1. Introduction

Human breast milk provides the optimum nutrition for infants, and is being designed to provide perfectly balanced nutrition to meet the needs of the growing infant in the first months after birth. About 50% of energy in human milk is provided by fatty acids in the milk triglycerides, which themselves are molecules comprised of mixtures of three fatty acids esterified to a glycerol backbone [1]. Triglyceride synthesis, rather than involving random esterification of three fatty acids to glycerol, involves specific positioning of fatty acids at the outer sn-1 and sn-3, and center sn-2 of the triacylglyceride. This stereospecific arrangement of fatty acids in human milk triglycerides influences the intestinal absorptions of fatty acids, which can be critical to the health and development of the infant. The human mammary gland has evolved with unusual pathways for acylation of fatty acids into triglycerides for secretion in milk, with these pathways resulting in a different triglyceride structure (triglyceride fatty acid arrangement) from the triglycerides in other human tissues and plasma [2], or common dietary fats and oils. This stereospecific positioning of fatty acids in human milk triglycerides involves preferential positioning of the saturated fatty acid palmitic acid (16:0) at the sn-2 position, rather than at the sn-1,3 positions, as is typical of human tissue and plasma lipids, and vegetable oils common in human diets, and in the fat blends used in the manufacture of infant formula [3]. Studies conducted two decades ago, together with very recent studies, have provided increasing evidence that this unusual positioning of 16:0 in human milk triglycerides has a significant role for infant health in different directions, such as fat and calcium absorption, bone health, intestinal flora and infant comfort. This review aims to unravel the relevance of human milk triglyceride sn-2 16:0 for intestinal health and inflammatory pathways and for other post-absorption effects.

2. Structured triglycerides

Palmitic acid (C16:0) is the major saturated fatty acid in human milk, accounting for 17–25% of the total fatty acids [4]. Equally important, over 70% of 16:0 is esterified at the milk triglyceride sn-2 position [2,9]. The major unsaturated fatty acid in human milk is oleic acid (18:1n-9) and this is mostly esterified at the triglyceride sn-1,3 (outer) positions, with the result that triglycerides with the structure 18:1n-9—16:0—18:1n-9 are a major triglyceride species in human milk and represent an estimated 11.8% of the total triglyceride species [2]. Early studies addressing the importance of the human milk triglyceride structure compared fat absorption in infants fed human milk with...
infants fed formulas containing lard (an unusual animal fat in which 16:0 is also high and esterified in the triglyceride sn-2 position), and infants fed lard that had been randomized to redistribute 16:0 equally across all three carbons on the triacylglyceride [10]. The latter studies showed that redistributing 16:0 from the sn-2 position of the formula triglyceride led to decreased fat absorption. Although two or more vegetable oils can be blended to give the same average amounts of 16:0, 18:1n-9 and 18:2n-6 in an infant formula as in human milk, the stereo-specific arrangement of vegetables oil triglycerides means that the 16:0 will be present almost entirely on the triglyceride sn-1,3 positions [3]. The development of structured triglycerides enables mimicking both the composition as well as the structure of human milk fat for infant formulas. Structured TG are achieved through an enzymatic process by which the 16:0—18:1n-9—16:0 is transformed to 18:1n-9—16:0—18:1n-9. The resulting product contains 17–25% palmitic acid with above 40% located at the center sn-2 position.

3. Importance of dietary 16:0 positioning in triglycerides for fatty acid and calcium absorption.

Studies done several decades ago have demonstrated the greater efficiency of fat absorption and softer stools in breast-fed infants compared to that of infants fed with formulas containing 16:0 from saturated vegetables; effect that was linked to the large amounts of 16:0 in human milk at the sn-2 position of the milk triglycerides [4–8]. Triglyceride digestion by endogenous lipases leads to hydrolysis of fatty acids from the triacylglyceride sn-1,3 linkages, to release two unesterified fatty acids and one sn-2 monoglyceride from each triglyceride into the intestinal lumen [11]. A role for the milk bile salt-stimulated lipase in completing hydrolysis of sn-2 monoglycerides with 16:0 released during triglyceride digestion is unlikely, since unesterified 16:0 is poorly absorbed [3]. Structuring 16:0 on the triglyceride sn-2 position of milk or formula fats improves 16:0 absorption [12,13], and plasma chylomicron triglycerides of breast fed infants are high in sn-2 16:0 [14,15]. In addition to low intraluminal solubility, unesterified 16:0 has an increased tendency to combine with divergent cations, such as calcium, to form insoluble soaps, which are malabsorbed [16]. Clinical evidence for this has been provided by studies to show increased fecal excretion of fatty acid soaps of 16:0 and calcium, accompanied by harder stools, in infants fed formula containing 16:0 from saturated vegetable oils rather than structured triglycerides containing β-16:0 [4–8]. Fig. 1 shows the correlation between the level of 16:0 in the milk or formula triglyceride sn-2 position and infant fatty acid and calcium absorption calculated as a modified Cohen’s effect size (F2) [17] using data from published studies with term [5–7] and preterm infants [4,8]. Since an effect size of over 0.8 is recognized as a large effect [17], β-16:0 with over 40% 16:0 at the sn-2 position would have a large beneficial effect on fatty acid and calcium. The results show that progressively increasing 16:0 at the sn-2 (and decreasing 16:0 at the sn-1,3 positions) of the formula triglyceride leads to a dose-dependent increase in 16:0 and calcium absorption (r = 0.95 and r = 0.78 for 16:0 and calcium, respectively). The reduction in fecal calcium and 16:0 as calcium soaps is accompanied by a decrease in the incidence of harder stools [6,18,19].

4. Bone health

Malabsorption of calcium in fatty acid soaps in infants fed formulas containing 16:0 rich vegetable oils has led to interest in the possible effects of milk and formula 16:0 on bone mineralization in young infants [6]. Recent advances in methods for assessment of bone strength parameters with the development of quantitative measures of bone using supersonic speed of sound (SOS) have recently been applied to this direction of research. Ultrasound bone sonometry is a non-invasive technique that enables quantitative longitudinal assessment of changes in bone parameters in the tibia or other bones in term and preterm infants from birth [20–22]. Litmanovitz et al. recently applied the bone SOS technology in a randomized, controlled, double-blind clinical study of bone parameters in term infants fed formula containing triglycerides with sn-2 16:0 from InFat® or standard vegetable oil blends with comparison to a non-randomized group of breast-fed infants [23]. Tibia bone SOS decreased during the first 3 months
after birth in all infants, consistent with the findings of studies using SOS to assess bone in term and preterm infants [24,25]. However, this recent study showed that infants fed 16:0 as β-16:0 had significantly higher bone SOS, at about 3 months of age than in infants fed formula with a standard vegetable oil blend (Fig. 2). The bone SOS measures for infants fed the β-16:0 formula were also comparable to those of the group of breast-fed infants [23]. These data confirm and extend studies by Kennedy et al. [6] who over a decade ago used dual-energy X-ray absorptiometry (DEXA) to show higher body bone mass in infants after 12 weeks of feeding with formula containing structured triglycerides enriched in sn-2 16:0 rather than a conventional formula. In contrast, Zuccotti et al. [26] recently reported no difference in left tibia bone SOS between exclusively breast-fed (n = 25) and formula fed (n = 12) infants at 4 months of age, or when assessed later at 12 months of age; however, the type of formula fed was unspecified. Clearly, more studies are needed to assess both the early and potential longer-term implications of the impact of dietary triglyceride composition and structure on bone mineralization and characteristics in infants.

6. Infant behavior

Early infant crying is considered to reflect basic, instinctive responses governed by neurochemical mechanisms similar to those that control feeding and drinking (i.e., spontaneous behaviors) (for a review, see Ref. [44]). Early infant crying follows a typical pattern over the day, with about 40% of crying episodes occurring between 16:00 and 22:00 h; it is only after the third month from birth that crying episodes become more distributed throughout the day [45,46]. The developmental regulation of crying coincides with the development of the circadian rhythm and forms in part the neurobiological/neuroendocrine basis for the link between crying behavior and the development of circadian rhythms. Crying behavior in young infants is, therefore, considered as modifiable by endogenous/exogenous stimuli that alter neurochemistry. However, this is complex since early infant crying includes not only spontaneous endogenous crying but also crying due to distress, such as separation from an individual to which the infant has become attached, hunger or other physical distress. Not unexpectedly, the duration of crying in very young infants is inversely correlated with the duration of sleep time [46,47].

In a recent open label study, the percent of daily time spent sleeping was not significantly different between the term gestation infants fed formula with β-16:0 and breast fed infants at 6 weeks (68.4% and 69.5% respectively) or 12 weeks of age (64.3% and 67.1% respectively) (unpublished data). Savino et al. (2006) provided the first evidence that triglycerides enriched in β-16:0 might impact infant crying. In this study, term gestation infants fed a formula containing partially hydrolyzed whey proteins, prebiotic oligosaccharides, and high 16:0 as β-16:0 (41% of the formula 16:0 in the triglyceride sn-2 position) showed significantly reduced crying compared to infants fed a control formula without β-16:0 hydrolyzed whey proteins or oligosaccharides [49]. However, the beneficial effects on crying in this study cannot be specifically attributed to the β-16:0. More recently, we found that among term infants fed formula with β-16:0 for the 12 weeks after birth there was a decreased percentage of infants that cried, and lower crying duration during the day and night, and especially in the evening and night hours, when compared to infants fed a standard formula with a similar ~20% 16:0 but from unmodified vegetable oil (Fig. 3). This difference in crying pattern between infants fed β-16:0 and those fed standard formula containing 16:0 from unmodified vegetable oils should probably be attributed to complex of mechanisms.
Several plausible mechanisms might link milk or structured triglycerides rich in sn-1,3 18:1n-9 and sn-2 16:0 to altered spontaneous crying in the first few weeks after birth. Structured triglycerides composed of sn-1,3 18:1n-9 and sn-2 16:0 will lead to uptake of unesterified 18:1n-9 and 16:0 into the intestinal enterocytes [16]. Several acylated molecules, such as the acyl ethanolamines and acylglycines, including palmitoyl and oleoyl ethanolamide are known to be potent signal molecules of the endocannabinoid system that contribute to regulation of relevant physiological processes, such as sleep and pain sensitivity [50], are thought to be involved in the circadian rhythm [51]. Notably, the endogenous opiateergic system is also known to be involved in spontaneous crying [for review see [44]]. Interestingly, the developmental regulation of crying coincides with the development of the circadian rhythm. This is perhaps a key, whereby alterations of circadian rhythm development or neuro-endocrine mechanisms therein may relate dietary variables that perturb these systems to crying behavior (see for e.g. the work of [52]. Lower crying behavior in the late afternoon among infants fed formula with β-16:0 is consistent with a neurochemical mechanism, interfacing with the development of circadian rhythm and limbic inhibition of spontaneous crying regulated via brainstem mechanism. Melatonin and fatty acid ethanolamides, including oleoyl ethanolamide, are possible targets for considerations as mediators of the effects of formula fats. Of interest, Banni et al. recently reported that feeding β-16:0 altered the endocannabinoid system and feed efficiency in post-weaning rats [53], suggesting that structured triglycerides may have effects on multiple physiological regulatory processes in young infants.

7. Summary

Research on the physiological importance to young infants of the unusual triglyceride structures in human milk, specifically the preferential acylation of 16:0 at the sn-2 position, with large amounts of 18:1n-9 at the sn-1,3 positions, is seeing regrowth of interest, made possible in part by technological advances which now make it possible to synthesize dietary triglycerides with the structure 18:1n-9—16:0—18:1n-9. Early work linked the enrichment of 16:0 at the sn-2 position of human milk triglycerides to a high efficiency of fatty acid absorption, prevention of calcium malabsorption and softer stools in breast-fed infants. Recent studies are now confirming and extending these observations to show that the sn-2 16:0 structure with 18:1n-9 on the triglyceride sn-1,3 positions also increases early bone mineralization and development, influences the composition of the intestinal microflora, may lower the extent and severity of intestinal inflammation in response to insult, and may also have neurobiological effects that include modulation of early infant crying. As discussed in this review of recent studies, the effect of triglycerides enriched in sn-2 16:0, and of β-16:0 itself is likely to extend well beyond fatty acid and mineral absorption, although much remains to be learnt regarding biological mechanism and potential implication for infant nutrition.

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References
