

Polyunsaturated fatty acids in maternal plasma and in breast milk

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Summary In order to explain processes underlying the transfer of fatty acids from the maternal compartment into human milk, the lipid content and the fatty acid composition of maternal plasma and milk have been analyzed in breastfeeding mothers at 1 day and 3 months of lactation.

The rise in milk lipids occurring during the study period was concomitant with a fall in plasma total fat content, mainly due to the decrease of triglycerides. Significant correlations between plasma and milk fatty acids at the two time points were observed only for linoleic (LA, 18:2 n-6) and (α -linolenic acid (α LNA, 18:3 n-3), while for arachidonic (AA, 20:4 n-6) and docosahexaenoic acid (DHA, 22:6 n-3) correlations were found only at one day and 3 months, respectively.

These data suggest that levels of the n-6 and n-3 18C polyunsaturated fatty acids in milk are closely dependent on their concentrations in maternal plasma, in turn related with the dietary intake, while the accumulation of AA and DHA in milk is the result of a sequence of transfer and metabolic processes. © 2002 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

The interest of nutritionists and pediatricians in human milk composition has grown considerably in the last several years. In fact since breast milk is universally accepted as the best food for the newborn, it should be used as reference in the preparation of formulas to be adopted when breast lactation is precluded.

In particular, in comparison with milk from other species, human milk contains appreciably greater amounts of long-chain polyunsaturated fatty acids (LC-PUFA), especially arachidonic acid (AA, 20:4 n-6) and docosahexaenoic acid (DHA, 22:6 n-3).¹ These compounds play key roles in tissue and organ growth and development, which take place very actively during the first months of extra-uterine life. The incorporation of LC-PUFA into membranes of specialized cells, i.e. the accumulation of DHA in brain cortical neurons, appears to affect the later cognitive development,² and could partly explain the developmental advantage of breastfed subjects.³

Several studies have been devoted to PUFA in human milk, but the relative contribution to the milk FA profile provided by fats in the maternal diet vs those in body stores is still under investigation. Thirty percent of individual FA in human milk are derived from maternal diet,^{4,5} the remaining 70% deriving from either body stores and liver metabolism.⁶ Since these last steps are influenced by usual dietary habits at middle-long term, we may speculate that diet is the main factor affecting the FA composition of human milk. On the other side, a direct evaluation of the relationships between FA in the maternal diet and in milk is biased by difficulties in obtaining reliable measures of dietary intakes and of food composition.

The relationship between the FA composition of the diet and that of plasma lipids has been confirmed by several reports.^{7,8} Thus, an indirect evaluation of the influence of dietary factors on breast milk PUFA may be obtained through the analysis of the correlations between PUFA in maternal plasma and those in milk. This study is aimed at assessing this type of relationships at different stages of lactation.

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METHODS

We have initially recruited 95 mothers, who gave birth to healthy, full-term (37–42 week gestation) infants. Women

suffering from any metabolic disorder (hyperlipidemia, insulin-dependent diabetes) and/or taking corticosteroids during pregnancy were excluded. Mothers gave informed consent and the design was approved by the Departmental Ethical Committee. All the mothers were enrolled at 1 day postpartum. Out of the initial group, 54 women breastfed up to 3 months, but only 22 gave consent to undergo blood drawing at the two time points.

Milk samples were obtained by the mothers by pooling breast milk (hindmilk) from all the feedings over 24 h at the first day and at 3 months of lactation, immediately frozen and delivered to the laboratory for analysis the day after. At the same day blood samples were collected from the fasting mothers, plasma was prepared by centrifugation, and kept at -20°C until analysis.

Serum triglyceride, total and HDL-cholesterol levels were measured by standardized enzymatic methods. LDL-cholesterol concentrations were estimated with the Friedewald formula.⁹

Total lipids from plasma and milk samples were extracted according to Folch¹⁰ with chloroform and methanol (2:1 v/v) containing butyl-hydroxytoluene (5 µg/ml) to prevent lipid oxidation, and the content in aliquots of the extract was determined microgravimetrically after solvent evaporation. Fatty acid methyl esters of total lipids prepared by transesterification with 3 N HCl-methanol (Supelco Belafonte, PA, USA) at 90°C for 1 h, were analyzed on a gas-chromatograph (GC 85.10 DANI, Monza, Italy, equipped with a flame ionization detector) using a capillary column (SP-2560 SupelcoTM 100 m length, 0.25 mm ID, 0.2 µm film thickness) with programming temperature (from 150 to 220°C , with 1°C per min increments up to 180°C and then 6°C per min increments), after addition of an internal standard for quantitation. Paired *t*-test and Spearman correlation coefficients were used to compare the individual FA levels at the different time points and to assess relationships between plasma and milk samples at the same time point.

RESULTS

The basic characteristics of the 22 mothers of the study are reported in Table 1. Socioeconomic indicators were coded according to the Italian Census (Central Statistics Institute (ISTAT), 1983). The 22 mothers are a representative group, since there are no significant differences with the other 73 as far as both basic characteristics and milk FA composition.

The plasma lipid profile of the mothers at 1 day and 3 months of lactation, described in Table 2, shows a fall of total and LDL-cholesterol and triglycerides during this period of lactation, together with a parallel significant rise in HDL-cholesterol. In particular, the decrease

Table 1 Basic characteristics of the 22 mothers who gave blood samples vs all the others initially recruited for the study ($n=22$ vs $n=73$; median and range)

| | <i>n</i> =22 | <i>n</i> =73 |
|---|------------------|------------------|
| Age at delivery (year) | 30.5 (23–42) | 30.0 (20–45) |
| Gestational age (weeks) | 39.3 (37.4–41.4) | 39.2 (37–41.6) |
| Height (cm) | 163.5 (158–170) | 163.5 (148–176) |
| Standard body weight (kg) | 55 (46–68) | 55 (45–80) |
| Intrapregnancy body weight increment (kg) | 12 (7–19) | 13 (4–20) |
| Pre-pregnancy BMI | 19.6 (17.9–27.2) | 20.7 (16.7–28.8) |
| End-pregnancy BMI | 24.8 (20.7–32.9) | 25.7 (20.3–33.3) |
| Primiparous (yes, not) | 15, 7 | 55, 28 |
| Education (<14 year, >14 year) | 8, 14 | 30, 43 |
| Social category (upper, lower) | 15, 7 | 43, 30 |
| Smoking (yes, not) | 6, 16 | 26, 69 |

Table 2 Plasma lipids in lactating women (mean \pm SE)

| | 1 day | 3 months |
|--------------------|--------------|-------------|
| Total cholesterol* | 197 \pm 9 | 152 \pm 6 |
| Triglycerides* | 159 \pm 12 | 43 \pm 10 |
| LDL-cholesterol* | 128 \pm 9 | 95 \pm 5 |
| HD-cholesterol* | 37 \pm 2 | 45 \pm 3 |

* $P < 0.05$.

observed in plasma total lipids (Fig. 1) is closely correlated with the change in triglycerides ($R=0.647$; $P=0.001$, not shown). These plasma modifications are associated with a marked increase in milk total lipids, which at 3 months reach concentrations three-fold higher than in colostrum.

The percentage fatty acid composition of plasma total lipids at 1 day and 3 months of lactation is reported in Table 3. The major changes concern the decrease of both total saturated and monounsaturated FA, in parallel with a significant increase of total PUFA and of the unsaturation index. Looking at individual fatty acids, a uniform reduction of major monounsaturated FA is observed, while trends of the individual FA in the other two classes are different. Although stearic acid (18:0) and its elongation products significantly increase from the first day up to the third month, total saturated FA decrease, due to the reduction of palmitic acid (16:0), the main saturated FA in plasma. The detailed analysis of PUFA shows that levels of LA (18:2 n-6) and of its main conversion products, γ -linolenic (18:3 n-6), dihomogamma-linolenic (20:3 n-6) and AA, rise from 1 day to 3 months. Among the n-3 FA, the decrements of α -LNA (18:3 n-3) and DHA are balanced by the increase of both the intermediate EPA (20:5 n-3) and docosapentaenoic acid (22:5 n-3), so that the total n-3 FA content remains unchanged. In Table 4, the FA

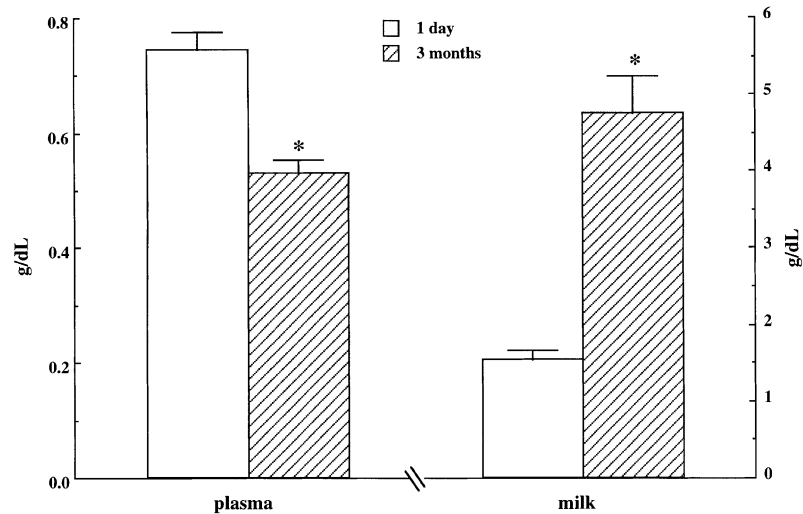


Fig. 1 Total lipids (g/dl) in maternal plasma and milk at 1 day and 3 months of lactation. The fall in plasma total lipids occurring during the first months of lactation is accompanied by a rise in milk FA content in the same period. Both changes are statistically significant ($P \leq 0.005$).

Table 3 FA % composition of maternal plasma total lipids at 1 day and 3 months of lactation (mean \pm SE)

| | First day | Third month |
|--------------------|-------------------|-------------------|
| <i>Fatty acids</i> | | |
| 14:0 | 0.87 \pm 0.08 | 0.90 \pm 0.09 |
| 16:0** | 26.80 \pm 0.35 | 22.38 \pm 0.34 |
| 18:0** | 6.22 \pm 0.20 | 7.73 \pm 0.21 |
| 20:0** | 0.23 \pm 0.01 | 0.28 \pm 0.01 |
| 22:0** | 0.50 \pm 0.03 | 0.66 \pm 0.03 |
| 24:0** | 0.40 \pm 0.03 | 0.55 \pm 0.02 |
| 16:1* | 1.91 \pm 0.11 | 1.50 \pm 0.12 |
| 18:1* | 22.46 \pm 0.55 | 20.48 \pm 0.64 |
| 18:1 n-7 | 1.60 \pm 0.03 | 1.55 \pm 0.05 |
| 20:1 | 0.17 \pm 0.01 | 0.15 \pm 0.01 |
| 22:1 | 0.05 \pm 0.01 | 0.04 \pm 0.01 |
| 24:1* | 0.83 \pm 0.04 | 0.92 \pm 0.05 |
| 20:3 n-9 | 0.13 \pm 0.01 | 0.15 \pm 0.01 |
| 18:2 n-6** | 26.66 \pm 0.81 | 30.26 \pm 0.89 |
| 18:3** | 0.22 \pm 0.02 | 0.38 \pm 0.03 |
| 20:3 | 1.54 \pm 0.06 | 1.66 \pm 0.08 |
| 20:4** | 6.11 \pm 0.34 | 7.30 \pm 0.23 |
| 22:4 | 0.20 \pm 0.01 | 0.20 \pm 0.01 |
| 22:5** | 0.28 \pm 0.02 | 0.16 \pm 0.01 |
| 18:3 n-3* | 0.40 \pm 0.03 | 0.31 \pm 0.01 |
| 20:5* | 0.31 \pm 0.03 | 0.55 \pm 0.08 |
| 22:5* | 0.25 \pm 0.04 | 0.38 \pm 0.02 |
| 22:6* | 1.85 \pm 0.12 | 1.51 \pm 0.11 |
| Saturated** | 35.02 \pm 0.39 | 32.50 \pm 0.36 |
| Monounsaturated* | 27.01 \pm 0.57 | 24.63 \pm 0.69 |
| Polyunsaturated** | 37.96 \pm 0.70 | 42.87 \pm 0.84 |
| UI* | 127.78 \pm 1.45 | 137.20 \pm 1.40 |
| n-6** | 35.01 \pm 0.73 | 39.97 \pm 0.85 |
| n-3 | 2.82 \pm 0.16 | 2.75 \pm 0.17 |

* $P \leq 0.05$.

** $P \leq 0.005$.

Table 4 FA % composition of milk total lipids at 1 day and 3 months of lactation (mean \pm SE)

| | Colostrum | Milk 3 months |
|--------------------|------------------|------------------|
| <i>Fatty acids</i> | | |
| 14:0* | 4.55 \pm 0.29 | 5.48 \pm 0.41 |
| 16:0** | 26.79 \pm 0.41 | 23.98 \pm 0.57 |
| 18:0* | 7.65 \pm 0.42 | 9.52 \pm 0.80 |
| 20:0* | 0.29 \pm 0.03 | 0.22 \pm 0.01 |
| 22:0 | 0.15 \pm 0.01 | 0.13 \pm 0.02 |
| 24:0* | 0.21 \pm 0.03 | 0.08 \pm 0.01 |
| 16:1 | 2.26 \pm 0.18 | 2.32 \pm 0.14 |
| 18:1 | 37.89 \pm 0.91 | 38.85 \pm 1.38 |
| 18:1 n-7 | 2.64 \pm 0.12 | 3.25 \pm 0.50 |
| 20:1** | 1.01 \pm 0.05 | 0.49 \pm 0.03 |
| 22:1** | 0.21 \pm 0.02 | 0.08 \pm 0.01 |
| 24:1** | 0.36 \pm 0.03 | 0.12 \pm 0.01 |
| 18:2 n-6* | 11.24 \pm 0.43 | 12.71 \pm 0.60 |
| 18:3* | 0.06 \pm 0.01 | 0.17 \pm 0.05 |
| 20:2** | 0.90 \pm 0.06 | 0.30 \pm 0.04 |
| 20:3** | 0.66 \pm 0.04 | 0.40 \pm 0.02 |
| 20:4** | 0.95 \pm 0.04 | 0.50 \pm 0.02 |
| 22:4** | 0.47 \pm 0.05 | 0.09 \pm 0.01 |
| 22:5** | 0.12 \pm 0.01 | 0.05 \pm 0.00 |
| 18:3 n-3 | 0.66 \pm 0.05 | 0.71 \pm 0.04 |
| 20:5 | 0.06 \pm 0.01 | 0.06 \pm 0.01 |
| 22:5** | 0.29 \pm 0.03 | 0.15 \pm 0.02 |
| 22:6** | 0.58 \pm 0.04 | 0.35 \pm 0.06 |
| Saturated | 39.65 \pm 0.91 | 39.41 \pm 1.16 |
| Monounsaturated | 44.37 \pm 1.00 | 45.11 \pm 1.07 |
| Polyunsaturated | 15.99 \pm 0.52 | 15.48 \pm 0.66 |
| UI | 84.28 \pm 1.24 | 80.70 \pm 1.61 |
| n-6 | 14.41 \pm 0.49 | 14.21 \pm 0.65 |
| n-3* | 1.58 \pm 0.08 | 1.27 \pm 0.08 |

* $P \leq 0.05$.

** $P \leq 0.005$.

composition of maternal milk total lipids at 1 day (colostrum) and 3 months (mature milk) is reported. Total PUFA levels do not change throughout lactation, but

together with a trend to the increase of the precursors of both the n-6 and the n-3 series, LA and α LNA, respectively, a statistically significant decrease of all their

long-chain polyunsaturated derivatives takes place. Only the percentage of EPA remains unmodified, but the relevance is limited due to the very low values. On the whole, total n-3 FA levels significantly decrease from colostrum to mature milk. The correlations found between maternal plasma and milk FA at 1 day and 3 months after delivery are reported in Figures 2 and 3, respectively. There are significant correlations between plasma and milk levels of the 18C precursors of both the n-6 and n-3 series in milk and maternal plasma at the two time points, and the degree of association is higher at 1 day of lactation ($R=0.515$, $P=0.01$ vs $R=0.465$, $P=0.02$ for LA, and $R=0.803$, $P=0.001$ vs $R=0.492$, $P=0.03$ for α LNA, respectively) (Figs. 2 and 3, Panels A and B). The maternal plasma/milk relationships for their major highly unsaturated and elongated products, AA and DHA, differ depending upon the time period considered (i.e. colostrum or 3 months). In fact, AA levels in plasma are significantly correlated to those in milk at the first day (Fig. 2, Panel C, $R=0.537$, $P=0.01$), but not at 3 months ($R=0.310$, $P=0.171$, not shown). On the other hand, the correlation between plasma and milk DHA levels is observed only at the third month (Fig. 3 Panel C, $R=0.515$, $P=0.01$) and not at the first day ($R=0.302$, $P=0.172$, not shown). Anyway even when significant, the correlations for AA and DHA are determined by the values from the same mother, showing the highest level of AA at colostrum and of DHA at 3 months, respectively.

DISCUSSION

A first observation deriving from this study regards the inverse trend in the total fat content of maternal plasma and milk from the first day to the third month of lactation. It is well known that pregnant women undergo an hyperlipidemic status¹¹ and, although we have no data about plasma lipid parameters in the last stages of pregnancy, we can assume that this status is maintained up to the beginning of lactation. At 3 months of lactation maternal plasma lipids are markedly reduced to levels far below those considered as physiological (e.g. 43 mg/dl of triglycerides). Preliminary data (not shown) from a small group of mothers indicate that plasma total lipids are higher at the end of lactation (6–9 months after delivery) than at 3 months, although not yet reaching the values measured at 1 day. These observations suggest a general association between the fall in plasma lipids, especially triglycerides, and the process of secretion of a triglyceride-rich fluid (milk),¹² with a trend to go back to pre-lactating values at the end of the breast-feeding period. During lactation, the reductions in maternal plasma fat content and PUFA concentrations are associated with fat transfer into the secreted milk.

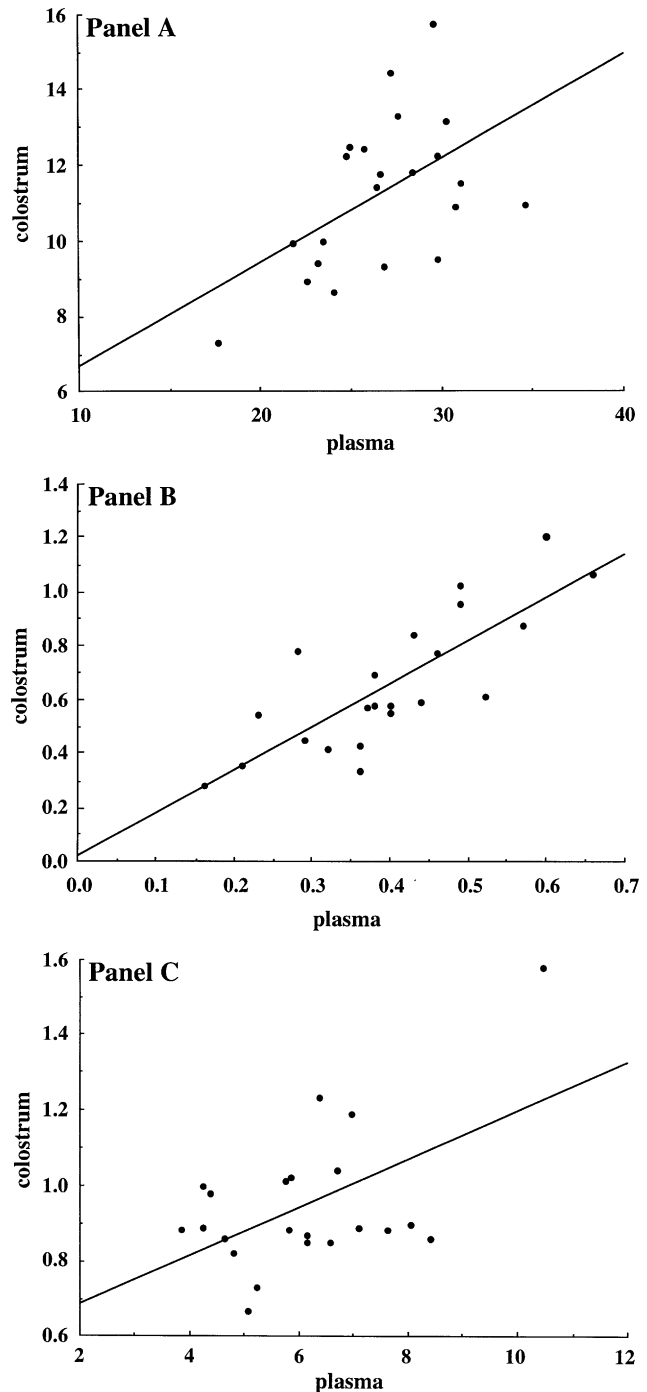


Fig. 2 Relationships between FA % levels in plasma and milk at the first day of lactation. Statistically significant correlations between the two compartment concentrations have been found for LA (Panel A: $R=0.515$; $P=0.01$), (α LNA (Panel B: $R=0.803$; $P=0.001$) and AA (Panel C: $R=0.537$; $P=0.01$).

As to plasma FA, the increase observed for LA from colostrum to 3 months may be associated with fat mobilization from the adipose tissue promoted by the lactation process.¹³ However, also the amount of LA esterified into stored triglycerides is necessarily dependent

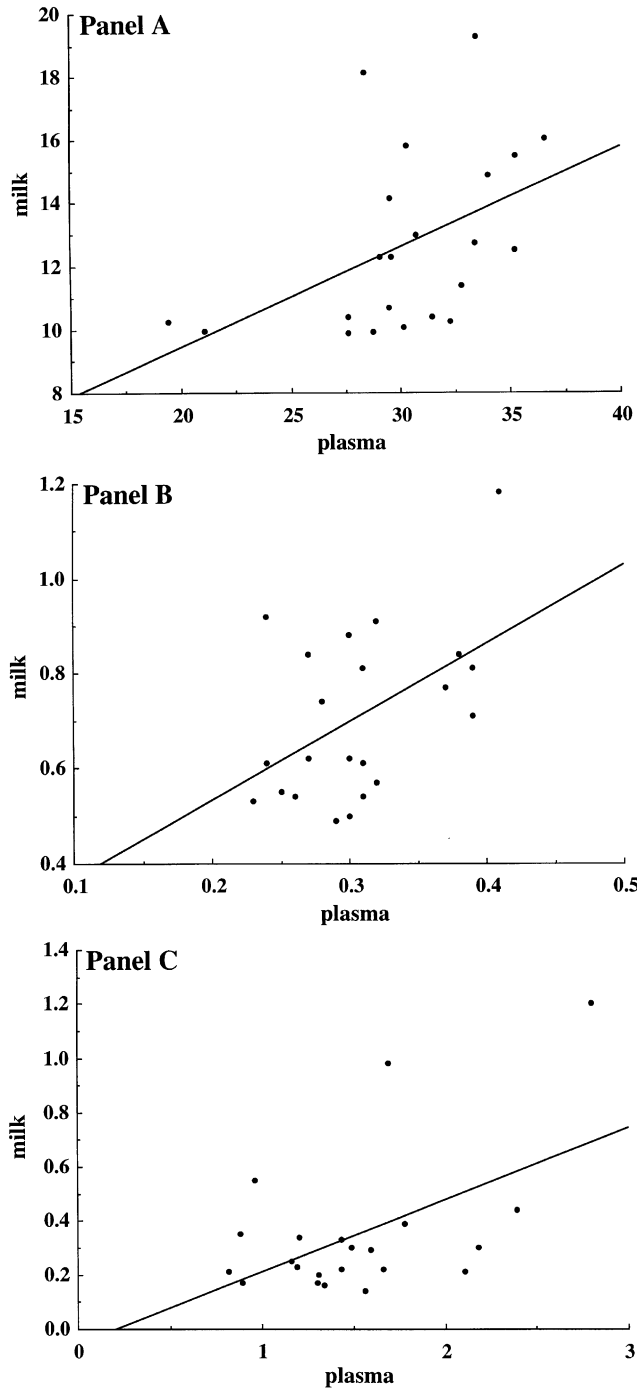


Fig. 3 Relationships between FA % levels in plasma and milk at the third month of lactation. Statistically significant correlations have been found for LA (Panel A: $R=0.465$; $P=0.03$), α LNA (Panel B: $R=0.492$; $P=0.02$) and DHA (Panel C: $R=0.515$; $P=0.01$).

upon the dietary intake, since this fatty acid cannot be synthesized in the human body.¹⁴

As to the LC-PUFA of both the n-6 and n-3 series, AA and EPA plus 22:5 n-3, their rise in plasma lipids suggests

the active conversion of the precursors LA and α LNA, respectively, to these products. The contemporary fall in DHA content may indicate that the last steps in the biosynthesis pathway for the n-3 FA, based on peroxisomal β -oxidation reactions,¹⁵ are not fully operating.

Since the plasma PUFA status is directly modulated by dietary PUFA, we may speculate a direct influence of dietary LA and α LNA on their levels in milk. Indeed our data suggest that the transfer of the two 18 C PUFA from maternal plasma does not appear to undergo differentiated transport processes for the n-6 and n-3 series. On the other hand, the percentage levels of the n-6 LC-PUFA in plasma and milk are associated at colostrum but not at 3 months, while the opposite is true for 22:6 n-3. We may hypothesize that additional steps in the diet-maternal plasma-mammary gland-milk pathway are working for the LC-PUFA. They appear to be different for FA of the n-6 and the n-3 series, and possibly aimed at counteracting plasma fluctuations and preserving their levels in milk.

We may conclude that physiological processes leading to increased availability of LA and α LNA from both diet and stored fats for transfer into human milk are operating in the first days of lactation. The dietary supply of the two essential FA may contribute to their circulating pools and should represent around 30% of their milk concentrations, according to data obtained with the use of stable isotopes.⁴ For the major LC-PUFA AA and DHA, intermediate metabolic steps, possibly occurring in the mammary gland, appear to take place to preserve their levels in milk.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

FA, fatty acids; LC-PUFA, long chain polyunsaturated fatty acids; LA, linoleic acid; α LNA, α -linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

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