Pregnant women's diet composition and transitional milk fatty acids: factor analysis

Składniki diety kobiet ciężarnych oraz kwasy tłuszczowe mleka pierwotnego: analiza czynnikowa

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Abstract

Introduction: *The variation in the nutrients contained in the milk is the result of changes to the breast metabolism, placenta metabolism and the diet of pregnant women. Various factors influence fatty acid composition which are one of the major components of woman's breast milk.*

In our research, we wanted to determine the relationship between the components of the diet of and the transitional milk fatty acid composition mothers who delivered healthy full-term babies, preterm and small for gestational age neonates.

Materials and methods: *The study group comprised of 95 healthy women who were divided into three subgroups: mothers of appropriate for gestational age (AGA) neonates (group A); mothers of preterm neonates (group B); and mothers who gave birth to small for gestational age (SGA) babies (Group C). The women's elements diet and the content of biochemical components were estimated based on the dietary questionnaire. The FAs in the mother's milk were analyzed using GCMS chromatography. The results of the studies of the dietary components and fatty acids of the milk underwent factor analysis.*

Results: *In group A, 10 correlations (5 positive and 5 negative) were found between the components of the mother's diet and the FAs in the milk (correlation varying from 0.285 - 0.366). In group B, only negative correlations were observed and these had higher absolute correlation values (0.354-0.500). The most correlations between dietary components and FAs in the milk were found in group C (0.537 - 0.800).*

Conclusion: *Nature of the correlations between the variables examined in groups A, B and C are different.*

Key words: **transitional milk** / **fatty acids** / **preterm infants** / **SGA infants** / / **AGA infants** / **factor analysis** /

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Streszczenie

Wstęp: *Zmiana składników w mleku kobiet karmiących jest rezultatem zmian w metabolizmie gruczołów piersiowych, funkcjonowaniu łożyska oraz diecie kobiet ciężarnych. Liczne czynniki modulują skład kwasów tłuszczowych, będących jednym z najważniejszych komponentów mleka kobiecego. W naszych badaniach chcieliśmy określić wzajemne relacje pomiędzy składnikami diety a kwasami tłuszczowymi mleka pierwotnego kobiet, które urodziły dzieci o czasie, przed czasem oraz urodziły dzieci małe w stosunku do wieku ciążowego.*

Materiały i metody: *Do badań zakwalifikowano 95 kobiet, które urodziły zdrowe dzieci o czasie (AGA) (grupa A), przed czasem (grupa B) oraz dzieci małe w stosunku do wieku ciążowego (SGA) (grupa C). Jakość i ilość składników diety matek była określana za pomocą kwestionariusza żywieniowego. Skład kwasów tłuszczowych mleka pierwotnego określono techniką chromatografii gazowej sprzężonej z detektorem mas. Wyniki badań składników diety oraz kwasów tłuszczowych mleka poddano analizie czynnikowej.*

Wyniki: *W grupie A otrzymano 10 korelacji (5 pozytywnych i 5 negatywnych) pomiędzy składnikami diety a kwasami tłuszczowymi mleka matek (współczynnik korelacji kształtował się na poziomie 0,285 – 0,366). W grupie B wszystkie otrzymane korelacje miały znak ujemny oraz charakteryzowały się większą wartością współczynnika korelacji (0.354 – 0.500). Największą liczbę korelujących ze sobą związków wykryto w grupie C. W grupie tej obserwowano także najwyższe wartości współczynników korelacji (0,537 – 0,800).*

Wnioski: *Natura korelacji pomiędzy badanymi zmiennymi w grupie A, B i C jest inna dla każdej z badanych grup.*

Słowa kluczowe: **mleko pierwotne** / **kwasy tłuszczowe** / **wcześniactwo** / / **SGA** / **AGA** / **analiza czynnikowa** /

Introduction

A woman's diet is said to be the most important factor in determining the components of breast milk. Various factors influence the nutritional value of the milk, including the mother's dietary habits, the method of feeding, caloric content of food and the mutual proportions of particular dietary components [1, 2]. One of the most important components of breast milk is fat. Fatty acids in particular are responsible for the correct development of a child's nervous system. They are also essential for the metabolism of neuron membranes and other cells as well as general biochemical metabolism [3-5]. The mammary glands need substrates for milk biosynthesis. The main source of those substrates is the diet because they are taken directly from the peripheral blood which distributes recently consumed components. Another source of substrates is tissue deposits. Their composition reflects the mother's diet over the last few weeks and even months. Out of all the dietary components, fats, proteins and carbohydrates have the greatest influence on the composition of fatty acids in the mother's milk. Fats included in the diet are used directly, albeit partially, as fatty acid precursors. These include fatty acids of different chain lengths and different saturation degrees [6-7]. Fatty acids (FA) may also be produced as the result of indirect transformations of non-fat compounds such as proteins and carbohydrates. The process of adjusting the composition of the milk according to the degree of development and/or maturity of the foetus and the child's condition at any given time is extraordinarily complex and is probably initiated during the gestation period [2]. The quantity and mutual proportions of FAs in the milk may be regulated according to the child's changing needs.

So far, neither the FA composition of the milk nor the diet composition changes occurring in mothers who gave birth in the period of time immediately preceding 38 weeks of pregnancy and in mothers whose children were born at term SGA have been analyzed.

The FA metabolism of breast milk at the interface of physiology and pathology of pregnancy is therefore not known. It is also not known relationships between the diet and transitional milk of mothers of AGA, SGA and preterm neonates. Therefore the objective of the study was to analyze the influence of a mother's dietary intake on the fatty acid composition of her transitional milk.

Materials and methods.

Study population

The study group consisted of 95 women selected randomly out of a total of 1,856 women who gave birth at the Provincial Specialist Hospital nr 1 in Tychy, Poland, in 2007. All the participants gave their written consent to take part in the research program. Women who participated in the research program were classified into three groups according to the following criteria:

Group A (n = 52): healthy mothers, routine and uneventful pregnancy, full-term delivery neonates (bw 10^{th} – 90^{th} percentile);

Group B (n = 31): mothers who gave birth prematurely - between 35-37 weeks (bw 10^{th} – 90^{th} percentile);

Group C (n = 12): mothers who gave birth to full-term but small for gestational age (SGA) neonates (bw <10^{th} percentile) (Table I).

The following exclusion criteria were applied to potential study participants:

- chronic diseases;
- pathologies during the course of pregnancy such as gestational high blood-pressure, infections during pregnancy, miscarriages and/or premature birth resulting in the death of the child, developmental anomalies in the foetus;
- AIDS and sexually transmitted diseases;
- lack of the mother's consent to take part in the research program or withdrawal of consent during the study.
- children whose weight exceeded the 90^{th} percentile (LGA).

The first ultrasound examination of pregnant women was performed between the 12^{th} and 14^{th} weeks of gestation, the second one between the 20^{th} and 22^{nd} weeks and the third one in the 32^{nd}-33^{rd} week. SGA neonates were identified and defined by the third and final ultrasound performed during pregnancy. Fetuses with in utero abdominal measurements below the 10^{th} percentile

Table I. Characteristic of the studied population. Results are mean (±SD).

	AGA (A)	Preterm (B)	SGA (C)
Age (y)	28.6 ± 4.7	27.7± 3.7	29.0 ± 5.1
BMI (kg/m²)	22.7 ± 3.8	23.2 ± 4.1	23.1 ± 4.8
Delivery (wk)	39.2 ± 1.2	34.65 ± 1.1	38.0 ± 1.0
Neonatal weight	3535.9 ± 392.9	2402 ± 424.5	2297 ± 158.9
Placental weight	499.2 ± 103.1	320.4 ± 98.6	358.6 ± 82.8
Mode of delivery	42n/12cs=54	21n/11cs=32	12n/11cs=23
Apgar score	9-10	9-10	9-10

of reference values were defined as SGA fetuses. SGA was confirmed at birth if the neonatal weight was below the 10^{th} percentile. All the fetuses had normal karyotypes and no malformations at birth. The mothers did not receive any dietary supplementation during pregnancy. The mothers in group B did not receive corticosteroids.

Maternal age, BMI, neonatal weights, mode of delivery and Apgar scores are presented in Table I.

The study was approved by the Bioethics Review Board of the Silesian University of Medicine in Katowice, Poland (L.dz. NN-6501-183/I/07).

Feeding questionnaire

The women's eating habits and the composition of their diet were estimated based on a dietary questionnaire designed by the National Food and Nutrition Institute in Warsaw, Poland. The questionnaire allowed the researchers to determine the daily consumption of each particular food component (proteins, carbohydrates, fats, fatty acids, vitamins) as well as the mothers' calorie consumption over a one month period. Before the research began every participant was clearly instructed and offered training on how to fill in the questionnaire as well as how to record the volume or the mass of food using standard household measures such as a spoonful, a glass etc. The questionnaire included a list of 124 products in the following food groups: milk and dairy products, eggs, meat, sausages, offal, fish, animal and vegetable fats, vegetables, fruit and fruit products, potatoes and potato-based products, seeds, legumes, cereals and cereal-products, pre-cooked ready meals, salty snacks, nuts and grains, sugar and sweets, soft drinks, alcohol, soup concentrates, sauces and spices. The study participants had to record how frequently they consumed each product, with the options being: daily, several times a week, once a week, 2-3 times a month, once a month or never. The questionnaire was filled in by the women three days after giving birth. The DIETA FAO program, which includes data for 1,067 typical Polish dishes and food products, was used to estimate the quantity of the aforementioned components.

Milk collection

The transitional milk samples were obtained between four and seven days after delivery. Approximately 10ml were obtained from each participant. The samples were stored in plastic test-tubes, cooled to -20°C and then transported (dry ice) to the laboratory. They were kept frozen until they were thawed out just prior to analysis. Gas chromatography-mass spectrometry method was used to analyze the fatty acids in the transitional milk.

Gas chromatography-mass spectometry (gc-ms) analysis

The lipids were extracted from the breast milk samples using chloroform-methanol (2:1 v/v containing 0.01% BHT) in accordance with Folch [8]. Organic extracts were evaporated at 40°C using nitrogen. After they were saponified with potassium hydroxide in methanol. They were subsequently methylated with hydrochloric acid in methanol. The fatty acids methyl esters (FAME) were analyzed by gas-liquid chromatography using a HP 6890 gas chromatograph equipped with MS detector. Helium was used as a carrier gas (20ml/s) at a head pressure of 43.4 psi. The oven temperature was programmed to 175°C for 40 minutes and was thereafter raised by 5°C per minute until it reached 220°C. The results were expressed as a percentage (% wt/wt) of all fatty acids detected with a chain length of between 8 and 24 carbon atoms.

Statistics

Analysis of the data from the questionnaires allowed us to identify and quantify a total of 60 components of the women's diets. These components subsequently became the subject of further study (Table II). Fatty acid (FA) analysis of the transitional milk was also conducted. 40 FA were isolated and analysed, including both saturated and unsaturated acids, with the chain length varying from 8 to 24 carbon atoms (Table III).

Factor analysis was used to analyze the relationship between dietary components and the fatty acid composition of the breast milk samples. The use of factor analysis meant it was possible to reduce the number of variables and to classify them. Subsequently, the most representative factors of variables were identified. To study the correlations it was decided to arbitrarily choose the variable within the factor which was the most effective means of measurement (which had a documented physiological role). If there was no factual basis for the selection of the variable most representative for the particular factor, the variable with the largest absolute value of the factor load was then chosen (variables carrying the greatest load of information) (Table IV, V). In both cases the following condition was satisfied FL>0.7 (FL – factor load).

Factor analysis of the food components of the mother's diet and the fatty acids in the transitional milk were carried out separately. Biochemical components of the mothers diet where the absolute value of the factor load was higher than 0.7 (FL>0.7) were subject to further statistical analysis. The same method was used to isolate FAs in the transitional milk. Spearman's rank correlation analysis was applied in this case. Statistical significance was set at $P<0.05$.

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Results

Based on factor analysis, 9 factors for the dietary components were determined and 8 factors for the fatty acids in the mothers' transitional milk were determined.

The first dietary factor that was identified (Table IV) consisted of 11 fatty acids (FL>0.7), out of which C12:0 (FL=0.945) had the highest value. C12:0 was therefore assigned to the correlation studies. In the other remaining factors - 2,3,4,5 and 6 - the variables singled out for study were: folic acid (0.826), C20:5 (0,961), C18:3 (0.806), a percent of energy coming from carbohydrates (-0.833) and beta carotene (0.951).The remaining three factors studied were – 7,8 and 9 – and were represented by the single variables: lactose (0.833), alcohol (0.724) and animal protein (0.736).

The first factor of the transitional milk that was identified (Table V) consisted of 4 fatty acids (FL>0.7), of which C20:1 (0.881) had the highest value. As far as the second factor was concerned, no variable out of the several dozen analysed reached FL>0.7. In factors 3 and 7 the variables designated to undergo correlation studies included C12:0 (0.894) and C24:0 (0.742). Factors 4,5,6 and 8 also underwent correlation studies, represented by the single variables C16:1 (0.843), C13:0 (0.940), C18:3n3 (0.800) and C18:1t (0,708).

All the above-mentioned dietary components and fatty acid components of transitional milk underwent correlation analysis in every studied group. In group A, 10 statistical correlations were identified at the level of significance P<0.05 (Figure 1).

These 10 correlations involved 7 dietary components and 5 components of the transitional milk. The dietary components correlated negatively with the fatty acids in the transitional milk in the following configurations: animal protein (AP)&C18:1t, AP&C20:1, C12:0&C20:1, C20:5&C13:0 and alcohol&C13:0. The remaining dietary components correlated positively with the transitional milk FAs in the following configurations: C12:0&C12:0, total fatty acids (TFA)&C12:0, C18:3&C12:0, percent of energy coming from carbohydrates (PEC)&C20:1 and PEC&C24:0. The absolute correlation coefficient values ranged between 0.285-0.366.

In group B, 6 statistically significant correlations were identified at the level of significance P<0.05 (Figure 2). They included 3 dietary components and 3 components of the transitional milk. In all the analyzed cases the dietary components correlated negatively with transitional milk FAs in the following configurations: animal protein&C18:3n3, C12:0&C20:1, C12:0&C24:0, TFA&C18:3n3, TFA&C20:1 and TFA&24:0. In group B the absolute correlation coefficient values were higher than in group A and ranged from 0.354 to 0.500.

In group C (similarly to group A), 10 statistically significant correlations were obtained at the level of significance P<0.05 (Figure 3). They included 5 dietary components and 3 components of the transitional milk. The dietary components correlated negatively with transitional milk FAs in the following configurations: AP&C18:1t, AP&C24:0, C12:0&C18:1t, C12:0&C24:0, TFA&C18:1t, TFA&C24:0, C18:3&C24:0, lactose (LAC)&C18:1t and LAC&C24:0. There was only one positive correlation observed in the following configuration LAC&C16:1. The absolute correlation coefficient values in group C were the highest of all the groups studied and ranged between 0.537-0.800.

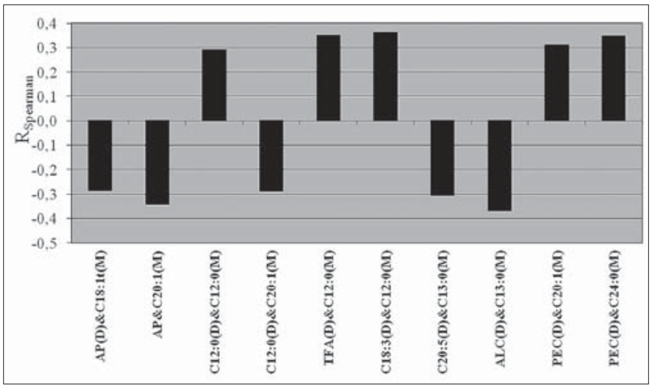

Figure 1. Correlation between dietary compounds and transitional milk fatty acids in the group of mothers who delivered at term. AP – animal protein, TFA – total fatty acids, LAC – lactose, ALC – alcohol, PEC – percent energy of carbohydrates, D – diet, M – milk. P<0.05.

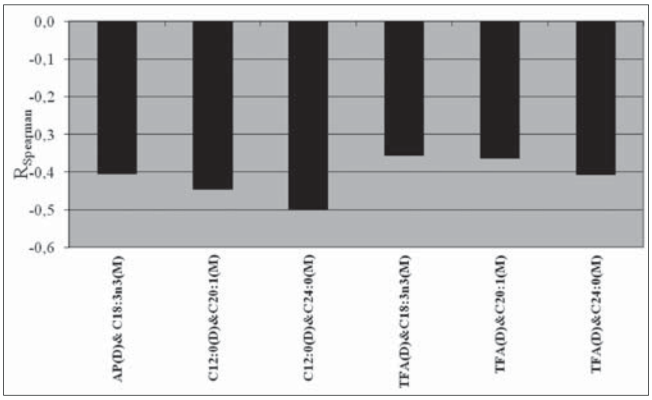

Figure 2. Correlation between dietary compounds and transitional milk fatty acids in the group of mothers who delivered preterm. AP – animal protein, TFA – total fatty acids, D – diet, M – milk. P<0.05.

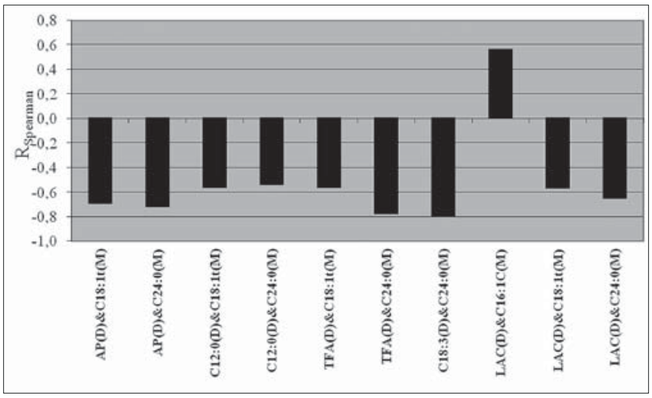

Figure 3. Correlation between dietary compounds and transitional milk fatty acids in the group of mothers who delivered small for gestational age neonates. AP – animal protein, TFA – total fatty acids, LAC – lactose, D – diet, M – milk. P<0.05.

The dietary elements and transitional milk FAs selected using factor analysis revealed a different number of correlations in each group studied. In the case of the dietary components, the components which correlated the most frequently were C12:0 (6 times), TFA (6 times) and protein (5 times). As far as fatty acids in the milk were concerned, the ones which correlated the most often were C24:0 (8 times), C20:1 (5 times) and C18:1t (5 times). The dietary components did not correlate evenly across the three

Table II. List of biochemical nutritional components included in the diet.

Nutritional component	
Fat	fat, cholesterol, C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C14:1, C15:1, C16:1, C17:1, C18:1, C20:1, C22:1, C18:2, C18:3, C18:4, C20:3, C20:4, C20:5, C22:5, C 22:6
Protein	Total protein, animal protein, vegetable protein
Carbohydrates	Total carbohydrates, lactose, saccharose, starch, cellulose
Vitamins	vit. A, retinol, beta-carotene, vit. E, thiamine, ryboflavin, niacin, vit. B6, vit. C, folic acid
Other	Water, ash, waste, energy, alcohol

Table III. List of fatty acids analyzed in transitional milk.

Fatty acids analyzed in transitional milk (n=40)	C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1, C15:0, C15:1, C16:0, C16:1t, C16:1, C17:0, C17:1, C18:0, C18:1t, C18:1c, C18:2c, C18:2cc, C18:3n6, C18:3n3, C20:0, C20:1, C20:2, C 20:3n6, C20:4n6, C20:3n3, C22:0, C22:1, C20:5n6, C24:0, C24:1, C22:5n3, C22:6n6, C21:0, C23:0, C18:3t, C22:2,

Table IV. Biochemical diet components with absolute value of factor load higher than 0.7 (FL>0.7).

Factor	Factor	Factor	Factor	Factor	Factor	Factor	Factor	Factor
1	2	3	4	5	6	7	8	9
C4:0	VIT C	C18:4	ENERGY KJ	%EN FAT	VIT A	**LACT**	**ALC**	**ANIMAL P**
C6:0	**FOLIC AC**	**C20:5**	FAT	**%EN CARB**	**BETA CAR**			
C8:0		C22:5	C20:0					
C10:0		C 22:6	C18:1					
C12:0			C22:1					
C14:0			C18:2					
C15:0			**C18:3**					

Table V. Fatty acids of transitional milk with absolute value of factor load higher than 0.7 (FL>0.7).

Factor	Factor	Factor	Factor	Factor	Factor	Factor	Factor
1	2	3	4	5	6	7	8
C20:0		C10:0	**C16:1C**	**C13:0**	**C18:3n3**	**C24:0**	**C18:1t**
C20:1		**C12:0**				C23:0	

groups (A, B and C). This phenomenon was most noticeable in the case of C12:0 and C13:0 which only correlated with FAs in group A. C18:3n3 correlated twice only in group B. C24:0 correlated 8 times altogether, 5 times in group C. 4 out of the 5 C18:1t correlations were found in group C. It must be added here that it was only in group C that a correlation between lactose in the diet and transitional milk FAs was evident.

The studies also showed differences in the degree of saturation and the chain length of the correlating milk FAs. Medium chain saturated acids correlated five times (all cases occurring in group A), long chain saturated acids correlated eight times, whereas long chain monounsaturated fatty acids correlated eleven times. Five out of the eleven incidences were connected with trans isomer FAs. Finally, polyunsaturated acid only correlated with the dietary components in two cases (both incidences occurring in group B).

Discussion

The results of previous studies have proven that the composition of fatty acids in the transitional milk depends not only on fat (including fatty acids in the diet) but also on other dietary components [5, 9-15]. The aim of our study was to investigate how various components of the maternal diet shape the fatty acid composition of breast milk. Factor analysis was used to analyze the data and this made it possible to identify the most significant dietary components and fatty acids in the transitional milk. The most significant dietary components included some selected FAs, folic acid, PEC, beta-carotin, lactose, alcohol and animal protein (Table IV). Similarly, in the case of the transitional milk, factor analysis identified the most significant fatty acids (Table V).

The correlated data showed some statistically relevant relationships between the dietary components and the fatty acids in the transitional milk in every group studied. The value and the direction of interactions of the variables differed between the groups. However, similarities were found within each group that was investigated.

The differences between the groups studied concerned the values of the correlation coefficients. The lowest values were observed in the group of mothers who gave birth to AGA neonates (group A). Their levels varied from 0.285 to 0.366. Higher correlation coefficient values were observed in the group of mothers who delivered prematurely (group B). The correlations observed in this group had an absolute value of between 0.362-0.500. The highest correlation coefficient values (with levels varying from 0.537 to 0.800) were observed in the group of mothers who gave birth to SGA neonates (group C). The study groups also differed in a sign of correlated components of the diet and FAs of the breast milk. In group A, half of the correlations were positive and the other half were negative (Figure 1). In groups B and C, all correlations with the exception of one were negative (Figure 2).

In no case was there was any repetition of the correlated pairs across any of the three groups studied. Only in groups B and C were two cases observed that involved the same pairs of correlated compounds. These related to C12:0 and TFA in the mother's diet and C24:0 and C20:1 in the transitional milk. In both cases, the correlations were negative.

The results of our studies indicate that the nature of the correlations between the variables examined in groups A, B and C are different. However, there are some similarities within the last two groups. This applies to both the direction of the correlations (almost all of them are negative) and in some cases identical pairs of correlated compounds. The mothers in groups B and C were those who gave birth prematurely but with a small degree of prematurity, and those who gave birth to children small for gestational age (SGA) at term, respectively. Thus, both groups appear to be similar in terms of the pathology of pregnancy - their degree is low. Analyzing the dynamics of changes in the composition of transitional milk, based on our results and in the context of literature data, it can be concluded that even "late prematurity" and SGA are sufficient stimulus for secretion of transitional milk of a modified composition. Moreover, nature of correlations between variables examined in groups A, B and C are different. It probably kind of adjustment of the composition of breast milk to the child's needs dependent at least partly of diet of mothers [16-17]. Preterm and SGA neonates have enzymatically underdeveloped digestive system so they cannot degrade many components with great efficiency [18-22]. The different correlation profiles between the groups may be explained not only by maternal diet during pregnancy but also by differences in placental function [23-26]. Referring the obtained results to well-known biochemical mechanisms considered in the context of not only maternal mammary gland or placenta, but also systemic processes in the mother and her child, it is difficult to explain the mutual relations between the numerous components presented in Figure 1-3.

It cannot be excluded that factor analysis has revealed the existence of hitherto unknown relationships between the dietary components of mothers and the fatty acids contained in their breast milk. In order to better understand the relationship between maternal diet and breast milk FAs, further research using factor analysis is required.

Conclusion

Nature of the correlations between the variables examined in groups A, B and C are different.

Oświadczenie autorów:

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