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Research Article

Correlation between Plasma Fatty Acid Profile of Lactating Women and Content in Mature Breast Milk

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Abstract

Introduction: long-chain polyunsaturated fatty acids (G.A) are of importance for the infant and that they reach mature breast milk (LMM) through blood circulation, from the digestion and absorption of ingested fat and/or from the mobilization of the mother's fat reserves; research affirms that most of the G.A. in blood plasma are of dietary origin, thus indicating that the content of these in the LMM is dependent on the amount circulating in the plasma.

Objectives: To evaluate the profile of G at the serum level and its influence on G content in the LMM.

Materials and Methods: The sample consisted of 50 women with at least 30 days of breastfeeding. 30 mL of LMM, collected from both breasts, were extracted from 7 to 9 a.m. by hand extraction and 2 mL of blood. The fatty acid profile for LM and plasma was done by gas chromatography, internal standard was used, and a reference standard (37-component FAME Mix) was used for the identification of FAs.

Results: Minimal correlation was found, such as linoleic acid, cis-docosahexaenoic acid and cis-eicosapentaenoic acid; low correlation with palmitoleic acid and in the rest of the A.G there is no correlation. Conclusions. There was no significant correlation between plasma G.A. content and LMM, its content seems to be mainly given by the nutritional needs of the baby and is directly related to a physiological process of the mammary gland.

Keywords: Breast Milk; Fatty Acids; Mammary Gland; Breastfeeding Woman; Baby; Plasma

Introduction

The recommendations of the WHO and UNICEF for optimal infant feeding are: "exclusive breastfeeding during the first six months of life (180 days) and initiate adequate and safe complementary feeding, from six months of age, maintaining breastfeeding until two years of age or more" [1].

Recent years have seen a growing recognition of the importance of promoting and supporting human milk feeding to optimize child growth and development, including neurological development [2].

Breast milk, due to its nutritional, immunological and bacteriological advantages, has been considered for the breastfeeding child as the ideal food [3]. It is a liquid resulting from complex biological processes, which is produced by the mammary gland, made up of various nutrients and substances, making it the complete and suitable food for a child to be fed during its first six months of life [4]. These elements that make up Mature Breast Milk (LMM), undergo various changes, according to the stages of lactation and can even change according to the mother's condition [5]. The production of LMM begins around 15 days after calving, it is characterized by having a stable composition of its components, making a contribution of 87% of fluids, 670 to 700 kcal/L, mostly from carbohydrates and fats, which are mostly in the form of lactose and fatty acids. respectively [4].

In LMM, lipids occur as emulsified globules in the aqueous phase. In general, the fat content of human milk can vary between 3.5 to 4.5%, with at least 7 forms of fatty acids, which would be determined by the amounts of individual fatty acids in the diet, with dietary changes in the mother being the factor that is most expected to affect the fatty acid composition in the final mature milk (clarifying that the final fatty acid composition is affected, but not the total amount of fat) [6].

Human milk provides essential or indispensable long-chain fatty acids, so called because they cannot be synthesized *again* through endogenous biochemical processes in the human body, which implies that these fatty acids must necessarily be obtained through the maternal diet [5]. Just as the mother receives essential fatty acids from food, the newborn must receive them from its mother through breast milk, which is why the mother's diet is very important to ensure the supply of these fatty acids [7].

It is estimated that the function of long-chain fatty acids is to allow the movement of proteins on the surface and within the lipid bilayer, which makes the activity of cell membranes occur more fluidly [8]. The fatty acids in human milk are derived from endogenous synthesis in the mammary gland and absorption from maternal plasma. Both the synthesis of fatty acids in the mammary gland and the fatty acids available in the maternal plasma are influenced by maternal nutrition [2]. These are formed from precursors of smaller chain size, through elongation and desaturation processes. Arachidonic acid (AA) and docosahexaenoic acid (DHA) are synthesized from the 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 PUFA in the diet [9]. By no metabolic route, polyunsaturated fatty acids can be converted from each other, which implies that their synthesis depends directly on the concentration of the respective precursors, being of essential importance a correctly balanced dietary intake, as mentioned above [7,8]. Essential fatty acids are necessary for normal growth and development, as well as for various physiological functions [10]. Alterations in the functionality of visual and nervous tissues have been observed in those children who have not received an adequate supply of polyunsaturated fatty acids during pregnancy and in the first months of life [8].

DHA, along with other long-chain polyunsaturated fatty acids, account for a third of all lipids in the brain's gray matter, and are considered important for the development of nervous tissue and synaptic membranes, and play an indispensable role in normal neuronal function. These fatty acids are part of the structure of the retinal cones and rods, which allows the proper formation of visual tissue [8].

Given the importance of polyunsaturated fatty acids for the proper development of infant children, it is necessary to know how the various variables can be related to the content of fatty acids of the LMM, one of them is the lipid profile of the mothers, since it has been previously mentioned that the concentrations of these in the maternal blood serum, can condition their synthesis and conversion, decreasing or increasing their availability and content in breast milk [11].

The sources of fatty acids in mature breast milk are the lipids circulating in the blood, derived from the digestion and absorption of ingested fat and the mobilization of fatty acids from adipose tissue [6]. It is claimed that most fatty acids derived from blood plasma are of dietary origin (> 80%) [12]. This circulation of fatty acids in plasma also infers the content of these in the LMM, even researchers say that this amount can differ according to lactation status, milk production and type of diet, this means that more than half of the fatty acids in milk are derived from blood plasma lipids [13].

To date, with respect to human milk fatty acids, they have considered the fatty acid balance, or the degree to which diet-induced increases in the consumption of specific fatty acids alter the content of other fatty acids in the LM [14,15]. Since then, numerous articles have described differences in human milk fatty acids and dietary interventions that combine to give strong evidence that maternal lipid intake is the most important factor contributing to human milk fatty acid variability [2,15-19].

The present research proposal is based on the possibility of evaluating how the fatty acid profile at the serum level can alter the fatty acid content in mature breast milk, allowing a knowledge focused on the development of nutritional strategies for the improvement in the consumption of foods source of these nutrients, as well as the identification of the importance of control and monitoring of the nutritional status of the breastfeeding mother in terms of of measurements of the lipid profile in plasma, therefore it is intended to evaluate the profile of fatty acids in plasma and its relationship with their content in mature breast milk.

Materials and Methods

Participants included 50 women, aged between 19 and 45 years, who had been breastfeeding their children for at least 1 month, who signed written consent for participation in the study, taking into account that the procedures used were carried out in accordance with internationally required ethical standards (Declaration of Helsinki and Ezequiel Emmanuel ethical requirements) and national (Resolution 8430 of 1993). for human studies. According to national regulations, this study was classified as "risk-free", since no direct intervention was carried out on the participants [20].

Inclusion criteria

Mothers who smoke or not, consume alcohol or not, omnivores, vegetarians or vegans, with normal delivery or cesarean section, with children born at term or prematurely and of socioeconomic status between 1 and 6.

Exclusion criterio

Mothers with pathologies that could affect the fatty acid profile (diabetes mellitus, thyroid disease, hypertension, metabolic syndrome, liver or kidney disorders or under any therapeutic control or with HIV), mothers who consumed contraindicated medications during breastfeeding, who were not simultaneously in the process of pregnancy, mothers with consumption of psychoactive substances and those who practice high-performance sports.

Breast milk simples

30 ml of LMM per mother were collected from both breasts, from 7 to 9 AM, by manual extraction, in 50 ml falcon tubes labeled with the mother's code, date of collection, time, age of the mother and baby; after manual homogenization; they were transported to the Food and Human Nutrition Laboratory (LANH) located in tower 1, laboratory 413 of the university research headquarters (SIU) of the University of Antioquia, in a high-density polyethylene refrigerator at room temperature where they were subsequently frozen at -22°C until processing.

Extraction of blood samples

The blood samples, as well as the breast milk samples, were analyzed with a gas chromatography detection test, in which the amount of each fatty acid present expressed in terms of area normalization percentages was quantified.

The methyl-esters of each fraction were analyzed by gas chromatography, using an Agilent Agilent 6890N chromatograph with flame ionization detector (FID), capillary column TR-CN100 60 m x 250 um x 0.20 um ID, split/splitless injector with a 100:1 ratio, injection volume 1.0 uL, injector temperature 260 ºC, Program temperature, 90ºC x 7 min, increasing a rate from 5ºC to 240 ºC and maintaining it for 15 min, detector temperature 300ºC, He entrainment gas at a flow of 1.1 mL/min. To identify the FAs, the retention times of the samples were compared with those of a reference standard (FAME Mix of 37 components: C4-C24, Supelco).

*The quantification of the lipid profile was done in the same way and with the same methodology as for the quantification of fatty acids in mature breast milk.

Fatty acid analysis in biological samples

Lipid extraction FOLCH method

Lipid extraction by the Folch method consists of: 40 µL of internal standard (triundecanoic acid, 50 mg/mL) is added to 100 µL of sample in a pyrex tube with a screw cap. 2 mL of chloroform/methanol (2:1) is added for the extraction of lipids and precipitation of the protein, it is mixed in vortex for 1 min, 1 mL of saturated sodium chloride is added, it is mixed again in vortex for 1 min. For the separation of the organic and aqueous phases, the tubes are centrifuged at 3400 rpm for 7 minutes. The organic phase is carefully aspirated with a pasteur pipette and transferred to another pyrex tube, while with the aqueous phase the extraction process is repeated 2 more times, adding 2 mL of chloroform. The organic phases are gathered and dried in a dry bath at 90ºC [21].

Fatty acid fraction (FFA) analysis

1 mL of hexane is added to the dry tube in order to solubilize the fatty acids present and proceed with the methylation of the fatty acids, where 1 mL of BF3 is added in 20% methanol, the tube is covered; it is mixed and put in a water bath at 80-90 ºC for 1 hour, after this time, the tube is allowed to cool to room temperature, 5 mL of saturated sodium chloride solution is added, the phases are allowed to separate, the upper phase (organic phase) is collected and transferred to an eppendorf tube, which contains a pinch of anhydrous sodium sulfate, 200 µL are taken and taken to a vial for gas chromatography análisis [22].

Gas chromatography (GC) analysis of FFA

The methyl-esters of each fraction will be analyzed by gas chromatography, using an Agilent Agilent 6890N chromatograph with flame ionization detector (FID), capillary column TR-CN100 60 m x 250 um x 0.20 um ID, split/splitless injector with a 100:1 ratio, injection volume 1.0 uL, injector temperature 260 ºC, Program temperature, 90 ºC x 7 min, increasing a rate from 5ºC to 240 ºC and maintaining it for 15 min, detector temperature 300ºC, He entrainment gas at a flow rate of 1.1 mL/min. For the identification of the FAs, the retention times of the samples will be compared with those of a reference standard (FAME Mix of 37 components: C4-C24, Supelco). The quantification will be carried out by normalization of areas. It was performed in triplicate [22-24].

Classification of plasma fatty acids

We searched MEDLINE (2014-2018), Embase (2014-2018), EBSCO (2014-2018), web of Science (2014-2018), El servier (2014-2018), Medes (2014-2018) and Dialnet (2014-2018) and the World Health Organization's International Clinical Trials Registry (WHO ICTRO) platform. with the following keywords: Serum fatty acids, blood serum compassion, human blood serum, blood fatty acids; to find scientific bases that would allow us a reference to classify fatty acids into normal, high and low.

Despite this, no scientific information was found that refers to a classification of these fatty acids, therefore, it was considered to make a classification from the data reported from the Food and Human Nutrition laboratory. Summarized in table 1, which presents the minimum and maximum values of the fatty acids studied in the blood serum of the 50 mothers.

	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
Ácido oleico (C18:1n9c)	<0,1	0,11 - 0,19	>0,2
Ácido linoleico (C18:2n6c)	<0,05	0,06 - 0,09	>0,1
Cis-8,11,14-eicosatrienoic acid (C20:3n6)	<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-docosahexaenoic acid (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 poly saturate (%)	<0,09	0,091 - 0,99	>0,1
Total grasa (%)	<0,5	0,49 - 0,59	>0,6

Table 1: Classification of Serum Fatty Acids Based on Laboratory Results.

Statistical análisis

The data collected were tabulated in the Microsoft Excel 2013 spreadsheet and analyzed under the SPSS version 25 program. Initially, a descriptive statistic of the content of fatty acids reported in both plasma and mature breast milk (averages and deviations) was performed. In the correlation process, the statistical analysis began with the evaluation of the normality of the continuous variables by means of the kolmogorov-smirnov test and finally the correlation between the content of fatty acids in the LM and the content of these in plasma was evaluated. Since the correlation data of whey fatty acids and mature breast milk are not distributed according to a normal curve, Spearman's correlation was the most appropriate to establish a degree of linear relationship between the two variables of interest tabulated in table 4.

Results

The amount of each fatty acid present in the mothers' serum shows that the highest content is for oleic acid (0.1669), followed by palmitic acid (0.1267), stearic acid (0.0923), linoleic acid (0.0680), docosahexaenoic acid (0.0292) and the lowest content is eicosatrienoic acid (0.0075), palmitoleic acid (0.0031), myristic acid (0.0027), lauric acid (0.0021), heptadecanoic acid (0.0018) and the lowest content of all is eicosapentaenoic acid (0.0005). table 2.

Fatty acid	Average in grams	Deviation
Lauric acid (C12:0)	0,0021	0,001
Myristic Acid (C14:0)	0,0027	0,001
Palmitic acid (C16:0)	0,1267	0,040
-Palmitoleic acid (C16:1)	0,0031	0,001
Heptadecanoic acid (C17:0)	0,0018	0,001
Stearic acid (C18:0)	0,0923	0,028
Ácido oleico (C18:1n9c)	0,1669	0,059
Ácido linoleico (C18:2n6c)	0,0680	0,022
Cis-8,11,14-eicosatrienoic acid (C20:3n6)	0,0075	0,002
(ARA) Arachidonic acid (C20:4n6)	0,0237	0,010
(EPA) Eicosapentaenoic acid (C20:5n3)	0,0005	0,001
(DHA) Cis-docosahexaenoic acid (C22:6N3)	0,0292	0,015
Total saturated	0,2257	0,070
Total monounsaturated	0,1699	0,059
Total poly saturada	0,1286	0,043
Total Fat	0,5196	0,167

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

Table 3 shows that according to the values established in minimum, normal and maximum for the study, 100% of the mothers are below the reference blood serum content of palmitoleic, followed by eicosatrienoic acid with 90% of the mothers, eicosapentaenoic acid in 80% of the mothers, myristic acid in 69.2% and stearic acid in 54%. the above being the fatty acids with the lowest presence in the blood serum of the mothers, since more than half of them are found with low concentrations of the aforementioned fatty acids.

With a percentage of sows greater than 30%, the fatty acids classified as normal amounts in serum are: oleic (82%), followed by total monounsaturated (78%), linoleic (76%), heptadecanoic (52%), lauric (42.2%), arachidonic (38%) and total saturated fat (34%).

The highest percentage of mothers classified above the reference values is 76% for palmitic acid, followed by total saturated (64%), cis-docosahexaenoic (DHA) (54%) and stearic acid (40%), these being the percentages greater than 30% of the participating mothers.

Nutritious	Percentage of mothers with low plasma FA content	Percentage of mothers with adequate plasma FA content	Percentage of 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%
Ácido oleico (C18:1n9c)	4%	82%	14%
Ácido linoleico (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-docosahexaenoic acid (C22:6N3)	22%	24%	54%
Total Saturated	2%	34%	64%
Total Monounsaturated	4%	78%	18%
Total Poliinsaturada	84%	16%	0%
Total Fat	52%	24%	24%

Table 3: Percentage of mothers with low, adequate or high content of fatty acids in serum according to classification according to the content reported by the food and human nutrition laboratory.

Table 4 shows the correlation between the content of FA in serum and its content in mature breast milk, it should be noted that only in some FA there is minimal correlation, such as linoleic acid, (DHA) cis-docosahexaenoic acid and (EPA) cis-eicosapentaenoic acid; low correlation with palmitoleic acid and in the rest of the fatty acids there is no correlation. The results of bilateral significance are indicating that there are statistically significant differences between the correlations, except in the total polyunsaturated fatty acids where the correlation does not present a statistically significant difference.

Fatty acids	Average in serumg/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
Heptadecanoic Acid (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
Ácido oleico (C18:1n9c)	0,167	1,469	0,449	-0,11
Total monounsaturated	0,174	1,595	0,434	-0,113
Ácido linoleico (C18:2n6c)	0,068	0,677	0,406	0,12
(ARA) Arachidonic acid (C20:4n6)	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 polyunsaturated	0,129	0,138	0	,980**
Grasa total g/100ml	0,519	5,672	0,424	-0,116

Table 4: Correlation of fatty acids in maternal serum and mature breast milk.

Discussion

Studies such as that of Chulei., *et al*. [25] show variations in FA content in mature breast milk dependent on the study population; other authors such as [26-29]. They indicate that factors such as nutritional status, maternal diet and breastfeeding time also influence the content of fatty acids in this food.

The total fat content in this study was higher (5.67g/100mL) than those reported by other studies [18,19,22] which date to the average content of 3.7g/100mL, likewise the content of saturated fatty acids present in mature breast milk in this study are of higher content (2.049) than that reported in other studies such as that of Galindo Gómez in which it is possibly associated with diets high in carbohydrates and low in carbohydrates. grease [30] followed by monounsaturated (1.595) and finally polyunsaturated (0.138) with similar values reported in the study by Thais Álvarez de Acosta [31].

According to the study by Galindo Gómez A, Alvarez de Acosta T and Jin H., *et al*. [30,32] the fatty acids with the highest presence found in LMM were oleic acid, followed by palmitic acid and linoleic acid, this being a FA with a majority presence, which is consistent with the results of FA present in this study. This is also the case of stearic acid, whose value is similar to the findings of other studies [33,34].

Likewise, fatty acids such as myristic (0.29), arachidonic (0.023), eicosapentaenoic acid (0.002), and docosahexaenoic acid (0.01) were found in low quantities, as reported by other studies [16-18].

In relation to the content of saturated and medium- or short-chain fatty acids in the LMM, we found that the amount of saturated fatty acids (SFA) (Table 4) is close to the average for Malaysian women [35] palmitic acid having the highest value as in this study, and according to the study by Nasser., *et al*. [36] It is significantly related to daily maternal intake or de novo synthesis in the mammary gland, which absorbs nutrients such as glucose, amino acids, lipids derived from digestion and adipose tissue, albumin-bound fatty acids, vitamins and minerals present in the maternal serum [37,38], these are carried to the extracellular fluid between the gland capillaries and the lactocytes, where they enter through the basement membrane and take the appropriate synthetic route [37].

The linoleic fatty acid reported in this study has a similar content in both LMM and serum, this is possibly related to what the author Burrows T expresses in his study, where he explains that the origin of this fatty acid depends directly on dietary consumption [39], a situation that was not corroborated in this investigation.

The values present in serum and LMM of (EPA) Cis-eicosapentaenoic acid and (DHA) Cis-docosahexaenoic acid, are similar in both matrices, the reason may be due to the fact that these are derivatives of linolenic acid, whose content as already mentioned is similar both in serum and in LMM, indicating that these could be derived from linolenic acid [40,41].

According to Smoczyński [42]. LCPUFA in LMM comes only from the diet and is absorbed until it reaches the mammary gland in the form of chylomicrons, if they do not come from the diet these are obtained from the body fat reserves of the mother's adipose tissue, which are transported by blood lipoproteins to the liver and then directed to the mammary gland in the form of VLDL (very low density lipoproteins) [43], the FAs present in the blood bound to lipoproteins cross the cell membrane through carrier proteins and, to a lesser extent, by passive diffusion [44].

Since no studies were found in which the plasma FA content of lactating women has been evaluated, a classification of the content in this matrix as low, adequate and high was developed according to the findings of this study, in order to analyze the FA in the plasma of lactating women and determine if these values are adequate or not; compared to the results of the study in obese adolescents from Bermúdez JA, in which values of SFA 0.0048g, AGMg 0.0188 and PUFAg 0.03304 were reported [45] lower than those found in this study, which deduces the non-comparative relationship due to the fact that in lactating mothers the importance of FA as LMM production is increased due to the numerous functions and processes that they perform in the development of the infant.

Regarding oleic acid, its whey content may be mainly due to the contribution of the diet, according to the study by Ettinger S [46], but it can also be synthesized by both animals and humans by the de novo route [47].

Of the polyunsaturated FAs the correlation is very good (0.980), showing a content of these in serum of 0.129g/100 and in LMM of

0.138g/100, this may be due to the fact that the technique used in the laboratory can measure the total content of LCPUFA in serum and only specify 4 (see table 4), likewise in the LMM the technique allows quantifying the total LCPUFA, but it is also more specific with respect to these, reporting a total of 11: Linoleic acid (C18:2n6c), Eicosadienoic acid (C20:2n6), Dihomogam linoleic acid (C20:3n6), Arachidonic acid (C20:4n6), Docosadieenoic acid (C22:2 n6), A-linolenic acid (C18:3n3), Eicosatrienoic acid (C20:3n3), Eicosapentaenoic acid (C20:5n3) EPA, Docosahexaenoic acid (C22:6n3) DHA, cis9, trans-11/trans-9 and cis-11-CLA (C18:2).

Finally, according to the author DJ Chapman, every mother's milk has an excellent nutritional value and is suitable for her baby, because the mother's body always prioritizes the baby's needs and, therefore, most of the FA are present in milk at an adequate and stable level, at the expense of maternal deposits [48], this concept under the condition that if the mother does not ingest the nutrients in her diet she will be able to obtain them from her fat tissue reserves, as long as these are adequate, otherwise wasting in the mother or deficiency of the nutrient in the LMM may occur [49].

The low or no correlation between serum FA content and LMM denotes that its content is conditioned more by an infant's nutritional need than by external factors, including, variations in the mother's diet can change the profile of FA but in serum without affecting GA content in LMM.

Conflict of Interest

The authors declare that they have no conflict of interest.

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No.

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