Review article

The influence of the position of palmitate in infant formula triacylglycerols on health outcomes

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Abbreviations

BF; breast fed

HM; human milk

OF; oligofructose

PA; palmitic acid

TG; triacylglycerol

Abstract

The purpose of this review is to discuss recent studies reporting on the influence of the position of palmitic acid in triacylglycerols in infant formula and relevant animal studies. Earlier experiments in rodents show that a diet with a higher proportion of palmitate at the sn-2 position of triacylglycerols improves dietary fat and calcium absorption compared to a diet with a lower sn-2 palmitate content. A high sn-2 palmitate diet increased fecal short chain fatty acids, reduced gut inflammation in a colitis model, and altered tissue endocannabinoid concentrations in laboratory rodents. Recent studies in infants confirm that formula with a high sn-2 palmitate content reduces stool fat, palmitic acid, fat soaps, palmitate soaps and calcium compared to formula with a low sn-2 palmitate content. These effects have been associated with improved bone strength, increased fecal bifidobacteria and reduced crying in infants. In some studies, findings with formula high in sn-2 palmitate match those seen in breastfed infants. However, in many studies high sn-2 palmitate formula remains inferior to breast feeding. It is concluded that infant formula high in sn-2 palmitate is superior to formula with low sn-2 palmitate but does not fully match human breast milk. Recent studies showing altered gut microbiota (human infants) and tissue endocannabinoids (rodent model) suggest the potential for marked physiological impact of high sn-2 palmitate that needs to be explored further in human trials.

Keywords

Infant, formula, breast milk, palmitic acid, calcium soap, sn-2 palmitate, β-palmitate, fatty acid

1. Introduction and historical background

The newborn infant has a high energy requirement and this is partially met by the high fat content (~50% of total energy) of human breast milk. Most of the fat in human breast milk is composed of triacylglycerols (TGs) in which three fatty acids are esterified to glycerol (i.e., 1,2,3-triacylglycerol). Breast milk contains an array of fatty acids, and the sixteen-carbon saturate palmitic acid typically contributes 20 to 25% of milk fatty acids or ~10 to 12.5% of total energy in breast milk [1]. Interestingly, 60 to 70% of this palmitic acid is esterified to the sn-2 position of human breast milk TGs [1]. This is sometimes called the β-position. In contrast, in most vegetable oils, including palm oil which is a rich source of palmitic acid, palmitic acid is esterified mainly at the sn-1 and sn-3 positions, with less than 20% in the sn-2 position [1]. In cows' milk only about 40% of palmitic acid is in the sn-2 position [1]. In the newborn infant lingual, gastric and pancreatic lipases all play important roles in digestion of milk fat TGs. The action of these endogenous lipases on TGs produces two free fatty acids and a 2-monoacylglycerol. Thus, the fatty acid at the sn-2 position is retained following TG digestion. The products of TG digestion form micelles with bile salts and are taken up by enterocytes for TG resynthesis and assembly into chylomicrons. Normally the process of free fatty acid and 2-monoacylglycetol absorption is efficient, but long-chain saturated fatty acids like palmitic acid can form insoluble calcium salts in the intestinal lumen, sometimes referred to as calcium soaps (Figure 1). This has the dual effect of preventing both calcium and saturated fatty acid absorption and the calcium soaps pass further along the intestinal tract (Figure 1). Here, they may contribute to stool hardening, constipation and subsequent infant discomfort (Figure 1). In this context, Quinlan et al. [2] showed that the biggest differences in stool fatty acid content between breast-fed and formula-fed infants was in soaps of palmitic and

77 stearic acids and they reported that fatty acid calcium soaps could account for as
78 much as one-third of the stool dry weight in infants. They argued that fatty acid
79 calcium soaps are major contributors to stool hardness. Thus, it has been
80 considered that having most palmitic acid in the sn-2 position could play a role in
81 promoting fat and calcium availability, and preventing stool hardness and
82 constipation in formula-fed infants (Figure 2).
83
84 A synthetic TG with palmitic acid in the sn-2 position has been used experimentally
85 for a number of years to study the effect of positional distribution of palmitic acid.
86 This synthetic TG is commercialized and is used in the infant formula industry.
87 Earlier studies demonstrated better absorption of palmitic acid from milk and from
88 the synthetic TG than from vegetable oils in rodents [3,4,5] and piglets [6], as
89 reviewed by Lien [7]. Innis et al. [8] reported that 50% of sn-2 palmitate from dietary
90 TGs is conserved through digestion and absorption in both breast fed and formula
91 fed infants. Both fat and calcium were better absorbed by preterm and healthy term
92 infants given formula with a significant proportion of palmitic acid at the sn-2 position
93 compared to formula with little sn-2 palmitic acid (but the same total amount of
94 palmitic acid) [9,10,11,12]. Furthermore, term infants given formula high in sn-2
95 palmitate had better fat absorption, higher bone mineral content, softer stools and
96 lower stool fatty acid calcium soap content than infants receiving formula with
97 palmitic acid not in the sn-2 position [13].
98
99 **2. Recent clinical research in infants**

Over the period 2013 to 2016, findings from a series of human studies were published that greatly enhance understanding of the impact of positional distribution of palmitic acid in TGs in the infant diet [Table 1] [14,15,16,17,18,19].

Bar-Yoseph et al. [14] reported findings from a randomized controlled trial of two formulas in term Chinese infants; the formulas had the same content of palmitic acid (20% of fatty acids) but one had 43% of palmitate at the sn-2 position and the other had 13%. Infants were included within 14 days of birth and the period of intervention was 6 weeks. Stool dry weight and fat content in the high sn-2 palmitate group were lower than in the control group (dry weight 4.25 g vs 7.28 g; fat 0.8 g vs 1.2 g). Stool palmitic acid, which represented ~50% of the fatty acid calcium soaps present, was lower in the high sn-2 palmitate group compared with the control group (0.3 g vs 0.7 g). These observations confirm those of earlier studies conducted in other settings [9,10,11,12,13]. It is important to note that a comparator group of breast-fed infants had a significantly lower stool dry weight, fat content, and saponified fat excretion compared with formula-fed infants, demonstrating the superiority of breast feeding over formula.

Litmanovitz et al. [15] compared the effect of 12 weeks feeding of term Israeli infants with formulas with different percentages of palmitic acid at the sn-2 position (14% or 43%) on anthropometric measures and bone strength, measured as bone speed of sound. At 12 weeks, the mean bone speed of sound was higher in the sn-2 palmitate group (2,896 ± 133 vs. 2,825 ± 79 m/s respectively) and was comparable with that of infants from the breast-fed comparator group (2,875 ± 85 m/s). Thus, in this study infants consuming formula with high sn-2 palmitate had changes in bone strength that were comparable to those of infants consuming breast milk and that were more favourable than the changes seen in infants consuming formula with

lower sn-2 palmitate. This effect might relate to better calcium availability when more palmitic acid is at the sn-2 position of dietary TGs. A second report from this study [16] indicated no difference between the two formula groups in stool frequency or consistency at either 6 or 12 weeks, but both formula groups showed lower stool frequency and harder stools than seen for infants in the comparator breast-fed group. At 12 weeks fewer infants in the high sn-2 palmitate group had hard stools compared to the low sn-2 palmitate formula group (0% vs 24%). The study found that the percentage of crying infants and total time spent crying each day were higher in the low sn-2 palmitate group than in the high sn-2 palmitate and breast-fed groups, which did not differ. It is possible that harder stools cause intestinal discomfort that lead to more infant distress and crying. An earlier study [20] had reported less crying in infants with colic who received a formula enriched in sn-2 palmitate and modified with respect to the contents of hydrolyzed whey proteins, oligosaccharides and lactose. The more recent study of Litmanovitz et al. [16] suggests that this finding may have been due to the sn-2 palmitate, rather than the other components.

Nowacki et al. [17] examined the effect of formula with high sn-2 palmitate (39% of palmitate) compared with low sn-2 palmitate (13%) on gastrointestinal tolerance, stool consistency, and stool fatty acid soap, palmitate soap and calcium concentrations in Taiwanese term infants; the study also included a group receiving high sn-2 palmitate and oligofructose and a breast-fed comparator group. Duration of the study was 28 days. Infants who received breast milk had lower stool total soaps, palmitate soaps and calcium than all formula-fed groups. Infants who were fed the formula with high sn-2 palmitate had lower stool palmitate soaps compared to those receiving the control formula. Stool total soaps and calcium were similar between low

and high sn-2 palmitate groups, as were stool frequency and consistency. Parental assessment of gastrointestinal tolerance did not differ between groups.

Yao et al. [18] evaluated the effects of a formula containing high sn-2 palmitate (36% of palmitic acid at sn-2 position), an identical formula supplemented with oligofructose at 2 concentrations (3 or 5 g/L), and a low sn-2 palmitate formula (12% sn-2 palmitate) on stool composition, stool characteristics, and fecal bifidobacteria in term Filipino infants. The intervention period was 8 weeks and breast-fed infants were included as a comparator. The high sn-2 palmitate group had 46% less stool palmitate soap and had softer stools than the low sn-2 palmitate group. Furthermore, the high sn-2 palmitate group had higher fecal bifidobacteria concentrations than the low sn-2 palmitate group and in this respect did not differ from breast-fed infants.

Yaron et al. [19] investigated whether palmitic acid positional distribution could affect gut microbiota in term Israeli infants. Infants received formula with high (44% of palmitic acid in sn-2 position) or low (14%) sn-2 palmitate or were breast-fed. At 6 weeks, the breast-fed and high sn-2 palmitate groups had higher fecal counts of lactobacilli and bifidobacteria than seen in the low sn-2 palmitate group. The study suggests that flow of palmitate soaps to the lower intestinal tract creates conditions that are less favourable for growth of lactobacilli and bifidobacteria, both of which are considered to be health-promoting.

The findings of these two recent studies [18,19] are interesting given an earlier report that a formula enriched in sn-2 palmitate, oligofructose and oligogalactose increased the proportion of bifidobacteria in feces of young infants [21]. Although this bifidogenic effect might be expected to relate to the prebiotic

oligosaccharides used, sn-2 palmitate may also have a role in promoting the growth of bifidobacteria.

3. Recent pre-clinical research in experimental animals

Lu et al. [22] studied the effects of diets low or high in sn-2 palmitate on colitis development in Muc2 deficient mice, a well-described animal model for spontaneous enterocolitis due to the lack of a protective mucus layer. Mice received one of the two diets for 5 weeks after weaning. The high sn-2 palmitate diet resulted in smaller intestinal erosions and less morphological damage compared with the low sn-2 palmitate diet. In addition, an immunosuppressive regulatory T cell response was enhanced by the high sn-2 palmitate diet; this may result in less inflammation and less mucosal damage. In this context, the high sn-2 palmitate diet resulted in higher mucosal expression of genes encoding peroxisome proliferator activated receptor gamma, an anti-inflammatory transcription factor, and several antioxidant proteins known to be involved in promoting an immunosuppressive regulatory T cell response and to protect against colitis.

Wan et al. [23] fed Sprague Dawley rats on diets providing 37% of fatty acids as palmitic acid but with different proportions of this in the sn-2 position of the dietary TGs. Diets were low (12% of palmitate in sn-2 position), medium (40%) or high (56%) in sn-2 palmitate. Total fecal fatty acids, fatty acid soaps, palmitate soaps and calcium all decreased with increasing amount of dietary sn-2 palmitate. Calculated calcium absorption was 43%, 54% and 61% for low, medium and high sn-2 palmitate groups. Fecal acetate, butyrate and total short-chain fatty acids all increased with increasing dietary sn-2 palmitate. While this may suggest an effect on gut microbiota, the amount of sn-2 palmitate in the diet did not affect fecal microbial richness or

198 diversity. However, there was an increase in some short chain fatty acid producing
199 bacteria genera in the feces of the rats fed the high sn-2 palmitate diet.
200 Carta et al. [24] fed Wistar rats diets containing 24% of fatty acids as palmitic
201 acid with low and high amounts of sn-2 palmitate (19% and 87% of palmitate,
202 respectively). Palmitic acid was higher in intestinal phospholipids from rats fed the
203 high sn-2 palmitate diet and this was linked to higher 2-palmitoyl-monoacylglycerol.
204 This diet also resulted in higher palmitic acid in visceral adipose tissue phospholipids,
205 where there was also higher palmitoylethanolamide and lower anandamide. On this
206 diet, higher palmitic acid in liver and muscle phospholipids was seen and there was
207 higher oleoylethanolamide in both tissues. Brain tissue also showed higher
208 oleoylethanolamide. Rats fed the high sn-2 palmitate diet had lower plasma tumour
209 necrosis factor concentrations 12 hours after intraperitoneal endotoxin injection, but
210 concentrations of interleukin-1 and interleukin-6 were not different between the two
211 groups. This is one of the few investigations to study in detail the effect of a high sn-
212 2 palmitate diet on tissue lipid composition. Through effects on endocannabinoids,
213 sn-2 palmitate could influence appetite, food intake and weight gain, energy
214 metabolism, inflammation and brain function amongst other physiological processes.
215 However, it is important to note that this study used a diet with a much higher
216 proportion of sn-2 palmitate than is used currently in infant formula and that some of
217 the reported effects were small. Therefore, the relevance of these findings to the
218 current main use of sn-2 palmitate in infant nutrition is unclear. However, this study
219 suggests that there may be nutraceutical uses of a very high sn-2 palmitate
220 preparation.
221

222 **4. Conclusions**

Earlier literature reported that inclusion of a high proportion of palmitic acid at the sn-2 position of TGs in infant formula improved fat and calcium absorption and resulted in softer stools [9,10,11,12,13]. Recent studies in infants confirm these observations and link them to improved bone strength, increased fecal bifidobacteria and reduced crying in infants (Figure 3). New studies in rodents report interesting findings on high dietary sn-2 palmitate and increased fecal short chain fatty acids, reduced gut inflammation in a colitis model, and altered tissue endocannabinoid concentrations. These observations suggest that high sn-2 palmitate may have important health benefits in the intestinal tract but that physiological impact may extend to several metabolic tissues and the brain. Such effects need to be explored further in infants. Taken together, the recent human infant and rodent research suggests a role for sn-2 palmitate in infant formula. However, in several studies in infants, high sn-2 palmitate formula remains inferior to breast feeding. It is also important to note that, despite the supportive research described herein, guidelines on the composition of infant formula do support the inclusion of high sn-2 palmitate [25,26]. This suggests that much more research is needed in this area.

Conflicts of interest

P.C.C. serves as an advisor to Danone/Nutricia, DSM, FrieslandCampina and Cargill. E.A.M. has no conflicts to declare.

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Figure captions

Figure 1. Intestinal handling of a triacylglycerol (TG) with palmitate at the sn-1 and/or sn-3 position. The palmitate freed by lipolysis forms insoluble salts with calcium reducing absorption of both palmitate and calcium resulting in increased fecal fat and calcium loss, hard stools and constipation. R indicates a fatty acyl chain.

Figure 2. Intestinal handling of a triacylglycerol (TG) with palmitate at the sn-2 position. The palmitate is retained in the 2-monoacylglycerol formed by lipolysis and is absorbed. If the fatty acids released from the sn-1 and sn-3 positions of the TG are unsaturated they do not form insoluble calcium salts and so remain available for absorption. R indicates a fatty acyl chain.

Figure 3. Scheme linking improved palmitic acid absorption to multiple physiological effects and health benefits in infants. Note that not effects demonstrated in rodent models only and not in infants are denoted by an asterisk.

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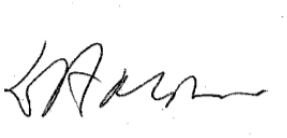

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