

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).

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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 mimic 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.

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/unerwuenschte-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.

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

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-monglyceride. Unsaturated FFA and monopalmitin are well absorbable. Saturated FFA (including PA) are involved in the re-synthesis of new TAG and/or formation of Ca²⁺ or Mg²⁺ 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

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