# The impact of selected environmental factors on cis-9, trans-11 octadecadienoate content in milk of breastfeeding women. Part II.

## Wpływ wybranych czynników środowiskowych na zawartość kwasu cis-9, trans-11 oktadekadienowego w mleku kobiet karmiących. Część II.

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**Wprowadzenie.** Karmienie piersią jest najlepszym sposobem dostarczania pokarmu niemowlętom i małym dzieciom. Liczne czynniki żywieniowe wpływają na stężenie kwasu cis-9, trans-11 oktadekadienowego, głównego izomeru CLA w tłuszczu mleka. Jednakże pozażywieniowe czynniki mogą także wpływać na jego ilości w mleku.

**Cel pracy.** Zbadanie zawartości tłuszczu i kwasu żwaczowego w mleku kobiecym w zależności od wybranych czynników środowiskowych i epidemiologicznych.

**Materiał i metoda.** Przebadano 67 próbek mleka kobiet karmiących. Wypełniały one ponadto ankietę dotyczącą zwyczajów żywieniowych i innych analizowanych parametrów. Analizę kwasów tłuszczowych wykonano techniką GC z zastosowaniem kolumny kapilarnej i detekcją płomieniowo-jonizacyjną. Wzorzec CLA FAME reference standard (Nu-Chek-Prep) posłużył do identyfikacji kwasu żwaczowego. Ocenę statystyczną wyników wykonano przy użyciu programu Statistica 8.0.

**Wyniki.** Zawartość kwasu żwaczowego była istotnie większa w mleku dojrzałym niż w siarze, czy w mleku przejściowym. Ponadto inne czynniki, takie jak ilości posiłków, płeć dziecka czy wykształcenie matki rzutowały na zawartość tłuszczu lub zawartość kwasu żwaczowego.

**Wniosek.** Uzyskane wyniki wskazują, że zarówno czynniki dietetyczne jak i epidemiologiczne mogą oddziaływać na zawartość tłuszczu i kwasu żwaczowego w mleku kobiecym.

**Słowa kluczowe:** *sprzężone dieny kwasu linolowego, CLA, mleko kobiece, karmienie piersią, żywienie matki*

**Introduction.** Breastfeeding is the optimal way of nutrition for newborns and infants. Numerous dietary factors can affect the concentration of cis-9, trans-11 octadecadienoate, the main isomer of CLA in milk fat. However, numerous different factors can affect its content as well.

**Aim.** To examine the content of fat and rumenic acid in human milk depending on the selected environmental and epidemiological factors.

**Material & method.** We investigated 67 milk samples of breastfeeding women. They were anonymously surveyed. The fatty acid analysis was made with GC with capillary column and flame-ionization detection. CLA FAME reference standard (Nu-Chek-Prep) was used to identify rumenic acid. The statistic evaluation was made with Statistica 8.0.

**Results.** The concentration of rumenic acid was significantly higher in mature milk in comparison to colostrum and intermediate milk. Other factors, as number of meals, child's gender or mother education influence the fat or rumenic acid content.

**Conclusion.** Our findings confirm that both dietary and environmental factors can affect the fat and rumenic acid content in human milk.

**Key words:** *conjugated linoleic acid, CLA, maternal milk, breastfeeding, maternal nutrition*



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Nadesłano: 10.06.2011
Zakwalifikowano do druku: 02.08.2011

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## Introduction

Maternal milk is recommended as optimal way of nutrition for newborns and infants. Human milk provides all nutrients essential for normal growth and development of the infant. Many factors, both dietary and environmental, can influence the composition of human milk. Among them the stage of lactation and maternal diet are essential [1, 2]. However, numerous epidemiological factors can affect its quality as well.

Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of linoleic acid with conjugated double bonds. Ruminant milk and meat of different species and dairy products are particularly rich in these compounds. However, these fatty acids are also present in human milk. CLA cause numerous physiological effects. They are also very important for infant health.

The aim of our study was to determine the influence of different dietary, anthropometric, social and environmental factors on the content of fat and cis-9, trans-11 octadecadienoate in human milk.

## Material and methods

This study involved 67 breastfeeding women, aged 20-42 who gave birth to one child or two children, mainly in The Gynecological-Obstetric Department of Central Hospital of the Ministry of Interior and Administration. All mothers agreed to provide samples of breast milk. Both colostrum and mature milk samples were examined. Mothers were anonymously asked about their anthropometric data, dietary habits during and after pregnancy and other environmental and social factors.

Samples of breast milk (50-150 ml) were collected by manual expression from both breasts. They were immediately frozen and stored at -20°C in sterile polypropylene containers. The milk samples were thawed only once and lipids extraction was prepared according to a modified procedure by Chin at al. [3]. The fatty acid analysis was made with GC with capillary column and flame-ionization detection, as described in part I.

The results were evaluated with Statistica 8 (StatSoft, Poland). The data were tested for normality with Shapiro-Wilk test. Because of the shortage of normal distribution the data were examined using a proper non-parametric test, e.g.: Mann-Whitney U rank test and Kruskal-Wallis test. P – value $\leqslant 0.05$ was considered significant.

## Results

All examined human milk samples contained measurable amounts of rumenic acid. The fat content in milk and the CLA content in milk fat and in milk, depending on the evaluated factors, are listed in Table I.

Nutritional factors, which were previously described (Part I), are not the only factors influencing the rumenic acid content in human milk. Dietary habits and the mode of nutrition are also important for the fat and rumenic acid concentration in breast milk. There was a significant difference in the cis-9, trans-11 CLA concentration among women who consumed three, four and five meals a day respectively.

Milks from mothers eating only three times a day had the smallest amount of rumenic acid both in milk fat and in whole milk, while those from mothers eating four meals a day were the richest sources of cis-9, trans-11 CLA (1.12±0.10 mg/g of fat versus 1.68±0.17 mg/g of fat, p=0.017 and 2.65±0.22 mg/100 ml of milk versus 4.03±0.40 mg/100 ml of milk, p=0.0187). Satiety did not cause any significant differences in the CLA concentration in milk but had great influence on the fat content in milk. Women eating to satiety produced milk richer in fat than those who finished their meals before being satiated (2.55±0.90% versus 2.26±0.93%, p=0.0257).

Table I. Content of fat and cis-9, trans-11 CLA depending on various factors
Tabela I. Zawartość tłuszczu i cis-9, trans-11 CLA w zależności od różnorodnych badanych czynników

| Factor /Czynnik | Frequency | FAT /Tłuszcz | | cis-9, trans-11 CLA | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | % | | mg/g of fat | | mg/100 ml of whole milk | | mg/100 g of whole milk | |
| | | mean±SD | p | mean±SD | p | mean±SD | p | mean±SD | p |
| Habitation /Miejsce zamieszkania | city | 2.29±0.73 | 0.0929 | 1.43±0.74 | 0.2871 | 3.18±1.67 | 0.4599 | 3.11±1.61 | 0.5037 |
| | village | 3.17±1.45 | | 1.14±0.51 | | 3.59±1.97 | | 3.52±1.85 | |
| Education /Wykształcenie | higher | 238±0.86 | 0.9263 | 1.55±0.79 | 0.0445* | 3.60±1.88 | 0.0215* | 3.51±1.81 | 0.0277* |
| | secondary | 2.38±0.88 | | 1.13±0.49 | | 2.55±1.05 | | 2.52±1.02 | |
| Childbirth /Poród | primipara | 2.27±0.64 | 0.7075 | 1.45±0.76 | 0.2031 | 3.28±1.83 | 0.3031 | 3.21±1.78 | 0.3136 |
| | multipara | 2.49±0.94 | | 1.17±0.58 | | 2.69±0.99 | | 2.66±0.98 | |
| Gender /Płeć | male | 2.14±0.55 | 0.0498* | 1.53±0.68 | 0.0439* | 3.33±1.80 | 0.6334 | 3.28±1.74 | 0.5190 |
| | female | 2.68±1.11 | | 1.24±0.75 | | 3.13±1.67 | | 3.06±1.62 | |
| State of lactation /Okres laktacji | colostrum | 2.13±0.50 | 0.8955 | 1.30±0.56 | 0.5057 | 2.79±1.19 | 0.6903 | 2.78±1.15 | 0.0454* |
| | mature | 2.62±1.12 | | 1.46±0.81 | | 3.61±2.00 | | 3.52±1.35 | |
| Season /Pora roku | winter | 2.66±1.64 | 0.1757 | 1.05±0.45 | 0.1165 | 2.34±0.66 | 0.1406 | 2.33±0.65 | 0.0778 |
| | spring | 2.26±0.65 | | 1.06±0.41 | | 2.40±0.71 | | 2.30±0.70 | |
| | summer | 2.16±0.68 | | 1.62±0.79 | | 3.51±2.00 | | 3.42±1.92 | |
| | autumn | 2.69±1.01 | | 1.38±0.74 | | 3.56±1.75 | | 3.51±1.66 | |
| Number of meals /Liczba posiłków | 3 a day | 2.51±1.04 | 0.4398 | 1.12±0.52 | 0.0170* | 2.65±1.11 | 0.0187* | 2.61±1.10 | 0.0267* |
| | 4 a day | 2.47±0.93 | | 1.68±0.88 | | 4.03±2.03 | | 3.89±1.96 | |
| | 5 a day | 2.07±0.63 | | 1.39±0.48 | | 2.92±1.61 | | 2.91±1.52 | |
| Satiety /Sytość | yes | 2.55±0.90 | 0.0257* | 1.35±0.72 | 0.5976 | 3.35±1.79 | 0.5976 | 3.31±1.73 | 0.6345 |
| | no | 2.26±0.93 | | 1.42±0.72 | | 3.10±1.61 | | 3.00±1.54 | |

There were significant differences in the CLA concentration in whole milk depending on the stage of lactation. The content of rumenic acid was significantly higher in mature milk as compared to colostrum (4.44±0.75 mg/100 g of milk to 2.78±0.23 mg/100g of milk) and to intermediate milk (4.44±0.75 mg/100 g of milk to 3.25±3.35 mg/100 g of milk). Molto-Puigmarti et al. indicate that rumenic acid constituted from 0.11% to 0.34% of all fatty acids in mature human milk and the mean concentration of this CLA isomer was 117.07 µg/ml [4]. We also observed growth in the fat ($r=0.2594$, $p<0.05$) and rumenic acid concentrations ($r=0.4604$, $p<0.05$) with the duration of lactation. These results are similar to those of Mojska et al, who observed a significantly lower lipid content in colostrum than in mature milk and an increase of total polyunsaturated fatty acids in mature milk in comparison with colostrum [2]. However Biernat et al. showed no differences in the total CLA content in colostrum and in mature milk [5] from women living in Wrocław.

We compared the fat and RA content in milk depending on the child's gender. Our results show that the fat concentration in milk of mothers who gave birth to a girl was higher as compared with the concentration in milk of mothers who gave birth to a boy. However we observed a reversed tendency in the cis-9, trans-11 octadecadienoic acid content in human milk: significantly higher amounts of RA in milk of women who gave birth to a boy.

Our results indicate that environmental factors also play an important part in the fat and rumenic acid concentration in milk. We compared the rumenic acid and fat content in milk of women living in a city (Warsaw), in small towns and in a village. The concentrations of measured values were not significantly different among the investigated groups. This can be explained by the lack of differences in maternal diet connected with the place of living. Glew et al. [6] investigating the trans fatty acid and CLA concentrations in breast milk of urban and nomadic women of northern Nigeria did not observe any significant differences either, even in spite of different dietary habits. Moreover, qualities of CLA in Nigerian women milk were lower than elsewhere in the world.

CLA has a great significance for newborn and infant health as a potent growth factor. The investigations of Chin et al. [3] examined that possibility for rats. They observed that feeding CLA to the dams during gestation and lactation did not influence the food intake or bodyweight of dams but improved the postnatal body weight gain of pups. The CLA concentration in milk increased in direct proportion to the dietary CLA intake. Also receiving the CLA-supplemented diet by pups after weaning led to a significantly higher body weight and improved feed efficiency. To our surprise we did not observe any correlation between the rumenic acid content in milk and child weight.

There were no significant differences in the fat and CLA concentrations in whole milk depending on the season of milk collection. Previously, we observed the seasonal fluctuations in the CLA and fat content in butters [7]. Similar results for different dairy products were obtained by Rutkowska et al. [8], Precht et al. [9] and Ledoux et al. [10] but only Mojska et al. reported the seasonal differences in the CLA concentration in human milk [2].

Among other social factors we ascertained that mothers' education influenced the cis-9, trans-11 CLA concentration in milk. Mean contents of rumenic acid in fat and in whole milk were significantly higher in the group of better educated women in comparison with the low educated (1.56 mg/g of fat versus 1.13 mg of fat, $p=0.040$, 3.60 mg/100 ml of milk versus 2.55 mg/100 ml of milk, $p=0.039$ and 3.51 mg/100 g of milk versus 2.52 mg/100 g of milk, $p=0.038$). It is possible that nutritional awareness and better dietary education go together with academic education. Our observations are in accordance with the results of Sochacka-Tatara et al., who proved that low educated women had improper nutritional habits before pregnancy [11].

## Conclusions

1. Both the diet of breastfeeding mothers and their nutritional habits have a great influence on the content of both fat and rumenic acid in their milk.
2. Higher concentration of rumenic acid in mature milk seems to confirm its significance for infant health.
3. It is necessary to make women aware of the impact of many various factors of their lifestyles on their children's health.

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