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CONTENT OF ODD-NUMBERED CARBON FATTY ACIDS IN THE MILK OF LACTATING WOMEN AND IN INFANT FORMULA AND FOLLOW-UP FORMULA

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Abstract. The objective of this study was to examine the relation between the content of pentadecanoic acid C15:0 (PA) and heptadecanoic acid C17:0 (HA) in human milk and the type of the mothers' diet and determine the content of these fatty acids in infant formulas and follow-up formulas available on the market. Thirty-four, healthy mothers, aged 24-33 y were take part in the study. Mothers were classified according to their diet status into one of two groups: diet low in dairy products (15) and standard diet (19). Analyzed 12 differences infant formulas, 6 infant formulas and 6 follow-on formulas. In the group of women whose diet was low in dairy products, the PA content of human milk fat reached on average 0.195 ±0.017% of total fatty acids, and the HA content – 0.202 ±0.012%. In the group of women eating a standard diet, a statistically significant increase was observed in the PA and HA content of milk fat which on average reached: PA – 0.406 ±0.019% and HA – 0.360 ±0.015% of total fatty acids. It was concluded that in respect of human milk, PA and HA could be regarded as biomarkers of milk fat consumption by lactating women. From among 12 investigated infant formulas (IF) and follow-up formulas (FF) which are available in retail, PA of 0.63 to 0.98% and HA of 0.37 to 0.60% of total fatty acids was found in only 4 formulas which contain bovine milk fat. In IFs which, according to the manufacturer's statement, do not contain milk fat, PA content was from 0.04 to 0.07% and HA – from 0.02 to 0.08% of total fatty acids.

Key words: pentadecanoic acid, heptadecanoic acid, human milk, diet, milk fat, infant formula

INTRODUCTION

According to present knowledge, breast feeding is the only nutritional option for infants and small children which guarantees their optimal health and development. It delivers health benefits for both the mother and the child. Due to its specific characteristics, human milk caters to the infant's nutritional needs until 6 months of age [Emmett

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and Rogers 1997, Das 2003]. The mother's milk contains an excellent combination of the necessary nutrients, immunological substances and enzymes. The composition of the human milk is ideally suited for meeting the infant's nutritional needs at every stage of lactation [Emmett and Rogers 1997, Koletzko et al. 2001].

Nevertheless, research has shown that the composition of human milk is unstable to a certain degree. This implies that the diet of lactating women can, to a certain extent, influence the content of some milk components, such as fatty acids. The research conducted by Francois et al. [1998] clearly indicates that the consumption of fats rich in certain fatty acids by women increases the content of those fatty acids in human milk shortly after consumption (from 6 to 24 hours). The above explains the varied fatty acid composition in the fat of human milk from women living in various parts of the world [Al-Othman et al. 1996, Borschel et al. 1986, Koletzko et al. 1988], which is characteristic of a given cultural region and determined by the woman's specific diet. One of the common components of lactating women's diet is the milk of ruminants and the resulting dairy products.

Saturated fatty acids – pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) – are synthesized by the bacteria of ruminal intestinal microflora in the rumen [Wu and Palmquist 1991]. Since these acids have an uneven number of carbon atoms in a molecule, they cannot be synthesized in the human body and are not effectively metabolized by humans [Smedman et al. 1999].

The HA and PA content of human tissue [Wolk et al. 1998, 2001, Baylin et al. 2002], muscles [Andersson et al. 2002] and blood serum [Brevik et al. 2005] is directly dependent on the content of milk fat in the diet. A positive correlation was also observed between the consumption of dairy products and the PA content of cholesterol esters and serum phospholipids [Smedman et al. 1999, Wolk et al. 2001]. This indicates that those odd-numbered carbon fatty acids can act as biomarkers of dairy product intake [Wolk et al. 2001, Brevik et al. 2005].

The objective of this study was the determine whether 15:0 and 17:0 acids can act as biomarkers of milk fat intake in relation to women's milk and to determine the content of these fatty acids in infant formulas and follow-up formulas available on the Polish market (most popular brands).

MATERIALS AND METHODS

Human milk

Mature human milk (milk after the 15th day of lactation, N = 15) from mothers who resorted to a milk-free diet due to the children's allergy to bovine milk. The mothers consumed only small quantities of milk fat in the form of margarine and butter mixes or margarine and yoghurt mixes, mainly for spreading on bread. Daily intake of dairy fat was lower than 5.3 g per day.

Mature human milk (N = 19) from women eating a standard, traditional diet. Daily intake of dairy fat was higher than 15.6 g per day.

Milk was collected between 7 a.m. and noon. Directly after collection, the samples were frozen and stored at –80°C. Fat was isolated by the Rose-Gottlieb method [AOAC... 1990, Method No 905.02] within 24 hours after milk collection.

Infant formulas

Infant formulas (IF) and follow-up formulas (FF) from four different manufacturers, available on the market under different brand names (marked from A to F), during the shelf-life period of 12-16 months after unit packaging.

The most popular brands were selected from a wide range of infant formulas available on the market. Infant formulas (IF) were marked with the digit 1, and follow-up formulas (FF) – with the digit 2. From among 12 different brands, the analysis involved: 2 hypoallergenic products (marked HA) and 4 products containing bovine milk fat (A.1, A.2, B.1 and B.2). The fat from IF and FF was isolated directly after the powdered product was dissolved.

Preparation of Fatty Acid Methyl Esters (FAMEs)

Isolated milk fat without water and solvent residues was converted into fatty acid methyl esters (FAMEs) in accordance with the European Standard EN:ISO 5509:2000.

Gas chromatography

FAMEs were separated according to the length of the hydrocarbon chain and the level of unsaturation with the involvement of high resolution gas chromatography HR-GS [EN:ISO 5508 1995]. The analysis was performed on a Hewlett-Packard gas chromatograph with a split/splitless injection port and a flame ionization detector (FID) on a Rtx 2330 column (Restek, Bellefonte, Pennsylvania, USA).

The qualitative and quantitative analysis of the PA and HA content of the investigated samples was performed with the use of FAME standards (Supelco Bellefonte, Pennsylvania, USA; Larodan Fine Chemicals, Malmö, Sweden). Milk fat fatty acids are expressed as percent of total fatty acids methyl esters.

RESULTS

All analysed samples of fat from mature human milk contained pentadecanoic acid C15:0 (PA) and heptadecanoic acid C17:0 (HA).

In the group of women eating a standard diet, the PA content of milk fat was from 0.34 to 0.45% of total fatty acids (on average 0.406 ±0.019%). The group of women whose diet was low in dairy products due to their children's bovine milk intolerance, the PA content of milk fat was from 0.15 to 0.24% (on average 0.195 ±0.017%; Table 1).

The HA (heptadecanoic acid 17:0) content of human milk fat is also closely related to the content of milk fat in the mother's diet. For women eating a standard diet, the average HA level was 0.36 ±0.015% of total fatty acids (from 0.31 to 0.41%), and in the group of women following a milk-free diet – on average 0.202 ±0.012% (from 0.17 to 0.24%; Table 1).

To verify the hypothesis on the equal level of average PA and HA content of fat in both groups of women, the Student's t-test was performed with the use of STATISTICA 5.0 software. Test results showed a statistically higher PA and HA content of milk of women eating a standard diet in comparison with the PA and HA content of milk of women whose diet was low in bovine milk fat.

Table 1. PA and HA content of human milk fat samples, percentage of total fatty acids
Tabela 1. Zawartość PA i HA w tłuszczu mleka ludzkiego, procent ogólnego składu kwasów tłuszczowych

	Standard diet (N = 19) Dieta standardowa (N = 19)		Nondairy diet (N = 15) Dieta bez tłuszczu mlecznego (N = 15)	
	PA	HA	PA	HA
	percentage of total FA – procent ogólnego składu KT			
Average – Średnia	0.406	0.360	0.195	0.202
Minimum	0.34	0.31	0.15	0.17
Maximum	0.45	0.41	0.24	0.24
SD	0.034806	0.027775	0.030675	0.022424
RSD, %	8.57	7.71	15.73	11.10
Δx (α = 95%)	0.019	0.015	0.017	0.012

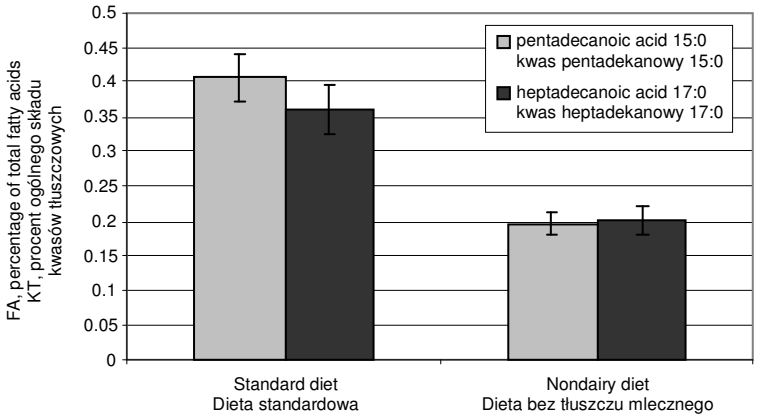

Fig. 1. Average content of pentadecanoic acid and heptadecanoic acid in the milk of women eating a standard diet and in the milk of women eating a diet low in milk fats
Rys. 1. Średnia zawartość kwasu pentadekanowego PA i kwasu heptadekanowego HA w mleku kobiet odżywiających się standardowo i w mleku kobiet z dietą ubogą w tłuszcz mleczny

The investigated infant formulas and follow-up formulas contained various quantities of PA and HA (Table 2), subject to the presence or absence of bovine milk fat in the fat content of the product (Fig. 2). The highest PA content of 0.9% and 0.97% of total fatty acids and HA – 0.60% and 0.57% was found in products A.1 and A.2 as well as in B.1 and B.2 with PA at 0.63% and 0.74% respectively; HA – 0.37% and 0.42% (Table 2), i.e. in infant formulas containing bovine milk fat.

In the remaining IF and FF products manufactured without bovine milk fat, based on skimmed bovine milk, the PA content did not exceed 0.07%, and HA content – 0.09%.

Table 2. Content of PA and HA isomers in the analysed samples of infant formula (1) and follow-up formula (2) products, percentage of total fatty acids
Tabela 2. Zawartość izomerów PA i HA w badanych próbkach mleka do początkowego (1) i następnego (2) żywienia niemowląt, procent ogólnego składu KT

Infant formulas Preparaty do żywienia niemowląt	PA	HA
	RSD = 8.08 %	RSD = 5.18 %
A.1	0.98	0.60
A.2	0.97	0.57
B.1	0.63	0.37
B.2	0.74	0.42
C.1.HA	0.06	0.07
C.2.HA	0.07	0.09
D.1	0.05	0.07
D.2	0.07	0.08
E.1	0.04	0.07
E.2	0.04	0.06
F.1	0.05	0.02
F.2	0.04	0.03

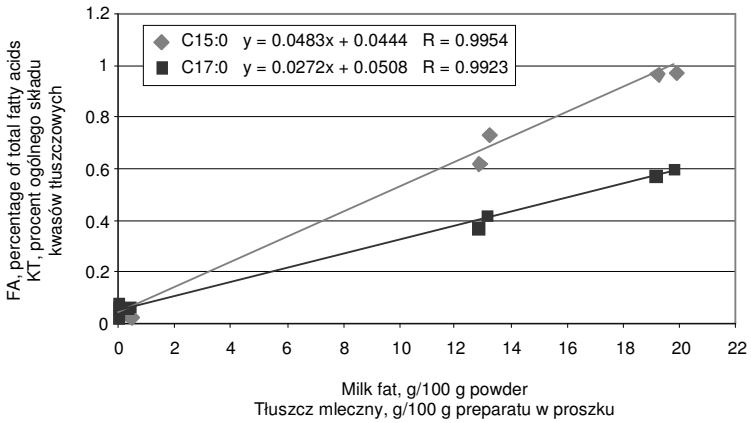

Fig. 2. Relationship between the PA (15:0) and HA (17:0) content in IFs and bovine milk fat content of the analysed products (manufacturer data)
Rys. 2. Zależność zawartości PA (15:0) i HA (17:0) w preparatach do żywienia niemowląt (IFs) od zawartości tłuszczu mleka krowiego w badanych preparatach (dane producentów)

The HA and PA content in the analysed IFs corresponds to the bovine milk fat content of these products as declared by the manufacturer (Fig. 2).

DISCUSSION

The obtained results indicate that the PA and HA content of human milk fat is dependent on the specific diet of lactating mothers. In the group of women eating a diet low in bovine milk and dairy products, the PA content was nearly 52% lower and the HA content was nearly 44% lower than in the milk of women following a standard diet. The above indicates that 15:0 and 17:0 acids may constitute biomarkers of milk fat intake in the diet of lactating women. These findings can be used to determine the content of milk fat in infant formulas and follow-up formulas based on their PA and HA content.

The question whether the presence of HA and PA, odd-numbered carbon saturated fatty acids, in infant formulas and follow-up formulas carries health benefits or the opposite is a separate issue.

It should be noted that throughout millennia, every new generation of the human species was fed with the mother's milk or the milk of domesticated ruminants. Therefore, the presence of PA and HA in the diet is natural for the infant, similarly to the presence of other milk fat components which are also found in human milk fat: cholesterol, vaccenic acid, conjugated linoleic acid (CLA) and sphingomyelin. Artificial formulas replacing human and ruminant milk in infant diets are a relatively new invention (dating back 2-3 generations). In view of the above, the priority goal is to ensure that the content of IF and FF resembles the composition of human milk characteristic of a given geographic region as closely as possible and that any differences in its composition are minimized.

Unfortunately, the recent years brought a reverse trend observed on an industrial scale where milk fat is completely replaced with plant, coconut or palm seed oil. As a result, the PA and HA content of most IF and FF products is several times lower than that of human milk.

The elimination of PA and HA from infant formulas seems to be completely unjustified from the point of view of a developing infant’s nutritional needs.

CONCLUSION

Content of odd-numbered SFAs (PA and HA) in human milk may be suitable biomarkers of milk and dairy fat intake in the diet of lactating women. Determination of PA and HA in powdered milk infant formulas can by used to determine the content of milk fat in this products. In IFs which do not contain milk fat PA content was under 0.07% and HA under 0.08% of total fatty acids.

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ZAWARTOŚĆ NIEPARZYSTOWĘGLOWYCH KWASÓW TŁUSZCZOWYCH W MLEKU KOBIET ORAZ W MLEKU POCZĄTKOWYM I NASTĘPNYM DO ŻYWIENIA NIEMOWLĄT

Streszczenie. Badano zależność pomiędzy występowaniem w mleku ludzkim kwasów pentadekanowego C15:0 (PA) i heptadekanowego C17:0 (HA) a rodzajem diety matek oraz oznaczono zawartość kwasów PA i HA w preparatach do początkowego i następnego żywienia niemowląt, dostępnych w sprzedaży. Badaniu poddano mleko ludzkie uzyskane od 34 zdrowych kobiet w wieku 24-33 lata. Matki podzielono na dwie grupy zgodnie ze spożywaną przez nie dietą: grupę kobiet z dietą ubogą w tłuszcz mleka krowiego (N = 15) i grupę kobiet odżywiających się przeciętnie (N = 19). W grupie kobiet spożywających dietę ubogą w produkty mleczne zawartość PA w tłuszczu mleka ludzkiego wynosiła średnio 0,195 ±0,017% ogólnego składu KT, natomiast HA osiągało średnio 0,202 ±0,012%. W grupie kobiet odżywiających się w przeciętny sposób zawartość PA i HA w tłuszczu mleka była statystycznie istotnie wyższa i wynosiła średnio: PA 0,406 ±0,019%,

HA 0,360 ±0,015% ogólnego składu KT. Tym samym stwierdzono, że dla mleka ludzkiego PA i HA mogą być biomarkerami spożycia tłuszczu mlekowego przez kobiety karmiące. Z pośród 12 badanych preparatów do początkowego IF i następnego FF żywienia niemowląt, dostępnych handlowo, tylko w czterech, zawierających w składzie tłuszcz mleka krowiego, stwierdzono obecności PA od 0,63 do 0,98% ogólnego składu KT oraz od 0,37 do 0,60% HA. W IFs, które zgodnie z deklaracją producenta nie zawierają tłuszczu mlekowego, zawartość PA wynosi od 0,04 do 0,07% ogólnego składu KT, a HA od 0,02 do 0,08%.

Słowa kluczowe: kwas pentadekanowy, kwas heptadekanowy, mleko ludzkie, dieta, preparaty do żywienia niemowląt, tłuszcz mleka krowiego

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