

**Palm oil and beta-palmitate in infant formula - A position paper by the
ESPGHAN Committee on Nutrition**

ESPGHAN Committee on Nutrition: ¶¶Jiri **Bronsky**; ¶¶Cristina **Campoy**; [†]Nicholas **Embleton**; §§Mary **Fewtrell**; &&Nataša **Fidler Mis**; **Konstantinos **Gerasimidis**; #Iva **Hojsak**; ¢Jessie **Hulst**; °°°Flavia **Indrio**; £Alexandre **Lapillonne**; &Christian **Molgaard**; ££Sissel Jennifer **Moltu**; ##Elvira **Verduci**; ¢¢Rakesh **Vora**; □Magnus **Domellöf**

¶¶ Department of Paediatrics, University Hospital Motol, Prague, Czech Republic;

¶¶ Department of Paediatrics, University of Granada, Spain;

[†] Newcastle Neonatal Service, Newcastle Hospitals NHS Trust and Newcastle University, Newcastle upon Tyne, UK;

§§ Childhood Nutrition Research Centre, UCL GOS Institute of Child Health, London, UK;

&& Department of Gastroenterology, Hepatology and Nutrition, University Children's Hospital, University Medical Centre Ljubljana, Slovenia;

** Human Nutrition, School of Medicine, Dentistry and Nursing, University of Glasgow, New Lister Building, Glasgow Royal Infirmary, Glasgow, UK

Children's Hospital Zagreb, Croatia, University of Zagreb School of Medicine;

¢ Department of Paediatric Gastroenterology, Erasmus MC, Sophia Children's Hospital, Rotterdam, The Netherlands;

°°° Ospedale Pediatrico Giovanni XXIII University of Bari Italy;

£ Paris Descartes University, APHP Necker-Enfants Malades hospital, Paris, France and CNRC, Baylor College of Medicine, Houston, Texas;

& Department of Nutrition, Exercise and Sports, University of Copenhagen, and Pediatric Nutrition Unit, Copenhagen University Hospital, Rigshospitalet, Denmark;

££ Department of Neonatal Intensive Care, Oslo University Hospital, Norway

Department of Pediatrics, San Paolo Hospital, Department of Health Sciences, University of Milan Italy

¢¢ Leeds teaching hospitals NHS trust, Leeds, UK;

⊠ Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden;

Secretary of CoN: # Iva Hojsak

Chair of CoN: ⊠Magnus Domellöf

Corresponding author:

Jiri Bronsky, MD, PhD,

Assoc. Prof. of Paediatrics Gastroenterology and Nutrition Unit

Department of Paediatrics, University Hospital Motol

V Uvalu 84, 15006, Prague 5, Czech Republic

Phone: +420224432001

Fax: +420224432020

Email: jiri.bronsky@gmail.com

Conflicts of Interest:

MF conducted a trial using beta-palmitate which was funded by Industry (Cow & Gate, now Nutricia; in 1995) and has received honoraria for attending two Consultancy meetings with Enzymotec (a company involved in the manufacture of beta-palmitate for infant formulas).

Authors report following conflicts of interest outside the submitted work: JB reports personal fees and non-financial support from AbbVie, Nutricia, Biocodex, personal fees from MSD, Nestlé, Ferring, Walmark. CC received research funding from ORDESA Laboratories and Abbott Nutrition. NE reports receipt of grants/research supports from National Institutes for Health Research (UK), Prolacta, Bioscience (US) and Danone Early life Nutrition. He also served as member of Advisory board for Danone Early life Nutrition and received payment/honorarium for lectures from Danone Early life Nutrition, Nestle Nutrition Institute, Baxter and Fresenius Kabi. KG reports personal fees from Nutricia, research grants and personal fees from Nestle and Nutricia and personal fees from Dr Falk. IH reports receipt of payment/honorarium for lectures from BioGaia, Nutricia, Nestle, GM pharma and receipt of payment/honorarium for consultation from Farmas, Chr Hansen. JH reports receipt of grants/research supports from Nutricia Advanced Medical Nutrition Netherlands and Danone Medical care (global). FI has participated as a clinical investigator and/or consultant and/or speaker for Arla Food, Biogaia, Nestle, Nestle Nutrition Institute, Wyeth, Danone and Abbott. AL received lecture fees and/or non-financial support from Baxter, Fresenius, Nestlé and Mead Johnson Nutrition. NFM acknowledges support of the Slovenian Research Agency (P3-0395: Nutrition and Public Health; L3-8213, L3-7538). CM reports receipt of grants/research supports from European Commission Innovation Fund Denmark, Nordea-fonden, Arla Foods, Chr. Hansen, USDEC, Gate Foundation. SJM reports receipt of grants/research supports from DSM Nutritional Products, she served as member of advisory board and received payment/honorarium for consultation from Baxter and received payment/honorarium for lectures from Baxter and Fresenius Kabi. EV reports grant/research support from Nutricia Italia Spa, Nestle Health Science - Vitaflo Italy, FoodAR srl Italy, PIAM Pharma and Integrative Care. RV reports no conflict of interest. MD has received speaker fees from Baxter, Fresenius, Semper, Abbvie, Nestlé and research support from Baxter and Prolacta.

Abstract

Background: Palm oil (PO) is used in infant formulas in order to achieve palmitic acid (PA) levels similar to those in human milk. PA in PO is esterified predominantly at the SN-1,3 position of triacylglycerol (TAG), and infant formulas are now available in which a greater proportion of PA is in the SN-2 position (typical configuration in human milk). Since there are some concerns about the use of PO, we aimed to review literature on health effects of PO and SN-2-palmitate in infant formulas.

Methods: PubMed and Cochrane Database of Systematic Reviews were systematically searched for relevant studies on possible beneficial effects or harms of either PO or SN-2-palmitate in infant formula on various health outcomes.

Results: We identified 12 relevant studies using PO and 21 studies using SN-2-palmitate. Published studies have variable methodology, subject characteristics and some are underpowered for the key outcomes. PO is associated with harder stools and SN-2-palmitate use may lead to softer stool consistency. Bone effects seem to be short-lasting. For some outcomes (infant colic, faecal microbiota, lipid metabolism), the number of studies is very limited and summary evidence inconclusive. Growth of infants is not influenced. There are no studies published on the effect on markers of later diseases.

Conclusions: There is insufficient evidence to suggest that PO should be avoided as a source of fat in infant formulas for health reasons. Inclusion of high SN-2-palmitate fat blend in infant formulas may have short-term effects on stool consistency but cannot be considered essential.

Key words: palm olein, palmitic acid, colic, constipation, growth, lipids

What is known:

- Palm oil (PO) is used as source of fat in infant formula in order to achieve palmitic acid (PA) levels comparable to human milk.
- PA in human milk is predominantly at the SN-2 position, in PO it is predominantly at the SN-1,3 position. SN-2-palmitate is used in some formulas to mimick PA position in human milk.

What is new:

- There is insufficient evidence to suggest that PO should be avoided as a source of fat in infant formulas for health reasons.
- Inclusion of high SN-2-palmitate fat blend in infant formulas may have short-term effects on stool consistency but cannot be considered essential.

ACCEPTED

Introduction

Lipids in human milk and infant formulas

Lipids in human milk serve as a major source of energy and essential fatty acids for the breastfed infants. They also facilitate absorption of fat-soluble dietary components and support gastrointestinal function, lipid and lipoprotein metabolism, neurodevelopment, and immune function (1, 2). Almost 100 % of human milk fat is composed by triacylglycerols (TAG). Fatty acids (FA) in human milk are either saturated (SFA, 35-40 %), monounsaturated (MUFA, 45-50 %) or polyunsaturated (PUFA, approx. 15 %) (1, 2). Palmitic acid (PA, C16:0) provides the major part of the total SFA content and its concentration is kept relatively constant in breastfeeding mothers (2, 3). Human milk TAG are predominantly esterified with PA in the SN-2 position and this configuration facilitates absorption in infants after digestion by human pancreatic lipase that is SN-1,3 specific. Nonesterified FA liberated from the SN-1 and SN-3 positions are quite well absorbed if they are unsaturated due to their water solubility. On the contrary, poorly absorbed saturated FA, such as PA tend to form calcium (Ca) soaps that are excreted in stool and increase stool hardness. However, pancreatic lipolysis of human milk TAG with PA esterified predominantly to the SN-2 position results in formation of water-soluble palmitoyl-monoglycerol. This reduces FA and Ca malabsorption and enables the breastfed infant to benefit from PA as source of fat (see Figure 1) (1, 2, 4-10).

The fat in infant formula comes mainly from vegetable oils. Palm oil (PO) is used in order to achieve PA levels similar to those in human milk. Recently, there has been an increasing discussion regarding the use of PO in food products, mostly due to environmental concerns but PO also has potential important health effects. In PO, PA is esterified predominantly at the SN-1,3 position of TAG. As the intestinal absorption of SN-1,3-palmitate is not optimal, there have been attempts to replace it, at least partly, in infant formulas with SN-2-

predominant TAG (beta-palmitate) which is the form present in human milk. A number of products using either a mixture of fat or commercial synthetic beta-palmitate (e.g. Betapol®, INFAT®, LipoMilk® or Zhejiang Beijia product) in order to achieve high SN-2 content are available on the market. Betapol® is produced by interesterifying a tripalmitin-rich PO fraction with a mixture of other fats by using the SN-1,3 specific lipase from *Rhizomucor miehei* (code SP-392; Novo Industries, Copenhagen, Denmark). INFAT® (Advanced Lipids, Karlshamn, Sweden) is produced by a patented enzymatic process which restructures the fat in a way that mimics the structure of PA in human milk (SN-2 predominant position).

Palm oil

PO is the most widely used vegetable oil in the world. It is obtained from an ancient tropical palm tree (*Elaeis guineensis*) and it was one of the major sources of dietary fats for centuries in most of West Africa (11). Palm kernel oil (PKO) is extracted from the seeds and edible PO from the mesocarp. PKO has a composition different from that of PO and is mainly used for non-edible purposes (4). Compared with most other vegetable oils, PO contains a high amount of saturated fat (8). Crude palm oil (known also as red palm oil), contains both compounds beneficial to health (such as TAG, vitamin E, carotenoids and phytosterols) as well as impurities (phospholipids, free fatty acids (FFA), gums, and lipid oxidation products). Both can be removed by refining processes, but the composition of the final product is dependent on the refining method (chemical or physical). High quality PO containing more than 95 % neutral TAG, less than 0.5 % FFA and a low impurity content is used in the food industry. Low-quality oils are used in non-edible industry (6).

PO represents approx. 1/3 of the world's vegetable oil production, and its consumption has increased rapidly in the past several decades (8). Malaysia and Indonesia are the main

producers of PO, but the *Elaeis guineensis* palm tree is now widespread throughout the tropical areas of America and South East Asia. Productivity of PO per unit area is 11, 10 and 7 times the yield of the other main vegetable oils, soybean, sunflower and rapeseed, respectively (4). Environmental and economic aspects of PO production (such as rainforest destruction, biofuels and child-labour) are widely discussed by journalists, consumers, public and industry via internet and social media (e.g. <https://www.theguardian.com/environment/palm-oil>). These aspects are beyond the scope of this paper.

PO has two major fractions. Palm olein (POL) (65 – 75 %) is the low-melting liquid fraction used mainly in cooking oil for frying and in margarines. The high-melting solid fraction, palm stearin (30 – 35 %), is present in shortenings and hydrogenated oils used as butter substitutes in some countries. PO is generally found in baked goods, cereals, confectionary fats, frozen meals, ice cream, industrial frying fats, margarines, non-dairy creamers, salad dressings, supplements/vitamins and other food products (6).

PO contains 50 % SFA, mostly PA (44 %) and lower amounts of stearic acid (5 %), 40 % MUFA, mostly oleic acid, and 10 % PUFA, mostly linoleic acid. Thus, PA is the principal constituent of refined PO. FA in PO (as in all vegetable oils) are mainly structured as TAG having oleic acid predominantly located at the SN-2 position, and PA mainly (over 70-80 %) located at the SN-1 and SN-3 positions. As in human milk, PA is also the main SFA naturally occurring in animal milk fats, often found at the SN-2 position (beta-position) of TAG - in cow's milk in approximately 40 % and in human milk in 60-80 % (3, 5-7).

Potential health effects of PO and PA in adults

High-fat diets, particularly those rich in SFA, have been linked to cardiovascular diseases (CVD), obesity, type 2 diabetes mellitus (T2DM) and cancer. However, studies on potential unhealthy effects of PO due to the high PA content, are controversial (6, 12, 13).

Moreover, PO is cholesterol free and POL, containing a substantial amount of oleic acid (48 %), was considered by some authors as a suitable substitute for olive oil in healthy human diets (6). PO has also been suggested as an alternative for partially hydrogenated fats in the food supply to reduce trans fat intakes (8). A lower atherogenic power of PO compared to animal fat is also hypothesized, due to the fact that in PO, PA is usually not present at the SN-2 position in TAG and it has been shown in animal experiments that higher percentages of PA at the SN-2 position are related to the most atherogenic profiles (6).

A meta-analysis of 30 papers including 32 clinical trials reported that PO significantly increased both low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol when compared with vegetable oils low in saturated fat, and that PO increased HDL cholesterol when compared to trans fat-containing oils (8). The authors of a recent review state that there is not enough evidence to conclude that PO is atherogenic and contributes to elevated serum cholesterol levels (14). A systematic review and meta-analysis of 51 dietary intervention trials (many of them included in the previously mentioned meta-analysis (8)) has shown that both favourable and unfavourable changes in blood lipid-related markers of CVD occurred when PO replaced the primary dietary fats (rich in stearic acid, MUFA and PUFA or myristic/lauric acids), whereas only favourable changes occurred when PO replaced trans fatty acids (15). The same author in a previous review concluded that the evidence on dietary PA or PO and the risk of cancer is not convincing and specific studies are limited (4). There

is one study showing promising data on potential anti-inflammatory effects of red palm oil and protection against ischemia and reperfusion injuries of the heart in an intensive care setting that merits further investigation (16). Another extensive review reports conflicting results regarding all considered outcomes (T2DM, CVD and cancer) mainly for methodological reasons (6).

There are data from studies on animals and tissue models showing potential negative health effects of PO compared to PO-non-supplemented diet, such as reduced insulin sensitivity and impaired glucose tolerance, lipotoxicity (negative effect of PA on mitochondrial function mediated by oxidative stress), inflammation in adipose tissue and pancreas, and supposed involvement of PA in regulation of tumour growth (cell proliferation, apoptosis, invasiveness) (6). On the contrary, there are number of animal studies showing potentially beneficial effects of PO on lipid profile (14).

Other potentially beneficial or harmful compounds of PO

PO is genetic-modification-free. Crude PO contains phytosterols, and is the richest natural source of carotenoids (vitamin A precursors), tocopherols and tocotrienols (vitamin E compounds) (6, 11, 17). The levels of these compounds are affected to various degrees during processing (personal communication, Specialised Nutrition Europe (SNE) - an association representing food manufacturers, including infant formula producers). The final levels of these compounds in the PO used in the oil blends for infant and follow-on formulas depends on the formula manufacturers' specifications to meet the nutritional profile of these foods. It is important to note that the infant and follow-on formulas fatty acid and vitamin profiles are considered in view of the contribution expected from all ingredients, not just PO. Tocotrienols are natural inhibitors of cholesterol synthesis and recent studies point to their

potential beneficial biological properties, such as protection against cancer, cardiovascular diseases, neurodegeneration, oxidative stress and immune regulation (6, 18-20).

On the other hand, PO may contain potentially harmful substances, such as phospholipids, FFA, gums, and lipid oxidation products (6). Glycerol-based process contaminants (glycidyl fatty acid esters (GE), 3-monochloropropanediol (3-MCPD), and 2-monochloropropanediol (2-MCPD) and their fatty acid esters) are found in PO, but also in other vegetable oils, margarines and some processed foods. The substances form during food processing, in particular, when refining vegetable oils at high temperatures (approx. 200 °C). The European Food Safety Authority (EFSA) points to potential health concerns (genotoxicity, carcinogenicity) of these compounds especially for young age groups. In their recent report, EFSA points out the fact that the intakes of 3-MCPD, especially in exclusively formula-fed infants, slightly exceeded the stipulated tolerable daily intake of 2µg/kg/day (21). Infant formulas that do not contain PO or POL have relatively low concentrations of 3-MCPD and glycidyl esters, however effective industrial mitigation strategies can substantially lower the content in PO/POL - based formula (22). Infants consuming solely infant formula may be particularly at risk of exposure to GE, however, in recent years, due to voluntary measures taken by producers, levels of GE in PO and fats have fallen substantially (<https://www.efsa.europa.eu/en/press/news/160503a>) (23). Oil blends used in infants and young children nutrition industry contain levels of contaminants (particularly 3-MCPD and GE) in line with regulation as low as is technically possible (SNE, personal communication). Several toolboxes are available and used by the suppliers to mitigate 3-MCPD esters and GE in oils (e.g. BLL toolbox - see <https://www.bll.de/de/lebensmittel/sicherheit/unerwunschte-stoffe-kontaminanten/3-mcpd-und-glycidyl-fettsaeureester/toolbox-minimierung-3-mcpd->

[glycidyl](#)). The EU has also recently implemented stricter regulations for foods for infants and young children versus general foods (24).

Some reports show that there may be non-essential trace elements and radionuclides present in PO, originating from water and soil on the palm plantations that may affect the health of consumers. However, data are conflicting, the available literature is limited and further research is needed to confirm or refute this suspicion (25).

The aim of this position paper is to review evidence for potential effects of PO and SN-2-palmitate used as source of fat in infant formula on the health of infants and children. Environmental effects of PO production will not be addressed in this paper.

Materials and Methods

We present the results of relevant studies (RCTs and large observational studies) on possible beneficial or harmful effects of either PO/POL or SN-2-palmitate (beta-palmitate) as a source of fat in infant formulas on the following outcomes: 1) Composition of stool; 2) Infantile colic; 3) Stool frequency and consistency; 4) Bone health and growth; 5) Metabolic effects (e.g. cardiovascular health, T2DM, hypertension and lipid profile).

The database Medline (via PubMed) and Cochrane Database of Systematic Reviews were searched for keywords for publications up to November 2017 - see Appendix 1 (Supplemental Digital Content, <http://links.lww.com/MPG/B600>).

Results

In total, we identified 12 relevant studies using PO/POL (5 on composition of stool, none on infantile colic, 2 on stool frequency and consistency, 3 on bone health and growth and 2 on metabolic effects) and 21 relevant studies using SN-2-palmitate (8 on composition of stool, 3 on infantile colic, 3 on stool frequency and consistency, 5 on bone health and growth and 2 on metabolic effects) - see Tables 1a, 1b. In the results section, studies are reported according to their primary outcome. Secondary outcomes are also mentioned in Tables 1a, 1b and in the discussion part of the manuscript. The majority of the identified studies are either industry supported or performed by employees of formula producers (also mentioned in Tables 1a, 1b). The quality of the studies is variable; some have methodological problems such as very low sample size or the use of multiple interventions (hydrolysed protein, oligosaccharides etc.).

1. PO/POL studies (see Table 1a)

1.1 Composition of stool (FA and calcium (Ca) content, intestinal microbiota)

We identified 5 RCTs on this topic (26-30), one of them composed of two subprojects (29) and two of them reporting results from the same cohort of patients (26, 30). All were performed on relatively small numbers of healthy term infants. All studies consistently reported lower fat and Ca absorption in infants using PO/POL-based formulas when compared to PO/POL-free formulas. Only one study reported equal fat absorption when soy protein-based formula was used, irrespective of its PO content (29). One study also described lower LC-PUFA absorption in POL-based formula (30). None of the studies reported on intestinal microbiota.

1.2 Infantile colic

No relevant study was identified.

1.3 Stool frequency and consistency

Both a large observational multicentre study and an un-blinded RCT (composed of 2 subprojects - one on breastfed and the second on formula-fed infants) have shown less frequent stools and harder stool consistency in term infants fed/weaned to formula containing POL when compared to non-POL formula (31, 32).

1.4 Bone health and growth

Three RCTs were identified - all in healthy term infants with several months of follow-up - focused on bone health and growth (33-35). Two of the studies reported lower bone mineralisation (measured by DEXA) in the group of infants fed PO/POL-based formula when compared to PO/POL-free formula (34, 35). Two of the studies used partially hydrolysed protein - based formula in both intervention and control arms (33, 34). All three studies consistently report no difference in anthropometric measurements between PO/POL group and PO/POL-free group.

1.5 Metabolic effects

No relevant intervention studies were found focused primarily on the effect of PO/POL-containing formula on predictors (markers) of metabolic diseases (cardiovascular health, T2DM, hypertension etc.) in infants and children. Two RCTs were identified that focused on lipid profile in healthy infants with several months of follow-up (36, 37). A lower serum TAG level at day 90 in the non-POL group when compared to the POL-group was found in the first study but there was no difference in TAG levels when compared to a human milk group in any of the formula groups (37). In the other study, infants consuming POL-based follow-up formula had lower increases in mean serum total cholesterol, LDL, and apo B by 12 months of age compared with infants ingesting the standard infant formula or whole cow milk (36).

2. SN-2-palmitate (beta-palmitate) studies (see Table 1b)

2.1 Composition of stool (FA and calcium (Ca) content, intestinal microbiota)

Altogether 8 RCTs were identified on this topic (38-45), three of them on preterm infants (39, 40, 44), the rest on term infants. The study by Carnielli et al. (39) was a subanalysis of a study previously published by the same group (40). The studies have consistently shown that a higher SN-2-palmitate proportion in formula is associated with improved absorption of Ca and fat, including palmitate. Only one study, presented as a congress abstract only, did not show any reduction in stool total FA soaps, palmitate soaps and total FA when increasing SN-2 palmitate in infant formula (42). One study has shown higher *Lactobacillus* and *Bifidobacteria* counts in the stool in high SN-2-palmitate group when compared to low SN-2-palmitate group (45).

2.2 Infantile colic

One DB-RCT, one single-blinded RCT and a large uncontrolled observational study were identified, all in term infants (46-48). The DB-RCT tested the effect of SN-2-palmitate alone (46), while the other studies used multiple interventions - partially hydrolysed SN-2-palmitate formula containing fructo- and galacto-oligosaccharides (47, 48). All studies have shown a reduction of crying episodes/frequency of colic, when SN-2-palmitate formula was used.

2.3 Stool frequency and consistency

Three DB-RCTs were identified, all in term infants (49-51). All of the studies used not only SN-2-palmitate, but also prebiotic oligosaccharides as the intervention, moreover, one of them used partially hydrolysed protein formula (49). The first study showed significantly increased defecation frequency and a trend to softer stools in the intervention arm, but there was no difference compared to standard formula (49). The second study did not show any difference from the control group when only SN-2-palmitate formula was used, however, the stool consistency score was significantly lower at day 28 when both SN-2-palmitate and

oligofructose enriched formula was used (50). In the third study, the SN-2-palmitate group had significantly softer stools than controls at week 8. Addition of oligofructose resulted in even fewer formed stools (51).

2.4 Bone health and growth

Four RCTs (one of them with unblinded follow-up) in healthy term infants were identified (52-56). Two of the studies were focused on bone health and both have shown short term positive effects of SN-2-palmitate formula on bone parameters - bone mineral content (BMC) and mean bone speed of sound (SOS) at week 12 (54, 55), however unblinded follow-up of 28 % of the original cohort until 10 years of age did not show long-term persistence of this effect (53). The other two studies focused on anthropometric parameters and did not show any significant difference at week 12 and/or 135 days of follow-up, respectively, between SN-2-palmitate and control formulas. The experimental formula in both studies was enriched also with prebiotic oligosaccharides and in one of the studies also contained partially hydrolyzed protein, in the other study acidified milk (52, 56).

2.5 Metabolic effects

No relevant intervention studies focused primarily on the effect of SN-2-palmitate formula on predictors (markers) of metabolic diseases (cardiovascular health, T2DM, hypertension *etc.*) in infants and children were found. Two studies on healthy term infants that focused on lipid profile were identified.

The first RCT showed higher (closer to breast-fed group) content of C16:0 FA in the SN-2 position of chylomicron TAG in infants fed SN-2-palmitate formula when compared to standard formula (57). In the other study higher formula SN-2 led to lower n-9-MUFA, but higher n-6-PUFA and n-3-PUFA in the infant plasma, higher C18:0 in LDL TAG, and higher apo B and lower apolipoprotein A-1 (apo A-1) (58).

Discussion

In cow milk and infant formulas, PA predominantly found in the SN-1 and SN-3 positions is hydrolyzed by pancreatic lipase and the resulting free PA may form Ca-FA complexes, which are poorly absorbed - this was previously confirmed by experiments in rodents and piglets (54, 59-65). The overall efficacy of fat absorption gradually increases both in preterm and term infants postnatally reflecting the functional development of the gut (66).

Several studies have shown that PO, as the predominant fat source, or PA present predominantly on SN-1,3 position, may negatively influence absorption of Ca and FA from infant formulas (26-30), and SN-2 palmitate positioning has generally an opposite effect (38, 40, 41, 43-45). One study presented as a congress abstract did not show any effect of SN-2 palmitate on reduction in stool total FA soaps, palmitate soaps and total FA (42). Despite varying quality of the studies, there is generally convincing evidence of differences in PA digestion and absorption related to positioning of PA on the TAG. No clinical conclusions can be directly made from these findings, but such changes may be relevant to underlying physiological mechanism for some clinical conditions, such as infantile colic or constipation and explain the observed effects on bone health. Moreover, different structure of TAG in infant formulas may influence intestinal microbiota, but the number of studies is limited and no clinically relevant conclusions are possible at the moment (45, 51, 52, 56).

There are two RCTs published so far on the effect of high SN2-palmitate formula on crying episodes in infantile colic (46, 48). The first study did not only evaluate the effect of SN-2-palmitate as the study formula also contained hydrolysed protein, a mixture of galacto- and fructo-oligosaccharides, and had different whey/casein ratio and carbohydrate content than control formula. Simethicone was added to standard formula in the control group. Whether the clinical effect was due to the PO content is therefore unclear (48). In the second study, the formulas differed only in SN-2-palmitate content and a reduction in crying was observed. No

pre- or probiotics were used (46). Possible beneficial effects on infant colic and other minor gastrointestinal problems were described in a large observational prospective trial, however the study formula contained fructo- and galacto-oligosaccharides, partially hydrolysed proteins and low levels of lactose apart from the SN-2-palmitate, and there was no control group (47). These findings are promising, but more data from well-designed RCTs are needed in order to draw conclusions on the effect of SN-2-palmitate in infant colic. In some of the studies not primarily focused on colic, minor GI problems (spit up, vomiting, "GI intolerance") were evaluated as secondary outcomes (29, 31, 32, 34). No difference was found between intervention and control group in any of the studies. Moreover, measurement of the primary outcome in trials focused on infant colic is often subject to discussion as it is very difficult to find an objective measurement for "crying episodes" and researchers have to rely on subjective evaluation by parents using various questionnaires. According to a recent consensus paper, limited data suggest that infant formula with a partial hydrolysate, galacto-oligosaccharides/fructo-oligosaccharides and added SN-2-palmitate may be of benefit in reducing infantile colic in formula fed infants in cases where cow's milk protein allergy is not suspected (67).

Previously, it was reported that formula-fed infants have harder stools than breastfed infants. Ca and FA soaps were the dominant factors significantly related to stool solids and hardness score across the breast- and formula-fed groups (68). Vandenplas and Salvatore in their review on functional gastrointestinal disorders in infants state that harder stools are frequent in infants fed formula containing POL or PO as the main source of fat (69). A large observational study and a RCT with two sub-studies were published on this topic (having stool frequency or consistency as primary outcome) suggesting that POL content in infant formulas may be responsible for this phenomenon (31, 32). However, in the observational

study, the two formulas differed also in other components (ratio of other oils, Ca and nucleotides content) (31). Also in the RCTs, tested and control formulas differed in other aspects (e.g. *whey* : *casein* ratio, content of nucleotides) (32).

A recent meta-analysis of RCTs indicated that infants fed POL-free formulas had significantly softer stools (difference in Mean Rank Stool Consistency score -0.355 , 95% CI of -0.472 to -0.239 , $p < 0.001$) than infants fed POL-predominant formulas. However, stool frequencies were similar between both groups ($p = 0.6$). Studies included in the meta-analysis had many differences in study design, infant age, formula types and composition. The meta-analysis did not include clinical data from infants fed human milk or SN-2-palmitate (70). Stool frequency and/or consistency was also mentioned as a secondary outcome in other studies (26, 29, 33, 34). Conclusions from these studies generally support the hypothesis that PO/POL content in formula may be associated with harder stools.

Several studies on the effect of SN-2-palmitate on stool consistency or frequency have been published (49-51). The study by Bongers et al. did not show significant difference in the effect of SN-2 formula on stool frequency in constipated infants (49). However, the authors used three different interventions at once (partially hydrolyzed protein, SN2-palmitate and prebiotics) and the study was considered to be underpowered for its outcomes (71). Two studies showed positive effect of SN-2-formula on stool consistency which seemed to be enhanced by adding prebiotic oligofructose (50, 51). Stool consistency or frequency is also reported as one of the outcomes in other studies on Ca/FA balance, bone health and growth, with conflicting results on the effect of SN-2-palmitate (40, 41, 46, 47, 52). Moreover, softer stools may not always be perceived positively by mothers. In the study by Kennedy et al. (54), a greater proportion of the mothers using the high-SN-2 formula were concerned about

runny stools at the age of 3, 6 and 12 weeks. The difference was not seen in the small group of infants who had started solids by 12 weeks but continued to receive the study formula. According to a recent consensus paper, a partially hydrolyzed infant formula with prebiotics and SN-2-palmitate may be considered as a dietary intervention for functional constipation in formula fed infants (67).

For the effect of PO/POL on bone health, the same pathophysiological background as for stool consistency changes was suggested (formation of Ca-FA complexes leading to poor Ca absorption). In an animal model, levels of intestinal calbindin-D9k (vitamin D-dependent Ca-binding protein) mRNA expression was higher in piglets fed PO-based formula when compared to formula with SN-2 predominant synthetic TAG (72). BMC, bone area (BA) and cortical BA in femur were lower ($p=0.002$, $p=0.005$, and $p=0.02$, respectively) in piglets fed human milk fat substitute with a modified TAG structure holding C16:0 predominantly in the SN-2-position compared with a control (63).

In healthy infants, average BMC and bone mineral density (BMD) significantly increases during infancy and body size is the dominant predictor of bone mineral status (73). Reference values of body composition obtained by DEXA both in preterm and term neonates were published (73-75), however there is a large variation in published normative data for BMC and BMD of both human-milk-fed and formula-fed infants (76).

Jones et al. have shown in a longitudinal observational cohort study (N=330) a positive association between breastfeeding in early life (particularly for 3 months or longer) and bone mass in 8-year-old children born at term (77). Schanler et al. have shown that although predominantly formula-fed preterm infants had significantly greater BMC values at 16, 25,

and 52 weeks, if the predominantly human-milk fed infants continue to receive human milk, radius BMC will "catch-up" to that of similar infants given formula in the post-hospitalization period (78). On the contrary, some studies find that human milk-fed infants have lower bone accretion than do formula-fed infants (with greater bone accretion when the mineral content of formula is higher).

Inclusion of PO in infant formula may be responsible for reduced bone mineral accretion, but other factors play a role, like maternal nutritional status (vitamin D, Ca) during pregnancy, type of infant feeding, Ca and phosphorus content of infant formula, infant vitamin D supplementation, diet, and physical activity during the toddler and preschool years (79). A small RCT on 67 infants indicates that during the first 6 months, bone mass accretion is lower in infants fed human milk or low-mineral (Ca and phosphorus) formula compared with infants fed moderate-mineral formula. However, the human milk-fed group had greater bone mass accretion during the second 6 months and by 12 months of age there were no differences among the feeding groups (80).

A RCT by Koo et al. showing that infants fed PO-based formula had significantly lower BMC and BMD at 3 and 6 months than PO-free formula (35) was challenged in 2004 by Clandinin et al. (76) due to lack of inclusion of a human milk control group. Infants fed human milk have BMC and BMD values well below either of the 2 study formulas and all are well within published normative values at both 3 and 6 months of age (the same is valid for the PO-based formula group after intervention). This questions the clinical significance of the data, as it is not clear whether bone mineral accretion higher than that found in breastfed infants is beneficial (76). Another RCT has shown higher BMC and greater 25-OH vitamin D serum levels in children fed PO-free formula when compared to PO formula. However, the

PO formula contained less Ca than the PO-free formula (34). A retrospective study that related DEXA performed at 4 years of age (N=178) with type of infant feeding identified by history has shown no significant differences in BMC or BMD (P = 0.51 and 0.89, respectively) among children who had exclusively consumed human milk (n = 57), an infant formula containing no PO (n = 56) or an infant formula containing PO (n = 65) during the first 4 months of life (81). This study was criticized due to its methodology (retrospective nature not controlling for many potential confounders, possible variability in measurement and underpowered sample size) (82). The authors reply to this criticism was that their study was designed to detect a difference of 0.52 SD of bone mineral content between feeding groups (82). A systematic review by Koo et al. included 9 publications with non-PO and PO comparison groups in infants between 28 - 42 weeks of gestational age and up to 192 days at study onset. The standardized results were consistently significantly (p < 0.05) positive in favour of the feeding with non-PO formulas with respect to increased intestinal fractional absorption of fat, PA and Ca and significantly higher BMC. The authors conclude that avoidance of PO or its substitution with synthetic TAG in infant formulas can prevent this detrimental effect (83).

Although a large RCT has shown possible short-term effects of SN-2 rich formula on bone health in infants (54), in an open-label extension of part of the original cohort, no significant effect was shown by DEXA at 10 years of age (53). Thus, it is questionable if the effect of high-SN-2 is long lasting. Another RCT has shown that palmitic structural distribution may influence (with borderline statistical significance) mean bone speed of sound (SOS) (a measure of bone density, micro-architecture, cortical thickness and elasticity) in term infants (55). In contrast, it has been suggested that SOS changes during infancy may be independent of the type of early diet (84).

A meta-analysis by Yu et al. (article in Chinese, only abstract evaluated by authors of this position paper) analyzed the effect of infant formula containing PA at the SN-2 position, formula containing PA at the SN-1, 3 positions and formula without PA on nutrient absorption, BMC and stool consistency in infants (85). Absorption of fat and Ca was lower, faecal excretion of Ca was higher, the BMC was reduced, and the incidence of hard stools was increased when the infant formula provided PA at the SN-1 and SN-3 positions as compared to formula with PA at the SN-2 positions or without PA. However, the authors stress that the conclusions should be used with caution because of the limited quality of evidence (85).

Published studies that did not include growth as primary outcome (26, 33-38, 43-45, 51, 52, 54-57) did not show any significant differences between PO/POL/SN-2-palmitate base formulas and controls. In an animal experiment, a small but significant improvement in most growth parameters was found in the rats fed beta-palmitate based diet when compared to controls (86).

No human intervention studies focused primarily on the effect of PO/POL/SN-2-containing formula on biomarkers of metabolic diseases (cardiovascular health, T2DM, hypertension etc.) as the primary outcome in infants and children were identified. Scarce data are available on lipid metabolism both from animal and human studies.

One study using a piglet model showed that mRNA levels of hepatic hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, and 7 α -hydroxylase (C7H) are higher ($P < 0.05$) and plasma total, HDL, and apo B-containing cholesterol are lower ($P < 0.05$) in formula-fed versus milk-fed piglets, irrespective of the formula TAG source (POL vs synthesized SN-2

predominant TAG). There was no difference in LDL receptor mRNA levels (87). This study shows that important components of lipid metabolism are altered by early diet in an animal model, but POL as source of fat in formula does not seem to play a role. In another study, plasma lipid percentages of C18:1 and C18:2n-6 were higher in piglets fed formula with MCT or coconut oil rather than formulas with C16:0 (from PO or synthesized triglyceride containing predominantly sn-2 C16:0), or sow milk, although the formulas contained similar C18:1 and C18:2n-6 (88).

In the study by Innis et al., only TAG levels at day 90 were lower in POL-free formula than in POL formula, however neither of the groups had different TAG levels when compared to breast-fed infants. Moreover, the study was primarily focused on the effect of n-6 and n-3 FA on growth, visual acuity and lipid profile in infants. Thus, no direct conclusions can be made on metabolic effects of POL by itself (37). The RCT by Fuchs et al. has shown that older infants fed lower fat formula have adequate total energy intake and normal growth and that the fat composition of the diets influenced serum lipid and lipoprotein profiles. However, the design of the study does not allow any POL-specific conclusions and POL as source of dietary fat may not necessarily be fully responsible for above-mentioned metabolic changes (36). Results of an RCT by Nelson and Innis suggest that $\geq 50\%$ of the dietary SN-2-palmitate is conserved through digestion, absorption, and chylomicron TAG synthesis in breast-fed and formula fed infants (57). In another study by Innis, post-prandial lipoprotein and unesterified fatty acids levels in term infants were different in children fed SN-2-predominant formula compared to low-SN-2 formula (58).

Summary

Despite available data on potential benefits of SN-2-palmitate and potential non-beneficial effects of PO/POL used in infant formulas (3, 89, 90), the current evidence remains inconsistent and does not allow definite conclusions to be drawn. Published studies have variable methodology, differ in subject characteristics and some of them are underpowered for the key outcomes. Many of the studies combine different interventions, such as partially hydrolysed protein, prebiotic oligosaccharides, and in some studies experimental and control formula differ in other aspects-like protein source and composition, carbohydrates or mineral content. Changes in Ca and PA absorption have been reported that may represent the physiological background for some clinical situations, such as infantile colic, constipation or lower BMC and BMD. PO/POL seem to be associated with harder stools, on the contrary, SN-2-palmitate use may lead to softer stool consistency. Bone effects seem to be short-lasting. For some of the outcomes (infant colic, faecal microbiota, lipid metabolism), the number of studies is very limited and summary evidence inconclusive. There are no studies published on the effect of PO/POL/SN-2 in infant formulas and long-term outcomes/markers of later diseases (CVD, T2DM, obesity, hypertension, cancer or long-lasting changes in lipid profile). Growth and infant health-related quality of life seems not to be influenced irrespective of PO/POL/SN-2 content of the formula (91). The majority of the studies are supported by (or performed by employees of) infant formula producers. Moreover, in several studies, high SN-2 palmitate formula remains inferior to breast feeding. Thus, due to the lack of high quality evidence and inconsistency in the findings of the studies presented here, current guidelines do not mandate the inclusion of high SN-2 palmitate in infant formulas (92, 93). EFSA successively rejected two health claim petitions for beta-palmitate in 2011 and 2014, respectively (3, 94, 95). There are also other potential health benefits of high dietary SN-2 palmitate suggested in animals, like reduced gut inflammation in a colitis model

and altered tissue endocannabinoid concentrations (7, 96, 97) that warrant further scientific attention.

Conclusions and recommendations

Based on available data, the ESPGHAN Committee on Nutrition:

- concludes that inclusion of high SN-2-palmitate fat blend in infant formulas may have short-term effects on stool consistency due to reduced formation of calcium soaps, but cannot be considered essential.
- concludes that there is insufficient evidence to suggest that PO/POL should be avoided as a source of fat in infant formulas for health reasons.
- recommends that all producers of infant formulas take measures to minimize levels of glycerol-based process contaminants in infant formulas.

The ESPGHAN Committee on Nutrition recommends further research on:

- possible long-term health effects of PO/POL/SN-2-palmitate based infant formulas in well-powered RCTs
- presence of non-essential trace elements and radionuclides in PO
- potential health benefits of high dietary SN-2 palmitate suggested in animals, such as reduced gut inflammation in a colitis model and altered tissue endocannabinoid concentrations
- the potential beneficial / harmful effects of other compounds in PO, like tocotrienols

Disclaimer

ESPGHAN is not responsible for the practices of physicians and provides guidelines and position papers as indicators of best practice only. Diagnosis and treatment is at the discretion of physicians.

List of abbreviations:

2-MCPD - 2-monochloropropanediol

3-MCPD - 3-monochloropropanediol

apo A-1 - apolipoprotein A-1

apo B - apolipoprotein B

ARA - arachidonic acid

BF - breastfed

BMC - bone mineral content

BMD - bone mineral density

C7H - 7alpha-hydroxylase

Ca - calcium

CF - control formula

CHF - casein hydrolysate-based formula

CVD - cardiovascular disease

DB-RCT - double-blinded randomised controlled trial

DEXA - dual-energy X-ray absorptiometry

DHA - docosahexaenoic acid

EF - experimental formula

EFSA - European Food Safety Authority

ESPGHAN - European Society for Paediatric Gastroenterology Hepatology and Nutrition

EU - European Union

FA - fatty acids

FFA - free fatty acids

GE - glycidyl fatty acid esters

HBP - high beta palmitate formula

HC - head circumference

HDL - high density lipoprotein cholesterol

HMG-CoA - hydroxymethylglutaryl coenzyme A

HO - high-oleic

LBP - low beta palmitate formula

LDL - low density lipoprotein cholesterol

MUFA - monounsaturated fatty acids

NF - new formula

NoPALM - palm oil/palm olein-free formula

OF - oligofructose

PA - palmitic acid

PALM - palm oil/palm olein-based formula

pHF - partially hydrolyzed whey protein

PKO - palm kernel oil

PO - palm oil

POL - palm olein

PUFA - polyunsaturated fatty acids

(DB)-RCT - (double-blinded) randomised controlled trial

SDS - standard deviation score

SF - standard formula

SFA - saturated fatty acid

SN-1,3 - palmitic acid placed predominantly on alpha and gamma position of triacylglycerol

SN-2 - palmitic acid placed predominantly on beta position of triacylglycerol (beta-palmitate)

SOS - mean bone speed of sound

SPF - soy protein-based formula

T2DM - type 2 diabetes mellitus

TAG - triacylglycerols

References

- 1 Delplanque B, Gibson R, Koletzko B, et al. Lipid Quality in Infant Nutrition: Current Knowledge and Future Opportunities. *J Pediatr Gastroenterol Nutr* 2015;61:8-17.
- 2 Koletzko B Human Milk Lipids. *Ann Nutr Metab* 2016;69 Suppl 2:28-40.
- 3 Zou L, Pande G, Akoh CC Infant Formula Fat Analogs and Human Milk Fat: New Focus on Infant Developmental Needs. *Annu Rev Food Sci Technol* 2016;7:139-65.
- 4 Fattore E, Fanelli R Palm oil and palmitic acid: a review on cardiovascular effects and carcinogenicity. *Int J Food Sci Nutr* 2013;64:648-59.
- 5 Lopez-Lopez A, Lopez-Sabater MC, Campoy-Folgozo C, et al. Fatty acid and sn-2 fatty acid composition in human milk from Granada (Spain) and in infant formulas. *Eur J Clin Nutr* 2002;56:1242-54.
- 6 Mancini A, Imperlini E, Nigro E, et al. Biological and Nutritional Properties of Palm Oil and Palmitic Acid: Effects on Health. *Molecules* 2015;20:17339-61.
- 7 Miles EA, Calder PC The influence of the position of palmitate in infant formula triacylglycerols on health outcomes. *Nutr Res* 2017;44:1-8.
- 8 Sun Y, Neelakantan N, Wu Y, et al. Palm Oil Consumption Increases LDL Cholesterol Compared with Vegetable Oils Low in Saturated Fat in a Meta-Analysis of Clinical Trials. *J Nutr* 2015;145:1549-58.
- 9 Lien EL, Yuhas RJ, Boyle FG, et al. Corandomization of fats improves absorption in rats. *J Nutr* 1993;123:1859-67.
- 10 Mu H, Hoy CE The digestion of dietary triacylglycerols. *Prog Lipid Res* 2004;43:105-33.
- 11 Boateng L, Ansong R, Owusu WB, et al. Coconut oil and palm oil's role in nutrition, health and national development: A review. *Ghana Med J* 2016;50:189-96.

- 12 Berraouan A, Abid S, Bnouham M Antidiabetic oils. *Curr Diabetes Rev* 2013;9:499-505.
- 13 Teng KT, Chang CY, Chang LF, et al. Modulation of obesity-induced inflammation by dietary fats: mechanisms and clinical evidence. *Nutr J* 2014;13:12.
- 14 Odia OJ, Ofori S, Maduka O Palm oil and the heart: A review. *World J Cardiol* 2015;7:144-9.
- 15 Fattore E, Bosetti C, Brighenti F, et al. Palm oil and blood lipid-related markers of cardiovascular disease: a systematic review and meta-analysis of dietary intervention trials. *Am J Clin Nutr* 2014;99:1331-50.
- 16 Bengmark S Nutrition of the critically ill - emphasis on liver and pancreas. *Hepatobiliary Surg Nutr* 2012;1:25-52.
- 17 Souganidis E, Lailou A, Leyvraz M, et al. A comparison of retinyl palmitate and red palm oil beta-carotene as strategies to address Vitamin A deficiency. *Nutrients* 2013;5:3257-71.
- 18 Ahsan H, Ahad A, Siddiqui WA A review of characterization of tocotrienols from plant oils and foods. *J Chem Biol* 2015;8:45-59.
- 19 De Silva L, Chuah LH, Meganathan P, et al. Tocotrienol and cancer metastasis. *Biofactors* 2016;42:149-62.
- 20 Meganathan P, Fu JY Biological Properties of Tocotrienols: Evidence in Human Studies. *Int J Mol Sci* 2016;17.
- 21 Chain) ECPEPoCitF Update of the risk assessment on 3-monochloropropane diol and its fatty acid esters. *EFSA Journal* 2018;16:15083, 48 pp. 2018.
- 22 Leigh J, MacMahon S Occurrence of 3-monochloropropanediol esters and glycidyl esters in commercial infant formulas in the United States. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 2017;34:356-70.

- 23 Chain) ECPEPoCitF Scientific opinion on the risks for human health related to the presence of 3- and 2monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. *EFSA Journal* 2016;14(5):4426, 159 pp. 2016.
- 24 EFSA panel on dietetic products naa Commission Regulation (2018/290) of 26 February 2018 amending Regulation (EC) No 1881/2006 as regards maximum levels of glycidyl fatty acid esters in vegetable oils and fats, infant formula, follow-on formula and foods for special medical purposes intended for infants and young children. *EFSA Journal* 2018;61, 64 pp. 2018.
- 25 Olafisoye OB, Oguntibeju OO, Osibote OA Trace elements and radionuclides in palm oil, soil, water, and leaves from oil palm plantations: A review. *Crit Rev Food Sci Nutr* 2017;57:1295-315.
- 26 Leite ME, Lasekan J, Baggs G, et al. Calcium and fat metabolic balance, and gastrointestinal tolerance in term infants fed milk-based formulas with and without palm olein and palm kernel oils: a randomized blinded crossover study. *BMC Pediatr* 2013;13:215.
- 27 Nelson SE, Frantz JA, Ziegler EE Absorption of fat and calcium by infants fed a milk-based formula containing palm olein. *J Am Coll Nutr* 1998;17:327-32.
- 28 Nelson SE, Rogers RR, Frantz JA, et al. Palm olein in infant formula: absorption of fat and minerals by normal infants. *Am J Clin Nutr* 1996;64:291-6.
- 29 Ostrom KM, Borschel MW, Westcott JE, et al. Lower calcium absorption in infants fed casein hydrolysate- and soy protein-based infant formulas containing palm olein versus formulas without palm olein. *J Am Coll Nutr* 2002;21:564-9.
- 30 Souza CO, Leite MEQ, Lasekan J, et al. Milk protein-based formulas containing different oils affect fatty acids balance in term infants: A randomized blinded crossover clinical trial. *Lipids Health Dis* 2017;16:78.

- 31 Alarcon PA, Tressler RL, Mulvaney A, et al. Gastrointestinal tolerance of a new infant milk formula in healthy babies: an international study conducted in 17 countries. *Nutrition* 2002;18:484-9.
- 32 Lloyd B, Halter RJ, Kuchan MJ, et al. Formula tolerance in postbreastfed and exclusively formula-fed infants. *Pediatrics* 1999;103:E7.
- 33 Borschel MW, Choe YS, Kajzer JA Growth of healthy term infants fed partially hydrolyzed whey-based infant formula: a randomized, blinded, controlled trial. *Clin Pediatr (Phila)* 2014;53:1375-82.
- 34 Borschel MW G-WS, Brabec BA, Ziegler EE, et al. Tolerance, Bone Mineral Content, and Serum Vitamin D Concentration of Term Infants Fed Partially Hydrolyzed Whey-based Infant Formula. *The Open Nutrition Journal* 2012;6:71-9.
- 35 Koo WW, Hammami M, Margeson DP, et al. Reduced bone mineralization in infants fed palm olein-containing formula: a randomized, double-blinded, prospective trial. *Pediatrics* 2003;111:1017-23.
- 36 Fuchs GJ, Farris RP, DeWier M, et al. Effect of dietary fat on cardiovascular risk factors in infancy. *Pediatrics* 1994;93:756-63.
- 37 Innis SM, Akrabawi SS, Diersen-Schade DA, et al. Visual acuity and blood lipids in term infants fed human milk or formulae. *Lipids* 1997;32:63-72.
- 38 Bar-Yoseph F, Lifshitz Y, Cohen T, et al. SN2-Palmitate Reduces Fatty Acid Excretion in Chinese Formula-fed Infants. *J Pediatr Gastroenterol Nutr* 2016;62:341-7.
- 39 Carnielli VP, Luijendijk IH, van Beek RH, et al. Effect of dietary triacylglycerol fatty acid positional distribution on plasma lipid classes and their fatty acid composition in preterm infants. *Am J Clin Nutr* 1995;62:776-81.

- 40 Carnielli VP, Luijendijk IH, van Goudoever JB, et al. Feeding premature newborn infants palmitic acid in amounts and stereoisomeric position similar to that of human milk: effects on fat and mineral balance. *Am J Clin Nutr* 1995;61:1037-42.
- 41 Carnielli VP, Luijendijk IH, Van Goudoever JB, et al. Structural position and amount of palmitic acid in infant formulas: effects on fat, fatty acid, and mineral balance. *J Pediatr Gastroenterol Nutr* 1996;23:553-60.
- 42 Lambidou M AB, Jochum F, Nomayo A, Stehle P. Effect of high beta-palmitate infant formula supplemented with galacto-oligosaccharides on stool fatty acid soaps. *Journal of pediatric gastroenterology and nutrition*. Conference: 49th annual meeting of the european society for paediatric gastroenterology, hepatology and nutrition, ESPGHAN 2016. Athens Greece. 2016;62:879.
- 43 Lopez-Lopez A, Castellote-Bargallo AI, Campoy-Folgoso C, et al. The influence of dietary palmitic acid triacylglyceride position on the fatty acid, calcium and magnesium contents of at term newborn faeces. *Early Hum Dev* 2001;65 Suppl:S83-94.
- 44 Lucas A, Quinlan P, Abrams S, et al. Randomised controlled trial of a synthetic triglyceride milk formula for preterm infants. *Arch Dis Child Fetal Neonatal Ed* 1997;77:F178-84.
- 45 Yaron S, Shachar D, Abramas L, et al. Effect of high beta-palmitate content in infant formula on the intestinal microbiota of term infants. *J Pediatr Gastroenterol Nutr* 2013;56:376-81.
- 46 Litmanovitz I, Bar-Yoseph F, Lifshitz Y, et al. Reduced crying in term infants fed high beta-palmitate formula: a double-blind randomized clinical trial. *BMC Pediatr* 2014;14:152.

- 47 Savino F, Cresi F, Maccario S, et al. "Minor" feeding problems during the first months of life: effect of a partially hydrolysed milk formula containing fructo- and galacto-oligosaccharides. *Acta Paediatr Suppl* 2003;91:86-90.
- 48 Savino F, Palumeri E, Castagno E, et al. Reduction of crying episodes owing to infantile colic: A randomized controlled study on the efficacy of a new infant formula. *Eur J Clin Nutr* 2006;60:1304-10.
- 49 Bongers ME, de Lorijn F, Reitsma JB, et al. The clinical effect of a new infant formula in term infants with constipation: a double-blind, randomized cross-over trial. *Nutr J* 2007;6:8.
- 50 Nowacki J, Lee HC, Lien R, et al. Stool fatty acid soaps, stool consistency and gastrointestinal tolerance in term infants fed infant formulas containing high sn-2 palmitate with or without oligofructose: a double-blind, randomized clinical trial. *Nutr J* 2014;13:105.
- 51 Yao M, Lien EL, Capeding MR, et al. Effects of term infant formulas containing high sn-2 palmitate with and without oligofructose on stool composition, stool characteristics, and bifidogenicity. *J Pediatr Gastroenterol Nutr* 2014;59:440-8.
- 52 Civardi E, Garofoli F, Longo S, et al. Safety, growth, and support to healthy gut microbiota by an infant formula enriched with functional compounds. *Clin Nutr* 2017;36:238-45.
- 53 Fewtrell MS, Kennedy K, Murgatroyd PR, et al. Breast-feeding and formula feeding in healthy term infants and bone health at age 10 years. *Br J Nutr* 2013;110:1061-7.
- 54 Kennedy K, Fewtrell MS, Morley R, et al. Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effects on stool biochemistry, stool characteristics, and bone mineralization. *Am J Clin Nutr* 1999;70:920-7.

- 55 Litmanovitz I, Davidson K, Eliakim A, et al. High Beta-palmitate formula and bone strength in term infants: a randomized, double-blind, controlled trial. *Calcif Tissue Int* 2013;92:35-41.
- 56 Schmelzle H, Wirth S, Skopnik H, et al. Randomized double-blind study of the nutritional efficacy and bifidogenicity of a new infant formula containing partially hydrolyzed protein, a high beta-palmitic acid level, and nondigestible oligosaccharides. *J Pediatr Gastroenterol Nutr* 2003;36:343-51.
- 57 Nelson CM, Innis SM. Plasma lipoprotein fatty acids are altered by the positional distribution of fatty acids in infant formula triacylglycerols and human milk. *Am J Clin Nutr* 1999;70:62-9.
- 58 Innis SM, Nelson CM. Dietary triacylglycerols rich in sn-2 palmitate alter post-prandial lipoprotein and unesterified fatty acids in term infants. *Prostaglandins Leukot Essent Fatty Acids* 2013;89:145-51.
- 59 de Fouw NJ, Kivits GA, Quinlan PT, et al. Absorption of isomeric, palmitic acid-containing triacylglycerols resembling human milk fat in the adult rat. *Lipids* 1994;29:765-70.
- 60 Innis SM, Dyer R, Quinlan P, et al. Palmitic acid is absorbed as sn-2 monopalmitin from milk and formula with rearranged triacylglycerols and results in increased plasma triglyceride sn-2 and cholesteryl ester palmitate in piglets. *J Nutr* 1995;125:73-81.
- 61 Lien EL, Boyle FG, Yuhas R, et al. The effect of triglyceride positional distribution on fatty acid absorption in rats. *J Pediatr Gastroenterol Nutr* 1997;25(2):167-74.
- 62 Tomarelli RM, Meyer BJ, Weaber JR, et al. Effect of positional distribution on the absorption of the fatty acids of human milk and infant formulas. *J Nutr* 1968;95:583-90.

- 63 Andersen AD, Ludvig SE, Damsgaard CT, et al. The effect of fatty acid positioning in dietary triacylglycerols and intake of long-chain n-3 polyunsaturated fatty acids on bone mineral accretion in growing piglets. *Prostaglandins Leukot Essent Fatty Acids* 2013;89:235-40.
- 64 Wan J, Hu S, Ni K, et al. Characterisation of Fecal Soap Fatty Acids, Calcium Contents, Bacterial Community and Short-Chain Fatty Acids in Sprague Dawley Rats Fed with Different sn-2 Palmitic Triacylglycerols Diets. *PLoS One* 2016;11:e0164894.
- 65 Innis SM, Dyer R, Nelson CM. Evidence that palmitic acid is absorbed as sn-2 monoacylglycerol from human milk by breast-fed infants. *Lipids* 1994;29:541-5.
- 66 Rings EH, Minich DM, Vonk RJ, et al. Functional development of fat absorption in term and preterm neonates strongly correlates with ability to absorb long-chain Fatty acids from intestinal lumen. *Pediatr Res* 2002;51:57-63.
- 67 Vandenplas Y, Alturaiki MA, Al-Qabandi W, et al. Middle East Consensus Statement on the Diagnosis and Management of Functional Gastrointestinal Disorders in <12 Months Old Infants. *Pediatr Gastroenterol Hepatol Nutr* 2016;19:153-61.
- 68 Quinlan PT, Lockton S, Irwin J, et al. The relationship between stool hardness and stool composition in breast- and formula-fed infants. *J Pediatr Gastroenterol Nutr* 1995;20:81-90.
- 69 Vandenplas Y, Salvatore S. Infant Formula with Partially Hydrolyzed Proteins in Functional Gastrointestinal Disorders. *Nestle Nutr Inst Workshop Ser* 2016;86:29-37.
- 70 Lasekan JB, Hustead DS, Masor M, et al. Impact of palm olein in infant formulas on stool consistency and frequency: a meta-analysis of randomized clinical trials. *Food Nutr Res* 2017;61:1330104.

- 71 Vandenplas Y, Benninga M, Broekaert I, et al. Functional gastro-intestinal disorder algorithms focus on early recognition, parental reassurance and nutritional strategies. *Acta Paediatr* 2016;105:244-52.
- 72 Devlin A, Innis SM, Wall K, et al. Effect of medium-chain triglycerides on calbindin-D9k expression in the intestine. *Lipids* 1996;31:547-9.
- 73 Koo WW, Bush AJ, Walters J, et al. Postnatal development of bone mineral status during infancy. *J Am Coll Nutr* 1998;17:65-70.
- 74 Koo WW, Walters J, Bush AJ, et al. Dual-energy X-ray absorptiometry studies of bone mineral status in newborn infants. *J Bone Miner Res* 1996;11:997-102.
- 75 Rigo J, Nyamugabo K, Picaud JC, et al. Reference values of body composition obtained by dual energy X-ray absorptiometry in preterm and term neonates. *J Pediatr Gastroenterol Nutr* 1998;27:184-90.
- 76 Clandinin MT, Larsen B, Van Aerde J. Comment on: Reduced bone mineralization in infants fed palm olein-containing formula: a randomized, double-blinded, prospective trial. *Pediatrics* 2004;114(3):899-900; author reply 899-900.
- 77 Jones G, Riley M, Dwyer T. Breastfeeding in early life and bone mass in prepubertal children: a longitudinal study. *Osteoporos Int* 2000;11:146-52.
- 78 Schanler RJ, Burns PA, Abrams SA, et al. Bone mineralization outcomes in human milk-fed preterm infants. *Pediatr Res* 1992;31:583-6.
- 79 Specker B. Nutrition influences bone development from infancy through toddler years. *J Nutr* 2004;134:691S-95S.
- 80 Specker BL, Beck A, Kalkwarf H, et al. Randomized trial of varying mineral intake on total body bone mineral accretion during the first year of life. *Pediatrics* 1997;99:E12.

- 81 Young RJ, Antonson DL, Ferguson PW, et al. Neonatal and infant feeding: effect on bone density at 4 years. *J Pediatr Gastroenterol Nutr* 2005;41:88-93.
- 82 Koo W. Re: neonatal and infant feeding: effect on bone density at 4 years. *J Pediatr Gastroenterol Nutr* 2005;41:681; author reply 81-2.
- 83 Koo WW, Hockman EM, Dow M. Palm olein in the fat blend of infant formulas: effect on the intestinal absorption of calcium and fat, and bone mineralization. *J Am Coll Nutr* 2006;25:117-22.
- 84 Zuccotti G, Viganò A, Cafarelli L, et al. Longitudinal changes of bone ultrasound measurements in healthy infants during the first year of life: influence of gender and type of feeding. *Calcif Tissue Int* 2011;89:312-7.
- 85 Yu ZB, Han SP, Zhu C, et al. [Effects of infant formula containing palm oil on the nutrient absorption and defecation in infants: a meta-analysis]. *Zhonghua Er Ke Za Zhi* 2009;47(12):904-10.
- 86 Bar-Maisels M, Gabet Y, Shamir R, et al. Beta Palmitate Improves Bone Length and Quality during Catch-Up Growth in Young Rats. *Nutrients* 2017;9(7).
- 87 Devlin AM, Innis SM, Shukin R, et al. Early diet influences hepatic hydroxymethyl glutaryl coenzyme A reductase and 7 α -hydroxylase mRNA but not low-density lipoprotein receptor mRNA during development. *Metabolism* 1998;47:20-6.
- 88 Innis SM, Quinlan P, Diersen-Schade D. Saturated fatty acid chain length and positional distribution in infant formula: effects on growth and plasma lipids and ketones in piglets. *Am J Clin Nutr* 1993;57:382-90.
- 89 Bar-Yoseph F, Lifshitz Y, Cohen T. Review of sn-2 palmitate oil implications for infant health. *Prostaglandins Leukot Essent Fatty Acids* 2013;89:139-43.
- 90 Havlicekova Z, Jesenak M, Banovcin P, et al. Beta-palmitate - a natural component of human milk in supplemental milk formulas. *Nutr J* 2016;15:28.

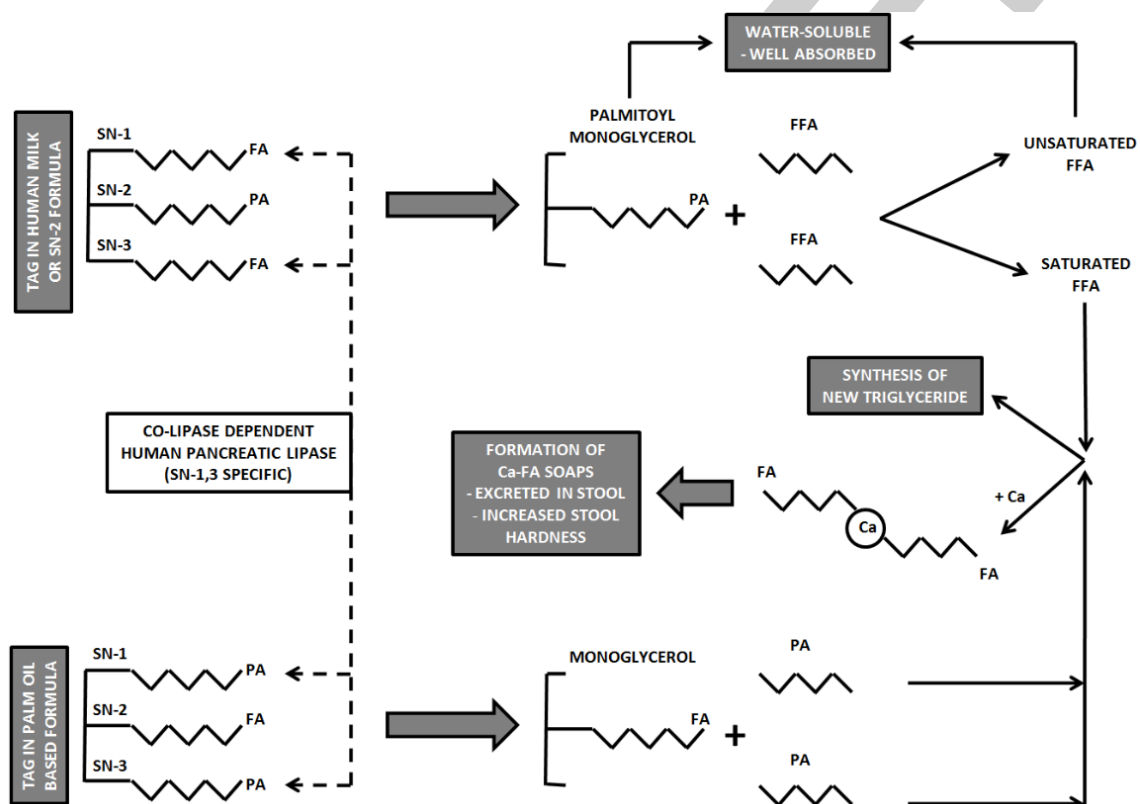
- 91 Hays NP, Mao M, Zhang L, et al. Infant feeding and health-related quality of life in healthy Chinese infants: results from a prospective, observational cohort study. *Health Qual Life Outcomes* 2016;14:116.
- 92 Alimentarius. C Standard for infant formula and formulas for special medical purposes intended for infants. Codex STAN 72–1981. Adopted as a worldwide Standard in 1981. Amendment: 1983, 1985, 1987, 2011, 2015 and 2016. Revision: 2007. 2016.
- 93 EFSA panel on dietetic products naa Scientific opinion on the essential composition of infant and follow-on formulae. *EFSA J* 2014;12:3760.
- 94 EFSA panel on dietetic products naa Scientific Opinion on the substantiation of a health claim related to beta-palmitate and increased calcium absorption pursuant to Article 14 of Regulation (EC) No 1924/2006. *EFSA J* 2011;9:2289–305.
- 95 EFSA panel on dietetic products naa Scientific Opinion on the substantiation of a health claim related to beta-palmitate and contribution to softening of stools pursuant to Article 14 of Regulation (EC) No 1924/2006. *EFSA J* 2014;12:3578–92.
- 96 Lu P, Bar-Yoseph F, Levi L, et al. High beta-palmitate fat controls the intestinal inflammatory response and limits intestinal damage in mucin Muc2 deficient mice. *PLoS One* 2013;8:e65878.
- 97 Carta G, Murru E, Lisai S, et al. Dietary triacylglycerols with palmitic acid in the sn-2 position modulate levels of N-acylethanolamides in rat tissues. *PLoS One* 2015;10:e0120424.

Figures

Figure 1 - Digestion and absorption of TAG and FA in human intestine

Legend: Colipase-dependent pancreatic lipase selectively hydrolyzes the FA at the SN-1 and 3 positions, yielding FFA and the 2-monoglyceride. Unsaturated FFA and monopalmitin are well absorbable. Saturated FFA (including PA) are involved in the re-synthesis of new TAG and/or formation of Ca^{2+} or Mg^{2+} soaps.

Abbreviations: FA = fatty acids; FFA = free fatty acids; PA = palmitic acid; Ca = calcium



Tables

Table 1a - List of studies evaluating formulas with PO/POL as source of fat

Table 1b - List of studies evaluating formulas with SN-2-palmitate as source of fat

Table
1a -
PO/POL
studies

Clinical outcome	First author, year	Ref. No.	Country	Study design	Sponsor	Type of sponsoring*	Study design	Population	Duration of intervention	Intervention	Comparison	Primary outcome	Secondary outcomes
Composition of stool (FA and calcium (Ca) content, intestinal microbiota)	Leite et al. 2013	26	Brazil	Blinded RCT, crossover	Abbott	E, G	Feeding two formulas for 14 days in a tolerance period, followed by a 4-day metabolic balance period in 17 of the male subjects.	33 healthy term infants (68-159 days of age)	14 days followed by a 4 day hospital metabolic balance study	Formula containing POL (44 % of total fat), PKO (21.7%) and canola oil (18.5%) as predominant fats (PALM)	Formula not containing POL, PKO or canola oil (NoPALM)	NoPALM group had higher Ca absorption (%) and retention (%) than PALM group, but absorption was not significant when Ca intake was used as a covariate. POL formula fed infants had lower fat and Ca absorption and higher fat and Ca excretion. The difference in percent fat absorption was explained by significantly (P <	NoPALM group versus PALM fed infants (p < 0.001). Formula and human milk intakes, growth, formula acceptability and adverse events were comparable between both groups.
	Nelson et al. 1998	27	USA	Blinded RCT, crossover	Abbott/Ross	S, G	Comparison of fat and Ca absorption of POL containing formula versus formula without POL. Fat and Ca levels in the two formulas were similar.	10 healthy infants (22-192 days of age)	72-96 hours	POL (45 % of fat) containing formula	Formula without POL	Stool consistency not determined in this study.	

Nelson et al. 1996	28	US	RCT, crossover	Abbott/Ross	S, G	Effect of POL-predominant formula on fat and Ca absorption. Half of infants admitted for 72-h metabolic studies and half of them performing stool collection at home.	11 term infants (27-161 days of age)	72-96 hours	Formula with mixture of 53 % POL and 47 % soy oil	Formula with mixture of 60 % soy oil and 40 % coconut oil	0.05) lower % absorption of palmitic (16:0) and stearic (18:0) acids. Both fat and Ca was less well absorbed in infants using experimental formula, presumably because of the formation of insoluble Ca soaps of unabsorbed PA. The difference in excretion of fat was explained by the difference in excretion of PA. Ca and fat absorption was less in infants fed CHF with PO compared to CHF without PO, (p < 0.01), but fat and Ca intake did not differ between the two groups. For infants fed SPF, fat and Ca intake did not differ between the feeding groups. Mean Ca absorptio	Stool consistency not determined in this study. Secondary outcomes are reported only for the soy-protein study: Infants averaged one to two stools per day in both groups. Mean rank stool consistency was 3.4 +/- 0.2 for PO and 3.2 +/- 0.2 for no PO group. The percentage of stools that were formed was significantly greater when infants
Ostrom et al. 2002	29	US	Two blinded RCTs, crossover	Abbott	E, S, G	"Casein hydrolysate study" and "soy protein study" comparing fat and Ca absorption in infants fed either casein hydrolysate-based or soy protein-based infant formulas with or without POL.	22 healthy, full term infants	72 hours	Casein hydrolysate-based (CHF) or soy protein-based (SPF) infant formulas with POL	CHF or SPF infant formulas without POL		

ACCEPTED

Souza et al. 2017	3	Brazil	DB-RCT, crossover	Abbott E, G	Feeding two formulas for 14 days in a tolerance period, followed by a 4-day metabolic balance period in 17 of the male subjects.	33 healthy term infants (68-159 ± 3 days of age)	14 days followed by a 4 day hospital ward metabolic balance study	Formula containing POL (44 % of total fat), PKO (21.7%) and canola oil (18.5%) as predominant fats (PALM)	Formula not containing POL, PKO or canola oil (NoPALM)	n was also significantly less when infants were fed SPF with PO than when fed SPF without PO (P < 0.05). Fat absorption did not differ between the two SPFs. PO, as the predominant fat, is associated with significantly lower absorption of Ca from infant formulas in which Ca salts are the source of Ca.	were fed PO formula than no PO formula (57 % vs 28 %; p<0.05). Percent of feedings with spit-up and vomit did not differ significantly between groups.	The absorption percentage of palmitic acid (C16:0) did not differ significantly, but this acid was excreted at significantly higher concentrations in the PALM (29.42 mg/kg/day) than in the NoPALM (12.28 mg/kg/day). Adverse events did not differ between groups (p > 0.05). Stool consistency not determined in this study.
-------------------	---	--------	-------------------	-------------	--	--	---	---	--	---	--	---

Stool frequency and consistency	Alarcon et al. 2002	3	US	1	A	Observational multicentre controlled study	Abbott E, G	Assessment of gastrointestinal tolerance (including stool frequency and consistency) of a new infant milk formula in healthy term infants (28-98 days of age)	6999 healthy term infants	2 weeks	Non-POL formula	Formula containing 45% of POL; Other formula; Human milk	Based on subanalysis of results: Less frequent stools and harder stool consistency (both $P < 0.001$) in infants fed formula containing 45% of POL when compared to non-POL formula	There were no statistically significant differences between Non-POL and POL-formula group for the incidence of GI intolerance indicators. Regurgitation not analyzed according to POL vs Non-POL.
	Lloyd et al. 1999 - part 1	3	US	2	A	Unblinded RCT	Abbott/Ross E	To compare the tolerance of two commercially available powder infant formulas that differ in composition. Measures of tolerance in exclusively breastfed infants weaned to an infant formula were evaluated.	82 healthy term breastfed infants	2 weeks	Cow milk-based formula with a whey:casein ratio of 60:40 and a fat blend of 45% palm olein, 20% soy, 20% coconut, and 15% high-oleic sunflower oils	Cow milk-based formula with a whey:casein ratio of 48:52 and a fat blend of 42% higholeic safflower, 30% coconut, and 28% soy oils; contained nucleotides	Healthy term breastfed infants weaned to POL-based formula had less frequent stools, fewer brown stools and more yellow stools, and firmer stools than did infants fed control formula without POL.	No significant differences in weight gain, spit-up or vomit between feeding groups.
	Lloyd et al. 1999 - part 2	3	US	2	A	Unblinded RCT	Abbott/Ross E	To compare the tolerance of two commercially available powder infant formulas that differ in composition. Measures of tolerance	87 healthy term formula-fed infants	2 weeks	Cow milk-based formula with a whey:casein ratio of 60:40 and a fat blend of 45% palm olein, 20% soy, 20% coconut, and 15% high-oleic sunflower oils	Cow milk-based formula with a whey:casein ratio of 48:52 and a fat blend of 42% higholeic safflower, 30% coconut, and 28% soy oils; contained nucleotides	Healthy term formula-fed infants randomized to POL-based formula experienced significantly firmer stools than controls.	No significant differences in weight gain, spit-up or vomit between feeding groups.

e in
exclusiv
ely
formula-
fed
infants
were
evaluate
d.

Bone health and growth	Borschel et al. 2014	3 3	US A	Blinded multicentre RCT	Abbott E, G	Effect of partially hydrolyzed whey-based infant formula with and without POL on growth of healthy term infants	209 healthy term infants	4 months	100% partially hydrolyzed whey formula containing 41% high-oleic safflower oil; 27% coconut oil; 29% soy oil; 1.5% mono- and diglycerides; 0.4% ARA, 0.15% DHA	100% partially hydrolyzed whey formula containing 46% POL; 26% soy oil; 20% coconut oil; 6% high-oleic safflower or high-oleic sunflower oil; 0.64% ARA; 0.32% DHA	There were no significant differences between groups in weight, length, HC, or weight, length or HC gains.	Infants fed PO-free pHF had significantly softer stools than those fed the PO-pHF except at 4 months of age. No statistically significant differences in the number of stools per day between groups during the study. Throughout the study, infants fed PO-free formula had predominantly green stools. Infants fed the EF had softer stools compared to infants fed CF. Incidence of spit-up/vomiting associated with feeding did not differ between groups. No differences were observed in formula tolerance or anthropometric measurements.
	Borschel et al. 2012	3 4	US A	Multicentre DB-RCT	Abbott E, G	Effect of two study formulas on tolerance, BMC and serum vitamin D concentration in healthy term infants	74 term infants (0-8 days of age)	56 or 84 days	Partially hydrolyzed cow's milk whey protein + high-oleic-safflower, soy, and coconut oils (EF)	Partially hydrolyzed cow's milk whey protein + POL, soy, coconut, high-oleic-safflower and high-oleic-sunflower oil (CF). The CF had less Ca and phosphorus than the EF. The vitamin D3 content was similar in both formulas.	Infants fed the EF exhibited significantly greater serum 25-OH vitamin D levels at 2 months of age and significantly greater BMC (assessed by DEXA) at 3 months compared to infants fed CF.	

																				Bone mineral content (BMC) and bone mineral density (BMD) measured by dual-energy X-ray absorptiometry (DEXA) were not different at baseline but infants fed PMF had significantly lower BMC and BMD at 3 and 6 months than infants fed MF. Mean daily intakes of total fat, saturated fat, monounsaturated fat, and cholesterol as well as mean serum total cholesterol was significantly higher in the infants fed cow milk, whereas mean LDL and apo B were lower in the infants fed the follow-up formulas. Infants consuming the infant formula or whole cow milk demonstrated greater	There were no significant differences between both groups in weight, length, head circumference, or formula intake throughout the study.
Koo et al. 2003	3	US	DB-RCT	Abbott/Ross	E, G	Effect of POL containing formula on bone mineralization in infants	128 healthy term infants (0-2 weeks of age)	6 months	Formula containing PO/coconut/soy/high-oleic sunflower oils (45/20/20/15 % oil) (PMF)	Milk-based formula without PO containing high-oleic safflower/coconut/soy oils (40/30/30 % oil) (MF)											
Metabolic effects	Fuchs et al. 1994	3	US	DB-RCT	Carnation Nutritional Products	G	Effect of dietary fat on cardiovascular risk factors in infancy.	104 healthy infants (4-6 months of age)	Until 12 months of age	One of two nearly identical (except of minor differences in micronutrients) lower fat follow-up formulas (36 % kcal from a fat blend of POL, corn, and safflower oils)	Whole cow milk or a standard infant formula (48 % to 49 % of energy as fat from butterfat or a soy-coconut oil blend, respectively)										Ponderal, linear, and head circumference growth was equivalent among feeding groups.

ACCEPTED

Innis et al. 1997	37	Canada	Multicentre RCT	Mead Johnson	E, G	Effect of infant formula containing POL on phospholipid and lipid profile, growth and visual acuity in term infants	172 term infants (0-14 days of age)	3 months	Formula containing POL, high oleic sunflower, coconut and soy oil (22.2% 16:0, 36.2% 18:1, 18% 18:2n-6, 1.9% 18:3n-3)	Formula containing coconut and soy oil (10.3% 16:0 18:6, 18:1, 34.2% 18:2n-6, 4.7% 18:3n-3); Breast-fed group	increases in mean serum total cholesterol, LDL, and apo B by 12 months of age compared with infants ingesting follow-up formula.	Plasma and red-blood-cell phospholipid DHA, AA, cholesterol and apolipoprotein B (apo B) were significantly lower in the formula- than breast-fed infants. There were no differences in visual acuity among the breast-fed and formula-fed infants. No significant relations were found between DHA and visual acuity, or AA and growth within or among any of the infant groups. The only difference were found at 1,2 or 3 months of age.
-------------------	----	--------	-----------------	--------------	------	---	-------------------------------------	----------	---	---	--	---

was lower TAG level in non-POL group at day 90 (p=0.014), but there was no difference in TAG levels when compared to breast milk group in any of the formula groups.

* Type of sponsoring: E = one of co-authors of the study was employee of the industry; S = study product supplied by industry; G = study supported by grant from/sponsored by industry; U = unclear role of sponsor

Table 1b - SN-2-palmitate (beta-palmitate) studies

Clinical outcome	First author, year	Ref. No.	Country	Study design	Sponsor	Type of sponsoring*	Study aim	Population	Duration of intervention	Intervention	Comparison	Primary outcome	Secondary outcomes
Composition of stool (FA and calcium (Ca) content, Bar-intestinal microbiota)	Bar-Yoseph et al.	38	China	Blinded RCT	Enzymotec	E, G	Effect of SN-2 palmitate on FA excretion in term formula-fed infants	171 term infants up to 14 days of age	6 weeks	Infant formula in which 43% of the PA was esterified to the SN2 position (INFAT®)	Infant formula containing a standard vegetable oil mixture (13% of the PA at SN-2 position); Breast-fed group	Significantly lower stool dry weight, fat and PA stool content in the SN2-palmitate formula-fed group compared to the control formula-fed group. Breast-fed infants had a	No significant differences were observed in any of the anthropometric measurements at baseline or at any visit during the study.

Carnie Ili et al. 1995 - subanalysis of study Carnie Ili et al. 1995 (ref. 40)	3	Netherlands	Blinded RCT, crossover	Nutricia	G	Effect of dietary TAG - FA positional distribution on plasma lipid classes and their FA composition in preterm infants.	7	preterm infants	1	week	Formula containing 76.1 % of PA at the SN-2 position (Betapol®)	Formula containing 87.3 % of PA at the SN-1,3 positions	significantly lower stool dry weight, fat content, and saponified fat excretion compared with formula-fed infants. Infants fed with SN-2-palmitate formula had higher percent ages of PA in plasma sterol esters, TAG, and FFA, and lower linoleic acid in TAG than control formula. SN-2-predominant TAG was associated with an improvement in the absorption of myristic, palmitic, and stearic acids and of mineral balance.	Plasma differences were consistent with enhanced absorption of PA from the SN-2 compared with the SN-1,3 positions. Fecal output was not significantly different by treatment. No differences were found in urine production or mean intestinal transit time.
Carnie Ili et al. 1995	4	Netherlands	Blinded RCT, crossover	Nutricia	G	Effect of SN-2 predominant formula on fat and mineral balance	12	preterm (28-32 week of gestation) infants at a postnatal age of 38 ± 7 days	2	weeks	Formula with PA esterified mainly at SN-2 TAG position (Betapol®)	Formula with PA esterified mainly at SN-1,3 position of TAG		

Carnie lli et al. 1996	4 1	Nethe lands	Blinded RCT	Nutricia G	Effect of SN-2 predomi nant formula on fat, fatty acid, and mineral balance	27 healthy term male infants (0-5 weeks of age)	5 weeks	Formula beta (24% palmitic acid, 66% esterified to β - position)(Betapol®)	Formula intermedi ate (24% palmitic acid, 39% esterified to the β - position) and regular formula (20% palmitic acid; 13% esterified to the β - position)	Fat absorpti on was highest in infants fed the beta formula , interme diate in those fed with the interme diate formula , and lowest in infants receivin g the regular formula . Fecal Ca excretio n was significa ntly lower in the beta group than in the other two groups. Breast- fed infants showed lower fecal excretio n of FA soaps and total FA than infants fed with regular infant formula . Howe ver, increasi ng SN-2 palmitate in infant formula did not reduce stool total FA soaps,	Infants fed the beta formula produce d a smaller amount of feces than regular formula group. Consiste ncy and the color of the feces was significan tly different among the groups. In the beta group, 2 infants had soft feces, 6 had runnysoft feces, and none had hard stools. All had yellow feces. The results for the safety paramet ers body weight and head circumfe rence showed no differenc es among the groups.
Lamb dou et al. 2016	4 2	Germa ny	RCT	No industry sponsor declared U	Fecal excretio n of FA soaps and total FA in infants fed with regular infant formula, SN-2 palmitate enriched formula and breastfe d infants	40 term infants	6 weeks	Infant formula with increased SN-2 palmitate	Breastfed infants; Infants fed regular formula		

												palmitate soaps and total FA.
												Feeding with SN-2-predominant formula reduced significantly the contents of total FA and PA in faeces. Faecal calcium in
												Groups A and C had diminished at 1 month whereas in Group B, it remained virtually unchanged. No differences between diet
												SN-2 palmitate predominant formula improved palmitate absorption, reduced the formation of insoluble Ca soaps in the stool and improved Ca absorption.
												The anthropometric parameters were not significantly different between the three groups throughout the study. No differences between diet groups were found for steady state gains in weight, length, or head circumference during the whole period on the assigned diets. The infant's sex was found to be unrelated to PA
Lopez - Lopez et al. 2001	4 3	Spain	Blinded RCT	Fundació Bosch Gimpera, Laboratoris Ordesa, CeRTA	G	The influence of dietary PA-TAG position on the FA, Ca and Mg contents of at term newborn faeces	36 term infants	2 months	Group B - formula "a" (19 % PA in SN-2-position) for 2 months and Group C - formula "a" during the first month and with formula "b" (44.5 % PA in SN-2-position) during the second month (Betapol®)	Group A - human milk (66 % PA in SN-2-position)		
Lucas et al. 1997	4 4	UK	Blinded RCT	Wyeth/Unilever	E, S	Effect of infant formula containing synthetic structure d TAG on palmitate, total fat and Ca absorption and Ca soap formation in the gut	24 preterm infants (less than 1500 g and less than 35 weeks of gestation)	3 weeks	Formula containing 74 % of SN-2 palmitate (Betapol®)	Two comparisons on diets (8.4 % and 28 % SN-2 palmitate, respectively)		

Author	Year	Country	Design	Intervention	Comparator	Population	Duration	Outcomes			
Yaron et al.	2013	Israel	RCT	Enzymotic	E, G	Effect of high SN-2-palmitate infant formula on the intestinal microbiota of term infants	36 term infants	6 weeks	High SN-2-palmitate formula (HBP group; 44% beta-palmitate from structured PO) (INFAT®) not containing pre- or probiotics	Breast-fed (BF group) and low SN-2-palmitate (LBP group, 14% beta-palmitate, based on standard unmodified PO; N=8) formula not containing pre- or probiotics	absorption, other individual or total FA absorption, soap excretion or fractional Ca absorption. At 6 weeks, no significant differences in weight and length were observed between the 2 formula-fed groups; however, the head circumference was lower in the HBP group than in the BF group (P < 0.01). The Lactobacillus counts at 6 weeks were not significantly different between the HBP and BF groups. The percent age of crying infants in the LBP group was significantly higher than in
Litmanovitz et al.	2014	Israel	DB-RCT	Enzymotic	E	Effect of high beta-palmitate formula on crying in term infants	63 healthy term infants	12 weeks	Formula with high SN-2-palmitate (HBP) (INFAT®)	Regular formula with a standard vegetable oil mix (LBP); Breastfed infants (BF)	The BF infants had significant

Savino et al. 2003	47	Italy	Observational prospective multicentre uncontrolled study	Unknown	U	Effect of high SN-2-palmitate formula on minor feeding problems	604 infants with minor gastrointestinal problems fed up to 90 days of age by study formula	2 weeks	Formula containing fructo- and galacto-oligosaccharides, partially hydrolysed proteins, low levels of lactose, high SN-2-palmitate and higher density	None	Reduction in frequency of colic (p < 0.005) occurred.	the HBP and BF groups both at weeks 6 and 12 (both P < 0.05). The infants fed HBP had significantly shorter crying durations when compared to LBP group (P = 0.047).
Savino et al. 2006	48	Italy	Single-blinded RCT	Numico	G	Effect of partially hydrolysed formula, with high SN-2-palmitate content and a mixture of galacto and fructo-oligosaccharides	267 infants below 4 months of age	2 weeks	Partially hydrolysed formula, with high SN-2-palmitate content and a mixture of galacto and fructo-oligosaccharides	Standard formula and simethicone	Reduction of crying episodes (both after 1 and 2 weeks) in infants fed experimental formula when compared to standard formula	tly higher stool frequencies and softer stools than the infants in both the formula groups at both 6 and 12 weeks postnatal. At 12 weeks, a significant reduction in hard stools was observed for the HBP group but not the LBP group. Reduction in frequency of regurgitation (p < 0.005) and increase in the daily number of stools in constipated children (p < 0.005) occurred.

Stool frequency and consistency	Bongers et al. 2007	49	Netherlands	DB-RCT, crossover	Nutricia G	Evaluation of a new infant formula in term infants with constipation	38 constipated term infants (3-20 weeks of age)	3 weeks + crossover follow-up	Formula containing high concentration of SN-2 palmitate (41%), a mixture of prebiotic oligosaccharides and partially hydrolyzed whey protein	Whey-based control formula (11.5% SN-2 palmitate) partly mixed with a formula based on hydrolyzed whey protein	Through out the study there were no serious adverse effects in either group. Both formulas were well tolerated. Weight gain was similar in both feeding groups.
reduction of crying episodes related to infantile colic										and simethicone (P < 0.0001).	Feeding with formula containing high concentration of SN-2-palmitate significantly increased defecation frequency (but there was no difference to standard formula (SF)) and had a trend to softer stool consistency when compared to SF. No difference was found in painful defecation or the presence of an abdominal or rectal mass between the two groups.

ACCEPTED

<p>Nowacki et al. 2014</p>	<p>5 0</p>	<p>USA</p>	<p>DB-RCT</p>	<p>Nestlé</p>	<p>E, G</p>	<p>Effect of infant formulas containing high SN-2 palmitate with or without oligofructose on stool fatty acid soaps, stool consistency and gastrointestinal tolerance in term infants</p>	<p>165 healthy term infants (25-45 days of age)</p>	<p>4 weeks</p>	<p>Formula containing high SN-2 palmitate plus 3 g/L of prebiotic oligofructose (SN-2+OF)</p>	<p>Control formula (CF); Formula containing high SN-2 palmitate (SN-2); Human milk-fed group (HM)</p>	<p>Stool consistency score at day 28 of the SN-2+OF group was lower than CF and SN-2 (P < 0.0001), but higher than the HM-fed group (P < 0.0001), moreover, SN-2 group was not different from CF. SN-2 group had lower stool palmitate soaps compared to CF (P = 0.0028) and SN-2+OF group had reduced stool palmitate soaps, total soaps and Ca compared to both CF and SN-2 (all P < 0.0001). The HM-fed group had lower stool palmitate soaps and Ca (all P < 0.0001). The parent assessment of GI tolerance was similar across all groups and the GI burden of each of the study feedings was low. All FF infants had urine osmolality and specific gravity values within the normal range.</p>
----------------------------	----------------	------------	---------------	---------------	-------------	---	---	----------------	---	---	---

ACCEPTED

Yao et al. 2014	5 1	USA	DB-RCT	Nestlé/ Wyeth	E, G	Effects of high SN-2 palmitate infant formulas with and without oligofructose on stool composition, stool characteristics, and bifidogenicity	300 healthy term infants (7-14 days of age)	8 weeks	High SN-2 palmitate formula (40% beta-palmitate) (Betapol®); SN-2+3 g/L OF—a high SN-2 palmitate formula supplemented with 3.0 g/L oligofructose (OF); SN-2+5 g/L OF—a high SN-2 palmitate formula supplemented with 5.0 g/L OF	Bovine milk-based term formula with a 100% vegetable fat blend; Human milk-fed group (HM)	for each comparison) than all formula-fed groups. At week 8 the SN-2 group had 46% less stool soap palmitate (P < 0.001) and softer stools than controls. At weeks 4 and 8, no differences were observed in GI tolerance among any of the feeding groups after adjusting for baseline scores. Mean z scores for weight-for-age, length-for-age, head circumference-for-age, and weight-length were significantly higher fecal
-----------------	--------	-----	--------	------------------	------	---	---	---------	---	---	--

Bone health and growth	Civardi et al. 2017	5	Italy	DB-RCT	Heinz Italia S.p.A.	E, S, G	Effect of infant formula enriched with functional compounds on safety, growth, and support to healthy gut microbiota	51 term neonates	135 days	Formula enriched with galactooligosaccharides (7 g/L), SN-2-palmitate (PA was 60 % of total FA, whose 39 % were esterified at the SN-2 position) and acidified milk (representing 50% of the whole milk in the formula)	Standard formula (SF) based on vegetable oils (including soya oil) and whey protein (enriched in alphalactalbumin)	Standard formula containing 12 % SN-2 palmitate . Both formulas contained similar salts, including Ca salts. The control formula had slightly less fat (39 vs 42 g/L); Breast-fed group	Infants fed the experimental formula had higher increase of bifidobacteria faecal counts. Gastrointestinal adverse effects, intestinal gas, bowel cramps and the mean number of stools per day were comparable between the 2 groups. The control formula subjects had significantly higher weight SDS than the breast-fed subjects, with intermediate values for the high SN-2 group. There were no significant differences in	bifidobacteria concentrations than controls at week 8, not differing from HM-fed infants.	Infants fed the experimental formula had higher increase of bifidobacteria faecal counts. Gastrointestinal adverse effects, intestinal gas, bowel cramps and the mean number of stools per day were comparable between the 2 groups. The control formula subjects had significantly higher weight SDS than the breast-fed subjects, with intermediate values for the high SN-2 group. There were no significant differences in
Fewtrell et al. 2013 - unblinded follow-up of RCT by Kennedy et al. 1999 (ref. 54)		5	UK	Unblinded follow-up of RCT	MRC UK and EU; Original RCT (ref. 54) sponsored by Nutricia	G	Long-term effects of formula containing synthetic TAG on bone mineralization evaluated by DEXA	Traceable subjects from original cohort (57 formula-fed and breast-fed)	10 years	Formula containing 50 % SN-2 palmitate (Betapol®)					

ACCEPTED

<p>Kenny et al. 1999</p>	<p>5 4</p>	<p>UK</p>	<p>DB-RCT</p>	<p>Nutricia</p>	<p>S</p>	<p>Effects of formula containing synthetic TAG on stool biochemistry, stool characteristics, and bone mineralization</p>	<p>323 healthy term neonates</p>	<p>3 months</p>	<p>Formula containing 50 % SN-2 palmitate (Betapol®)</p>	<p>Standard formula containing 12 % SN-2 palmitate. Both formulas contained similar salts, including Ca salts. The control formula had slightly less fat (39 vs 42 g/L); Breast fed group</p>	<p>Infants fed formula containing 50 % SN-2 palmitate had higher (but similar as breast-fed infants) whole-body BMC, evaluated by DEXA, softer stools at 6 and 12 wk, and a lower proportion of stool soap FA than infants fed standard formula.</p>	<p>es between the groups in the number of children who reported a previous fracture, nor in estimated current daily Ca intake. The stools of infants receiving the high-SN-2 formula contained less total FA (P=0.013), however there were no significant differences for the nonsoap FA. A greater proportion of the mothers using the high-SN-2 formula were concerned about runny stools and reported more colic at the age of 3 wk. Duration of crying was not significantly different. Among the formula-fed infants, there</p>
--------------------------	----------------	-----------	---------------	-----------------	----------	--	----------------------------------	-----------------	--	---	--	--

ACCEPTED

Litmanovitz et al. 2013	5	Israel	DB-RCT	Enzymotic	E	Effect of high beta-palmitate formula on bone strength in term infants	83	term infants	3 months	High SN-2 palmitate formula (43 % of PA on SN-2 position; HBP group) (INFAT®) based on standard vegetable oil mix of PKO, rapeseed oil, sunflower oil, and PO or structured PO	Regular formula (14 % of PA on SN-2 position; LBP group) based on standard vegetable oil mix of PKO, rapeseed oil, sunflower oil, and PO or structured PO; Breast-fed group	There were no significant differences in anthropometry throughout the study. There were no significant differences in respiratory tract infections, visits to the family doctor, hospital outpatient visits, or hospital admissions. Anthropometric data during study visits showed no significant differences between the two formula groups. There was a twofold difference in maternal smoking between the two formula groups (not statistically significant) - in further analysis not found to affect the change in bone SOS.
-------------------------	---	--------	--------	-----------	---	--	----	--------------	----------	--	---	--

Author	Year	Country	Study Design	Sponsor	Blinding	Population	Intervention	Comparison	Outcomes
Schmelzle et al. 2003	5/6	Germany	DB-RCT	Numico	E, G	154 term infants (0-2 weeks of age)	Infant formula (NF) containing partially hydrolyzed whey protein, modified vegetable oil with a high (41%) SN-2-palmitate content, prebiotic oligosaccharides, and starch	Standard formula (SF) not containing hydrolyzed protein or prebiotic oligosaccharides	During the first 6 weeks, NF girls gained more weight and head circumference than the SF girls. These velocity differences were not maintained through the 12-week study period. Infants fed EF, SF, or breast milk had 15.8%, 8.3%, and 28.0% 16:0 in the chylomicron triacylglycerol 2 position (P < 0.05). There were no significant differences in the weight, length, or HC between the 3 groups during the whole follow-up (120 days).
Nelson et al. 1999	5/7	Canada	RCT	Ross	S, G	87 full term infants	Experimental formula (EF) (fatty acid composition similar to SF, but made with synthesized TAG with 30% SN-2-palmitate) (Betapol®)	Formula containing 25-27% 16:0 with 29% 16:0 at the TAG	Effect of positional distribution of fatty acids in infant formula triacylglycerols on plasma lipoprotein fatty acids
Innis et al. 2013	5/8	Canada	RCT	No industry sponsor declared	U	Healthy infants (120 days of age)	Formula containing 25-27% 16:0 with 29% 16:0 at the TAG	Formula containing 16:0 with 5% 16:0 at the TAG	Effect of dietary TAG rich in SN-2 palmitate on

post-prandial lipoprotein and unesterified fatty acids in term infants

SN-2 position

TAG SN-2 position; Breastfed group

but higher n-6-PUFA and n-3-PUFA in the infant plasma, higher 18:0 in LDL TAG, and higher apo B and lower apolipoprotein A-1 (apo A-1).

* Type of sponsoring: E = one of co-authors of the study was employee of the industry; S = study product supplied by industry; G = study supported by grant from/sponsored by industry; U = unclear role of sponsor

ACCEPTED