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RESEARCH ARTICLE

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A Concept Formula With Large, Milk Phospholipid-Coated Lipid Droplets Enriched With Milk Fat Decreases Palmitic Acid and Calcium Levels in Stools of Healthy, Term Infants

Bert J. M. van de Heijning, Stefanie Schoen, Marieke Abrahamse-Berkeveld, Anita C. S. Hokken-Koelega, and Eline M. van der Beek*

This study evaluates the impact of an infant formula comprising large, dairy milk phospholipid-coated lipid droplets containing a vegetable-dairy lipid mixture (Concept-Mix) with 6% of fatty acid (FA) as *sn*-2 palmitic acid (PA) on infant stool characteristics. Stool samples from infants fed Concept-Mix or standard formula with 97% vegetable lipids with 2% of FA as *sn*-2 PA (Control-Veg) are evaluated at baseline, 13 and 52 weeks of age. Overall, mean stool fat content decreases from 8 to 10 w% at baseline to <2 w% at 52 weeks. At 13 weeks, PA and Calcium (Ca) content, as well as FA sum are lower in Concept-Mix than Control-Veg stools, with values closer to breastfed infants. The increased dietary *sn*-2 PA content is regarded as instrumental for this fecal PA reduction. Indeed, in their proof of principle study in rats fed formula-based diets using Control-Veg, Concept-Mix, or Concept formula with 95% vegetable lipids (Concept-Veg) comprising 2%, 6%, and 2% of FA as *sn*-2 PA, they demonstrate that PA absorption is the highest for Concept-Mix. Hence, a concept formula with large, milk phospholipid coated lipid droplets enriched with milk fat beneficially affects PA and Ca absorption in infants, likely due to its increased *sn*-2 PA content.

***Practical applications*: The results of these studies show the merit with regard to *sn*-2 palmitic acid (PA) and Calcium absorption of increasing the *sn*-2 PA content in the fat blend of infant formula by including dairy milk fat.**

1. Introduction

Exclusive human milk feeding is considered as the optimal form of nutrition for infants up to six months of age by the World Health Organization.[1] Fat provides up to half of total energy in human milk,[2] and dietary fat absorption is highly efficient (≈97%) already in six-week-old breastfed infants.[3]

In human milk, palmitic acid (PA) is the most abundant saturated fatty acid (22% of total fatty acids, FA), and ≈70% of the total PA is present at the *sn*-2 position of TG, with reported ranges between 51% and 88%.[4–6] However, most infant formulas are based on vegetable lipids and contain ≈18% PA, of which the vast majority (>85%) is located at the *sn*-1 or *sn*-3 position. During digestion, PA at the *sn*-1 or *sn*-3 position, unlike PA at the *sn*-2 position, is enzymatically hydrolyzed yielding free PA. Free PA can bind to calcium ions (Ca^{2+}) in the intestinal lumen forming insoluble Ca-soaps which cannot be absorbed and are excreted with the feces. The formation and fecal presence of Ca-soaps in infants fed with vegetable lipids-based formula low in *sn*-2 PA results in an increased stool consistency (i.e., to lead to "harder stools") compared to breastfed infants.[7,8] Although fat absorption is very efficient already early in infancy,[3] it has been shown that a higher PA content at the *sn*-2 position in formula positively impacts the PA and Ca absorption from the diet,[9,10] which consequently affects growth and bone development.

The introduction of cow's milk fat, in which ≈40% of total PA is naturally present at the *sn*-2 position, or the use of modified TG with increased *sn*-2 PA (≈39% to 44% of total PA), brought the FA structural composition of formula and, as a consequence, its functional properties closer to human milk.[11–14]

Lipids in human milk appear as fat globules with a volume-based mode diameter of 3–5 μm. These milk fat globules mainly consist of triglycerides (TG) in their core and are surrounded by a tri-layered milk fat globule membrane (MFGM) consisting of phospholipids and associated glycolipids, proteins, and cholesterol, which is formed during the synthesis and secretory process in the mammary gland.[15,16] In a standard infant formula the

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fat usually originates from vegetable oil blends and is present as lipid droplets with a mode diameter of ≈0.5 µm, i.e., much smaller than in human milk, and without a (natural) membrane coating.[17]

Inspired by human milk, we developed a new concept formula (Nuturis) comprising large lipid droplets (mode diameter of 3–5 µm) coated with dairy milk phospholipids, by adaptations to the manufacturing process and including dairy milk phospholipids and other MFGM components at human milk concentration levels in the formulation.[18] Compared to current standard formulas, the introduction of this concept brings formula closer to human milk regarding the lipid droplet size, composition and coating. It is postulated that the introduction of the large, dairy milk phospholipid-coated lipid droplets may also bring the physiological properties of the concept formula closer to those of human milk.

We previously demonstrated that the aforementioned concept formula with large, dairy phospholipid-coated lipid droplets containing vegetable and dairy lipids (Concept-Mix) is safe, well tolerated, and supports an adequate growth in healthy infants.[19] Moreover, we observed a lower stool consistency, i.e., softer stools, in infants consuming this Concept-Mix formula versus a standard formula (97% vegetable lipids, Control-Veg). Based on the findings described above, it was hypothesized that a Concept-Mix formula with a mixture of vegetable and dairy lipids, and therefore an increased *sn*-2 PA content will positively affect dietary fat and Ca absorption in infants of the aforementioned study. Furthermore, a positive association of the decreased PA and Ca stool content with a lower stool consistency was anticipated.[19] Next, to test the hypothesis raised and to disentangle the impact of fat source from lipid droplet characteristics (size and coating, i.e., the Nuturis concept), each of which may affect fat absorption, a fat balance study was conducted in adult rats, an animal model frequently used for fat digestion studies in general.[20]

2. Experimental Section

2.1. Clinical Study

A randomized, controlled, double-blind trial was conducted to investigate the effects of the new concept formula on growth, tolerance and safety in healthy term infants. The primary outcome parameter of the study was the daily weight gain (g d^{-1}) from enrollment until 17 weeks of age. Secondary outcome measures included length, head circumference, tolerance parameters, plasma parameters, and adverse events. The primary and secondary outcome measures of the study, along with details on the design and execution of the trial, i.e., the Mercurius study, were already published.[19] The current report focuses on exploratory parameters of the clinical study: the stool fat and Ca content and its FA composition. A post-intervention follow-up visit at week 52 was added to the initial protocol.

2.2. Participating Centers

The study was conducted in 17 study centers in four countries including the Netherlands (six centers: Erasmus University Medical Centre/Sophia Children's Hospital, Rotterdam; Albert Schweitzer Ziekenhuis, Dordrecht; Amphia Ziekenhuis, Breda; Isala Zwolle, Zwolle; Medisch Spectrum Twente, Enschede; Jeroen Bosch Ziekenhuis, Den Bosch), Belgium (seven centers: Algemeen Stedelijk Ziekenhuis, Aalst; Universitair Ziekenhuis, Brussels; AZ Sint Vincentius, Antwerpen; Clinique et Maternité Sainte-Elisabeth, Namur; Centre Hospitalier Régional de la Citadelle, Liège; Private Practice Dr Franckx, Mollem-Asse; Heilig Hart Ziekenhuis, Roeselare), France (three centers: CHU de Nantes, Nantes; CHU Angers Unité de Néonatologie, Angers; Hopital Nord-Ouest, Villefranche-sur-Sâone), and Singapore (KK Women's and Children's Hospital, Singapore). After approval was obtained by country, also approval of the independent local ethics review boards at all participating centers was obtained (details in Table S1, Supporting Information).

The study was conducted according to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)-good clinical practice (GCP) principles and in compliance with the principles of the Declaration of Helsinki and with the local laws and regulations of the countries where the study was performed. The study was registered in the Dutch Trial Register as NTR3683 (www.trialregister.nl).

2.3. Subjects and Study Design

Healthy term infants, with a gestational age between 37 and 42 weeks, a postnatal age up to 35 d, a birth weight between the 10th and 90th percentiles according to the Dutch Growth Charts,[21] a head circumference at enrollment within normal range for age and sex [within 2 SD according to WHO Growth Standard[22]], and either fully formula-fed or fully breastfed were eligible for participation. Exclusion criteria were defined as illnesses that could interfere with the study, special dietary needs, diagnosed maternal hepatitis B or HIV, participation in any other study, or investigator's uncertainty about the ability of the parents to comply with the protocol requirements. Written informed consent was obtained from all parents/guardians before enrollment. The study was designed as a randomized, double-blind, controlled, prospective, multicountry, (growth) equivalence trial, with a nutritional intervention until the infants age of 17 weeks and a post-intervention follow-up visit at the infants' age of 52 weeks.

Formulas were coded by the sponsor (Danone Nutricia Research) as letter codes A, B, C, and D; the investigators, the infants' parents, and the sponsor employees involved were blinded to the formulas. The randomization sequence was generated based on region (Europe/Asia), sex (male/female), and infants' age at randomization (≤14 d/>14 d) as strata (PLAN procedure in SAS statistical software; Enterprise Guide version 4.3) by a statistician from the sponsor not involved in the conduct of the study. The generated randomization sequence was uploaded in the eCRF (electronic case report form) and, after enrollment and input of subject data, formula-fed infants were randomly assigned by the system to receive one of the two formulas. Breastfed infants served as a reference group and were enrolled if the mother intended to (fully) breastfeed for at least 13 weeks and all other eligibility criteria were met. During the study, infants

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Table 1. Composition of the clinical study products.

per 100 mL milk/per 100 g powder[a)]	Control-Veg formula	Concept-Mix formula
Energy [kcal]	66/484	66/484
Fats [g]	3.4/24.7	3.4/24.7
vegetable lipids [g]	3.3/24.1	1.7/12.7
animal lipids [g]	0.1/0.5	1.6/12.0
Saturates; SFA [g]	1.5/10.9	1.4/10.6
16:0; PA [g]	0.58/4.26	0.57/4.16
sn-2 PA [g]	0.067/0.49	0.202/1.48
18:0; SA [g]	0.1/0.77	0.2/1.46
Monounsaturates; MUFA [g]	1.3/9.5	1.2/8.8
Polyunsaturates; PUFA [g]	0.6/4.2	0.6/4.1
18:2 *n*−6; LA [g]	0.45/3.29	0.45/3.28
18:3 *n*−3; ALA [g]	0.082/0.61	0.083/0.61
20:4 *n*−6; ARA [mg]	11/82	12/85
20:5 *n*−3; EPA [mg]	1.4/10	1.8/13
22:6 *n*−3; DHA [mg]	6.4/47	6.6/48
Dairy phospholipids [mg]	–	55/400
Soy phospholipids [mg]	4.5/33	–
Protein [g]	1.3/9.7	1.3/9.7
whey protein [g]	0.8/5.8	0.8/5.8
casein [g]	0.5/3.9	0.5/3.9
Carbohydrates [g]	7.3/53.9	7.3/53.9
lactose [g]	7/51.5	7/51.5
scGOS/lcFOS (9:1) [g]	0.8/5.88	0.8/5.88
Calcium [mg]	47/345	47/345

[a)] Formula with 13.6 g powder/100 mL prepared milk; scGOS; short-chain galacto-oligosaccharides; lcFOS; long-chain fructo-oligosaccharides.

were fully formula-fed or fully breastfed; only water, rehydration solutions, drops or syrups (vitamins, minerals, medicines) were allowed additionally.

The enrollment (baseline) visit was planned ≤35 d of age, followed by visits at 5, 8, 13, and 17 weeks of age, the end of the intervention, and a follow-up visit at age 52 weeks.

2.4. Study Products

The clinical study products (**Table 1**) were manufactured per good manufacturing practices (ISO 22000) and were compliant with EC Directive 2006/141 by Nutricia Cuijk B.V. (Cuijk, the Netherlands). The study products were iso-energetic, containing similar amounts of macronutrients and 0.8 g/100 mL of the specific prebiotics mixture of short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) in a 9:1 ratio.

The Control-Veg formula was a 97% vegetable lipids-based standard IF containing lipid droplets with a volume-based mode diameter of 0.5 μm and proteins as main emulsifiers. The Concept-Mix formula contained a mixture of vegetable (52%) and dairy lipids (48%), including dairy phospholipids, and its lipid droplets had a volume-based mode diameter of 3–5 μm and were coated predominantly by a monolayer of dairy phospholipids following an innovative production process[18] (Nuturis, patent EP2825062A1). For clarity, in the current paper the origin of the lipid source is included in the name of the intervention products, whereas these were originally described as "Control" and "Concept."[19]

The Control-Veg and Concept-Mix formulas contained equal amounts of fats (24.7 g/100 g powder) mainly present as TAG, and similar total levels of saturated (SFA), mono-unsaturated (MUFA), and poly-unsaturated fatty acids (PUFA; Table 1). The lipid moiety of both formulas comprised a fat blend of several vegetable oils (Control-Veg) or a blend of vegetable oils and cow's milk fat (Concept-Mix). Although different fat sources were used, the levels of linoleic acid (LA) and α-linolenic acid (ALA), total $n-3$ and $n-6$ levels, as well as their ratios were similar for both study products (LA/ALA ratio of 5.4 and $n-3/n-6$ ratio of ≈5). Total PA content in both intervention products was ≈0.58 g/100 mL (i.e., ≈18% of total FA), but the proportion of PA located at the *sn*-2 position of TG was threefold higher in Concept-Mix than in Control-Veg formula: 202 versus 67 mg/100 mL (i.e., 6 vs 2% of total FA and 36 vs 12% of total PA). No information is available on the *sn*-distribution over the glycerol backbone of TG for the other FA in the formulas. Hence, to conclude, the key differences between the study products were lipid sources used (differing in *sn*-2 PA content) next to size and coating of the lipid droplets.

2.5. Stool Characteristics

As part of the tolerance assessments, parents recorded in a diary the stool consistency of each stool passed in the week preceding the study visits at age of 5, 8, 13, and 17 weeks. No stool consistency was scored at baseline and at 52 weeks. Stool consistency was scored by the parents for each stool passed, on a four-point scale (1 = watery, 2 = soft, 3 = formed, 4 = hard) using pictures of the "Amsterdam" stool form scale with a proven excellent inter- and intra-observer reliability.[23]

Stool samples were collected by the parents at baseline during the intervention at 13 weeks of age, and at the post-intervention follow-up time point 52 weeks. The outcomes presented here restrict to the stool consistency scored for the visit at time point 13 weeks, and to the analyses of the stool samples collected at baseline, and at age 13 and 52 weeks. Stool samples were kept in the home freezer (−20 °C) until handed-in at the study site at the next visit and stored at −80 °C. Stool samples were analyzed at the University of Pécs, Hungary, for the following exploratory outcome parameters: total fatty acid content (i.e., the sum of all FA detected), and the FA composition with focus on PA and Ca content.

Stool FA content and composition were determined by capillary gas chromatography (GC) of FA methyl esters (FAME) formed. The preparatory method used was a slight modification of the method previously described.[24] In short, the methanol : toluene (4:1) solution used for lipid extraction was changed into a methanol:hexane (4:1) solution, and hexane instead of toluol solvent was used to dissolve the internal standard tridecanoic acid methyl ester. FAME were analyzed by a Thermo Trace 1310 GC equipment with a programmable temperature vaporizing (PTV) injector and a flame ionization detector (FID), using an Agilent

J&W DB-23 capillary column (60 m × 0.25 mm inner diameter with a 0.25 mm film thickness). The temperature program was as follows: an initial temperature of 50 °C for 0.1 min, followed by a temperature increase of 50 °C min^{-1} up to 173 °C, a 10 min isothermic period, a temperature increase of 25 °C min^{-1} up to 221 °C, an 8 min isothermic period, a temperature increase of 10 °C min^{-1} up to 250 °C, and a 24.62 min isothermic period. Peak identification was by comparison with authentic mixtures of weighed FAME coded GLC-463, 643, and 642 (NuChek Prep, Elysian, MN) and the Supelco 37 FAME Mix (Supelco, Bellefonte, PA). Stool FA contents were expressed as weight percent of total fatty acids (%FA).

Stool Ca content was analyzed by atomic absorption spectrophotometry (AAS, Varian) after freeze-drying of the sample and mineral acid digestion in a 2:1 mix of conc. HNO_3 and H_2SO_4 for 15 min at 100, 200, and 300 °C.

2.6. Preclinical Study

2.6.1. Animals and Housing

All experimental animal procedures were approved by an external, independent animal experimental ethics committee (DEC-Consult, Soest, the Netherlands) and complied with national legislation and the principles of good laboratory animal care following the EU-directive for the protection of animals used for scientific purposes.

Adult male Wistar rats (n = 10, 180–200 g, 7–8 weeks old) were obtained from an SPF facility (Harlan, Horst, the Netherlands) and were housed conventionally in the animal facility of IntraVacc (Bilthoven, The Netherlands) with a 12/12 L/D cycle (lights on at 07:00 h), and room conditions controlled for temperature and humidity. As data on individual diet intake and excretion were to be assessed, animals were housed individually in macrolon cages with wood shaving bedding (including cage enrichment). Rats were weighed and handled at least weekly and checked for general health (a.o. for soft feces or diarrhea); rats had ad libitum access to the semisynthetic AIN93-G-based feed (see below) and tap water.

2.6.2. Rat Diets

Because infant formula powder is not compliant to the nutritional requirements of rats, specific formula powder-based study diets were prepared as follows: formula powder (283 g powder kg^{-1} feed, containing 70 g fat) was supplemented with specific protein and carbohydrates to meet levels as present in the AIN93-G reference diet.[25,26] AIN93-G was chosen as it contains more fat (7 vs 4 w%) than the M-version, resulting in a higher, but still healthy fat intake. Importantly, the formula powder was the sole source of the fat moiety (7 w%) of the rodent diet (**Table 2**). The final diet obtained was not pelleted to preserve the lipid droplet structure, but was offered to the animals as daily freshly prepared dough balls (30 g diet powder) upon mixing with 6 g water (i.e., 16 w%). Fresh dough balls were provided daily on the cage floor and the remaining diet was removed from the cage the next day.

Following these preparative procedures three different formula-based rat diets were evaluated: a) a diet containing a standard formula with 97% vegetable lipids (Control-Veg diet), b) a diet containing the concept formula with a mix of 52% vegetable and 48% dairy lipids (Concept-Mix diet), and c) a diet containing the concept formula with 95% vegetable lipids (Concept-Veg diet). The latter diet was included in this experiment to allow the discrimination between the impact of lipid droplet structure and lipid sources used, because it was created based on an formula having the large, dairy milk phospholipid-coated lipid droplets like the Concept-Mix, but now comprising 95% vegetable lipids instead (Concept-Veg diet). The formula powder used for the rat diet a and b were identical to the Control-Veg and Concept-Mix formulas evaluated in the clinical study[19] as described in Table 1. As indicated above, dietary requirements of rats differ from infants, and specific adjustments were made to provide adequate nutrition to the animals during the study. Table 2 shows the fat sources used and FA composition of the three rat diets tested.

All formula-based diets contained the scGOS/lcFOS prebiotic mixture as about one-third of total fibers present (50 g kg^{-1}), in addition to a substantial amount of lactose (about 25 w% of total carbohydrates, ≈150 g kg^{-1}), which cannot be digested by adult animals and is hence treated as "fiber." Previously, it was demonstrated that rat diets with 30 w% lactose were well-tolerated by adult rats and did not cause abdominal discomfort or any effect on stool consistency, but resulted in a tripled cecum size and weight.[27]

2.6.3. Study Design and Outcomes

A cross-over, open study design was used for evaluation of the three formula-based rat diets. After a two-week acclimation period on AIN93-G pellets, animals consumed each study diet in random order (i.e., Latin square) for two weeks with one-week wash-out in-between on standard AIN93-G pellets (Figure S2A, Supporting Information). During the last 72-h of each two-week period, individual animals were placed in a clean cage and fat intake was assessed each 24 h by back-weighing the dough ball leftovers. Feces produced in this 72-h period were collected from the bedding, air-dried and weighed.

Fat content in the rat diets was checked for according to the Roese-Gottlieb method (AOAC Intl. Methods 905.02). FA composition of the diet and dried feces was analyzed at Danone Nutricia Research in lipid extracts[28] with samples being spiked prior to lipid extraction with 19:0 as internal standard to enable quantification, whereupon FAME were prepared and processed according to Morrison.[29] FAME were analyzed by gas chromatography[30,31] using a CP-SIL88 column 50 m × 0.25 mm inner diameter with a 0.22 µm film thickness. A flame ionization detector (FID) was used, and a chromatography protocol starting at 150 °C for 3.75 min, upon which the temperature was raised to 220 °C at 22 °C min-1, which was maintained for 14.07 min.

Absorption efficiency of individual FA and total fat was calculated from the balance of FA intake and excretion, and was expressed as the percentage of total FA intake. Fecal Ca-content was not analyzed in the animal study.

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Table 2. Composition of the formula-based rat diets.

(g kg^{-1} diet)	Control-Veg diet	Concept-Veg diet	Concept-Mix diet
Control-Veg formula (see Table 1)	283		
Concept-Veg formula[a)]		283	
Concept-Mix formula (see Table 1)			283
Lipid droplet modal diameter [µm]	0.5	3–5	3–5
Caseinate	150	150	150
Corn starch	290	290	290
Sucrose	100	100	100
Maltodextrin	100	100	100
Cellulose	32	32	32
AIN vitamin and mineral mix	45	45	45
Energy [kcal kg^{-1}]	3850	3850	3850
Proteins [w%/En%]	18/19	18/19	18/19
Carbohydrates [w%/En%]	63/64	63/64	63/64
Fats [w%/En%]	7/17	7/17	7/17
Fibers [w%/En%]	5/0	5/0	5/0

	g kg−1	w%	g kg−1	w%	g kg−1	w%
Total fat	69.9	6.99	69.9	6.99	69.9	6.99
animal fat	1.4	2.0	3.9	5.7	33.9	48.6
vegetable fat	68.2	97.6	65.9	94.3	35.9	51.4
dairy phospholipids	–	–	1.1	1.6	1.1	1.7
soy phospholipids	0.09	0.01	–	–	–	–
FA profile	g kg^{-1}	%FA	g kg^{-1}	%FA	g kg^{-1}	%FA
4:0	0.03	0.04	0.06	0.10	0.93	1.39
6:0	0.14	0.20	0.17	0.24	0.65	0.98
8:0	1.33	2.00	1.30	1.96	0.79	1.19
10:0	1.02	1.53	1.02	1.54	1.19	1.81
12:0	7.78	11.70	7.58	11.40	3.51	5.29
14:0	3.25	4.89	3.37	5.04	4.58	6.90
16:0 PA	12.06	18.10	12.25	18.40	11.78	17.7
16:1 (*n*−7)	0.11	0.19	0.14	0.23	0.62	0.95
18:0 SA	2.18	3.26	2.38	3.58	4.13	6.22
18:1 (*n*−9) OA	25.04	37.60	24.82	37.30	23.06	34.7
18:2 (*n*−6) LA	9.31	14.00	9.06	13.60	9.28	14.00
18:3 (*n*−3) ALA	1.71	2.58	1.69	2.53	1.72	2.59
20:0	0.23	0.34	0.23	0.34	0.19	0.31
20:1 (*n*−9)	0.31	0.49	0.31	0.47	0.28	0.45
20:3 (*n*−6)	0.03	0.03	0.03	0.04	0.03	0.04
20:4 (*n*−6) ARA	0.23	0.35	0.24	0.35	0.24	0.35
20:5 (*n*−3) EPA	0.03	0.04	0.04	0.06	0.04	0.06
22:0	0.17	0.27	0.17	0.26	0.19	0.31
22:1 (*n*−9)	0.08	0.11	0.08	0.11	0.06	0.10
22:5 (*n*−3)	0.01	0.01	0.03	0.03	0.03	0.03
22:6 (*n*−3) DHA	0.13	0.20	0.13	0.20	0.14	0.21
24:0	0.06	0.08	0.06	0.08	0.06	0.09
24:1 (*n*−9)	0.03	0.06	0.03	0.06	0.03	0.06
Other	1.19	1.80	1.33	2.00	1.18	1.78
sn-2 PA	1.4	2.1	1.4	2.1	4.2	6.3
FA summary	g kg^{-1}	%FA	g kg^{-1}	%FA	g kg^{-1}	%FA

(*Continued*)

Table 2. (Continued).

	g kg−1	w%	g kg−1	w%	g kg−1	w%
Saturates; SFA	30.85	44	31.41	45	30.00	45
Monounsaturates; MUFA	26.89	39	26.60	38	24.90	37
Polyunsaturates; PUFA	11.89	17	11.60	17	11.60	17
n−6:*n*−3 ratio	5.10	5.00	4.90			
LA:ALA ratio	5.43	5.38	5.40			

[a] Concept-Veg formula: 95% vegetable lipids in large, dairy phospholipid-coated lipid droplets, w%: weight percentage, En%: energy percentage.

2.6.4. Statistical Methods

In general, the clinical data are presented as medians (Q1; Q3), and the animal data as means ± SD. For all statistical tests performed $p < 0.05$ was considered statistically significant.

The primary outcome of the clinical study was the daily weight gain (g d^{-1}) from enrollment until 17 weeks of age. To demonstrate equivalence in daily mean weight gain between the randomized groups, a required sample size of 176 randomized infants (88 per group) was calculated for two one-sided statistical tests (with $\alpha = 0.05$, power = 0.80, and dropout/noncompliance rate = 20%). The a priori assumptions included a margin of equivalence of ≈3 g d^{-1}[32] and an equal within-group standard deviation (SD) of 6.0 g in weight gain in both the Control-Veg and Concept-Mix groups. The estimated difference in mean daily weight gain between the two groups was assumed to be zero.

Differences between Control-Veg and Concept-Mix groups for total FA, PA, and Ca stool content at baseline, 13 and 52 weeks were investigated using Wilcoxon rank sum test. No statistical comparisons to the breastfed reference group were performed. Subjects were included in the analyses when at least one stool sample was present at one of the time points. For the post hoc exploration of associations between stool PA content and stool consistency at week 13, as well as between Ca stool content and the stool consistency, a pooled data analyses in a subgroup was performed: subjects with both i) stool PA and/or stool Ca data collected at 13 weeks of age, and ii) diary data on stool consistency recorded for at least 3 d and within 15 d of the stool sample collection. As at the time the stool sample was collected at baseline or at 52 weeks of age no diary had to be completed, no associative analyses were performed for these time points. The association between either PA or Ca content and stool consistency was analyzed using the subjects' mean stool consistency score, calculated over all stools over all days within the diary at age 13 weeks, as dependent variable (in a linear regression model). Statistical analyses were conducted using SAS (SAS version 9.4_TS1M3 or higher in SAS Life Science Analytics Framework version 4.7.3 or higher) for LIN X64 (SAS Institute Inc., Cary, NC). No correction for multiple testing was applied.

The preclinical study was powered based on fat absorption data[33,34] using adult rats which showed a variance (σ) of about 5%. Two-sided testing with a relevant effect (μ) set at 5%, an α (type-I error) cut-off of 5% and a β (type-II error) cut-off of at 20% (i.e., a power of 80%) required ten animals, with each animal testing each diet (cross-over design). Data obtained in the fat balance tests were statistically evaluated using SPSS (v19.0) by multifactorial ANOVA with Tukey HSD ("honestly significant difference") post hoc testing for significance.

3. Results

3.1. Clinical Study

3.1.1. Subject Characteristics

From October 2012 to December 2013, 313 subjects were screened for eligibility, 223 subjects were randomized to one of the infant formulas (two subjects did not meet the eligibility criteria) and 88 infants were included in the breastfed reference group. The study flowchart (Figure S1, Supporting Information) shows 108 fully formula-fed infants were randomized to receive until 17 weeks of age Control-Veg formula and 115 received Concept-Mix formula. Eight randomly assigned infants (five in Control-Veg and three in Concept-Mix group) did not consume any study product and were excluded from the "all subjects treated" (AST) population. A total of 168 randomly assigned subjects completed the intervention period, of which 81 subjects were in the Control-Veg and 87 subjects in the Concept-Mix group, i.e., dropout rate was 25%. In total, 69 breastfed infants completed the study up to 17 weeks of age (22% dropout). The dropout rate (Control-Veg 25% compared with Concept-Mix 24%) and reasons for early termination were similar between both formula groups. The predominant reason for early termination in the formula groups was the occurrence of a (serious) adverse event (S)AE: 17 subjects in the Control-Veg group, of which one subject terminated early because of an SAE possibly related to the study product (cow's milk intolerance), and 24 subjects in the Concept-Mix group. Other reasons for termination were nonspecified (Control-Veg and Concept-Mix 1 subject each), protocol violation (Control-Veg 1 subject), or withdrawal of the informed consent (Control-Veg 1 subject).

Demographic data were not different between the formula groups for the "per-protocol" (PP) as well as "intention-to-treat" (ITT) populations (data not shown).

The number of infants for which the exploratory parameter "stool composition" was analyzed was 98 for the Control-Veg group, 101 for the Concept-Mix group, and 75 for the breastfed reference group (**Table 3**). The age of the infants at baseline stool sample collection was identical in the Concept-Mix and Control-Veg groups (9 d), and 27 d in the breastfed reference group (Table 3). Stool samples at 13 weeks were collected before study product intake stopped (99.3%) and before solid food was introduced

Table 3. Infant age at stool sample collection for FA and Ca analysis.

Infant age [weeks]	Concept-Mix $n = 101$	Control-Veg $n = 98$	Breastfed $n = 75$
At baseline			
n	93	82	63
median (Q1; Q3)	1.3 (0.6; 3.0)	1.4 (0.6; 2.9)	3.9 (1.6; 4.7)
At 13 weeks			
n	78	70	53
median (Q1; Q3)	12.4 (12.0; 13.0)	12.6 (12.1; 12.9)	12.6 (12.1; 12.9)
At 52 weeks			
n	53	46	43
median (Q1; Q3)	52.6 (51.7; 53.4)	52.6 (51.7; 53.9)	53.6 (52.1; 54.6)

Note: *n* = number of subjects of the analyzed population. Q1; Q3: the first and third quartile, i.e., the 25th and 75th percentile of the data.

(93.2%), indicating that at 13 weeks almost all infants were fully formula-fed.

3.1.2. Stool Parameters

Total stool FA content (w% in wet stool) decreased over time in all groups from baseline to 52 weeks (**Figure 1**, top panel). At baseline and 52 weeks, no differences between Concept-Mix and Control-Veg groups were found in total FA content. During the intervention period at 13 weeks of age the total FA detected in wet stool was statistically significantly different ($p < 0.001$) in Concept-Mix (median (Q1; Q3): 3.9 (2.8; 5.2) w% in wet stool) in comparison to Control-Veg (6.1 (4.6; 7.8) w% in wet stool), suggesting a lower total fat excretion in the Concept-Mix-fed infants. The stool fat content was apparently higher, though not statistically tested, in both formula groups than in the breastfed reference group.

Only at 13 weeks the PA stool content (as % of total FA) was statistically significantly different ($p < 0.001$) in Concept-Mix (median (Q1; Q3): 42.3 (40.5; 44.1) in comparison to Control-Veg group: 65.1 (62.5; 66.7), and closer to human milk (Figure 1, middle panel). The reverse held for SA (stearate, 18:0) levels in stools which were at week 13 higher in Concept-Mix (median (Q1; Q3): 25.9 (24.8; 28.0) than in Control-Veg group: 11.8 (11.2; 12.6); see Table S2 (Supporting Information).

During the intervention period at 13 weeks of age, Ca stool content was statistically significantly different ($p = 0.014$) in Concept-Mix (median (Q1; Q3): 0.9 (0.6; 1.2) mg/100 mg in comparison to Control-Veg group: 1.1 (0.7; 1.4) mg/100 mg, i.e., congruent with the changes observed in PA stool concentration (Figure 1, lower panel). Ca stool content in both formula groups was apparently higher (though not statistically tested) than in the breastfed reference group at all time points (Figure 1, lower panel, and Table S2, Supporting Information, which summarizes the stool analyses).

3.1.3. Association between Stool Consistency, PA and Ca Content

The majority of infants across both formula intervention groups had a stool consistency categorized as "soft" (data not shown). In the Concept-Mix group the percentage of subjects with a consistency score of "watery" was higher compared to the Control-Veg group from 5 to 13 weeks of age, and as such, stool consistency in the Concept-Mix group was more similar to the breastfed reference group. Linear regression analysis was used to investigate the association of the PA or Ca stool content and the subject's mean stool consistency at age 13 weeks. The results of the regression analyses (**Table 4**) indicated that stool consistency increased (i.e., stool got harder) with an increase of PA or Ca content.

3.1.4. Preclinical Study

All rats ($n = 10$, no dropouts) tolerated the formula-based diets well: no changes in animal health or well-being, and no pasty or soft feces were observed. Figure S2 (Supporting Information) shows the body weight development (panel B) and food intake (panel C) of each individual rat. Animals accrued 232–367 g in weight during the ten weeks the total experiment lasted. There were no indications for differences in preference for any of the three diets, or for apparent differences in their effect on body weight gain. Average dry weight food (65–75 g) and total fat intake (5–6 g) during the 72-h test period was not different between diets. The weight of dry weight feces (mean ± SD) produced per 72 h did not differ statistically significant between diets, and amounted 5.8 ± 0.8 g in the Concept-Mix group, 6.9 ± 1.4 g in the Concept-Veg group, and 6.7 ± 1.2 g in the Control-Veg group. Total fecal FA content (w%; **Figure 2**, top panel) was also not statistically significantly different for the Concept-Mix diet (1.46 ± 0.5%) compared with Concept-Veg (1.77 ± 0.8) and Control-Veg diets (1.90 ± 0.7%).

Total fat absorption efficiency, expressed as percentage calculated from fat intake and fecal fat content, was high for all intervention diets: 98.5 ± 0.6% in the Concept-Mix diet, 97.6 ± 0.9% for Control-Veg diet (Figure 2, middle panel). Despite the relative similar PA content in all three diets (16.6%–18.8% of total FA), the fecal PA levels (%w) were statistically significantly lower in animals fed the Concept-Mix diet compared to those fed either of the 95%–97% vegetable lipids comprising diets (Figure 2, bottom panel).The PA absorption efficiency in rats fed the Concept-Mix diet (97.3 ± 1.3%) was not statistically significantly different from the Control-Veg diet (94.3 ± 2.5%). No statistically significant differences in absorption efficiency were observed between diet groups for any of the other FA; mostly having absorption levels between 90% and 99% (data not shown).

4. Discussion

PA (16:0) provides the major part of the total saturated FA content, and PA concentration is relatively constant (≈22%FA) in human milk,[4] where 70% of the PA present is esterified to the *sn*-2 position of the TAG. Infant formulas use either vegetable oils (in particular palm oil) or cow's milk fat as the main source of PA to mimic human milk PA content. The vegetable fat sources used in formula have the majority of PA esterified at the *sn*-1 and *sn*-3 position of the TG compared to human milk or cow's milk fat, which may affect the amount of free PA in the gut lumen after TG digestion by human pancreatic lipase which is *sn*-1,3-specific.

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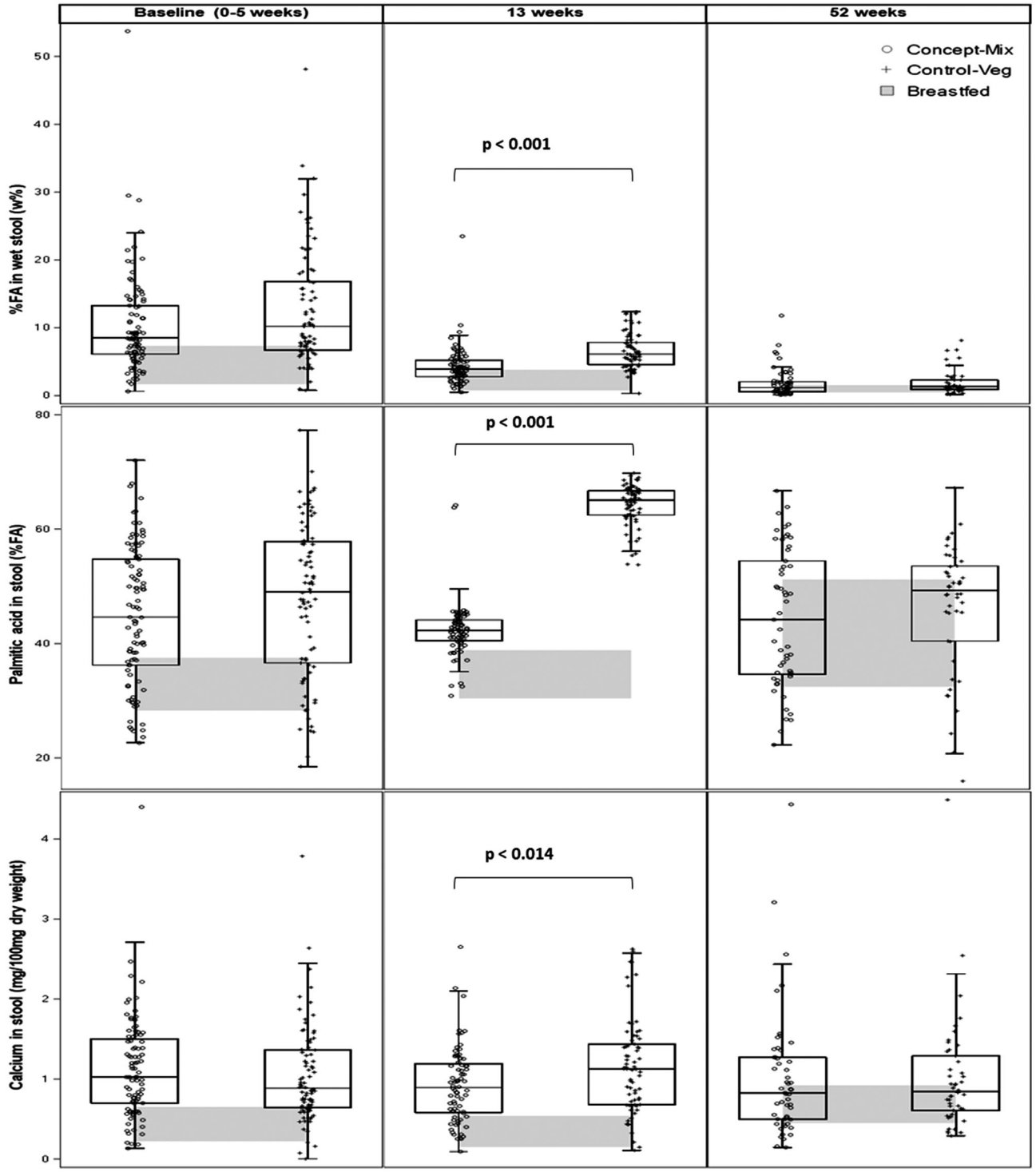

Figure 1. Stool parameters at baseline and at age 13 and 52 weeks in Concept-Mix and Control-Veg groups: %FA (top), palmitic acid (PA, middle), and calcium content (bottom). Shown are boxplots (median and IQ (Q1; Q3) ranges, whiskers at 1.5*(Q1–Q3)). Gray bars represent Q1–Q3 range of the Breastfed reference group. Q1 and Q3 = quartiles up to, respectively, the 25th and 75th percentiles of the data obtained. Wilcoxon rank sum test was used for comparisons between the Concept-Mix and Control-Veg groups at 13 weeks. Number of subjects per time point for Concept-Mix, Control-Veg, and Breastfed groups: %FA (top): Baseline: $n = 90/79/63$; 13 weeks: $n = 76/70/51$; 52 weeks: $n = 53/46/43$; palmitic acid (middle): Baseline: $n = 93/82/63$; 13 weeks: $n = 78/70/53$; 52 weeks: $n = 53/46/43$; calcium (bottom): Baseline: $n = 92/82/58$; 13 weeks: $n = 76/70/50$; 52 weeks: $n = 53/46/43$.

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Table 4. Statistical analysis of association between stool consistency score[a)] and stool parameters PA and calcium content at infant age 13 weeks.

Stool parameter	Regression parameters		
	Intercept	Slope (β)	95% CI of β
PA (% FA)	1.482	0.006	0.002; 0.010
Calcium (mg/100 mg)	1.687	0.131	0.038; 0.224

Notes: Analysis model used: mean stool consistency = intercept + slope × stool parameter. CI, confidence interval; [a)] see the Experimental Section.

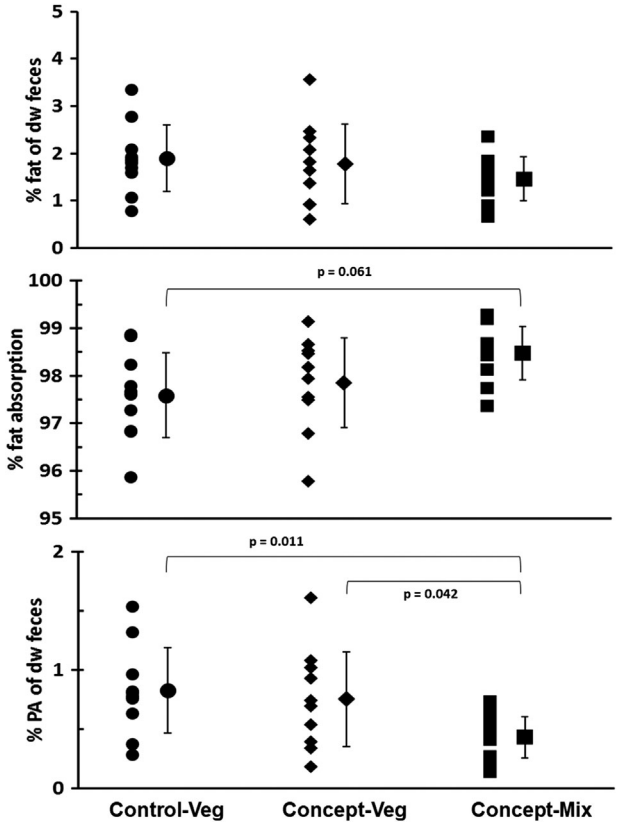

Figure 2. Outcomes of the fat balance study in rats (n = 10) after 14 d on each of the formula-based test feeds: individual data and means (± SD) of fat absorption, total fat, and fecal (PA) content in dry weight (dw) feces. Multifactorial ANOVA with post hoc Tukey HSD test. Control-Veg diet, diet based on standard formula with 97% vegetal lipids; Concept-Veg diet, diet based on concept formula with large, dairy phospholipid-coated lipid droplet comprised of 95% vegetable lipids; Concept-Mix diet, diet based on concept IF with large, milk phospholipid coated lipid droplets comprised of a mix of 52% vegetable and 48% animal lipids.

The freed PA forms unabsorbable soaps with Ca, resulting in a lower PA and Ca absorption and potentially harder stools. Therefore, a formula with fat sources with a higher *sn*-2 PA content is expected to show a higher absorption of PA and lower amounts of PA ending up in the feces, thus helping to prevent FA and Ca malabsorption (see for reviews in refs. [35] and [36]).

Indeed, in the clinical study it was observed that infants fed a concept formula with large, phospholipid-coated lipid droplets containing a 48/52% mixture of dairy and vegetable lipids (Concept-Mix) had lower total FA, PA, and Ca stool levels at 13 weeks of age, compared to those fed a standard formula with small lipid droplets consisting of 97% vegetable lipids (Control-Veg). Further analyses at time point 13 weeks showed that levels of Ca and PA in stool were weakly positively associated with the reported stool consistency scores.

Based on these observations the hypothesis was raised that the higher *sn*-2 PA TAG content in the Concept-Mix formula, i.e., the fat source, rather than the lipid droplet characteristics (size and coating) could be causative for the effects observed on stool PA levels. Subsequent testing of this hypothesis in a preclinical setting confirmed that the observed effects on stool PA content were, in line with the hypothesis, most likely explained by the presence of the dairy lipids and the concomitant increased *sn*-2 PA TAG content rather than the alterations in lipid droplet characteristics. Finally, our observations on stool lipids and Ca levels in infants are in line with the findings from previous studies evaluating formulas with similar levels of *sn*-2 PA TAG content.[35,36]

The very low and in time decreasing stool fat content in all clinical study groups suggested that fat absorption early in life is very high and matures quickly over time in both formula- and breast-fed infants. These results are in line with previously reported absorption efficacy data in (pre)term infants which were previously reported to increase from 92% in neonates to >97% at age 13–19 weeks.[3]

Although both intervention formulas provided in the current clinical study contained equal amounts of FA and Ca, we observed a lower sum of all FA, PA, and Ca stool content at 13 weeks of age in infants which were fed Concept-Mix formula compared to those fed Control-Veg formula. The lack of relevant differences in these stool outcome parameters at baseline as well as at 52 weeks of age, i.e., at times when no study product was provided and the infant's diet was not controlled and probably diverse, suggests that the effects found at 13 weeks, when the vast majority of infants received study product as sole source of nutrition since their first week of life, can most likely be attributed to the type of intervention formula provided.

As reported previously,[19] the median age at start of study product intake was at 7 d for Concept-Mix and at 5.5 d for Control-Veg groups (see also Table S3, Supporting Information). Parents were instructed to use the study product as sole source of nutrition for their infant until 17 weeks of age: The median (Q1, Q3) duration the study products were used was 113 (104–119) d for the Control-Veg group, and 110 (96–118) d for the Concept-Mix group. So, at time point week 13 all infants in the formula groups were formula-fed for ≈12 weeks. Introduction of complementary solid foods started after week 17, i.e., at the end of the intervention period. The median (Q1, Q3) start date of solid feeding for Control-Veg was at 122 (118–126) d and for Concept-Mix at 123 (116–127) d. The differences in stool composition between the study groups at 13 weeks are hence likely to be related to differences in the study product characteristics. No quantitative food data at 52 weeks are available; all subjects are assumed to consume solid foods when the last stool sample was collected. So any changes in gut function or digestion due to the introduction of solid foods can be expected to be similar between the study groups.

As shown previously,[19] the daily weight gain (g d^{-1}) in either formula group was equivalent, although the daily formula

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intake (mL d^{-1}) was lower in infants consuming the Concept-Mix formula with median (Q1; Q3) values of 790 (745; 878) mL d^{-1} compared to the Control-Veg formula at 13 weeks of age with 837 (767; 947) mL d^{-1}, resulting in an approximate difference of about 1.6 g d^{-1} fat intake between the groups (≈5% of the daily 27–28 g fat intake). It is unlikely that this lower fat intake can (fully) explain the observed lower stool levels of total FA and PA in the Concept-Mix compared to the Control-Veg group as observed at the infants' age of 13 weeks (Figure 1).

One of the "landmark" differences between the intervention formulas of the current study (Table 1) was the three times higher *sn*-2 PA levels in the Concept-Mix versus Control-Veg formula (35.4 vs 11.6% of total PA, i.e., 6% vs 2% of total FA). As previously shown, the presence of TAG with a higher *sn*-2 PA content in formula precludes the formation of Ca-soaps, as digestion in this case results in PA-monoglycerides which are readily absorbed by the body, thus resulting in an increased fat absorption, a reduced stool saponified FA, and a reduced Ca content in stool.[7,14,37,38] Although no saponified FA or Ca-soaps were assessed in the current study, it is likely that the differences in *sn*-2 PA levels of the intervention formulas affected total fat and PA absorption, and in line with its previously reported role in Ca-soap formation, very likely also explains the related difference in Ca absorption observed at 13 weeks. As previously described for infant formulas with increased *sn*-2 PA content,[7,14] compared to the Control-Veg group, the stool parameters of the Concept-Mix group were more like those seen in infants of the breastfed reference group, which is known to have PA preferentially esterified (up to 70%) at the *sn*-2 position of TG.[4] Supportive to these observations in infants, the animal fat balance study also showed lower PA levels in the feces of the rats fed the high level of *sn*-2 PA (Concept-Mix diet) compared to the rats fed the lower *sn*-2 PA levels (Control-Veg and Concept-Veg diets). No relevant differences were observed in any of the other stool FA levels between the Control-Veg and Concept-Veg groups. To conclude, based on these insights it indeed seems plausible that the observed differences in stool parameters between the infants of the Concept-Mix and Control-Veg groups in the current study can be (mainly) attributed to the different *sn*-2 PA levels present in the formulas.

Apart from PA, other saturated FA (SFA) and monounsaturated FA (MUFA) can be involved in the Ca-soap formation as well.[7,39] Two other SFA (12:0 and 14:0) also showed lower levels in the stool of the Concept-Mix group, but are only present at low levels and as such, are unlikely to make a substantial impact on the reported outcomes (Table S2, Supporting Information). Also, their *sn*-position at the TAG backbone is unknown, as only a change in the *sn*-2 position is relevant and possibly affecting soap formation. Both medium-chain FA may have "aided" in the effect observed on stool consistency which is mainly driven by PA. The same holds for the MUFA oleic acid (OA,18:1n-9) which also shows lower levels in the Concept-Mix group at week 13 (Table S2, Supporting Information).

The observed higher levels of the SFA stearic acid (SA,18:0) at week 13 in stools from the infants fed Concept-Mix formula may be related to the almost double amount of this FA present in the formula (Table 1) and a higher retention of SA in the gut lumen due to the potential formation of Ca-soaps with free SA. Also, no information is available on the steric distribution of SA in the fat blend of either formula. Although SA levels in the stools of both formula groups (11.8%FA in Control-Veg and 25.9 in Concept-Mix) are substantial and could be physiologically relevant, and impact the extent of soap formation, stool consistency scores were still lower in the Concept-Mix group. Hence, the different SA levels in the formulas do not interfere with the hypothesis raised that the observed differences in stool composition may primarily be driven by the presence of *sn*-2 PA.

As reported previously,[19] the majority of stools were categorized as soft in both formula groups, a feature most likely related to the presence of the scGOS/lcFOS mixture (0.8 g/100 mL), a specific prebiotic 9:1 mixture consisting of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides, which has been shown to have a stool-softening effect.[40] The prebiotic scGOS/lcFOS mix is present in both formulas, still, infants fed Concept-Mix formula had lower stool consistency values than the Control-Veg group and closer to the breastfed reference group during the intervention period (at 5, 8, 13, and 17 weeks of age), particularly for the occurrence of watery stools.[19] The lower stool consistency observed for the Concept-Mix group on top of the scGOS/lcFOS effect, is most likely due to the increased level of dietary *sn*-2 PA which has been shown to reduce the formation of calcium soaps and has a stool-softening effect.[7–9] This is also in line with the association found between stool Ca (and PA) content and stool consistency in the association analysis of the current study. Hence, the observed differences in stool consistency between Control-Veg and Concept-Mix formulas of the current study, may be partly explained by the observed differences in Ca and PA stool levels.

Although the clinical and preclinical data of the current study have provided some key insights regarding the contribution of the fat source and fat droplet characteristics, some limitations need to be addressed. In the clinical study, the daily weight gain (g d^{-1}) in both formula groups was equivalent although the intake (in mL day^{-1}) was lower for infants consuming the Concept-Mix versus Control-Veg formula at 13 and 17 weeks of age.[19] However, daily formula intake corrected for body weight, and expressed per kg body weight, was similar between Concept-Mix and Control-Veg formula-fed groups during the entire study period. The slightly lower intake of Concept-Mix formula resulted in a lower daily fat intake (≈1.6 g d^{-1}) which may have contributed to the differences found in stool total FA content. The latter cannot be confirmed, because no proper fat balance study was performed in the infants, nor were 24-h stools collected. So, the stool data obtained from the clinical study can only be considered as a proxy for fat absorption and were used therefore for hypothesis generation only. Moreover, the reduced PA levels observed in the Concept-Mix group are unlikely caused by the lower stool FA content, since it is expressed as percentage of total FA and not as stool weight.

Both formulas tested in the clinical study contained the prebiotic scGOS/lcFOS mixture with a known stool-softening effect.[40] Testing formulas without this prebiotic may have resulted in more pronounced effects in the association analyses performed: the scGOS/lcFOS mixture may have as such partly masked the effects on stool consistency.

Although the evaluation of stool parameters was an exploratory outcome of the clinical study, the sample size of the formula groups, with stool sample collection in the vast majority of enrolled infants, was higher or similar (ranging from 46 to 93)

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compared to previous studies (n = 23, 66, or 16) which had the primary objective to evaluate stool characteristics and were powered to this end.[7,8,41] A more recent study planned 183 infants per group (including a 20% dropout) to detect a clinically relevant difference in stool consistency of 0.5,[42] and came to the same conclusion testing two levels of dietary *sn*-2 PA enrichment.

Some baseline stool samples were collected within 1–2 d after study product intake had started, which may have affected stool analyses outcome. Also, seven subjects (9.0%) in the Concept-Mix group, four in the Control-Veg group (5.7%), and two in the breastfed group (3.8%) received complementary feeding before the stool sample at 13 weeks was collected. Only one subject in the Concept-Mix group had stopped product intake before sample collection at 13 weeks.

The intervention formulas of the clinical study differed in more than one aspect and lacked a head-to-head comparison of the presence of the dairy lipids and the inherent increase in *sn*-2 PA content, implying that the observed differences could not be attributed to the single effect of presence of dairy lipids or altered lipid droplet structure. We used an adult rat model to evaluate possible effects of lipid droplet characteristics versus the possible contribution of *sn*-2 PA content in the concept. The primary focus was on PA levels, but retrospectively, it would have been even more informative if Ca stool measurements had been obtained in the rat model as well. Although rats, besides pigs, are regarded a viable model for gastrointestinal human fat digestion in general, the absence of a gall bladder and gastric lipase may impede direct comparison of rat and human data.[20,33,34] In addition, it is of importance to state that the adult rat model is not specifically validated for its translational potential on human infant fat absorption in the gut. However, for our objective to disentangle the "general" impact of differences in fatty acid composition versus lipid droplet characteristics on fat digestion, we regarded this experimental model to be appropriate in the current study. In the animal study coprophagia might have occurred in the 72-h feces collection period which may have confounded the outcomes reported. Coprophagia is a normal behavioral feature in rodents more likely to occur in case of fasting, or nutrient restriction.[43,44] The rats in the current study were provided amply and ad libitum with daily freshly prepared feeds that fully met their nutritional requirements. Rats were on the diet for 12 d at the start of each of the balance studies, and were in steady state between intake, excretion and coprophagia. It is in this status of equilibrium we assessed for 72-h the fat intake and the fat excretion in the feces produced. So coprophagia is not regarded to be a significant confounding factor for the balance outcomes.

5. Conclusions

The current study clearly suggests that infants consuming the Concept-Mix formula comprising large, milk phospholipid-coated lipid droplets (Nuturis) enriched with milk fat comprising an increased *sn*-2 PA content show an efficient fat absorption, in particular of PA, and an improved Ca absorption, closer to that of breastfed infants, when compared to those fed standard formula. These effects are most likely explained by the presence of the increased *sn*-2 PA content of the evaluated Concept formula rather than the altered lipid droplet structure.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

B.J.M.v.d.H., S.S., and M.A.-B. are employees of Danone Nutricia Research, or were (E.M.v.d.B.) at the time of study conduct. No other conflicts of interest are to be disclosed.

Author Contributions

B.J.M.v.d.H.: conceptualization; formal analysis; methodology; visualization; writing – original draft; writing – review & editing. S.S.: conceptualization; data curation; investigation; methodology; writing – original draft; writing – review & editing. M.A.-B.: conceptualization; methodology; supervision; writing – original draft; writing – review & editing. E.M.v.d.B., : conceptualization; supervision; writing - review & editing. A.C.S.H.-K.: conceptualization; methodology; supervision; writing - review & editing. All authors contributed to and approved the final draft of the manuscript.

Data Availability Statement

The clinical data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. The preclinical data can also be made available on request.

Keywords

fat absorption, infant formula, lipid droplets, milk phospholipids, *sn*-2 palmitic acid, stools

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