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► **To cite this version:**

Laurent Beghin, Xavier Marchandise, Eric Lien, Myriam Bricout, Jean-Paul Bernet, et al.. Growth, stool consistency and bone mineral content in healthy term infants fed sn-2-palmitate-enriched starter infant formula: A randomized, double-blind, multicentre clinical trial.. Clinical nutrition (Edinburgh, Scotland), 2018, Clinical nutrition (Edinburgh, Scotland), 10.1016/j.clnu.2018.05.015 . hal-02149330

HAL Id: hal-02149330

<https://hal.univ-lille.fr/hal-02149330>

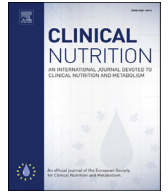
Submitted on 6 Jun 2019

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Randomized Control Trials

Growth, stool consistency and bone mineral content in healthy term infants fed *sn*-2-palmitate-enriched starter infant formula: A randomized, double-blind, multicentre clinical trial[☆]



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ARTICLE INFO

Article history:

Received 10 November 2017

Accepted 25 May 2018

Keywords:

Stool consistency

Growth

Bone mineral content

Healthy infants

Infant formula

SUMMARY

Background: Palmitate in breast milk is predominantly located in the triacylglycerol *sn*-2 position, while infant formulae contain palmitate predominantly in the *sn*-1 and *sn*-3 positions. During digestion, palmitate in the *sn*-1 and *sn*-3 positions is hydrolyzed to free palmitic acid that can subsequently complex with calcium to form insoluble soaps; this may partially explain why formula-fed infants have harder stools than breast-fed infants.

Methods: This large ($n = 488$) randomized, double-blind, multicentre trial investigated whether increasing the *sn*-2 palmitate content of infant formula improves stool consistency and bone mineral content (measured by dual-energy x-ray absorptiometry), without affecting growth or health. From ~1 week to 4 months of age, infants were exclusively fed one of three formulae: *i*) control formula (CF; 16% of total palmitate at *sn*-2; $n = 162$), *ii*) experimental formula 1 (EF1; 43% of total palmitate at *sn*-2; $n = 166$) or *iii*) experimental formula 2 (EF2; 51% of total palmitate at *sn*-2; $n = 160$).

Results: Intention-to-treat analysis showed softer stools in both EF groups (vs. CF) at ages 2 weeks and 1 and 2 months ($p \leq 0.01$), but not 3 and 4 months. At 4 months, all groups had similar growth outcomes while bone mineral content was significantly higher in EF1 ($p = 0.0012$) and EF2 ($p = 0.0002$) compared with CF. Comparison of reported adverse events up to 12 months revealed no differences among groups. All 3 infant formulae exhibited equally good digestive tolerance.

Conclusions: Formulae enriched in *sn*-2 palmitate fed in early infancy are safe, improve stool consistency (from 2 weeks to 2 months) and increase bone mineral content (at 4 months).

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[☆] The study was registered on [Clinicaltrials.gov](https://clinicaltrials.gov) (Registration Number: NCT02332967; URL: <https://clinicaltrials.gov/ct2/show/NCT02332967>) after the study was completed.

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1. Introduction

Breast milk is the optimal food for healthy infants and provides an adequate supply of all nutrients to support growth [1]. Triacylglycerols in breast milk provide 45%–55% of the energy intake of breast-fed infants as well as essential fatty acids and other lipids that play important roles in optimal development [2]. Palmitate constitutes about 25% of the fatty acids of breast milk triacylglycerols and approximately 70% of this is at the *sn*-2 position [3]. Inversely, palmitate in most infant formulae is predominantly at the *sn*-1 and *sn*-3 positions of the triacylglycerol backbone [4]. This difference in triacylglycerol structure is of importance in palmitic acid and total lipid absorption. The pancreatic lipase-colipase system is highly selective for the *sn*-1 and *sn*-3 triacylglycerol positions, resulting in the generation of two free fatty acids and an *sn*-2 monoacylglycerol. All *sn*-2 monoacylglycerols are well absorbed as are saturated fatty acids of chain length <14 carbons and all unsaturated fatty acids [5]. However, free long-chain saturated fatty acids, such as palmitic acid, form calcium soaps which are insoluble at body temperature and are excreted in the feces [6,7]. Thus, palmitic acid absorption is greater in breast-fed infants than in formula-fed infants [8]. The percentage of fed calcium that is also absorbed is greater in breast-fed than in formula-fed infants [9]; fecal calcium-palmitate soaps are associated with hard stools, and this may, at least in part, explain the greater stool softness in breast-fed infants as compared to formula-fed infants [6]. In addition to triacylglycerol structure, human milk oligosaccharides are also likely to play a role in the greater stool softness in breast-fed infants [10].

Infant formulae using structured triacylglycerols (named structured lipids in the rest of the paper) have been developed in order to increase the proportion of palmitate at the *sn*-2 position and to test possible beneficial effects on lipid and mineral balance [8,11,12]. These studies have demonstrated that palmitic acid and calcium absorption are improved by introduction of structured lipids. A number of studies have reported an 8–40% reduction in fecal total fatty acid-calcium soaps or palmitate-calcium soaps when formulae containing structured lipids are fed [13–15] while some [8,13,14], but not all [15,16], studies have reported softer stools in infants fed formulae with an increase in *sn*-2 palmitate and a concomitant reduction in *sn*-1 and *sn*-3 palmitate.

Bone maturation has also been assessed with formulae containing structured lipids in two studies. Both studies compared high and low levels of *sn*-2 palmitate (50% vs 12% [13] and 43% vs 14% [17], respectively) in infant formulae fed from <2 weeks after birth until 12 weeks of age. These studies reported significantly higher values of bone mineral content [13] and bone speed of sound [17] (a measure of bone strength) in infants fed formulae with a high *sn*-2 palmitate content compared to infants fed a low *sn*-2 palmitate formula. However, these studies were characterized by a high rate (58%) of study non-completion [13], and relatively small number of infants assessed [17].

The current study is a randomized, double-blind, controlled, multicentre trial which compared three infant formulae with differing content of *sn*-2 palmitate. The main purpose of this trial was to evaluate weight gain and stool consistency during the first 4 months of life. We hypothesized that stools would be softer in the groups receiving formulae enriched with *sn*-2 palmitate, whereas weight gain would be comparable among all groups. Secondary objectives were an evaluation of bone mineral content, body composition, digestive tolerance and safety of the three formulae.

2. Subjects and methods

2.1. Study design and ethical approvals

This was a randomized, double-blind, multicentre clinical trial with three parallel groups ($n = 488$). Infants between 1 and 7 days of age were enrolled and randomly assigned to exclusively receive one of three whey-predominant intact protein formulae: *i*) 16% of total palmitate in the *sn*-2 position as a control formula (CF; $n = 162$), *ii*) 43% or *iii*) 51% of total palmitate in the *sn*-2 position as enriched experimental formula 1 (EF1; $n = 166$) and 2 (EF2; $n = 160$) respectively. The primary objectives of the trial were first to evaluate whether weight gain of infants fed a formula enriched with *sn*-2 palmitate (EF1 with 43% or EF2 with 51% of total palmitic acid content) during the first 4 months of life was non-inferior to that of infants receiving CF, and then to evaluate whether the stools were softer in EF1 and EF2 groups vs. CF. The secondary outcomes were to assess other growth parameters, body composition, bone mineral content, digestive tolerance and safety among groups.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the European regulatory authority (N° EudraCT 2007-A0018-50), French regulatory authority (N° DGS 2007-0175) and the local ethics committee (Comité de Protection des Personnes Nord-Ouest IV de Lille, France CPP 07/32 in 2007). Written informed consent was obtained from both parents after an explanation of the study procedures to the parents by the study paediatrician. Written informed consent was obtained from both parents for each infant before inclusion. The study was conducted from August 2007 to December 2010.

2.2. Study population and randomization

Infants were recruited in private maternities from the Lille metropolitan area by ambulatory paediatricians of the GREPA-Nord (Groupe de Recherche et d'Etudes en Pédiatrie Ambulatoire-Research and Study Group in Ambulatory Paediatrics from the North of France). Infants were screened and recruited on the basis of the following inclusion criteria: *i*) healthy, full-term (37–42 weeks gestation) newborn; *ii*) mother electing not to breastfeed beyond the 7th day of life and to feed infant formula exclusively up to 4 months of age; *iii*) birth weight between 2500 and 4500 g; and *iv*) singleton birth. Exclusion criteria were: *i*) congenital illness or malformation that may affect normal growth; *ii*) significant pre-natal and/or post-natal disease; *iii*) hospitalization within the first 2 weeks of life after leaving the maternity ward; *iv*) antibiotic treatment at the time of enrolment; *v*) parents who were not expected to comply with the study protocol; and *vi*) current participation in another interventional clinical trial. The formula-fed infants who met the eligibility criteria were randomized equally (1:1:1) to receive CF, EF1 or EF2. Stratified randomization was performed using sex and mode of delivery as stratification factors. Randomization was carried out using a computer software program (TrialBalance, a Nestlé software).

2.3. Study formulae

Study formulae were manufactured by the Nestlé Product Technology Center in Konolfingen, Switzerland. They were in powder form and consisted of a mix of proteins, carbohydrates and fats with vitamins and minerals in amounts intended for full nutritional support of infants from birth to six months and in accordance with the compositional criteria laid down by the European Union [18] for

infant formulae and follow-on formulae. The composition of the study formulae is provided in Table 1. Energy, protein, carbohydrate, fat, total and individual fatty acids, and mineral content were similar among the 3 formulae. While the concentrations of palmitate as a percentage of total fatty acids had only minor variations across the 3 formulae, the structural distribution of palmitate differed substantially. Specifically, the proportion and absolute amount of palmitate in the *sn*-2 position in CF were 16% and 1.2 g/L, respectively, whereas the values were 43.0% and 3.1 g/L in EF1, and 50.9% and 3.3 g/L in EF2. Consequently, the levels of palmitate in the *sn*-1 and *sn*-3 positions for CF, EF1 and EF2 were 6.0 g/L, 4.0 g/L, and 3.2 g/L, respectively. The high *sn*-2 levels in EF1 and EF2 were achieved by using two different grades of InFat™ (structured triacylglycerols blend produced by Advanced Lipids AB, Karlshamn, Sweden). The formulae did not contain prebiotics.

The study formulae were labeled by the manufacturer using a single letter per formula group (A, B or C), the identity of which was known only to the manufacturer. All individuals involved in the trial including parents/caregivers, sponsor, investigators and study staff were blinded to the identity of the study formulae. Code break envelopes were provided to the study sites so that the code could be broken in the event of an emergency. The tin label included guidance for the parents on the daily volume of formula intake required by the infant, which depended upon age, weight and appetite. Parents were also encouraged to take advice from investigators, if required.

2.4. Study visits and duration of study feedings

After randomization, infants were fed their assigned study formulae exclusively until introduction of complementary feeding

Table 1

Selected nutrient composition, fatty acid profiles and positional distribution of palmitic acid of the study formulae.

	CF	EF1	EF2
Energy ^a (kcal/L)	673.3	672.0	672.0
Protein (g/L)	14.1	14.2	14.2
Whey/casein ratio	60/40	60/40	60/40
Carbohydrate ^b (g/L)	75.5	75.2	75.2
Fat ^c (g/L)	35.0	34.9	34.9
Total fatty acids (g/L)	32.7	32.7	32.7
Fatty acids (% by weight of total fatty acids)			
Lauric acid (C12:0)	9.7	9.2	10.6
Myristic acid (C14:0)	4.3	4.1	4.5
Palmitic acid (C16:0)	22.0	21.7	19.8
Stearic acid (C18:0)	3.7	3.8	3.7
Oleic acid (C18:1)	36.1	35.3	34.8
Linoleic acid (C18:2)	17.4	19.3	19.3
Alpha-linolenic acid (C18:3)	2.1	2.1	2.3
Total PA (g/L)	7.2	7.1	6.5
PA in <i>sn</i> -2 position			
(g/L)	1.2	3.1	3.3
(% of total palmitic acid)	16.0	43.0	50.9
PA in <i>sn</i> -1, <i>sn</i> -3 positions			
(g/L)	6.0	4.0	3.2
(% of total palmitic acid)	84.0	57.0	49.1
Calcium (mg/L)	484.7	493.9	493.9
Phosphorus (mg/L)	268.6	273.8	273.8
Magnesium (mg/L)	62.9	64.2	64.2
Vitamin D (µg/L) ^d	10.0	10.0	10.0

CF: control formula; EF1: experimental formula 1 with 43% of total palmitate at the *sn*-2 position; EF2: experimental formula 2 with 51% of total palmitate at the *sn*-2 position; PA: palmitic acid.

^a Macronutrient contribution to total energy was similar in all 3 formulae: 8% protein, 45% carbohydrate and 47% fat.

^b Primarily lactose; all 3 formulae had the same amount and composition of carbohydrates.

^c 100% vegetable oil blend was used in CF; the same 100% vegetable oil blend was used in EF1 and EF2 with one difference: enzymatic interesterification was employed to increase the proportion of palmitic acid in the *sn*-2 position.

^d Values for Vitamin D represent the label declaration.

at 4–6 months of age. The study intervention formally ended at 6 months. Clinic visits were performed at the following time intervals after enrolment: 1–7 days of life (V_0 , baseline), 2 weeks (V_1), 1 month (V_2), 2 months (V_3), 3 months (V_4), 4 months (V_5), 6 months (V_6) and 12 months (V_7). Primary outcomes (weight gain and stool consistency) were analysed using data collected from 0 to 4 months of age; the follow-up visits at 6 and 12 months were conducted to collect further data for the secondary outcomes. All study subjects received a follow-up formula without enrichment in *sn*-2 palmitate from 6 to 12 months of age. All infants underwent a bone mineral content and body composition measurement with dual-energy x-ray absorptiometry (DXA) at V_5 (month 4). Parents were given a diary to record the volume of study formula consumed for the 3 days immediately prior to each of the clinic visits in order to assess the comparability of formula intake among groups. For microbiota assessment, parents were also instructed to collect at home stool from their infant at baseline (3–7 days) and at 4 months of age before nutritional diversification (1–7 days after V_5).

2.5. Anthropometry

Weight, recumbent length and head circumference were measured at each visit by the study paediatrician. Infants were weighed without clothing or diaper on an electronic infant scale and measurements were recorded to the nearest 10 g. Recumbent length was measured on a standard paediatric length board to the nearest 1 mm. Head circumference was measured using a non-elastic plastic coated measuring tape to the nearest 1 mm. Z-scores for weight-for-age, length-for-age, head circumference-for-age and weight-for-length were computed using the 2006 World Health Organization (WHO) Child Growth Standards [19]. A co-primary outcome of this trial was weight gain expressed as the mean of weight gain (g/day) during the first 4 months of life.

2.6. Digestive tolerance

Digestive tolerance indices, including stool characteristics (consistency and frequency), spitting-up and vomiting as well as behavioral patterns including crying and fussiness, were recorded by the parents/caregivers in a diary provided to parents by study staff. The parents were asked to fill in the tolerance record for the 3 days immediately prior to each of the clinic visits. Prior to visit 1 they were asked to fill in the record for the first 3 days when the infant was fed the study formula.

Stool consistency was a co-primary outcome. Stool consistency was recorded by parents using stools pictures derived from the modified Bristol Stool Form Scale for Children [20]. For the purpose of the study, the 7 categories in the Bristol stool chart were merged to 5 categories: (i) hard = separate hard lumps, like nuts; (ii) formed = sausage-shaped but lumpy/like sausage but with cracks on the surface; (iii) mushy soft = like smooth sausage, smooth and soft/soft blobs with clear-cut edges; (iv) runny = fluffy pieces with ragged edges, a mushy stool; (v) watery = watery, no solid pieces, entirely liquid. Stool frequency was recorded as number of stools per 24 h. The average values of stool consistency and stool frequency over the 3-day observation periods were computed for each subject for use in statistical analyses.

2.7. Bone mineral content and body composition

At V_5 (month 4), all infants underwent a bone mineral content and body composition measurement with dual-energy x-ray absorptiometry (DXA) (Hologic Discovery, Waltham MA, USA).

Whole-body scan time was 65–90 s, and the radiation dose was <0.3 mSv. Total body image acquisition and analysis were obtained following manufacturer's instructions, with the two X-ray energies minimizing errors due to irregular soft tissue mass and body contours and providing high resolution [21]. After body scanning, a specialized physician interpreted each scan image using the manufacturer software to provide data on bone mineral content, and on body composition as lean and fat mass.

2.8. Safety assessment

A physical examination was conducted at each clinic visit. In addition, any illness (change of health condition) and/or medication prescribed to the child since the last visit were recorded. If the child had been hospitalized between visits, the reason and the number of days were recorded. All these items/events were recorded by the study investigator during each clinic visit. In addition, parents were asked to record the episodes of diarrhea, cough, fever, skin rash and antibiotics usage occurring between visits in a specific diary. Standard definitions were provided to help parents record these events. The investigator reviewed the completed diary at each clinic visit.

2.9. Statistics

Sample size was calculated on the basis of an O'Brien-Fleming [22] design with 2 stages (interim analysis at information rate of 21%). In order to detect a clinically relevant difference in stool consistency of 0.5 (standard deviation of 1.2) with a power of 90% and overall type I error of 5% (two comparisons, EF1 vs. CF and EF2 vs. CF), 146 infants per feeding group were required. To compensate for an expected 20% dropout rate, enrolment of approximately 183 infants per group was planned. For the one-sided test on body weight gain, a sample size of approximately 105 infants per group was required (non-inferiority margin of -3 g/day, standard deviation of 6.1 g/day).

Statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, N.C., USA). The evaluation of the primary variables was performed in the intention-to-treat (ITT) data set and in the per protocol (PP) data set; the primary analysis was the ITT analysis. Data were described by calculating mean and standard deviation (SD) values for continuous variables and frequency and percentages for categorical variables.

The primary outcomes were growth as measured by weight gain per day during the first 4 months of life (from V1 to V5) and stool consistency at V5 (4 months). Weight gain during the first 4 months of life was analyzed using an analysis of covariance (ANCOVA) model adjusted for baseline weight, treatment, and sex. Weight gain in EF1 or EF2 was considered non-inferior to CF if the lower bound of the 2-sided 97.5% CI for the difference between EF1 and CF or EF2 and CF was above the non-inferiority margin of -3 g/day. Non-inferiority of EF1 or EF2 vs. CF in terms of weight gain was also assessed using a linear mixed model incorporating more dependent variables and correcting p-values according to Bonferroni. ANCOVA was also performed to compare stool consistency among feeding groups (p-values corrected according to Dunnett).

Secondary outcomes were evaluated in the ITT population. An analysis of variance (ANOVA; adjusted for sex) was conducted to compare volume of formula intake, digestive tolerance and behaviors, and of the body composition (DXA) outcomes (lean mass, fat mass, and percent fat mass) among feeding groups. An analysis of covariance (ANCOVA; adjusted for sex and baseline weight) was conducted to compare bone mineral content (DXA) among the feeding groups. For both the ANOVA and ANCOVA, p-values were corrected according to Dunnett. Adverse events/morbidity data were analyzed using Fisher's exact tests and reported as odds ratios with 95% CIs and p-values.

Deblinding of randomization occurred after statistical analyses were performed.

3. Results

3.1. Study population

Disposition of infants is presented in Fig. 1. A total of 488 infants were randomized (CF, $n = 162$; EF1, $n = 166$; EF2, $n = 160$). At 4 months, the drop-out rate was 16.6% (81 infants), with no significant difference among the three study groups (15.4% for CF, 13.9% for EF1, and 20.6% for EF2). At 12 months, the drop-out rate was 22.3% (109 infants), with no significant difference among the three study groups (20.4% for CF, 20.5% for EF1, and 26.3% for EF2). Concerning the reasons for drop-out, the incidence of reported adverse events was significantly higher in the EF1 group (42.1%) as compared to the EF2 group (25.0%) Chi-squared test; $p = 0.0001$. The others reasons for drop out (consent withdrawn, lost to follow-up, other reasons) were not different among groups.

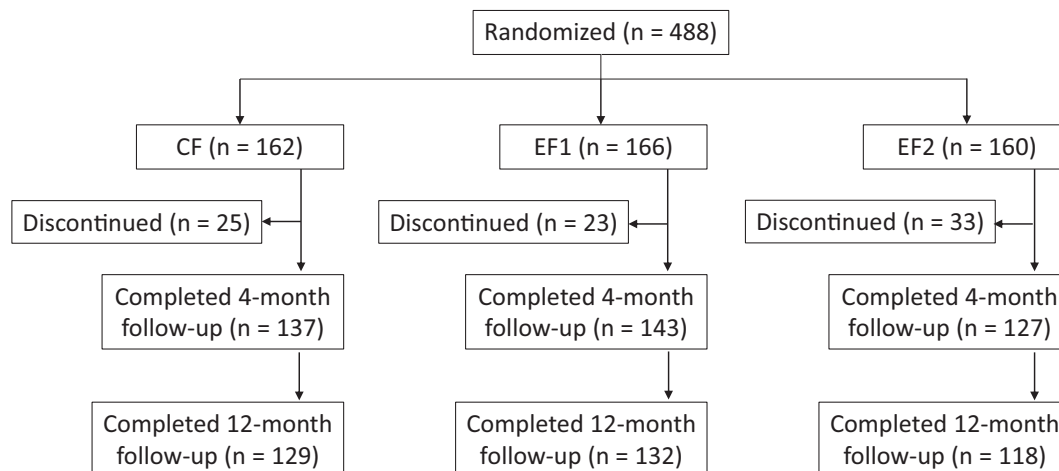


Fig. 1. Enrolment and discontinuation of the study participants. CF: control formula; EF1: experimental formula 1 with 43% of total palmitate at the *sn*-2 position; EF2: experimental formula 2 with 51% of total palmitate at the *sn*-2 position.

Table 2
Demographic and baseline characteristics of the study population.

	CF	EF1	EF2	p-value ^a
n	162	166	160	
Sex (% female)	51.9	50.6	51.3	0.967
Age (days) ^b	4.1 ± 1.3	4.1 ± 1.3	4.2 ± 1.2	0.483
Gestational age (weeks) ^b	39.3 ± 1.2	39.3 ± 1.2	39.2 ± 1.1	0.762
Delivery mode (% vaginal delivery)	84.6	81.9	84.4	0.771
APGAR scores ^c				
1 min (%)	96.3	93.4	95.6	0.488
5 min (%)	98.1	96.4	98.1	0.615
10 min (%)	99.3	100.0	99.3	0.772
Weight (kg) ^b	3.4 ± 0.4	3.4 ± 0.4	3.4 ± 0.4	0.609
Length (cm) ^b	49.5 ± 1.8	49.7 ± 2.0	49.7 ± 2.0	0.649
HC (cm) ^b	34.5 ± 1.4	34.6 ± 1.3	34.6 ± 1.8	0.852

CF: control formula; EF1: experimental formula 1 with 43% of total palmitate at the *sn*-2 position; EF2: experimental formula 2 with 51% of total palmitate at the *sn*-2 position; HC: head circumference; SD: standard deviation.

^a Fisher's exact test for proportion or ANOVA for comparison of means among 3 study groups for ITT population.

^b Values are presented as mean ± SD.

^c Values are presented as percentage of infants having the APGAR score of 10 at 1, 5 and 10 min.

The ITT population included all infants randomized to the study groups. The per-protocol (PP) population differed from the ITT population by the exclusion of infants with major protocol violations, including non-compliance with inclusion/exclusion criteria. Demographic and baseline characteristics of the study groups (ITT population) are presented in Table 2. Delivery was mainly vaginal (83.6%) and mean age at enrolment was 4.1 days (±1.3SD). Smoking status, the consumption of alcohol and the education level of the mothers during pregnancy as well as their age, height, weight at the end of pregnancy were similar among groups. There were no significant differences in age at enrolment, sex, mode of delivery, and APGAR scores (at 1-, 5- and 10-min) among the study groups. Baseline anthropometric measures, including weight, length and head circumference, were also similar among groups.

3.2. Formula intake

Mean daily formula intake was similar among groups at all study visits. At 4 months of age, mean (±SD) intake was 800 (±143) mL, 768 (±125) mL, and 796 (±118) mL for CF, EF1 and EF2 groups, respectively (p = 0.08).

3.3. Growth

Weight gain (g/day) between V1 (2 weeks of age) and V5 (4 months of age) among infants in the ITT population is presented in Table 3. For the overall study population, the mean (±SD) weight gain was 27.8 (±5.7) g/day. For girls, the mean weight gain was 26.1 (±5.3) g/day whereas for boys, it was 29.7 (±5.5) g/day, which were similar to mean WHO standards for infant weight gain (between birth and 4 months) of 26 g/day and 30 g/day, respectively.

Table 3

Comparison of weight gain (g/day) from enrolment until the age of 4 months among the study groups.

	All (n = 488)	CF (n = 162)	EF1 (n = 166)	EF2 (n = 160)	EF1 vs. CF	EF2 vs. CF
	Mean ± SD				LS mean difference [97.5% CI] ^a	
Weight gain for all infants	27.8 ± 5.7	27.2 ± 5.5	28.1 ± 6.3	28.0 ± 5.2	0.75 [-0.95, 2.45]	0.83 [-0.92, 2.57]
Weight gain for boys	29.7 ± 5.5	29.1 ± 5.3	30.3 ± 6.0	29.6 ± 5.2	1.25 [-1.27, 3.76]	1.25 [-1.27, 3.76]
Weight gain for girls	26.1 ± 5.3	25.7 ± 5.2	25.9 ± 5.8	26.7 ± 4.9	0.26 [-2.07, 2.59]	1.06 [-1.26, 3.39]

CF: control formula; CI: confidence interval; EF1: experimental formula 1 with 43% of total palmitate at the *sn*-2 position; EF2: experimental formula 2 with 51% of total palmitate at the *sn*-2 position; LS: least square; SD: standard deviation.

^a Analysis of covariance (ANCOVA) among 3 study groups adjusting for baseline weight and sex for ITT population.

Adjusted mean weight gain did not significantly differ between either EF1 and CF (mean difference = 0.75 g/day; 2-sided 97.5% CI = -0.95, 2.45) or EF2 and CF (mean difference = 0.83 g/day; 2-sided 97.5% CI = -0.92, 2.57). Further, because the lower bounds of the 2-sided 97.5% CIs for the differences between the EF groups and the CF group did not fall below the non-inferiority margin of -3 g/day, both EF1 and EF2 were shown to be non-inferior to CF. Similar results were observed when weight gain data were analyzed separately for boys and girls. Additional assessment of non-inferiority using a linear mixed model showed a mean difference in weight gain between EF1 and CF of 0.89 g/day (2-sided 97.5% CI = -2.00, 3.77), and between EF2 and CF of 0.44 g/day (2-sided 97.5% CI = -2.55, 3.44), confirming non-inferiority of EF1 and EF2 compared to CF. Results were similar in the PP population.

Further comparison of anthropometric parameters at 4 months (V5) among the study groups showed that weight and head circumference did not differ significantly among groups, while length was slightly but statistically significantly higher in the two EF groups compared with the CF group (62.4 ± 2.0 cm for CF; 63.0 ± 2.5 cm for EF1 and 63.0 ± 2.2 cm for EF2; p = 0.024). Mean z-scores for weight-for-age, length-for-age, head circumference-for-age and weight-for-length were not significantly different among the study groups at any study visit up to 12 months of age (Fig. 2).

3.4. Stool characteristics and microbiota assessment

Stool consistency scores from baseline to 4 months are presented in Fig. 3. Mean stool consistency scores differed significantly among the 3 groups at 2 weeks (V1), 1 month (V2) and 2 months (V3). The stool consistency scores for the EF1 group were significantly greater (softer stools) than those of the CF group at 2 weeks (p < 0.001) and at 1 and 2 months (p ≤ 0.01). During the same period, the EF2 group also had consistently higher stool consistency scores than the CF group (p < 0.001). At 3 and 4 months, stool consistency scores did not differ significantly among the 3 study groups. No significant differences in stool frequency were reported at any visit. Due to technical reasons, microbiota assessment was not performed.

3.5. Digestive tolerance and behavior

Infants' digestive tolerance and behavior were reported by parents in the diary 3 days prior to each visit from V1 (2 weeks) to V5 (4 months). Digestive tolerance summarized as mean frequencies of flatulence, spitting-up, vomiting and colic over this period was similar among the 3 groups. With regard to infants' behavior, while the mean duration of crying was similar among groups, parents perceived infants to be less restless and irritable in the EF2 group (32.9%) compared with the CF group (37.4%; p = 0.016).

3.6. Bone mineral content and body composition

Comparisons of body composition and bone mineral content at 4 months (V5) are presented in Table 4. Body composition

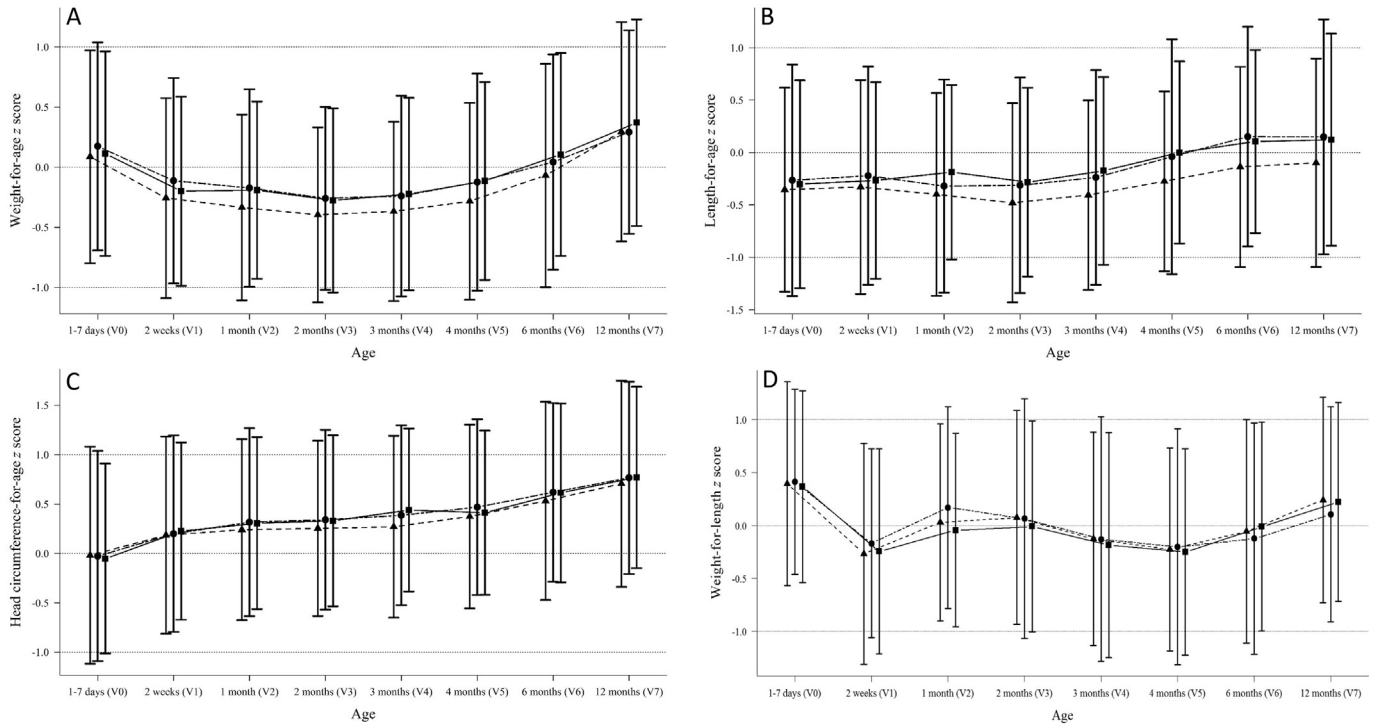


Fig. 2. Anthropometric z-scores based on the World Health Organization reference data. Triangle symbols/dashed line = CF, Circle symbols/dot-dashed line = EF1, Square symbols/solid line = EF2. EF1: experimental formula 1 with 43% of total palmitate at the sn-2 position; EF2: experimental formula 2 with 51% of total palmitate at the 2 position; HC: head circumference; for ITT population.

parameters including lean mass, fat mass and ratio of fat mass to total body mass were not significantly different among the study groups. Bone mineral content was significantly higher in both the EF1 (by 6%) and EF2 (by 7%) groups, compared with the CF group.

Adverse events and antibiotic use

There was a lower incidence of reported AEs involving the respiratory system in both the EF1 (36.8%) and EF2 (32.5%) groups,

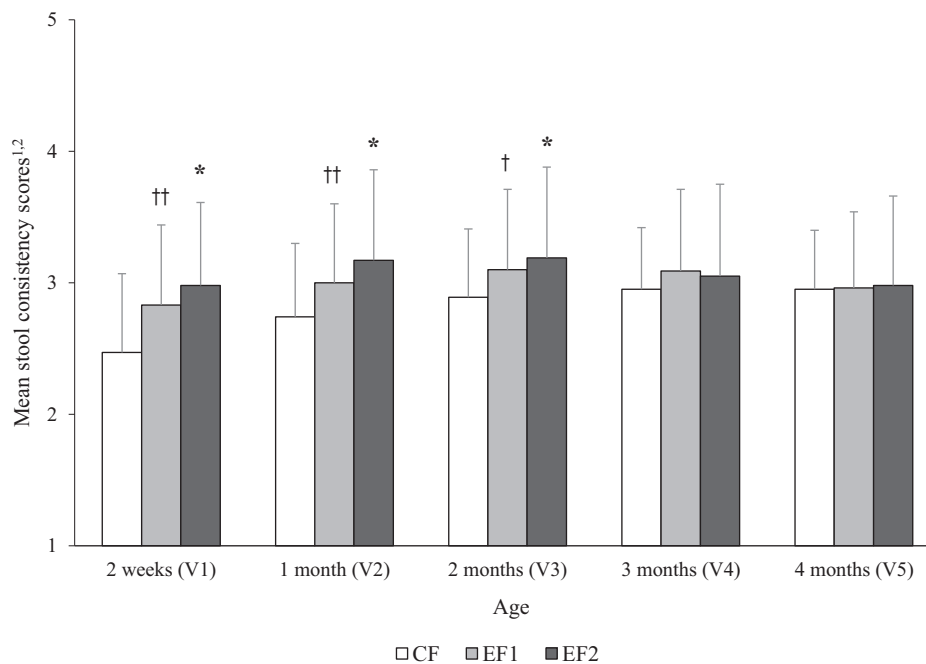


Fig. 3. Stool consistency scores from enrolment until age 4 months among feeding groups. ¹Stool consistency scores were determined using a 5-point scale: 1 = hard, 2 = formed, 3 = soft, 4 = liquid, 5 = watery. ²Values are means (\pm SD). EF1 significantly different from Control: [†] $P \leq 0.01$, ^{††} $P \leq 0.001$; EF2 significantly different from Control: [†] $P \leq 0.001$. CF: control formula; EF1: experimental formula 1 with 43% of total palmitate at the sn-2 position; EF2: experimental formula 2 with 51% of total palmitate at the 2 position; for ITT population.

Table 4
Comparisons of body composition and bone mass at the age of 4 months among the study groups.^a

	CF (n = 119)	EF1 (n = 124)	EF2 (n = 111 ^c)	p-value ^b	
				EF1 vs. CF	EF2 vs. CF
Lean mass (kg)	4.5 ± 0.4	4.5 ± 0.4	4.5 ± 0.5	0.87	0.97
Fat mass (kg)	2.3 ± 0.4	2.4 ± 0.5	2.4 ± 0.5	0.23	0.05
Fat mass/total body mass (%)	33.1 ± 4.5	33.5 ± 3.9	33.9 ± 3.5	0.56	0.24
Bone mineral content (g)	112 ± 19	119 ± 20	121 ± 20	0.0012	0.0002

CF: control formula; EF1: experimental formula 1 with 43% of total palmitate at the *sn*-2 position; EF2: experimental formula 2 with 51% of total palmitate at the *sn*-2 position; SD: standard deviation.

^a Values are presented as mean ± SD for ITT population.

^b Analysis of variance (ANOVA; adjusted for sex) among 3 study groups with pairwise group comparisons at 4 months of age for lean mass, fat mass and fat mass/total body mass. Analysis of covariance (ANCOVA; adjusted for sex and baseline weight) among 3 study groups with pairwise group comparisons at 4 months of age for bone mineral content.

^c n = 110 for fat mass/total body mass.

compared to the CF group (46.3%), and the difference between the EF2 and CF groups reached statistical significance ($p < 0.011$). The incidence of reported AEs involving the gastrointestinal tract was significantly higher in the EF1 group as compared to the CF group (53.0% vs. 39.5%, $p < 0.01$), but was not significantly different between the EF2 and CF groups (39.4% vs. 39.5%). There was no significant difference of antibiotic use among groups.

4. Discussion

The current study, reporting weight gain, stool consistency, and bone mineral content in healthy full-term infants, is the largest ($n = 488$) reported randomized, controlled trial to date investigating the comparative effects of infant formulae with differing palmitate positional distribution. We assessed three formulae which differed in their content of *sn*-2 palmitate: 16%, 43% and 51% of total palmitate content. In a reciprocal manner, the palmitate present at triacylglycerol positions *sn*-1 and *sn*-3 was substantially reduced in the formulae containing structured lipids compared to the control formula.

Stool consistency and weight gain were the primary study outcomes. Formula-fed infants typically have harder stools than breast-fed infants [6] and mothers of formula-fed infants tend to have more concerns about stool hardness and to more often seek professional advice than mothers of breast-fed infants [23]. A primary cause of hard stools during formula feeding is the generation of saturated fatty acids soaps, and one means of reducing soap formation is to replace fat blends containing palm oil (high in *sn*-1 and *sn*-3 palmitate) with a structured lipid-containing fat blend enriched in *sn*-2 palmitate. Kennedy et al. [13] demonstrated that such a formula resulted in a significant 37% reduction in stool fatty acid soaps with a 50% proportion of this being comprised of palmitate and calcium. This study also demonstrated that stools were softer (more like breast-fed infants) when a structured lipid-containing formula was fed. Our results demonstrated that infants fed structured lipid formulae had softer stools than controls until the age of 2 months, in agreement with previous literature [8,14].

Weight gain was of interest as a co-primary outcome since fatty acid absorption is partially dependent on triacylglycerol structure. In a balance study in term infants, Carnielli et al. [8] found that fat loss was elevated in a control formula when compared to a formula containing high *sn*-2 palmitate levels, suggesting a difference in calorie absorption. In the current study no significant effect on weight gain was detected when feeding formulae that were different in their triacylglycerol structure (i.e., 16%, 43% and 51% of total palmitate content at *sn*-2 position). All three groups showed similar sex-dependent gains in body weight and other anthropometric parameters over the first 4 months of life and demonstrated normal growth when compared to the WHO 2006 child growth standards [19]. These results demonstrate that the infants receiving

all three formulae absorbed and retained sufficient calories to sustain normal growth.

Data from several studies [8,11,12] indicate that increasing *sn*-2 palmitate content of infant formulae to levels close to the *sn*-2 palmitate content of human breast milk (up to 70% of total palmitate) improves the efficiency of calcium uptake from infant formulae. At 3 months, an increase in bone mineral content [13] and bone speed of sound assessed by quantitative ultrasound measurements [17] were observed in randomized clinical trials. The results of the present study agreed with these previous results: a significantly higher bone mineral content was found at age 4 months in infants fed either of the high *sn*-2 palmitate formulae compared to those fed the standard formula. More important than the proportion or absolute amount of formula *sn*-2 palmitate is the absolute amount of *sn*-1 and *sn*-3 palmitate (directly related to the generation of insoluble calcium-palmitate soaps). The 51% and 43% *sn*-2 palmitate formulae had substantially less palmitate in the *sn*-1 and *sn*-3 positions than the control formula (3.2 g/L, 4.0 g/L, and 6.0 g/L, respectively). Decreased levels of *sn*-1 and *sn*-3 palmitate will generate less free palmitate during digestion and lower levels of palmitate-calcium soaps lost in the feces. The present study confirms the relationship between bone mineralization and the amount of formula palmitate in the *sn*-1 and *sn*-3 positions as well as in the *sn*-2 position.

The strengths of this study are: (i) the multicentre design and enrolment of infants by primary care paediatricians, which provides a sample that is representative of infants living in rural and township areas; (ii) the large sample size ($n = 488$) compared to previous studies [13–15], which allowed a higher statistical power in the ITT analyses; (iii) a low drop-out rate (16.6%), which did not differ among the three infant study groups; (iv) the use of validated tools such as DXA and the stool consistency chart; and (v) the fact that each group had similar overall volumes of formula intake. Limitations that should be considered include: (i) the lack of a DXA measurement at baseline, although ethically, it was not possible to do this exam at delivery; and (ii) the lack of a breast-fed reference group.

In conclusion, this study demonstrates that feeding infant formulae with increased levels of *sn*-2 palmitate and a concomitant decrease in *sn*-1 and *sn*-3 palmitate supports normal infant growth, results in softer stools during the first 2 months of life and increased bone mineral content at 4 months of age, and is well tolerated. Thus, feeding formulae containing high *sn*-2 palmitate is safe and provides positive outcomes to infants in terms of stool consistency and bone mineralization.

Financial support

This study was sponsored by Nestlé Nutrition, through a research contract to Lille University Hospital. Authors employed by

the sponsor contributed to study design, data analysis, interpretation of findings, and preparation of the manuscript.

Conflicts of interest

NDG and JJ are employees of, and EL is a consultant for, Nestlé Nutrition. PS is an employee of Nestlé Health Science. The other authors report no conflicts of interest.

Author contributions

LB and DT formulated the study question, designed and conducted the research, interpreted the findings and contributed to the writing of the manuscript. XM interpreted DEXA data and contributed to the writing of the manuscript. EL and NDG contributed to the interpretation of the findings and the writing of the manuscript. SMB, JPB, JFL, FJ, VM, JCR, and JM included subjects, collected data locally and contributed to the interpretation of the findings. JJ performed statistical analysis and contributed to the writing of the manuscript. PS contributed to the design and organization of the study and the interpretation of the findings.

All authors approved the final manuscript.

Acknowledgments

We thank the parents who participated in this study. We also thank Annie Ple from CIC-1403-Inserm-CHU for logistical assistance; Dr Patrick Gele from CRB-CIC-1403-Inserm-CHU for biological sample storage and management; Brigitte Poras, José Cano-Morales, Stéphane Deschilder, Nathalie Duquesnoy, Jeanne-Marie Descamps-Ben, Valérie Held, Isabelle Chardon, and Laurence Crinon for DEXA acquisition. Dr Georges Lion is gratefully acknowledged for DEXA interpretation. We gratefully acknowledge the Nestlé Biostatistics & Data Management team for statistical analysis.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.clnu.2018.05.015>.

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