acid (C12:0)	< 0.0020	0,0021 - 0,0029	>0.0030
Myristic Acid (C14:0)	<0.0030	0,0031 - 0,0034	>0.0035
Palmitic acid (C16:0)	<0.09	0,10 - 0,09	>0.1
Palmitoleic acid (C16:1)	<0.03	0,02 - 0,05	>0.06
Heptadecanoic acid (C17:0)	<0.001	0,0001 - 0,002	>0.003
Stearic acid (C18:0)	<0.09	0,091 - 0,99	>0.1
Oleic acid (C18:1n9c)	<0.1	0,11 - 0,19	>0.2
Linoleic acid (C18:2n6c)	<0.05	0,06 - 0,09	>0.1
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Cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n3)	<0.001	0,011 - 0,029	>0.003
CIS-4,7,10,13,16,19-Acid docosahexaenoic (C22:6n3)	<0.020	0,021 - 0,029	>0.030
Total saturated (%)	<0.09	0,091 - 0,19	>0.2
Total monounsaturated (%)	<0.1	0,11 - 0,10	>0.2
Total polyunsaturated (%)	<0.09	0,091 - 0,99	>0.1
Total Fat (%)	<0.5	0,49 - 0,59	>0.6

Table 2: Average in grams of fatty acids present in serum of the mothers.

Table 3. displays the established values in the minimum, normal, and high ranges of fatty acids in the plasma of the mothers participating in this study. This classification indicates that 100% of the mothers are below the reference content in blood serum for palmitoleic acid, followed by eicosatrienoic with 90% of mothers, eicosapentaenoic with 80%, myristic with 69.2%, and stearic acid with 54%. These are the fatty acids with the lowest content in the mothers' blood serum, as more than half of them have low concentrations in these fatty acids.

Nutrient	*Mothers with low plasma FA content (%)*	*Mothers with adequate plasma FA content (%)*	*Mothers with high plasma FA content (%)*
Lauric acid (C12:0)	46,2	44,2	9,6
Myristic Acid (C14:0)	69,2	13,5	17,3
Palmitic acid (C16:0)	18	6	76
Palmitoleic acid (C16:1)	100	0	0
Heptadecanoic acid(C17:0)	34	52	14
Stearic acid (C18:0)	54	6	40
Oleic acid (C18:1n9c)	4	82	14
Linoleic acid (C18:2n6c)	18	76	6
Cis-8,11,14-eicosatrienoic acid (C20:3n6)	90	10	0
(ARA) Arachidonic acid (C20:4n6)	46	38	16

(EPA) Eicosapentaenoic acid (C20:5n3)	80	20	0
(DHA) Cis- acid docosahexaenoic (C22:6n3)	22	24	54
Total Saturated	2	34	64
Total Monounsaturated	4	78	18
Total Polyunsaturated	84	16	0
Total Fat	52	24	24

Table 3: Classification according to the content reported by the food and human nutrition.

Fatty acids in the serum that exhibited normal contents greater than 30% in the mothers' plasma were oleic (82%), monounsaturated (78%), linoleic (76%), heptadecanoic (52%), lauric (42.2%), arachidonic (38%), and total saturated fat (34%).

In 30% of participating mothers, values above the reference range were found for certain fatty acids such as palmitic acid (76%), total saturated fat (64%), cis-docosahexaenoic acid (DHA) (54%), and stearic acid (40%).

Table 4 shows the correlation between the fatty acid content in serum and its content in mature human milk (MHM). Some fatty acids exhibit low correlation, such as linoleic acid, cis-docosahexaenoic acid (DHA), and cis-eicosapentaenoic acid (EPA). There is a low correlation with palmitoleic acid, and no correlation is observed with the rest of the fatty acids. The results of bilateral significance indicate statistically significant differences between the correlations, except for total polyunsaturated fatty acids (PUFAs), where the correlation does not show statistically significant differences.

Fatty acids	***Average serum g/100mL***	***Average in breast milk g/100mL***	***Bilateral Significance (spearman)***	***Ratio coefficient (spearman)***
Myristic Acid (C14:0)	0,003	0,29	0,342	-0,137
Palmitic acid (C16:0)	0,127	1,093	0,445	-0,11
Acid heptadecanoic (C17:0)	0,002	0,014	0,113	-0,227
Stearic acid (C18:0)	0,092	0,319	0,522	-0,093
Total, Saturated	0,226	2,049	0,482	-0,102
Palmitoleic acid (C16:1)	0,003	0,1	0,148	0,207
Oleic acid (C18:1n9c)	0,167	1,469	0,449	-0,11
Overall, monounsaturated	0,174	1,595	0,434	-0,113
Linoleic acid (C18:2n6c)	0,068	0,677	0,406	0,12
(ARA) Arachidonic acid (S20:4n6)	0,024	0,023	0,95	-0,009
(EPA) Cis-eicosapentaenoic acid (C20:5n3)	0,000	0,002	0,292	0,152
(DHA) Cis-docosahexaenoic acid (C22:6n3)	0,029	0,01	0,933	0,012
Total polyunsaturates	0,129	0,138	0	,980**
Total fat g/100ml	0,519	5,672	0,424	-0,116

Table 4: Correlation of fatty acids in whey and milk mature mother.

Discussion

Due to the absence of studies assessing the fatty acid content in lactating women's plasma, a classification of the content in this matrix was developed based on the minimum, normal, and high contents found in the plasma of donor mothers. This aimed to analyze the content of fatty acids in the mothers' plasma and determine the adequacy of these values. A comparison was made with the results from other studies, such as Bermúdez JA [16], which reported fatty acid values in obese adolescents as AGS 0.0048g, AGM 0.0188g, and AGPI 0.03304g, lower than those found in this study. This suggests a non-comparative relationship between these two life stages, as the importance of fatty acids for MHM production is elevated in lactating mothers, while reported values in adolescents are used for other vital processes.

Studies like Chulei R, Xiaofang L, et al. [17] report variations in fatty acid content in MHM depending on the study population. Other authors like Marín M, Kovács A, and Ortega FD [18-20] indicate that factors such as nutritional status, maternal diet, and lactation duration also influence fatty acid content in this milk. In this study, the total fat content was higher at 5.67g/100mL compared to other studies like Kim H, et al. and Ojo-okunola A, et al. [10, 11], which reported an average content of 3.7g/100mL. Similarly, saturated fatty acid content in MHM in this study exhibited a higher lipid content, averaging at 2.049g/100mL, in comparison to studies like Galindo Gómez, which may be associated with high carbohydrate and low-fat diets [21]. This was followed by monounsaturated fatty acids at 1.595g/100mL and lastly polyunsaturated fatty acids at 0.138g/100mL, with similar values reported in other studies [21, 22].

According to studies by Galindo Gómez and Alvarez de Acosta [21, 22], the most prevalent fatty acids found in MHM are oleic acid, palmitic acid, and linoleic acid, which align with the results of fatty acids reported in this study. The case of stearic acid also showed a similar value to those reported by other studies [23].

Furthermore, the content of fatty acids (Table 4) such as Myristic 0.29g/100mL, Arachidonic 0.023g/100mL, Eicosapentaenoic 0.002g/100mL, and Docosahexaenoic 0.01g/100mL were found in low quantities, as reported in the investigations of Gaete G. M, et al., Innis SM, and Kim H, et al [8, 10].

Relating the content of saturated fatty acids (SFAs), medium-chain, and short-chain fatty acids in MHM (Table 4), the SFA content is close to the average in Malaysian women at 0.25g/100mL [24], while for palmitic acid in both studies, it holds the highest value. The linoleic fatty acid content in both MHM and serum is similar to that reported in studies, which may be related to what Duran AS, Masson SL [23] express, stating that the presence of this fatty acid in MHM depends on the maternal dietary intake, a factor not corroborated in this research due to the absence of food consumption analysis among the participating mothers.

According to Smoczyński [25], PUFAs in MHM originate solely from the diet, being esterified into triglycerides until reaching the mammary gland via maternal serum in the form of chylomicrons. When not sourced from the diet, triglycerides are transported from the liver to the mammary glands in the form of very low-density lipoproteins (VLDL), they can also be transported by albumin and in smaller quantities by passive diffusion, emphasizing the need for these to be ingested in the human diet from foods.

EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic Acid) are synthesized by the body through processes of elongation and desaturation of the essential fatty acid α-linolenic acid (ALA), and their ratio depends entirely on the conversion rate [8]. In this study, the content of EPA and DHA in the serum and MHM of participating mothers is similar in both matrices, possibly due to their derivation from α-linolenic acid, which is found in similar quantities in both serum 0.068g/100mL and MHM 0.677g/100mL.

It is also noteworthy that PUFAs have been extensively studied for their biological functions in better development, growth, and particularly in immune function during the first two years of life [23]. Table 4 displays the positive correlation between their content in maternal serum and content in MHM (0.980), with a serum content of 0.129g/100mL and MHM content of 0.138g/100mL. This finding is reasonable and considers the capacity to meet the infant's nutritional needs with this ideal food. Additionally, oleic acid provides the necessary liquidity for the formation, transport, and metabolism of MHM fat globules [5]. Serum content may primarily derive from the mothers' diet but can also be synthesized via de novo pathway [25].

Finally, according to Kathleen Copp [26], a mother's milk possesses excellent nutritional value because the mother's body always prioritizes the baby's needs. Hence, most fatty acids are present in the milk at an adequate and stable level, at the expense of maternal reserves. This concept is based on the condition that if the mother does not ingest the nutrients in her diet, she can obtain them from her fat tissue reserves as long as they are adequate; otherwise, the mother may experience emaciation or nutrient deficiency in MHM [1].

The low or non-correlation between fatty acid content in serum and MHM denotes that the content in the latter is more conditioned by the infant's nutritional need than external factors. Even variations in the mother's diet can change the profile of fatty acids in the serum without affecting the fatty acid content in MHM.

This study aimed to assess the fatty acid (FA) content in the plasma of lactating women. While no prior studies specifically evaluated this content in lactating women, a classification of plasma GA content was developed based on data from donor mothers. There was no significant correlation between plasma fatty acid content and mature breast milk. The content appears to be primarily influenced by the nutritional needs of the infant and is directly related to a physiological process in the mammary gland.

In summary, our findings indicate that the fatty acid content in the plasma and breast milk of breastfeeding women varies depending on factors such as maternal diet, nutritional status, and breastfeeding duration. Saturated, monounsaturated, and polyunsaturated fatty acids are present in breast milk at adequate levels to meet the infant's nutritional needs. However, it is essential to note that the fatty acid content in whey does not directly correlate with the content in breast milk, suggesting that the latter is more influenced by the nutritional needs of the infant than external factors.

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