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Polyunsaturated Fatty Acid Composition of Human Colostrum Lipids in Slovenia: Regional Differences

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Summary

The fatty acid composition of human milk in Slovenia has not been investigated previously. In the present investigation the fatty acid composition of colostrum lipids from 41 Slovenian women recruited the third day post partum in three Slovenian hospitals (Celje, Koper and Ljubljana) was analysed. The coefficient of variation for the measurement of individual fatty acids participating more than 0.1 % was from 2 to 6 %. According to regional areas, there were significant differences ($P<0.05$) in polyunsaturated fatty acids contents of colostrum lipids both of n-6 [linoleic (C18:2), γ-linolenic (C18:3)] and n-3 series [α-linolenic (C18:3), octadecatetraenoic (C18:4), eicosatrienoic (C20:3) and docosahexaenoic acid (C22:6)]. The linoleic acid represented between 12.6 % (Koper) and 17.4 % of total fatty acids (Ljubljana) in colostrum lipids. The α-linolenic acid accounted from 0.7 % (Koper) to 1.1 % (Ljubljana) and the average linoleic to α-linolenic acid ratio was 16.8. The lowest portion of docosahexaenoic acid in colostrum lipids was in Ljubljana (0.35 %), whereas the highest in Celje (0.49 %, $P<0.05$). Differences in fatty acid composition of colostrum lipids in various regions of Slovenia are presumably due to regional differences in dietary habits.

Key words: long-chain polyunsaturated fatty acids, human colostrum

Introduction

Human milk is recognised as the most optimal diet for newborn infants (*1*). During the past few years special attention has been given to the nutritional and physiological value of the long-chain polyunsaturated fatty acids (LCP, ≥20 C-atoms) in infant nutrition, particularly to docosahexaenoic (C22:6 n-3, DHA) and arachidonic acid (C20:4 n-6, AA) that are essential structural components of the lipid matrix of cellular and subcellular membranes (*2,3*). Besides structural properties, some LCP fatty acids are precursors for the synthesis of eicosanoids, e.g. prostaglandins and leukotrienes, with important regulatory functions (*4,5*). LCPs are derived from linoleic (C18:2 n-6) and α-linolenic acids (C18:3 n-3) in a series of enzymatic desaturation and elongation steps (*6*). In premature infants the supply with n-3 LCP fatty acids, specially DHA, has been related to development of visual acuity (*7,8*) whereas n-6 LCP fatty acids, specially AA, appear to affect early body growth (*9,10*). Fatty acid composition of human milk is affected by mothers long and short term diet (*11,12*).

In Slovenia no investigations have been performed to measure the fatty acid composition of human milk. In this study we examined and characterised colostrum lipid composition from women living in three regions of Slovenia, with special attention to polyunsaturated fatty acids.

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Material and Methods

Human milk sampling and analysis

Samples (cc. 5 mL) were collected the third day after term delivery (colostrum) in the morning from women (n=41) living in three regions of Slovenia (Celje, Koper and Ljubljana). All human milk samples were collected during the months of June-August 1996 and frozen within 15 minutes after collection on dry ice and transferred to the laboratory, where they were stored at –80 °C for maximum two months until analysis.

Frozen colostrum samples were thawed at room temperature and fatty acids methyl esters prepared by *in situ* transesterification of fatty acids (*13*) and separated by capillary gas chromatography, using a Hewlett Packard gas chromatograph 5890 series 1, equipped with a flame ionisation detector and a Carbowax capillary column (25 m × 0.32 mm × 0.3 μm). The procedure was earlier described (*14*). The precision and accuracy of the analytical method was checked by analysis of BRC Certified Reference Material (No. 164, anhydrous milk fat) (*15*) and a very good agreement was obtained.

Dietary assessment

The habitual intakes, specially of fish, meat and fat sources during the period of one year before the delivery were assessed by food frequency questionnaire (*16*).

Ethical consideration

The study protocol was approved by the Ethical Committee of the Medical Faculty of the University of Ljubljana. From all participating women a written consent was obtained after a careful explanation of the study.

Statistical

Statistical analyses were performed by two-way analysis of variance (ANOVA). The level of significance was set at P<0.05. All analyses were performed using the statistical software SAS (*17*).

Results

There was no difference in contents of total saturated, monounstaurated and polyunsaturated fatty acids in colostrum lipids between Slovenian women living in Celje, Koper or Ljubljana region. Fig. 1 presents mean contents of total saturated, monounsaturated and polyunsaturated fatty acids in colostrum lipids of all 41 Slovenian women.

Mean contents of n-6 and n-3 polyunsaturated fatty acids of colostrum lipids of three regions are shown in Figs. 2 and 3. The polyunsaturated fatty acids showed the most observable differences in the content of parent essential fatty acids, linoleic (C18:2 n-6) and α-linolenic acids (C18:3 n-3). Colostrum lipids from women living in Ljubljana could be distinguished from those of the other two regions by higher linoleic acid content (17.35 *vs*. 12.62–14.72 %, P<0.01), higher α-linolenic acid content (1.07 *vs*. 0.93 in Celje *vs*. 0.70 % in Koper, P<0.001), higher sums of n-6 fatty acids (21.20 *vs*. 16.77 % in Koper, P<0.05) as well as of n-6 plus n-3 fatty acids (23.70 *vs*. 19.00 % in Koper, P<0.05). There were also differences in the contents of γ-linolenic (C18:3 n-6) and octadecatetraenic acid (C18:4 n-3). From the LCP fatty acids there were only differences in the contents of n-3 LCP fatty acids, eicosatrienoic acid (C20:3 n-3) and DHA. Colostrum lipids from women living in Celje contained higher content of eicosatrienoic acid (0.15 *vs*. 0.11 % in Koper, P<0.05). Women from Celje region had also almost 30 % higher content of DHA (0.49 %) in colostrum lipids, compared to those from Ljubljana region (0.35 %, P<0.05), whereas the amount in Koper was in between (0.41 %).

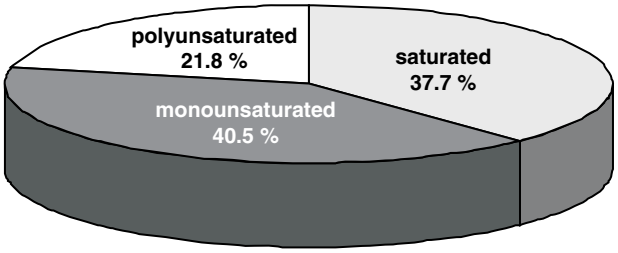

Fig. 1. Average fatty acid composition of colostrum lipids of Slovenian women (n=41, means)

Table 1. Correlations between contents % of n-6 and n-3 polyunsaturated fatty acids in colostrum lipids of 41 Slovenian women (P=0.0001)

Fatty acid	Fatty acid	r
18:2 n-6	18:3 n-3	0,82
18:3 n-6	18:4 n-3	0,66
20:2 n-6	20:3 n-3	0,74
22:4 n-6	22:5 n-3	0,72
22:4 n-6	22:3 n-3	0,54
$\sum$ n-6 LCP	$\sum$ n-3 LCP	0,77

No significant correlations between parent essential fatty acids (linoleic acid, C18:2 n-6 and α-linolenic acid, C18:3 n-3) and their respective long-chain derivative (≥ 20 C-atoms) contents were noted. However, there were positive correlations between several n-6 and n-3 fatty acids, *i.e.* linoleic and α-linolenic acid, n-6 LCP and n-3 LCP fatty acids (Table 1). There was also a correlation between AA (C20:4 n-6) and its elongation product, docosatetraenoic acid (C22:4 n-6) (r=0.74, P=0.0001), as well as between eicosatrienoic acid (C20:3 n-3) and its elongation and desaturation product, DHA (C22:6 n-3) (r=0.57, P=0.0001).

Discussion

The saturated, monounsaturated and polyunsaturated fatty acid contents in colostrum lipids of Slovenian women were within the range reported for colostrum lipids in France (*18*), Germany (*19*), Italy (*20*), Norway (*21*), Poland (*22*), Spain (*23*) and Sweden (*24*). Compared to colostrum composition in countries mentioned, the colostrum lipids of Slovenian women contain relatively

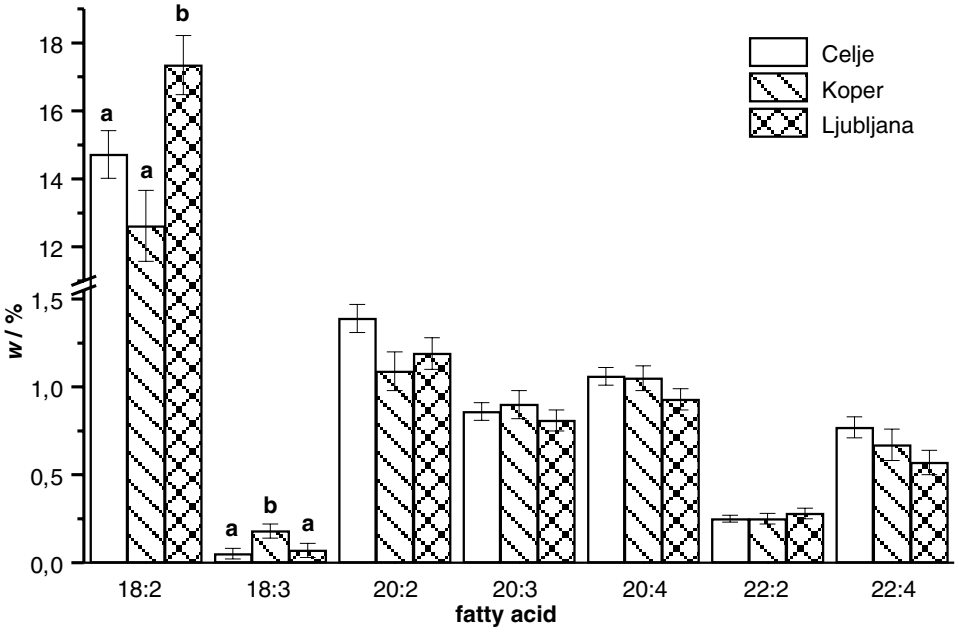

Fig. 2. The n-6 polyunsaturated fatty acid composition of colostrum lipids in Slovenia according to regional areas (estimated mean ± SEM)
[a,b,c] Statistically different at $P<0.05$

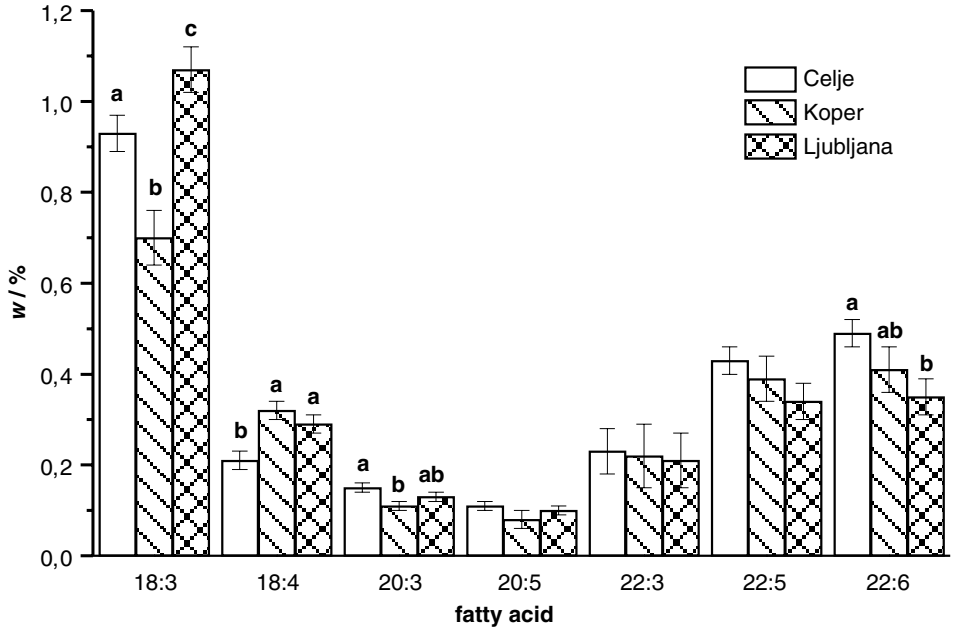

Fig. 3. The n-3 polyunsaturated fatty acid composition of colostrum lipids in Slovenia according to regional areas (estimated mean ± SEM)
[a,b,c] see under Fig. 2

low amount of total saturated fatty acids, median amount of monounsaturated fatty acids and high amount of polyunsaturated fatty acids.

The present study shows some differences in colostrum content of polyunsaturated fatty acids between women living in Celje, Koper and Ljubljana region (Figs. 2 and 3). Differences in contents of parent essential fatty acids, linoleic and α-linolenic acid, as well of other polyunsaturated fatty acids are most probably due to differences in dietary intakes of these fatty acids. It is known that linoleic and α-linolenic acid cannot be synthesized *de novo* in humans, but must be provided by diet. The study of Martin *et al.* (*25*) on relationship of human colostrum triglycerides and white adipose tissue composition showed that interindividual variation of linoleic acid content of human colostrum triglycerides largely depends on body-stores composition. Comparison of the fatty acid composition of human milk lipids of vegan

and vegetarian women with that of omnivore women showed higher contents of linoleic and α-linolenic acid in milk lipids of vegan and vegetarian women, most probably due to higher dietary intakes of these fatty acids (*26,27*). With stable isotope techniques it has been confirmed that most of the linoleic acid in human milk originate from body stores and only about 30 % comes directly from diet (*28*). Other long chain polyunsaturated fatty acids in human milk can additionally be obtained from diet and body stores and to a minor extend from endogenous synthesis (*29*). It was measured that about 11 % of dihomo-γ-linolenic acid and only about 1.2 % of AA present in mature human milk originate from endogenous conversion of dietary linoleic acid not intermediary deposited (*28*). Due to this facts, the long term nutrition is important, because it affects composition of body stores (*11*).

LCP fatty acids are minor, but very important component of human milk lipids. Differences in DHA content in colostrum lipids of women living in Celje and Ljubljana region could be due to differences in dietary intakes (*30,31*), specially of sea fish, that are a rich source of n-3 LCP fatty acids (*32*). The correlation between DHA and eicosatetraenoic acid content in colostrum lipids observed in our study has not been reported in the literature. The positive correlations found between n-6 and n-3 fatty acids (Table 1) are in agreement with literature data (*21,25*). The positive correlation between linoleic and α-linolenic acid highlights the physiological importance of balance between these two essential fatty acids (*21*). Tight correlation between n-6 and n-3 LCP fatty acids has also been reported in transitional (*18*) and mature human milk (*18,33*). This relationship shows that constant balance between n-6 and n-3 LCPs is maintained through lactation (*18*). The lack of correlations between parent essential fatty acids and their long-chain polyunsaturated product fatty acids suggests that maternal dietary intake of linoleic and α-linolenic acid has no influence on milk LCP fatty acids levels in humans (*18*). Indeed, very low levels of LCP fatty acids have been found in human milk of vegetarian women in comparison to women on omnivorous diet (*27*). A large body of scientific evidence suggests that n-3 LCP fatty acids exert beneficial effects in maintaining healthy physiological systems and in balancing the effects of n-6 fatty acids on eicosanoid-mediated disorders involving arrhythmia, thrombosis, blood pressure, lipid metabolism and immune functions in adulthood (*34*). Whether LCP fatty acid should be added to infant formula is one of the most debated, unresolved issues in infant nutrition. Recent extensive study of Lucas *et al*. (*35*) showed no beneficial effect of LCP fatty acids on cognitive and motor development or growth.

Conclusions

The present study gives a view on polyunsaturated fatty acid composition of colostrum lipids of Slovenian women living in Celje, Koper and Ljubljana regions. The average fatty acid composition of human colostrum lipids in Slovenia is comparable to that in other European countries. There are some differences in polyunsaturated fatty acid composition of colostrum lipids of women living in different regions of Slovenia. The correlations found between fatty acids are in agreement with results of other studies.

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Regionalne razlike udjela polinezasićenih masnih kiselina u lipidima kolostruma dojilja na području Slovenije

Sažetak

Dosada se u Sloveniji nije ispitivao sastav masnih kiselina u majčinom mlijeku. U radu je analiziran sastav masnih kiselina u mastima kolostruma 41 Slovenke trećeg dana nakon poroda u tri slovenske bolnice (Celje, Koper i Ljubljana). Koeficijent varijacije, prilikom mjerenja pojedinih masnih kiselina kojih je bilo više od 0,1 % masenih udjela, iznosio je od 2 do 6 %. Ovisno o regionalnom području postoje signifikantne razlike ($P<0,05$) u udjelu polinezasićenih masnih kiselina u lipidima kolostruma, i to: kod n-6 [linoleinska (C18:2), γ-linolenska (C18:3)] i kod n-3 [α-linolenska (C18:3), oktadekatetraenska (C18:4), eikosatrienska C20:3) i dokosaheksaenska kiselina (C22:6)]. Linoleinska kiselina iznosila je između 12,6 % masenih udjela (Koper) i 17,4 % masena udjela (Ljubljana) od ukupnih masnih kiselina u lipidima kolostruma. α-linoleinska kiselina iznosila je od 0,7 % masenih udjela (Koper) do 1,1 % masenih udjela (Ljubljana), a prosječni odnos linoleinske kiseline prema α-linolenskoj kiselini iznosio je 16,8. Najmanji je udjel dokosaheksaenske kiseline u lipidima kolostruma nađen u Ljubljani (0,35 % masenih udjela) dok je najviši bio u Celju (0,49 % masenog udjela, $P<0,05$). Razlike u sastavu masnih kiselina u lipidima kolostruma u raznim slovenskim regijama vjerojatno su posljedica različita načina prehrane.