The role of long-chain unsaturated fatty acids in infant feeding

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1 Introduction

Research on the physiological and food chemistry of fatty acids as compounds extends back a long way. In 1848, Arthur Görgey identified the fatty acid components of coconut oil. As early as 1929, the Burr couple demonstrated the essentiality of dietary fats in small animal experiments. By completely eliminating fats from the diet for a few months, they induced a new deficiency disease in rats, which caused tail death. This was easily prevented or cured by adding 2 percent (3 drops) lard to the animal's diet. In the following year, they were also able to demonstrate the vital need for a specific fatty acid, n-6 linoleic acid (C18:2n-6).

In the middle of the last century, infants fed with infant fed formula (IFF) containing fats of various origins were reported to have essential fatty acid (EFA) -deficiency disease, which was reversed by C18:2n-6 supplementation (2 % calorie). Intravenous fat-free feeding also caused biochemical changes similar to oral feeding in infants, which were reversed by standard IFF containing both C18:2n-6 and α-linolenic acid (C18:3n-3). This supports the view that C18:3n-3 is solely sufficient as a source of n-3 polyunsaturated fatty acid (PUFA) in infants, which is not contradicted by the suggestion that docosahexaenoic acid (C22:6n-3) may be conditionally essential for very low birth weight preterm infants.

Taking into consideration the complex metabolism of fatty acids, the following flowchart (**Figure 1**) is a simplified and summarized metabolic pathways of fatty acids with different carbon chain lengths and degrees of unsaturation from saturated stearic acid (C18:0). During metabolism, elongation occurs rapidly, while desaturation steps occur more slowly, and these steps determine the rate of metabolism (rate-determining step). All three discussed (n-9, n-6, and n-3) of fatty acid groups compete with the same enzymes during their metabolism. However, in mammals (and thus in humans), in the absence of Δ12- and Δ15-desaturase enzymes, members of different fatty acid groups cannot be converted into each other. However, the conversion between n-6 and n-3 long-chain polyunsaturated fatty acids (LCPUFA) occurs in algae, terrestrial plants, and simpler animal organisms, and research into these processes of potential food technology relevance is still ongoing.

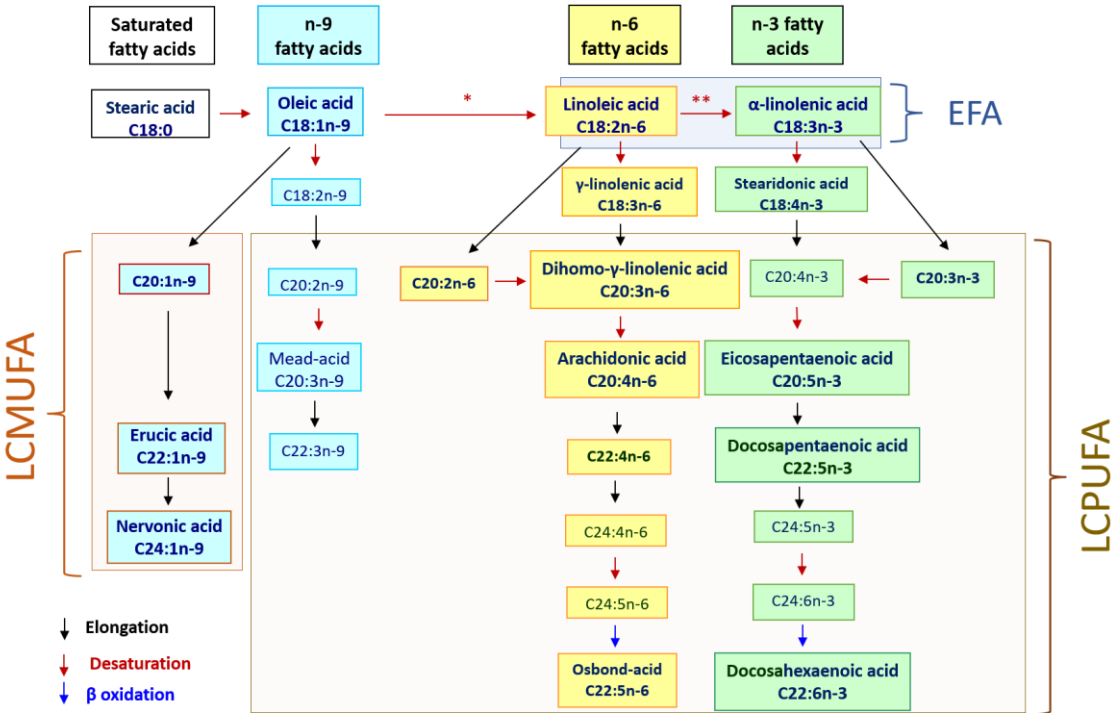

Figure 1: A simplified flowchart of the formation of long-chain unsaturated fatty acids (fatty acids in bold are used in this paper, LCMUFA: long-chain monounsaturated fatty acids; LCPUFA: long-chain polyunsaturated fatty acids; EFA: essential fatty acids; *: Δ12-desaturase enzyme, **: Δ15-desaturase enzyme, absent in mammals)

EFAs play different roles in nutrition. While C18:2n-6 accumulates in most mammalian organisms, C18:3n-3 is rarely found in tissue lipids in amounts comparable to those of C18:2n-6. The conversion of 18:3n-3 to n-3 LCPUFAs becomes less efficient in the classically accepted pathway up to 22:6n-3 according to the order of formation of n-3 PUFAs, making 22:6n-3 the least efficiently formed n-3 LCPUFA in humans. Studies suggest that conversion efficiency decreases with increasing age in infants and becomes even less efficient in adults.

Fatty acids, as constituents of various lipids, are involved in the structure of cell membranes and influence the fluidity of cell membranes. Membranes rich in saturated fatty acids are more rigid, whereas unsaturated fatty acids increase the fluidity of cell membranes and consequently increase the number of receptors and the affinity of receptors for substrates (hormones, growth factors, and other proteins), and can also be precursors of many secondary messenger molecules. C18:2n-6 plays an important role in the regulation of epidermal water permeability, prevents epidermal damage and excessive water loss. This fatty acid can also reduce serum total cholesterol and LDL cholesterol levels, and is also thought to have a protective effect against

atherosclerosis. The role of LCPUFA metabolites is made even more important by the fact that various prostaglandins and leukotrienes, are produced from dihomo-γ-linolenic acid (C20:3n-6); from arachidonic acid (C20: 4n-6) endocannabinoids, hydroxy, dihydroxy and epoxy derivatives, lipoxins, pro-inflammatory prostaglandins and thromboxanes can be produced; while n-3 LCPUFAs are precursors for resolvins, marezins, and other pro-inflammatory prostaglandins, thromboxanes and leukotrienes.

The above-mentioned LCPUFA metabolites contribute significantly to the structure of neuronal membranes, accounting for approximately 20% of the brain's dry matter. At the same time, the importance of long-chain monounsaturated fatty acids (LCMUFA), such as nervonic acid (C24:1n-9), is highlighted by the fact that the content of this fatty acid in total cerebellar white matter lipids more than doubles in breastfed infants during the first 20 weeks of life. While C20:4n-6 and C22:6n-3 play a dominant role in the phosphoglycerides of human placental membranes in both preterm (PT) and full-term (FT) infants, C24:1n-9 accounts for more than half of the unsaturated fatty acids of the sphingomyelin fraction in both groups and can therefore be considered as a key building block in myelin membrane sphingolipids.

Since the amount of LCPUFA in plasma lipids, red blood cell membrane and brain tissue of young children is strongly determined by dietary LCPUFA intake, the fatty acid composition of human milk (HM) has also become a focus of interest.

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2 Aims

- Since our literature search did not provide daily data on the fatty acid composition of human milk (HM) during the very early stages of lactation, we aimed to investigate the fatty acid composition of colostrum (C) and mature human milk (MHM) during the first month of lactation. Therefore, HM samples were obtained on each day of the first week of lactation and then on days 14 and 28 of lactation from mothers of healthy term newborns.
- In our studies we also investigated one of the classic questions in neonatal nutrition, whether there are differences in the fatty acid composition of the HM of women who gave birth to FT and PT newborns.
- Many years after our original observations were reported, we re-analysed our available data on the changes in LCMUFA metabolites (C20:1n-9, C22:1n-9 and C24:1n-9) in early HM. Our aim was to investigate the changes in these FAs, which have recently acquired independent physiological significance, in PT and FT HM samples during lactation and to determine whether there are differences between PT and FT HM groups.
- C22:6n-3 is the new mandatory component of IFFs in Europe. The purpose of our narrative review was to summarise the data behind the new European mandatory dietary recommendations that C22:6n-3 should be added to infant formula by at least 20 mg/100 kcal (4.8 mg/100 kJ).

3 Methods

Fatty acid analysis was performed in the chromatography laboratory of the Department of Paediatrics of the University of Pécs, mostly by myself, sometimes with the help of PhD students, medical students of the Undergraduate Research Society, or with the support of a laboratory assistant.

3.1 Determination of lipid content and total fatty acid composition of breast milk

Although there are recommended analytical methods for the extraction of lipids for all food groups, they are under continuous technical evaluation, as the costs, extraction efficiency, toxicity, availability, and quality of the final product must be taken into account. A full qualitative analysis of all lipids in a HM sample requires more than one instrument due to the complexity of the sample, and the choice of instrument depends on the objectives of the study.

3.1.1 Determination of the lipid content of breast milk samples by gravimetric method

In the laboratory, all HM samples were stored in a freezer and thawed once, immediately before analysis.

The lipid content of the mammary glands was determined by a slightly modified Folch extraction using a methanol/chloroform mixture to determine the chloroform soluble lipid content of the sample.

3.1.2 Determination of the fatty acid composition of breast milk

For the fatty acid analysis, the fatty acids were separated from the ester partner during transesterification and all the fatty acids were converted to methyl esters under acid catalysis.

Fatty acid methyl esters were determined by split injection (ratio 1:15) using a high-resolution capillary column gas-liquid chromatograph (Finnigan 9001, Finnigan/Tremetrics Inc., Tex., USA).

A 40 m long (50% cyanopropyl)-methylpolysiloxane stationary phase capillary column (DB-23, J&W Scientific, Calif., USA) with a wall thickness of 0.25 mm and a layer thickness of 0.25 μm was used for the measurements. The qualitative identification of peaks on the chromatograms was based on

standard mixtures (Supelco FAME 37, Alles). The relative response factor method was used, for quantification.

3.2 Statistical analysis

Data were analyzed using different versions (7.5-28) of the IBM (Windows) SPSS (SPSS Inc., Chicago, AL) statistical software package.

3.2.1 Changes in the fatty acid composition of breast milk during lactation

The fatty acid compositions of the total HM samples were calculated as weight percent (w/w%) of fatty acids with chain lengths between 12 and 24 carbon atoms. The data were expressed in median and interquartile range (distance between the first and third interquartile range), as the distributions did not correspond to a normal distribution, especially for fatty acids present in low concentrations. The results were compared using the Mann-Whitney test, and the significance level chosen was $p < 0.05$.

3.2.2 Comparison of milk from women who gave birth to term and preterm newborns

Two-way analysis of variance was performed with age at gestation and day of lactation as the two factors. If this test indicated a significant difference, comparisons between days of lactation were evaluated using a non-parametric Wilcoxon signed rank test, while differences between total and preterm milk at each time point were evaluated using a Mann-Whitney U test.

3.2.3 Changes in the long chain monounsaturated fatty acid composition of breast milk during lactation

We re-analysed the previous unpublished LCMUFA metabolites C20:1n-9, erucic acid (C22:1n-9) and C24:1n-9 in our HM study databases. The Mann-Whitney U-test was used to assess the difference between groups and the Wilcoxon signed rank test was used to control for differences within groups. To get a better overview of the changes in FA composition, the data of these three LCMUFA were mathematically aggregated into an estimated "pooled LCMUFA value". A logarithmic (Ln) trend line was fitted to both PT HM and FT HM data for the total LCMUFA metabolites and aggregated LCMUFA values.

4 The fatty acid composition of the human milk

4.1 Introduction

4.1.1 The role of polyunsaturated long-chain fatty acids

It has long been known that LCPUFA intake significantly affects the visual and cognitive development of infants, and the LCPUFA content of HM has been associated with atopic involvement in infants.

The fatty acid composition of HM depends on a number of factors, such as gestational age (GA), duration of lactation, number of maternal pregnancies, maternal diabetes and many other factors that were only partially identified at the time of our studies. In addition, the contribution of LCPUFA to the fatty acid composition of HM shows significant variability not only between populations living in different locations and following different diets, but also between different periods of lactation in the same mother.

The availability of LCPUFA for neonatal development depends on the amount of LCPUFA stored in maternal tissues at birth, the amount of LCPUFA intake from the diet, and the ability of the neonate to synthesize LCPUFA from their shorter-chain precursors. Whereas there is an ongoing discussion on whether synthesis of n-3 LCPUFA exclusively from C18:3n-3 may be sufficient to meet the requirements of the human body, the vast majority of n-3 LCPUFA supplementation experiments during pregnancy, lactation and infancy have also included preformed C22:6n-3.

While HM contains the most important LCPUFAs, including C20:4n-6 and C22:6n-3, many of the IFFs available at the time of our studies did not contain these fatty acids at all.

Although LCPUFA-supplemented HM-replacement formulae were available in many countries, the need for dietary LCPUFA intake in preterm infants was not universally accepted. LCPUFA-supplemented HM-substituting IFFs were considered a specialty, as witnessed by the significantly higher price and lack of availability of social insurance support.

4.1.2 The role of monounsaturated long-chain fatty acids

Not only C20:4n-6 and C22:6n-3, but also one of the LCMUFAs, C24:1n-9, are among the most important structural building blocks of the central nervous system. In their pioneering study, Babin and colleagues

suggested that C24:1n-9 levels in the sphingomyelin fraction of red blood cells could be used as an indicator of brain maturation. They reported that, regardless of the type of infant feeding, C24:1n-9 levels in red blood cell sphingomyelin lipids of PT infants (32 weeks of AG) were consistently elevated until the calculated term (week 37 of AG), suggesting that C24:1n-9 is efficiently metabolised from C18:1n-9 and incorporated into membrane sphingomyelin. Likewise, in healthy infants born at term, serum phospholipid C24:1n-9 levels were significantly elevated between day 2 and 4 months of age, while C24:1n-9 levels in HM were significantly reduced.

By investigating the concentrations of different fatty acids in the developing brain, it was detected that while the major LCPUFAs, C20:4n-6 and C22:6n-3, showed a rapid increase in the phosphatidylethanolamine (PE) fraction, C24:1n-9 also increased very rapidly in sphingomyelin lipids during the first eight years of life, an observation that further supports the importance of C24:1n-9 in myelination.

In a recent animal study, six-week administration of LCMUFA-rich plant-based oil (total C20:1n-9 + C22:1n-9 + C24:1n-9 content about 35%) (*Acer truncatum*, Bunge seed oil) in Sprague-Dawley rats resulted in improved cognitive function and brain remodelling. These experimental data support a role for C24:1n-9 and/or its precursors in structural brain development and maturation of brain functions such as learning and memory.

Studies on the composition of HM have addressed not only the possible developmental roles of n-3 and n-6 LCPUFAs, but also n-9 MUFAs. When HM samples were collected daily from eight healthy Chinese mothers between postnatal days 3 and 30, it was observed that not only was highest C24:1n-9 but also the n-9 metabolites C20:1n-9 and C22:1n-9 were the highest in C. After this, their contribution to HM fatty acid composition decreased significantly with the progression of lactation.

We found only six studies comparing HM from mothers of PT and FT newborns in the first month of lactation that also reported n-9 FA metabolites longer than C18:1n-9, however we did not find a daily approximation of HM sampling.

4.2 Changes in the fatty acid composition of breast milk during the first month of breastfeeding: daily data during the first week

4.2.1 Patients

HM samples were collected from 18 healthy breastfeeding women residing in Pécs, Hungary. The study included only mothers of healthy, full-term infants who were not twins. Samples were collected daily during the first week and on days 14 and 28.

4.2.2 Experimental results

Age of mothers (29.4 ± 4.0 years, mean ± SD), length of pregnancy (39.1 ± 1.6 weeks), infants' birth length (51.3 ± 2.8 cm) and weight (3537 ± 528 g), and early psychosomatic development of the infants were in accordance with physiological values. Six mothers gave birth for the first time, whereas 12 were not primiparous. Data from the food consumption frequency questionnaire did not show any self-limiting dietary intake, and the contribution of fish or fish products to the maternal diet was low.

Values of the physiologically most important saturated fatty acid, palmitic acid (C16:0), showed a time-dependent decrease over the study period. Total saturated fatty acids (SAT) increased significantly by day 7 of lactation, while monounsaturated cis-fatty acids (MUFA) decreased significantly during the first 2 weeks of lactation.

The n-6 essential fatty acid, C18:2n-6, was constant in HM fatty acid composition throughout the study period. In contrast, the values of C20:2n-6, C20:3n-6, C20:4n-6, docosadienoic acid (C22:2n-6), docosatetraenoic acid (C22:4n-6) and n-6 docosapentaenoic acid (C22:5n-6) significantly decreased almost daily. As a result, the total n-6 PUFA decreased significantly between days 1 and 4 and the total n-6 LCPUFA decreased with increasing lactation duration.

The n-3 essential fatty acid C18:3n-3 showed a significant increase during the first 2 weeks of lactation. In contrast, significant decreases were observed in C20:3n-3 and C22:5n-3, while C22:6n-3 values did not decrease consistently during the first month of lactation. Total n-3 LCPUFA showed a significant decrease during the study period, whereas total n-3 PUFA increased significantly between days 1 and 14.

Linear correlation analysis revealed a significant positive correlation between C18:3n-3 and C22:6n-3 on day 4, but significant negative correlations on days 7 and 14 of lactation. During the study period, there were no significant correlations between C18:2n-6 and C18:3n-3 and between C18:2n-6 and C20:4n-6, but significant and positive correlations were observed between C20:4n-6 and C22:6n-3 on days 1, 5 and 6 of lactation.

4.2.3 Discussion

We reported a relatively low variability in the fatty acid composition of HM samples during the very early stages of lactation. The fatty acid composition of HM is influenced not only by the current diet of the lactating mother but also by the long-term LCPUFA status of the mother. The relatively low variability in early HM fatty acid composition suggests that these compositional data may serve as additional indicators for studying the effective modification of maternal LCPUFA status during pregnancy.

4.3 Fatty acid composition of breast milk was different in mothers of preterm and full-term newborns during the first three weeks of breastfeeding.

4.3.1 Patients

HM samples were collected from 18 breastfeeding mothers living in Pécs. Healthy mothers of singleton newborns were included. The ethics committee of our university approved the study protocol and all mothers were informed about the studies in writing. Two of the newborns born full-term were born to primiparous mothers, while eight were born to multiparous women. With regards to preterm newborns, only one had a first-time mother, while seven had multiparous mothers.

There was no difference in maternal eating habits between the two groups. Fish consumption was generally low, with the majority of mothers in both groups consuming fish 1-3 times a month.

Hand-pressing hindmilk was collected from the mothers daily during the first week after birth, and then on days 14, 21 and 28.

4.3.2 Experimental results

The age of the mothers was the same, while the gestational age differed significantly between the two groups (38.5 (2.7) weeks versus 28.0 (4.2) weeks, median, (IQR)).

The fat content (g/L) of breast milk was not different between milk samples from mothers who delivered a term and a preterm newborn. SAT increased only in the milk of mothers with preterm newborns, whereas this value did not change in mothers with mature newborns with lactation progression. Total cis MUFA levels did not change significantly over the study period. Total *trans* isomeric fatty acids did not show any change in the milk of women with mature newborns during the first three weeks of lactation, but significantly decreased in the milk of women with preterm newborns between days 1 and 4.

Not only the total n-3 LCPUFA, but also the total n-6 LCPUFA decreased significantly in the milk of women who gave birth to both term and preterm newborns during the study period.

The amounts of SAT and MUFA were not different in the milk of women who gave birth to preterm and term newborns during the study period.

Moreover, the amount of total trans fatty acids was significantly higher in the milk of women who gave birth to preterm infants than in that of mature newborns on the first day of lactation, but no difference was observed at later time points. Additionally, the values of essential C18:2n-6 and C18:3n-3 did not differ between the two groups during the study.

In contrast, the major LCPUFAs, C20:4n-6 and C22:6n-3, were significantly higher in the milk of women who gave birth to preterm newborns than in term newborns. In addition, the levels of C20:4n-6 and C22:6n-3, intermediary metabolites involved in the synthesis of C20:4n-6 and C22:6n-3, were also significantly higher in milk samples from mothers who gave birth to preterm than those from mothers who gave birth to term newborns. The total n-3 LCPUFA and total n-6 LCPUFA values were both significantly higher in milk samples from mothers who gave birth to preterm newborns than in those from mothers who gave birth to mature newborns on days 4, 7, 14 (total n-3 LCPUFA only) and day 21.

4.3.3 Discussion

In our study, we found that C20:4n-6 and C22:6n-3 contributed significantly more to the fatty acid composition of HM in mothers who delivered preterm infants than in term infants. The differences were detectable as early as the second or third day of lactation and persisted throughout the study period (i.e. until day 28 of lactation). In addition, the levels of the most important perinatal LCPUFA, C22:6n-3, were not only slightly higher in the milk of mothers who delivered preterm infants than in those born term, but at least a twofold difference was detectable throughout the study period.

Since the age, body mass index, parity and diet of the mothers did not differ between the two groups, the differences in fatty acid composition observed appear to be related to preterm birth.

The less depleted maternal LCPUFA stores after a significantly shorter gestation period may explain the greater contribution of LCPUFAs to the fatty acid composition of HM after preterm delivery than after term delivery, since the LCPUFA content of HM is closely related to the restoration of maternal LCPUFA stores during lactation.

It would also be tempting to speculate about some adaptive mechanisms that may have evolved during evolution to ensure a higher LCPUFA requirement in preterm infants compared to term infants. However, the high survival rate of low-birth-weight preterm infants is a very recent development in human history; therefore, evolutionary changes cannot explain the differences observed in our study.

4.4 Temporal change of long-chain monounsaturated fatty acids in breast milk

4.4.1 Background

There is no generally accepted classification of LCMUFA metabolites in the literature, with some authors classifying MUFA isomers with carbon numbers between 20 and 22 as LCMUFAs, while others refer to MUFA isomers with carbon numbers between 20, 22 and 24 as "Σ 20:1n-9, 22:1n-9, 24:1n-9" or VLC-MUFAs. In this study, LCMUFA was used to characterize the total amount of metabolites with carbon chain lengths longer than C18:1n-9, namely C20:1n-9, C22:1n-9 and 24:1n-9.

In our review of the literature, we did not find any daily follow-up study that examined the FA composition of PT and FT HM. Most studies sampled shorter periods, but different authors used different definitions of lactation stages (C, TM, and MHM). Some studies used a relatively wide sampling intervals, where the distribution of lactation periods was not uniform; however, there was a significant overlap between the ranges of the breastfeeding phase in the publications.

4.4.2 Origin of the data

Quantitative changes in this understudied family of fatty acids in HM were reevaluated as lactation progressed, based on previous databases from our research group. The newborns of mothers who gave birth to preterm infants did not suffer from any conditions other than those normally associated with preterm birth (e.g. congenital malformation, metabolic disease). In all studies, the mothers were aged approximately 30 years and the average maternal BMI was within the normal range.

4.4.3 Experimental results

We found a significant decrease not only for the three individual LCMUFA metabolites, but also for the calculated total LCMUFA values (Figure 2) with

increasing duration of lactation. For each monounsaturated fatty acid (C20:1n-9, C22:1n-9 and C24:1n-9), the highest values were found in C, followed by decreasing values in transitional milk (TM) and MHM.

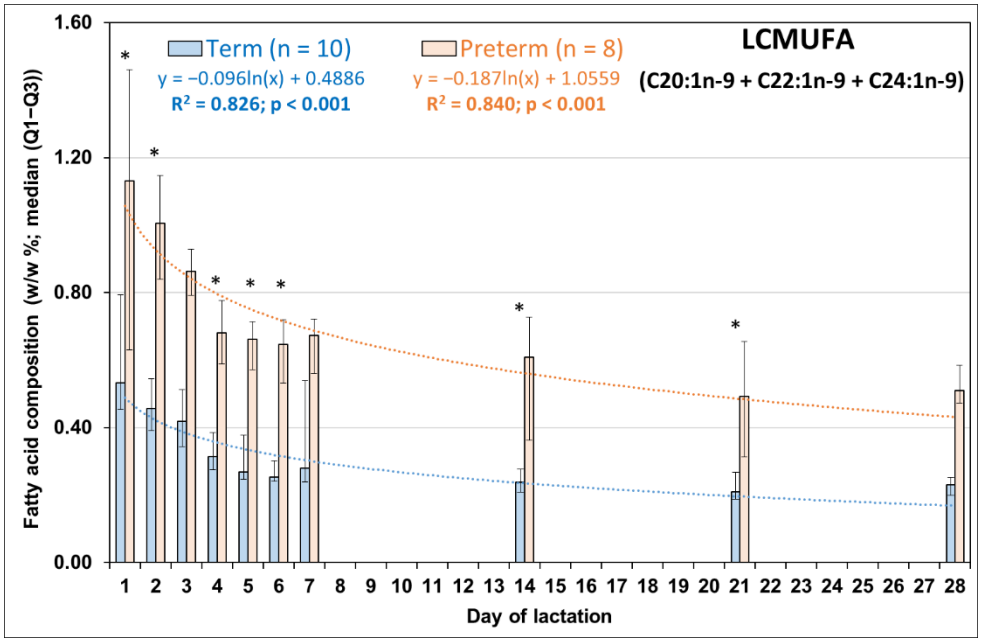

Figure 2: Contribution of total long-chain monounsaturated fatty acids (LCMUFA) to the fatty acid composition of breast milk from mothers who gave birth to full-term (n = 10) and preterm (n = 8) infants (*: indicates a significant difference between full-term and preterm milk, Mann-Whitney U test, $p < 0.05$)

4.5 The role of docosahexaenoic acid in infant formulae: a narrative review

4.5.1 Docosahexaenoic acid: a new mandatory ingredient in infant formulae in Europe

C22:6n-3 supplementation for healthy infants is regulated by the European Union (EU) in the form of a mandatory food composition regulation, so all infant formulae and follow-on formulae available and purchased in the EU must contain at least 20 mg/100 kcal (4.8 mg/100 kJ) and no more than 50 mg/100 kcal (128 mg/100 kJ) of C22:6n-3. Therefore, C22:6n-3 has become a mandatory nutrient ingredient in complementary foods, along with vitamins, trace elements, essential amino acids and EFAs.

4.5.2 Literature overview on docosahexaenoic acid in infant feeding

The PubMed database contains more than 18,000 articles on C22:6n-3, including more than 1800 randomised controlled trials (RCTs). If we limit the

search to articles potentially related to infant feeding - search term: "docosahexaenoic acid with (infant or human milk or formula)" - we can identify nearly 2,000 articles, including more than 400 RCTs (about 20%), and concluding that this metabolite is the core interest of several ongoing clinical trials.

4.5.3 Docosahexaenoic acid in infant formulae

The most important medical priority in infant feeding is to protect, support and promote breastfeeding. Efforts to modify the composition of IFF are justified only if (a) there is an unavoidable need to use IFF, and (b) the modification will have a positive effect on the growth and development of infants fed IFF or will prevent an adverse health outcome.

4.5.3.1 The contribution of infant formula to the feeding of term infants

The World Health Organisation (WHO) and the United Nations Children's Fund recommend that children should be breastfed within the first hour of birth and exclusively breastfed for the first 6 months of life - which means that they should not be given any other food or fluids, even water. However, according to the WHO's current official public database, only 48% of babies aged 0-5 months worldwide are exclusively breastfed.

Between 2005 and 2019, the retail sales of all standard milk formulae increased by 54.5% to 10.8 kg/child in 77 different countries, compared to 17.8% to 29 kg/child in the 24 European countries included in this database.

Unfortunately, infant formula is a common source of nutrition for healthy full-term infants worldwide, particularly in Europe. In addition to the primary goal of protecting, supporting and promoting breastfeeding, improving the nutrient composition of infant formulae can serve as a secondary, complementary goal in supporting infant growth and development.

The data presented also clearly indicate that infant formulae are an important source of nutrients for healthy, mature infants. Therefore, research to improve the nutrient composition of infant formulae may be warranted.

4.5.3.2 The impact of docosahexaenoic acid in infant formulae

According to a Cochrane review, 4 of the 9 studies that examined visual acuity reported positive effects, while the remaining 5 did not. Of the 11 studies that examined neurodevelopmental outcomes, 4 of them reported positive effects, while the remaining 7 did not. No beneficial or adverse effects

of supplementation were reported in 13 studies that measured physical growth. A meta-analysis of five RCTs showed that the supplemented group had lower body weight but not height or head circumference at 12 months of age, while there was no difference at 18 months of age. The authors of the Cochrane review concluded that “Most of the included RCTs reported no beneficial effects or harms …” and “Routine supplementation of full-term infant milk formula with LCPUFA cannot be recommended this time”.

RCT studies have identified a variety of genetic, environmental, dietary and methodological factors influencing the efficacy of C22:6n-3 supplementation of formula for FT infants, which makes it understandable why no high-level evidence has been found to date and suggests that such evidence is unlikely to presented in the near future.

4.5.4 Current considerations for docosahexaenoic acid in infant formulae

The regulatory inclusion of C22:6n-3 in the FA formulation of infant formulae in Europe was preceded by three scientific opinions of the European Food Safety Authority (EFSA), which considered C22:6n-3 a conditionally essential FA for infants and established an adequate intake of 100 mg per day for infants aged 7-24 months. The observed intake of C22:6n-3 from HM and an intake of 100 mg C22:6n-3 per day were both considered adequate for the majority of 0–6-month-old infants. They also reviewed the available data on the effect of adding C22:6n-3 to the HM-replacement formula on various health outcomes and concluded that C22:6n-3 should be added to the HM-replacement formula. Indeed, the lack of evidence for adverse effects may further support the recommendation.

The recommendation for the mandatory inclusion of C22:6n-3 in infant formula is currently the focus of scientific attention in Europe, mainly because there is no clear opinion on the inclusion of the biologically most important n-6 LCPUFA, C20:4n-6, in infant formula. As the Regulation states, "Other LCPUFAs (20 and 22 carbon) may be added". As C20:4n-6 also appears to be necessary for optimal neurodevelopment, it is currently under debate whether formulae for mature infants should contain C20:4n-6 in addition to C22:6n-3. The mandatory inclusion of C22:6n-3 in IFFs has also raised the question of the optimal intake level of the classic n-6 EFA, C18:2n-6. C20:4n-6 alone is more effective in preventing clinical symptoms of EFA deficiency than C18:2n-6. Moreover, it has very different biological functions compared to C22:6n-3; however, the majority of studies include both C22:6n-3 and

C20:4n-6, when testing for the enhancement of C22:6n-3 specifically. Maintaining an appropriate balance of C22:6n-3 and C20:4n-6 in the formula of preterm infants is an established goal, but the optimal composition of the supplements has yet to be determined.

5 Summary

There were previously no daily data on changes in FT HM fatty acid composition in the very early stages of lactation, and limited data series were available over the first few weeks of lactation. In our study, we reported a decreasing proportion of n-3 and n-6 LCPUFA metabolites in the process of conversion to C MHM. We also found a significant positive correlation between the values of the two most important LCPUFA, C20:4n-6 and C22:3n-6, during the early lactation period. For dietary recommendations, these observations may provide further evidence for the importance of LCPUFA compounds, particularly C22:6n-3, in the diet of lactating mothers.

Our studies suggest that the fatty acid composition of milk from women who delivered term neonates and women who delivered preterm neonates differed markedly not only in individual values of several n-6 and n-3 LCPUFA metabolites, but also in total n-3 and n-6 LCPUFA at different time points during lactation. The decrease in LCPUFA values observed in the milk of mothers who have given birth to a preterm infant with advancing lactation, provides additional evidence for the practice of ensuring adequate LCPUFA supply to expectant mothers.

Although long-chain monounsaturated fatty acids such as C24:1n-9 play a less obvious role in the perinatal period than C20:4n-6 and C22:6n-3, our results suggest a possible importance of this group of compounds in the early postnatal period. In our study, we found significantly higher levels of C20:1n-9, C22:1n-9, C24:1n-9 and total LCMUFA values in milk samples from mothers who delivered a preterm than a full-term newborn at almost all time points examined. The levels of C20:1n-9, C22:1n-9, C24:1n-9 and total LCMUFA values decreased significantly during lactation. To our knowledge, a precise description of this decrease has not been previously reported in the literature.

In our review article on the overall topic of this thesis, we have described the role of C22:6n-3 in infant feeding and infant formula. We have also outlined the development process that has led to the current mandatory addition of the most important n-3 LCPUFA compound, C22:6n-3, to infant formulae in Europe.

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6 List of publications

The data in chapters 6.1-6.4 reflect the state of the MTMT database on 11 April 2024 in the following format: (**Impact Factor (IF); Quarterly distribution (Q1...Q4);** Total References (TR), Independent References (IR))

6.1 The following peer-reviewed publications in English were used as the basis for this thesis

1. **Marosvölgyi T.,** Dergez T., Szentpéteri J. L., Szabó É. and Decsi T. (**2023**) Higher availability of long-chain monounsaturated fatty acids in preterm than in full-term human milk *Life* 13, 1326. DOI: 10.3390/life13061326 (**IF: 3,200; Q2;** TR: 0, IR: 0)
2. Decsi T., **Marosvölgyi T.,** and Szabó É. (**2023**) Docosahexaenoic Acid in Formulas for Term Infants: The Way from Pioneer Idea to Mandatory Dietary Recommendation *Life* 13, 1205. DOI: 10.3390/life13051205 (**IF: 3,200; Q2;** TR: 0, IR: 0)
3. Kovács A., Funke S., **Marosvölgyi T.,** Burus I., and Decsi T. (**2005**) Fatty acids in early human milk after preterm, and full-term delivery *J Pediatr Gastroenterol Nutr* 41, 454; DOI: 10.1097/01.mpg.0000176181.66390.54 (**IF: 2,077; Q1;** TR: 69, IR: 63)
4. Minda H., Kovács A., Funke S., Szász M., Burus I., **Marosvölgyi T.,** and Decsi T. (**2004**) Changes of fatty acid composition of human milk during the first month of lactation: a day-to-day approach on the first week. *Ann Nutr Metab,* 48 202-9; DOI: 10.1159/000079821 (**IF: 1,067; Q2;** TR: 44, IR: 35)

6.2 The following peer-reviewed publications in Hungarian were used as the basis for this thesis

1. **Marosvölgyi T.,** Kovács A., Lohner Sz., Funke S., Burus I. és Decsi T. (**2006**) Az anyatej zsírsavösszetétele koraszülöttet és érett újszülöttet szülő anyákban a szoptatás első három hetében. *Orvosi Hetilap* 147, 1459. (IF: -; Q3; TR: 6, IR: 6)
2. Kovács A., Minda H., Funke S., Szász M., Burus I., **Marosvölgyi T.** és Decsi T. (**2004**) Az anyatej zsírsavösszetételének változása a szoptatás első hónapjában. *Gyermekgyógyászat*, 55, 460-466. (IF: -; Q-; TR: 0, IR: 0)

6.3 The following oral and poster presentations were used as the basis for this thesis

1. Szabó É., **Marosvölgyi T.**, Dergez T. és Decsi T. (**2022**). A nervonsav magasabb aránya a koraszülöttet szült anyák anyatejmintáiban az érett tejhez képest. *Gyermekgyógyászat* 73, 378 Magyar Gyermekorvosok Társasága 2022. évi Nagygyűlése
2. **Marosvölgyi T.**, Dergez T., Szabó É., and Decsi T. (**2022**) Contribution of nervonic acid to the fatty acid composition is substantially higher in preterm than in term human milk. *J Ped Gastroenterol Nutr* 74:S2 pp. 996-996., 54th Annual Meeting of ESPGHAN
3. Decsi T., Kovács A., Funke S., **Marosvölgyi T.**, and Burus I. (**2003**) Fatty acids in early human milk following preterm and full-term delivery. 44th Annual Meeting of the European Society for Paediatric Research, Bilbao, Spanyolország
4. Kovács A., Funke S., **Marosvölgyi T.,** Burus I. és Decsi T. (**2003**) Az anyatej zsírsavösszetétele koraszülöttet és érett újszülöttet szült anyákban. Magyar Gyermekorvos Társaság Évi Nagygyűlése, Szeged
5. **Marosvölgyi T.,** Funke S., Kovács A., Burus I., és Decsi T. (**2003**) Az anyatej zsírsavösszetétele koraszülöttet és érett újszülöttet szült anyákban. A Magyar Gyermekorvosok Társasága és a Magyar Gasztroenterológiai Társaság Gyermekgasztroenterológiai Szekciójának XX. Tudományos Ülése, Szolnok
6. Decsi T., Kovács A., Funke S., **Marosvölgyi T.,** and Burus I. (**2003**) Daily comparison of fatty acid composition of early human milk following preterm and full-term delivery. (poszterprezentáció) *J Ped Gastroenterol Nutr* **36(4), S:577** The 36th Annual Meeting of ESPGHAN, Prága, Cseh Köztársaság
7. Decsi T., Kovács A., Funke S., **Marosvölgyi T.** és Burus I. (**2003**) Fatty acids in early human milk following preterm and full-term delivery *Pediatr Res* 54 600 Annual Meeting of the European Society for Paediatric Research, Bilbao, Spain
8. **Marosvölgyi T.,** Kovács A., Funke S., Burus I. és Decsi T. (**2002**) Az anyatej zsírsavösszetételének változása a laktáció első hónapjában. Magyar Táplálkozástudományi Társaság XXVII. Vándorgyűlése, Eger
9. Minda H., Kovács A., Funke S., Szász M., Burus I., **Marosvölgyi T.**, and Decsi T. (**2002**) Fatty acids in human milk during the first month of lactation. *Pediatr Res* 52, 781 Annual meeting of Europian Societys for Pediatric Research, Utrecht, Belgium
10. **Marosvölgyi T**., Kovács A., Funke S., Burus I., Minda H. és Decsi T. (**2002**) Az anyatej zsírsavösszetételének változása a szoptatás első hónapjában. Magyar Gyermekorvos Társaság Évi Nagygyűlése, Tatabánya, Gyermekgyógyászat 53 (Supplementum 1)

6.4 The following peer-reviewed publications in English were not used as the basis for this thesis

1. **Marosvölgyi T*.,** Mintál K*., Farkas N., Sipos Z., Makszin L., Szabó É., Tóth A., Kocsis B., Kovács K, Hormay E., Lénárd L., Karádi Z. and Bufa A. (**2024**) Antibiotics and probiotics-induced effects on the total fatty acid composition of feces in a rat model *Sci Rep* **14**, 6542 DOI: 10.1038/s41598-024-57046-6 (**IF: 4,600; D1**; TR: 0, IR: 0)
2. Hatem O*., Kaçar Ö. F.*, Kaçar H. K., Szentpéteri J. L., **Marosvölgyi T.** and Szabó É. (**2024**) Trans isomeric fatty acids in human milk and their role in infant health and development, *Front Nutr* 11:1379772. DOI: 10.3389/fnut.2024.1379772 (**IF: 5,000; Q1**; TR: 0, IR: 0)
3. Balogh-Hartmann F., Páger C., Bufa A., Madarászné-Horváth I., Verzár Z., **Marosvölgyi T.** and Makszin L. (**2023**) Microfluidic analysis for the Determination of Protein Content in Different Types of Plant-Based Drinks, *Molecules* 28, 6684; DOI: 10.3390/molecules28186684 (**IF: 4,600; Q1**; TR: 0, IR: 0)
4. Ordnung, M., Mank, M., Stahl, B., Kurz, D., **Marosvölgyi, T.,** Decsi, T., Rothenbacher, D., Genuneit, J. and Siziba, L. P. (**2023**) Potential sex differences in human milk fatty acids and their association with atopic dermatitis: Results of the Ulm SPATZ health study, *Pediatr Allergy Immun* 34, e13992; DOI: 10.1111/pai.13992 (**IF: 4,54; Q1;** TR: 1, IR: 1)
5. Kőrösi, L., Molnár, S., Teszlák, P., Dörnyei, Á., Maul, E., Töpfer, R., **Marosvölgyi, T.,** Szabó, É., and Röckel F. (**2022**) Comparative study on grape berry anthocyanins of various teinturier varieties, *Foods* 11, 3668; DOI: 10.3390/foods11223668 (**IF: 5,561; Q1;** TR: 1, IR: 1)
6. Mező, E., Hartmann-Balogh, F., Madarászné Horváth, I., Bufa, A., **Marosvölgyi, T.,** Kocsis, B., and Makszin L. (2022) Effect of culture conditions on fatty acid profiles of bacteria and lipopolysaccharides of the genus Pseudomonas – GC-MS analysis on ionic liquid-based column, *Molecules* 27, 6930; DOI: 10.3390/molecules27206930 (**IF: 4,927; Q1;** TR: 2, IR: 2)
7. Decsi, T., **Marosvölgyi, T.,** Muszil, E., Bódy, B., and Szabó, É. (**2022**) Long-chain polyunsaturated fatty acid status at birth and development of allergy: a systematic review Life, 12, 526. DOI: 10.3390/life12040526 (**IF: 3,251; Q2;** TR: 1, IR: 1)
8. Mintál, K., Tóth, A., Hormay, E., Kovács, A., László, K., Bufa, A., **Marosvölgyi, T.,** Kocsis, B., Varga, A., Vizvári, Z., Cserjési, R., Péczely, L., Ollmann, T., Lénárd, L., and Karádi Z. (**2022**) Novel probiotic treatment of autism spectrum disorder associated social behavioral symptoms in two rodent models, *Sci Rep* 12, 5399 DOI: 10.1038/s41598-022-09350-2 (**IF: 4,996; D1;** TR: 14, IR: 13)
9. Szabó, Z.*; **Marosvölgyi, T.***; Szabó, É., Koczka, V., Verzár, Z., Figler, M., and Decsi, T. (**2022**) Effects of repeated heating on fatty acid composition of plant-based cooking oils, *Foods* 11, 192. DOI: 10.3390/foods11020192 (**IF: 5,561; Q1;** TR:16, IR:16)
10. Szabó, Z., Koczka, V., **Marosvölgyi, T.,** Szabó, É., Frank, E., Polyák, É., Fekete, K., Erdélyi, A., Verzár, Z., and Figler M. (2021) Possible Biochemical Processes Underlying

the Positive Health Effects of Plant-Based Diets - A Narrative Review, *Nutrients* 28, 2593. DOI: 10.3390/nu13082593. (**IF: 6,706; D1;** TR: 14, IR: 14)

11. Szabó, É., Marosvölgyi T., Szilágyi, G., Kőrösi, L., Schmidt, J., Csepregi, K., Márk, L., and Bóna, Á. (**2021**) Correlations between total antioxidant capacity, polyphenol and fatty acid content of native grape seed and pomace of four different grape varieties in Hungary, *Antioxidants* 9, 1101. DOI: 10.3390/antiox10071101. (**IF: 7,675; Q1;** TR: 20, IR: 19)
12. Mező, E., Bufa, A., Páger, C., Poór, V., **Marosvölgyi, T.,** Kilár, F., and Makszin, L. (**2021**) The Role of Ionic Liquid Interaction in the Separation of Fatty Acid Methyl Esters – Polyunsaturated Geometric Isomers in GC-MS, *Separations* 8, 38. DOI: 10.3390/separations8040038 (**IF: 3,344; Q3;** TR: 1, IR: 0)
13. Siziba, L. P., Lorenz, L., Brenner, H., Carr, P., Stahl, B., Mank, M., **Marosvölgyi, T.,** Decsi, T., Szabó, É., Rothenbacher, D., and Genuneit, J. (**2021**) Changes in human milk fatty acid composition and maternal lifestyle-related factors over a decade: a comparison between the two Ulm Birth Cohort Studies, *Br J Nutr* 12, 1. DOI: 10.1017/S0007114520004006. (**IF: 4,125; Q2;** TR: 8, IR: 4)
14. Szabó, Z., **Marosvölgyi, T.,** Szabó, É., Bai, P., Figler, M., and Verzár Z.: (**2020**) The potential beneficial effect of EPA and DHA supplementation managing cytokine storm in COVID-19, *Front Physiol* 19, 752. DOI: 10.3389/fphys.2020.00752. (**IF: 4,566; Q2;** TR: 41, IR: 39)
15. Siziba, L. P., Lorenz, L., Stahl, B., Mank, M., **Marosvölgyi, T.,** Decsi, T., Rothenbacher, D., and Genuneit, J. (**2020**) Human milk fatty acid composition of allergic and non-allergic mothers: The Ulm SPATZ Health Study, *Nutrients* 12, 1740; DOI:10.3390/nu12061740. (**IF: 5.719; D1;** TR: 3, IR: 2)
16. Weiss-Hersh, K., Garcia, A. L., **Marosvölgyi, T.,** Szklenár, M., Decsi, T., and Rühl, R. (**2020**) Saturated and monounsaturated fatty acids in membranes are determined by the gene expression of their metabolizing enzymes SCD1 and ELOVL6 regulated by the intake of dietary fat, *Eur J Nutr* 59, 2759-2769, DOI: 10.1007/s00394-019-02121-2. (**IF: 5,619; Q1;** TR: 18, IR: 18)
17. Siziba, L. P., Lorenz, L., Stahl, B., Mank, M., **Marosvölgyi, T.,** Decsi, T., Rothenbacher, D., and Genuneit, J. (**2019**) Changes in Human Milk Fatty Acid Composition During Lactation: The Ulm SPATZ Health Study, *Nutrients* 20, E2842. DOI: 10.3390/nu11122842. (**IF: 4,546; D1;** TR: 17, IR: 11)
18. Logan, C. A., Brandt, S., Wabitsch, M., Brenner, H., Wiens, F., Stahl, B., **Marosvölgyi, T.,** Decsi, T., Rothenbacher, D., and Genuneit, J. (**2017**) New approach shows no association between maternal milk fatty acid composition and childhood wheeze or asthma, Allergy 72, 1374-1383. DOI: 10.1111/all.13161 (**IF: 6,048; Q1;** TR: 17, IR: 11)
19. Kiss, B., Szántó, M., Szklenár, M., Brunyánszki, A., **Marosvölgyi, T.,** Sárosi, E., Remenyik, É., Gergely, P., Virág, L., Decsi, T., Rühl, R., and Bai, P. (**2015**) Poly(ADP) ribose polymerase-1 ablation alters eicosanoid and docosanoid signaling and metabolism in a murine model of contact hypersensitivity, *Mol Med Rep* 11, 2861-2867. DOI: 10.3892/mmr.2014.3044 (**IF: 1,559; Q3;** TR: 14 IR: 6)
20. Mihályi, K., Györei, E., Szabó, É., **Marosvölgyi, T.,** Lohner, S., and Decsi, T. (**2015**) Contribution of n-3 long-chain polyunsaturated fatty acids to human milk is still low in

Hungarian mothers, *Eur J Pediatr* 174, 393-398. DOI: 10.1007/s00431-014-2411-6 (**IF: 1,791; Q1;** TR: 16, IR: 14)

21. Tárnok, A., **Marosvölgyi, T.,** Szabó, É., Györei, E., and Decsi, T. (**2014**) Low n-3 Long-Chain Polyunsaturated Fatty Acids in Newly Diagnosed Celiac Children With Preexisting Type-1 Diabetes. *J Pediatr Gastroenterol Nutr* 60, 255-258. DOI: 10.1097/MPG.0000000000000561 (**IF: 2,4; Q1;** TR: 7 IR: 7)
22. Lohner, S., Vágási, J., **Marosvölgyi, T.,** Tényi, T., and Decsi, T.: Inverse association between 18-carbon trans fatty acids and intelligence quotients in smoking schizophrenia patients Psychiatry Res, 215:1 pp. 9-13., DOI: 10.1016/j.psychres.2013.10.011 (**2014 IF: 2,467; Q1;** TR: 2 IR: 2)
23. Mihály, J., **Marosvölgyi, T.,** Szegedi, A., Köröskényi, K., Lucas, R., Törőcsik, D., Garcia, L. A; Decsi, T., and Rühl, R. (**2014**) Increased FADS2-derived n6-PUFAs and reduced n3-PUFAs in plasma of atopic dermatitis patients, *Skin Pharmacol Physiol* 27, 242-248. DOI: 10.1159/000358290 (**IF: 2,366; Q1;** TR: 9, IR: 5)
24. Weiss, K., Mihaly, J., Liebisch, G., **Marosvölgyi, T.,** Garcia, A. L., Schmitz, G., Decsi, T., and Ruhl, R. (**2014**) Effect of high versus low doses of fat and vitamin A dietary supplementation on fatty acid composition of phospholipids in mice, *Genes Nutr* 9, 368. DOI: 10.1007/s12263-013-0368-0 (**IF: 2,794; Q1;** TR: 11, IR: 9)
25. Lohner, S., Fekete, K., **Marosvölgyi, T.,** and Decsi, T. (**2013**) Gender differences in the long-chain polyunsaturated fatty acid status: systematic review of 51 publiations *Ann Nutr Metab* 62, 98-112. DOI: 10.1159/000345599 (**IF: 2,744; Q1;** TR: 146 IR: 144)
26. Decsi, T., Campoy, C., Demmelmair, H., Szabó, E., **Marosvölgyi, T.,** Escolano, M., Marchal, G., Krauss-Etschmann, S., Cruz, M., and Koletzko, B. (**2011**) Inverse Association between trans Isomeric and Long-Chain Polyunsaturated Fatty Acids in Pregnant Women and Their Newborns: Data from Three European Countries, *Ann Nutr Metab* 59, 107-116. DOI: 10.1159/000332912 (**IF: 2,257; Q1;** TR: 15, IR: 13)
27. Weiss, K., Mihály, J., Liebisch, G., **Marosvölgyi, T.,** Schmitz, G., Decsi, T., and Rühl, R. (**2011**) Effect of synthetic ligands of PPAR α, β/δ, γ, RAR, RXR and LXR on the fatty acid composition of phospholipids in mice *Lipids* 46, 1013-1020., DOI: 10.1007/s11745-011-3593-6 (**IF: 2,129; Q2;** TR: 18, IR: 16)
28. **Marosvölgyi, T.,** Horváth, G., Dittrich, A., Cseh, J., Lelovics, Z., Szabó, É., Decsi, T., and Figler, M. (**2010**) Fatty acid composition of plasma lipid classes in chronic alcoholic pancreatitis, *Pancreatology* 10, 580-585. DOI: 10.1159/000289466 (**IF: 2,128; Q1;** TR: 9, IR: 8)
29. Fekete, K., **Marosvölgyi, T.,** Jakobik, V., and Decsi, T. (2009) Methods of assessment of n-3 long-chain polyunsaturated fatty acid status in humans: a systematic review, *Am J Clin Nutr* 89, 2070S-2084S. DOI: 10.3945/ajcn.2009.27230I (**IF: 6,307; D1;** TR: 103 IR: 97)
30. **Marosvölgyi, T.,** Campoy, C., Koletzko, B., Szabó, É., Jakobik, V., Jimenez, M., Demmelmair, H., and Decsi, T. (**2009**) Trans isomeric and LCPUFA are inversely correlated in erythrocyte membrane lipids at mid-gestation, *Adv Exp Med Biol* 646, 159-163., DOI: 10.1007/978-1-4020-99173-5_18 (**IF: 2,02; Q1;** TR: 1 IR: 0)
31. Szabó, É., **Marosvölgyi, T.,** Kozári, A., Erhardt, É., Soltész, G., and Decsi, T. (2009) Long-chain polyunsaturated fatty acids in a diabetic teenager during and after nine repeated

episodes of diabetic ketoacidosis *Pediatr Diabetes* 10, 209-212. DOI: 10.1111/j.1399-5448.2008.00487.x (**IF: 2,628; D1;** TR: 4 IR: 3)

32. Ruhl, R., Koch, C., **Marosvölgyi, T.,** Mihály, J., Schweigert, F. J., Worm, M., and Decsi, T. (**2008**) Fatty acid composition of serum lipid classes in mice following allergic sensitisation with or without dietary docosahexaenoic acid-enriched fish oil substitution, *Brit J Nutr* 99, 1239-1246. DOI: 10.1017/S0007114507862374 (**IF: 2,764; Q1;** TR: 14 IR: 8)
33. Decsi, T., Szabó, É., Burus, I., **Marosvölgyi, T.,** Kozári, A., Erhardt, É., and Soltész, G. (**2007**) Low contribution of n-3 polyunsaturated fatty acids to plasma and erythrocyte membrane lipids in diabetic young adults. *Prostaglandins Leukot Essent Fatty Acids* 76, 159-164., DOI: 10.1016/j.plefa.2006.12.003 (**IF: 2,000; Q2;** TR: 26 IR: 19)
34. Decsi, T; Szabó, E; Kozári, A; Erhardt, É; **Marosvölgyi, T;** and Soltész, G (2005) Polyunsaturated fatty acids in plasma lipids of diabetic children during and after diabetic ketoacidosis, *Acta Paediatr* 94, 850-855., DOI: 10.1080/08035250510028254 (**IF: 1,277; Q2;** TR: 12, IR: 9)
35. Lasztity, N., Hamvas, J., Biró, L., Németh, E., **Marosvölgyi, T.,** Decsi, T., Pap, A., and Antal, M. (2005) Effect of enterally administered n-3 polyunsaturated fatty acids in acute pancreatitis - a prospective randomized clinical trial, *Clin Nutr* 24, 198-205. DOI: 10.1016/j.clnu.2004.12.008 (**IF: 2,296; Q1;** TR: 82, IR: 81)
36. Horváth, O., **Marosvölgyi, T.,** and Vörös, J. (2004) Microenvironmental effects on equilibrium and photoredox chemistry of bromo complexes in reverse micelles, *Prog Colloid Polym Sci* 125, 17-23. DOI: 10.1007/b13488 (**IF: 1,11; Q2;** TR: 1, IR: 1)