Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effects on stool biochemistry, stool characteristics, and bone mineralization[1–3]

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ABSTRACT

Background: The low *sn*-2 palmitate content of infant formulas results in formation of fatty acid calcium soaps in the stools and reduced calcium absorption.

Objective: Our objective was to test the hypotheses that increasing the proportion of *sn*-2 palmitate in formula for term infants would result in greater skeletal mineral deposition and reduced stool hardness.

Design: Healthy term neonates were randomly assigned to receive standard formula (n = 103) or formula containing 50% *sn*-2 palmitate (high–*sn*-2 formula; n = 100) for 12 wk. One hundred twenty breast-fed infants were also studied. The main outcome measures were *1*) radial (single-photon absorptiometry) and whole-body (dual-energy X-ray absorptiometry) bone mineral content (WBBMC) at 12 wk and *2*) stool frequency, volume, and consistency at 6 and 12 wk. Secondary outcome measures included stool fatty acid content.

Results: Infants receiving high–*sn*-2 formula had higher WBBMC (128.1 ± 9.7 compared with 122.7 ± 10.1 g, adjusted for size and sex), softer stools at 6 and 12 wk, and a lower proportion of stool soap fatty acids than did infants receiving the control formula. Breast-fed infants had adjusted WBBMC values (128.3 ± 9.1 g) similar to those of infants fed high–*sn*-2 formula and significantly higher than those of infants fed the control formula.

Conclusions: Changing the stereoisomeric structure of palmitate in infant formula resulted in higher WBBMC, reduced stool soap fatty acids, and softer stools more like those of breast-fed infants. The greater bone mass measured could be important if it persists beyond the trial period; this merits further investigation. *Am J Clin Nutr* 1999;70:920–7.

KEY WORDS Term infants, full-term infants, synthetic triacylglycerol, palmitate, stool characteristics, stool biochemistry, bone mineralization, infant formula

INTRODUCTION

Triacylglycerol is the main source of energy in both breast milk and infant formula, providing ≈50% of dietary energy (1). However, the stereoisomeric structure of human milk triacylglycerol differs significantly from that of infant formula triacylglycerol (2). Palmitate constitutes about one-quarter of the fatty acids in human milk, with 70% in the *sn*-2 (middle) position on the glycerol backbone. In this position, palmitate is generally not hydrolyzed by pancreatic lipase, and the remaining 2-monoacylglycerol, which forms mixed micelles with bile salts, is well absorbed (3–5). In contrast, palmitic acid in the *sn*-1 and *sn*-3 positions, the predominant form found in cow milk and infant formulas, is hydrolyzed by pancreatic lipase; the resulting free palmitic acid may form calcium–fatty acid complexes, which are poorly absorbed (6, 7). This would be expected to result in lower fatty acid and calcium absorption from infant formulas than from human milk, despite similar total fatty acid contents. The formation of calcium soaps in the gut may partly explain the substantial differences in bowel habit and stool consistency between breast- and formula-fed infants (8, 9). Parents of breast-fed infants report fewer concerns about the hardness of their infants' stools and consequently seek less advice on this topic from health professionals (10).

Previous studies that have looked at the effect of altering the *sn*-2 palmitate content of infant formulas have produced conflicting results (2, 4, 11, 12). However, synthetic triacylglycerols are now available with various proportions of palmitate in the *sn*-2 position (13–15), making it more feasible to examine the influence of fatty acid configuration on outcome. In preterm infants, use of a formula containing synthetic triacylglycerol resulted in improved fatty acid and calcium absorption (13, 14) and a reduction in the formation of insoluble calcium soaps in

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the stools (13), whereas in term infants it reduced fatty acid and calcium excretion and resulted in softer, less-formed stools (15).

In this study, we hypothesized that because increasing the proportion of palmitate in the *sn*-2 position in a formula for term infants should reduce the excretion of palmitate and associated calcium soaps in the stool and increase calcium absorption, reduced stool hardness, reduced parental concern regarding stool hardness, and greater skeletal mineral deposition should result.

SUBJECTS AND METHODS

Subjects and trial design

Two hundred three formula-fed infants were recruited from the Rosie Maternity Hospital in Cambridge, United Kingdom. All subjects were term infants (≥37 wk gestation) with birth weights above the 5th percentile. Subjects were enrolled within the first 8 d of life (after the mother had unequivocally decided to formula feed) and were randomly assigned to receive 1 of 2 formulas by using a double-blind random permuted block allocation with dietary assignments identified by bar code. Subjects completed the study at 12 wk. A reference group of 120 term breast-fed infants with birth weights above the 5th percentile were enrolled at 10 wk of age and completed the study at 12 wk. Ethical approval was obtained from the Cambridge Health Authority and local Medical Research Council ethics committees.

Trial diets

The composition of the trial diets is shown in **Table 1**. Both formulas were supplied by Nutricia (Cuijk, Netherlands) and had similar concentrations of palmitate as a percentage of total fatty acids. However, the experimental formula with the synthetic triacylglycerol (Betapol; Loders Croklaan BV, Wormerveer, Netherlands; high–*sn*-2 formula) contained 50% of the palmitate in the *sn*-2 position compared with 12% in the *sn*-2 position in the control formula. The Betapol fat blend was prepared by enzymatic interesterification of fractionated palm oil with the fatty acids derived from high-oleic sunflower oil. The remainder of the fat in the high–*sn*-2 formula and that in the control formula was a blend of 4 vegetable oils. Both formulas contained similar salts, including calcium salts (≈45% of calcium from calcium carbonate, 52% from milk, and the remainder from calcium chloride and water). Analytic values showed that attempts to standardize the compositions of the 2 formulas were generally successful, although the control formula had slightly less fat (and therefore energy) than the high–*sn*-2 formula (39 compared with 42 g/L). The packaging of each formula was identical except for differences in the bar codes; both investigators and parents were blinded to the dietary allocation. Data analyses were performed with the dietary groups coded and the code was not broken until all analyses had been completed.

Data collection

The main outcomes were bone mineral content (BMC) at 12 wk of age, measured by single-photon absorptiometry (SPA) and dual-energy X-ray absorptiometry (DXA), and stool characteristics (assessed by 7-d diaries at 6 and 12 wk of age). Secondary outcome measures were parental concerns regarding infant stools (at 3, 6, and 12 wk of age), infant growth, clinical events, tolerance, and safety. Stool biochemistry was assessed in a subset of infants.

TABLE 1
Composition of study formulas (per 100 mL)[1]

	High–*sn*-2 formula	Control formula
Energy		
(kJ)	305	293
(kcal)	73	70
Protein (g)	1.6	1.6
Fat (g)	4.2	3.9
Carbohydrate (g)	7.1	7.1
Lactose (g)	7.1	7.1
Fatty acids (% by wt of total fatty acids)		
8:0	1.5	1.6
10:0	0.4	0.4
12:0	12.4	10.3
14:0	4.7	4.9
16:0	20.1[2]	19.6[3]
18:0	3.1	3.9
18:1n−9	42	41.3
18:2n−6	13	12.1
18:3n−3	1.6	1.9
Other fatty acids	1.2	4.0

[1] Other constituents of high–*sn*-2 and control formulas, respectively: calcium (mg) 57, 54; phosphorus (mg) 33, 32; sodium (mg) 23, 23; potassium (mg) 74, 76; chloride (mg) 44, 45; magnesium (mg) 6.3, 6.3; iron (mg) 0.57, 0.51; zinc (mg) 0.49, 0.45; iodine (μg) 10, 10; manganese (μg) 5.2, 5.1; copper (μg) 45, 44; vitamin A as retinol (μg retinol equivalents) 95, 92; vitamin D (μg) 1.1, 1.1; vitamin E (mg tocopherol equivalents) 1.1, 1.1; vitamin K (μg) 5, 5; thiamine (μg) 40, 40; riboflavin (μg) 100, 100; vitamin B-6 (μg) 40, 40.

[2] Approximately 50% in the *sn*-2 position.

[3] Approximately 12% in the *sn*-2 position.

Main efficacy outcomes

Bone data. BMC and bone mineral density (BMD) of the distal forearm were measured by using SPA (Lunar SP2; Lunar Radiation Corp, Madison, WI) at age 12 wk. Two traverses were made in the same position at the distal one-third site of the right ulna—with the forearm wrapped in a strip of compound with a density equivalent to tissue. The radiation exposure for each scan was <0.18 μSv.

Whole-body BMC and BMD were measured by using DXA at 12 wk of age (Hologic QDR 1000/W; Hologic Inc, Waltham, MA). Infants were measured naked in a supine position without sedation on a pediatric platform that attenuated the normal beam. Each scan took ≈10 min and the radiation exposure per scan was 3.5 μSv. Quality-control scans were performed each day by using the manufacturer's spine phantom. Scans were analyzed by using Hologic v5.61P software. DXA was not available when the study commenced; the number of infants who had DXA scans was therefore less than the number who had SPA scans.

Stool characteristics. At 6 and 12 wk of age, mothers kept infant stool diaries for 7 d. Each stool passed by the infant was coded for consistency and volume by using previously validated color photos for stool consistency (runny, mushy, formed, or hard) and diagrams for stool volume (8).

Secondary efficacy outcomes

Parental concerns about stools. Mothers completed a questionnaire using point analogue scales (9) concerning different aspects of their infants' stools at 3, 6, and 12 wk of age. Mothers of breast-fed infants completed the stool diary and questionnaire

at age 12 wk only. At each visit, mothers were asked if they had sought any advice from a health professional regarding their infants' bowel movements, and, if so, this was recorded.

Stool biochemistry. Stool samples were collected at age 6 wk from a subgroup of 41 formula-fed infants (21 in the control formula and 20 in the high–*sn*-2 formula groups) and 22 breast-fed infants. Breast-fed infants in this subset were not necessarily the same as those measured at 10 and 12 wk (although all were born at term with birth weights above the 5th percentile); if the mother was still breast-feeding at 10 wk she was invited to complete the measurement portion of the study. Mothers collected all their infants' stools until they had obtained the required volume for analysis (generally for 24–48 h, depending on the frequency of the baby's bowel movements). The samples were collected from the home and freeze-dried. Stool fatty acid soap analysis was then performed as described previously (8).

Briefly, nonsoap fatty acids and neutral lipids were first extracted from the freeze-dried sample by refluxing it for 16 h with petroleum ether (40–60 °C boiling point). This procedure does not extract fatty acid soaps. The residue was dried, mixed with 20 mL acidified petroleum ether, and allowed to stand for 2 h to solubilize the fatty acid soaps by converting them to free fatty acids. The sample was then refluxed with petroleum ether for 16 h and the acid-soluble lipids were recovered quantitatively. A known amount of 17:0 was added as an internal standard and preparative thin-layer chromatography was performed to separate the fatty acid fraction of the lipids. Plates were developed in petroleum ether:diethyl ether:formic acid and the lipid bands were visualized under ultraviolet light by spraying the plate with a methanolic solution of 2,7-dichlorofluorescein. The fatty acid bands were recovered, converted to methyl esters, and analyzed by capillary gas chromatography.

Safety and tolerance outcomes

Growth data. Formula-fed infants were measured at enrollment and at 3, 6, and 12 wk of age. Breast-fed infants were measured at 10 wk (enrollment) and 12 wk of age only. Weight was measured to the nearest 10 g by using SECA infant scales (North Bend, WA) and length to the nearest 1 mm by using a horizontal stadiometer. Triceps and subscapular skinfold thicknesses were measured to the nearest 0.1 cm by using Harpenden skinfold calipers (Holtain Ltd, Crymych, United Kingdom). Head circumference and midupper arm circumference were measured to the next millimeter by using encircling paper tapes (Child Growth Foundation, London).

Clinical events. At 3, 6, and 12 wk of age, data were collected about the infants' general health, including the number of upper respiratory tract infections, lower respiratory tract infections requiring antibiotics, visits to the family doctor, hospital outpatient visits, hospital admissions, and the use of medications.

Tolerance data. At 3, 6, and 12 wk of age, mothers were asked to report daily for 7 d the duration of crying and whether they thought their baby had colic, and to keep diaries of the amount of formula consumed. The age of weaning, consumption of any solids during the third, sixth, or twelfth week of life and adverse events were also recorded. Any reason given by the mother was recorded if an infant was withdrawn from the study or was changed to a different formula. We attempted to collect outcome data and to see all infants at 12 wk of age for bone densitometry even if they had not completed consumption of 12 wk of the formula randomly assigned to them.

Statistical analyses

Sample size (120 per group) was calculated on the basis of our own and published data to detect plausible differences in stool hardness and constipation between the study groups at 5% significance and 80% power. This sample size would enable a plausible 0.365-SD difference in radial BMC between randomly assigned groups to be detected at 5% significance and 80% power. For the DXA measurements, 40 infants per group would permit the detection of a 0.6-SD difference. Data from a pilot study (10) also confirmed that the sample size would be adequate to detect differences in parental concerns about stool hardness.

Differences between randomly assigned groups were examined by using Student's *t* test for normally distributed data, nonparametric tests for nonnormally distributed data, and chi-square tests for categorical data. Analyses were performed on an intention-to-treat basis (as randomized), then separately for those infants who had received the study formula for the entire 12 wk. Analysis of variance was used to examine differences in outcome variables between the 3 groups (2 formula-fed groups plus the breast-fed reference infants) with post hoc pairwise comparisons by Bonferroni test.

BMC and BMD measurements were analyzed both as unadjusted values (ie, the values produced by the machine) and also after correction for bone and body size and sex. BMC is highly influenced by both bone and body size and provides little meaningful information without reference to these variables (16, 17).

RESULTS

Randomized trial

One hundred infants were randomly assigned to the high–*sn*-2 formula and 103 to the control formula. There were no significant differences in birth, demographic, or anthropometric data between the 2 groups at randomization (**Table 2**).

Main efficacy outcomes

Bone densitometry. There were no significant differences between the 2 formula groups in radial BMC (0.088 compared with 0.098 g/cm^2 for the high–*sn*-2 and control formula groups, respectively) or bone width (0.517 compared with 0.521 cm for the high–*sn*-2 and control formula groups, respectively) measured by SPA either before or after adjustment for body size (current weight and length) and sex. Unadjusted whole-body BMC and BMD measured by DXA were not significantly different between the 2 formula groups (**Table 3**). However, after appropriate adjustments for sex and current body size (weight, length, and bone area at 12 wk), infants receiving control formula had lower BMC than infants receiving high–*sn*-2 formula. Similarly, BMD was greater for infants receiving the high–*sn*-2 formula than for those receiving the control formula.

Further analysis of subjects who completed 12 wk of the trial diet. Some infants seen at 12 wk had not continued to receive the study formula. When the analyses were confined to those who completed 12 wk of the study diet, the differences in whole-body BMC and BMD were greater (Table 3). Results from SPA were unchanged compared with those when data from all infants were analyzed.

Stool characteristics. Infants consuming the high–*sn*-2 formula passed more runny and fewer formed stools at both 6 and 12 wk of age. There were no differences between groups in the number of

TABLE 2
Baseline characteristics of subjects according to feeding group

	High–*sn*-2 formula (*n* = 54 M, 46 F)	Control formula (*n* = 65 M, 38 F)	Breast-fed (*n* = 65 M, 55 F)
General characteristics			
Birth weight (kg)	3.48 ± 0.46[1]	3.57 ± 0.42	3.48 ± 0.39
Gestation (wk)	39.9 ± 1.3	40 ± 1.3	39.9 ± 1.3
Age when meconium passed (d)	1.08 ± 0.3	1.06 ± 0.3	1.16 ± 0.4[2]
Age at randomization (d)	6.1 ± 1.8	5.6 ± 2.0	(10 wk)
Maternal characteristics			
Age (y)	27.9 ± 5.3	26.5 ± 5.4	31.6 ± 4.5[3]
Number of pregnancies	2.2 ± 1.3	2.4 ± 1.4	2.2 ± 1.4
Percentage from social classes 1 and 2 (%)[4]	27	18	78[3]
Anthropometric measures at randomization			
Weight (kg)	3.44 ± 0.46	3.53 ± 0.42	—
Length (cm)	51.3 ± 2.2	51.4 ± 1.9	—
Head circumference (cm)	35.1 ± 1.4	35.4 ± 1.3	—
Midupper arm circumference (cm)	10.5 ± 0.8	10.6 ± 0.7	—
Triceps skinfold thickness (mm)	4.9 ± 1.0	5.0 ± 0.9	—
Subscapular skinfold thickness (mm)	5.1 ± 1.3	5.1 ± 1.4	—

[1] $\bar{x}$ ± SD.
[2,3] Significantly different from other groups (ANOVA): [2] $P < 0.05$, [3] $P < 0.001$.
[4] The 2 highest social classes.

stools per week or the mothers' estimation of total stool volume per week or volume per stool. Thirty-seven infants (20 infants in the high–*sn*-2 and 17 in the control formula group) who completed 12 wk of the trial formula had started solids (at a median of 10 wk): differences in stool consistency between these infants fed different formulas were reduced compared with those who remained exclusively formula fed.

Secondary efficacy outcomes

Parental concerns about stools. Use of the high–*sn*-2 formula did not influence the proportion of mothers expressing concern about how often their infants passed a stool (at 3 wk: 15% of high–*sn*-2 formula group mothers compared with 18% of control formula group mothers; NS; at 6 wk: 25% compared with 16%; NS; at 12 wk: 28% compared with 31%; NS) or stool hardness (at 3 wk: 29.8% of high–*sn*-2 formula group mothers compared with 34.1% of control formula group mothers; NS; at 6 wk: 32.9% compared with 24.7%; NS; at 12 wk: 17.9% compared with 18.9%; NS). However, a greater proportion of the mothers using the high–*sn*-2 formula were concerned about runny stools at the age of 3 wk (41.9% compared with 16.5%; $P < 0.001$), 6 wk (39.3% compared with 18.0%; $P = 0.002$), and 12 wk (30.3% compared with 16.2%; $P = 0.04$). This difference was not seen in the small group of infants who had started solids by 12 wk but continued to receive the study formula. There was no significant difference between groups in the number of parents requesting advice from health professionals regarding their infants' stools.

Stool biochemistry. Total and individual fatty acids (8:0–24:1) were expressed as percentages (% by wt) of each sample (**Table 5**). The stools of infants receiving the high–*sn*-2 formula contained 24.8% fatty acids total, and those of the control group 34.9% (95% CI: 17.9, 22.4; $P = 0.013$). The soap and nonsoap portions of each sample were analyzed for individual fatty acids from 12:0 to 20:0. There were no significant differences between the formula groups for the nonsoap fatty acids. There were highly significant differences between the groups in soap saturated fatty acids; in particular, the proportions of stearate (18:0), palmitate (16:0), and myristate (14:0) were significantly lower in the group receiving the high–*sn*-2 formula than in the control group.

Safety and tolerance outcomes

Growth. Among the formula-fed infants, there were no significant differences in anthropometry at the time of randomization, or at 3, 6, or 12 wk of age (**Table 6**). Infants receiving control formula who completed 12 wk of the trial diet were slightly heavier and longer than the infants receiving the high–*sn*-2 formula, but the difference was not significant at the 5% level.

Clinical events. A total of 43 formula-fed infants stopped receiving the study formula before the end of the 12-wk study: 20 in the high–*sn*-2 formula group and 23 in the control formula group. Eight infants in the high–*sn*-2 formula group and 9 in the control formula group were switched to a casein-dominant formula because it was felt that the infant was hungry, 2 in the high–*sn*-2 formula group and 1 in the control formula group switched formulas because they were "very constipated," and 1 in the high–*sn*-2 formula group and 5 in the control formula group switched formulas because of vomiting. The mothers of 9 infants receiving the high–*sn*-2 formula and 8 receiving the control formula gave no specific reason for switching formulas.

There were no significant differences between randomly assigned groups in the number of reported upper respiratory tract infections, lower respiratory tract infections requiring antibiotics, visits to the family doctor, hospital outpatient visits, or hospital admissions.

Tolerance. At 3 and 12 wk of age, infants receiving control formula consumed more formula than did those receiving high–*sn*-2 formula (3 wk: 716 ± 132 compared with 674 ± 136 mL/d; 95% CI: 3, 81; $P = 0.04$; 12 wk: 827 ± 142 compared with 768 ± 154 mL/d; 95% CI: 10, 106; $P = 0.02$). However, energy intakes were not significantly different between groups

at any time point. Forty-four infants started solids before 12 wk of age (25 in the high–*sn*-2 formula group and 19 in the control formula group). The median age at which solids were introduced was 10 wk for both groups. Research staff were not involved in the decision to introduce solids (current United Kingdom recommendations are that solids should be introduced between 4 and 6 mo of age).

At each visit, mothers were asked "Do you think that your baby has colic?" Mothers of infants receiving high–*sn*-2 formula reported more colic at 3 wk of age than did those receiving control formula (32% compared with 18%; $P = 0.02$); however, this difference had disappeared by 6 wk. At 12 wk both groups reported a similar rate of colic. The reported duration of crying each day was not significantly different between groups at any age.

Comparison of formula-fed groups with breast-fed reference group

As expected, mothers of breast-fed infants were older and of significantly higher social class than were the mothers of formula-fed infants (Table 2).

Main efficacy outcomes

Bone densitometry. There were no significant differences in radial BMC or BMD between the breast-fed reference group and the formula-fed groups. However, breast-fed infants had adjusted whole-body BMCs and BMDs that were similar to those of the high–*sn*-2 formula-fed infants and significantly higher than those of the infants receiving control formula (Table 3).

Stool characteristics. At 12 wk, breast-fed babies passed more runny or watery stools than either formula group (Table 4). Breast-fed infants also passed a significantly greater number of stools per week and the mothers' estimation of volume per individual stool and total stool volume per week was significantly greater.

Secondary efficacy outcomes

Parental concerns about stools. Twelve percent of breast-feeding mothers were concerned about runny stools compared with 30% of those using the high–*sn*-2 formula ($P = 0.009$) and 16% using the control formula ($P = 0.6$). Breast-feeding mothers made fewer requests for advice regarding their infants' stools than did the mothers using either formula ($P = 0.006$).

Stool biochemistry. The stools of breast-fed infants contained less fatty acids than did those of the high–*sn*-2 formula-fed group (Table 5). Their stools also had significantly lower saturated fatty acid content in soaps (12:0–20:0) when compared with the high–*sn*-2 formula-fed group (Table 5).

Growth. There were minor differences in anthropometry between the formula- and breast-fed groups (Table 6). Formula-fed infants had greater subscapular skinfold thicknesses than breast-fed infants (7.3 compared with 6.8 mm; 95% CI: 0.2, 0.9; $P < 0.04$).

DISCUSSION

We found that, as hypothesized, the addition to infant formula of synthetic triacylglycerol with a greater proportion of palmitate in the *sn*-2 position resulted in a lower proportion of palmitic acid in stool fats, reduced stool hardness, and stool characteristics and biochemistry intermediate between that of breast-fed infants and those fed a standard formula. Of most biological interest was the higher whole-body bone mass in infants fed high–*sn*-2 formula. Palmitic acid in the stools forms soaps with calcium, resulting in poor calcium absorption. Our own (14) and other previous studies (4, 13, 15) showed an increase in calcium absorption with greater *sn*-2 palmitate in the diet. However, no study had established whether the extra available calcium had any significant biological effect on the infant rather than simply resulting in an increase in calcium excretion. It is for this reason that we focused on bone mineralization rather than calcium absorption.

TABLE 3
Bone densitometry results (by dual-energy X-ray absorptiometry) according to feeding group

Bone measures	High–*sn*-2 formula ($n = 42$ for all, 34 for completers)	Control formula ($n = 40$ for all, 37 for completers)	Difference between formula groups (95% CI)	Breast-fed ($n = 69$)
All subjects				
Unadjusted figures				
BMC (g)	125.8 ± 18.5[1]	125.8 ± 23.3	0.005 (−9.2, 9.3)	128.0 ± 19.9
BMD (g/cm^2)	0.244 ± 0.022	0.236 ± 0.022	0.008 (−0.00097, 0.02)	0.245 ± 0.022
Bone area (cm^2)	507 ± 72	531 ± 62	−24 (−54, 6)	520 ± 49
Adjusted figures				
BMC (g)	128.1 ± 9.7	122.7 ± 10.1	5.4 (1.0, 9.8)[2]	128.3 ± 9.1[3,4]
BMD (g/cm^2)	0.244 ± 0.019	0.235 ± 0.019	0.009 (0.0006, 0.02)[5]	0.246 ± 0.017[3,6]
Subjects who completed the study				
Unadjusted figures				
BMC (g)	127.0 ± 19.0	126.4 ± 23.5	0.6 (−9.6, 10.7)	128.0 ± 19.9
BMD (g/cm^2)	0.247 ± 0.022	0.236 ± 0.023	0.01 (0.00047, 0.02)[5]	0.245 ± 0.022
Bone area (cm^2)	513 ± 53	533 ± 63	−20 (−48, 7.8)	520 ± 49
Adjusted figures				
BMC (g)	129.2 ± 9.3	123.0 ± 9.1	6.2 (1.9, 10.5)[7]	128.7 ± 9.1[3,8]
BMD (g/cm^2)	0.247 ± 0.017	0.235 ± 0.018	0.012 (0.004, 0.02)[9]	0.246 ± 0.017[3,10]

[1] $\bar{x}$ ± SD. BMC, bone mineral content; BMD, bone mineral density; completers, those who completed 12 wk of the study formula. Adjusted figures are adjusted for current weight, length, bone area, and sex.

[2,5,7,9] Significant difference between formula groups (Student's *t* test): [2] $P = 0.05$, [5] $P = 0.04$, [7] $P = 0.02$, [9] $P = 0.009$.

[3] Breast-fed group and group fed high–*sn*-2 formula significantly different from control formula group, $P < 0.02$ (Bonferroni test).

[4,6,8,10] Significant difference between 3 feeding groups (ANOVA): [4] $P = 0.01$, [6] $P = 0.006$, [8] $P = 0.004$, [10] $P = 0.003$.

TABLE 4
Mothers' estimation of stool consistency and volume at 6 and 12 wk for all infants according to feeding group

Stool characteristic	High–*sn*-2 formula 6 wk (*n* = 84)	High–*sn*-2 formula 12 wk (*n* = 75)	Control formula 6 wk (*n* = 87)	Control formula 12 wk (*n* = 73)	Breast-fed[1] (*n* = 104)
Stool consistency[2]					
Hard or formed (%)	0 (0, 35)	7 (0, 40)	33 (0, 73)[3]	20 (0, 57)	0 (0, 0)[4]
Mushy (%)	50 (23, 88)	57 (32, 92)	60 (22, 88)	69 (40, 92)	0 (0, 0)[4]
Runny or watery (%)	14 (0, 50)	7 (0, 29)	0 (0, 0)[5]	0 (0, 0)[6]	100 (100, 100)[4]
Stool consistency score[2,7]	3.0 (2.7, 3.3)	3.0 (2.8, 3.4)	3.3 (3.0, 3.7)[5]	3.2 (3.0, 3.6)[8]	2.0 (2.0, 2.0)[4]
Total stool volume per week (mL)[2]	53 (38, 85)	60 (40, 95)	69 (45, 94)	68 (45, 95)	173 (95, 243)[9]
Volume per stool (mL)[10]	6.5 ± 2.8	7.3 ± 3.1	6.9 ± 3.4	6.9 ± 2.7	8.7 ± 3.5[9]
Stools per week[10]	11.9 ± 9.6	11.3 ± 8.1	12.8 ± 7.2	12.4 ± 7.3	—

[1] Data collected at 12 wk only.
[2] Median (25th, 75th percentile).
[3,5] Significantly different from high–*sn*-2 formula at 6 wk (Mann-Whitney *U* test): [3] $P = 0.004$, [5] $P < 0.001$.
[4] Significantly different from both formula groups, $P < 0.05$ (Kruskal-Wallis test).
[6,8] Significantly different from high–*sn*-2 formula at 12 wk (Mann-Whitney *U* test): [6] $P < 0.001$, [8] $P = 0.003$.
[7] Calculated by scoring each stool (5 = hard, 1 = watery) and dividing by the number of stools passed in the week.
[9] Significantly different from both formula groups, $P < 0.02$ (Bonferroni test).
[10] $\bar{x}$ ± SD.

We presented both unadjusted bone densitometry data and also these data after bone and body size and sex were corrected for. BMC is strongly influenced by both bone and body size and provides little meaningful information without reference to these variables. BMD is used in an attempt to correct BMC for size. However, this assumes that BMC and bone area are directly proportional, which is often not the case, with the result that BMD fails to fully correct for body size. For this reason, we adjusted BMC for bone area, weight, and height (16, 17).

High–*sn*-2 formula-fed infants and those who were breast-fed had whole-body BMC and BMD values ≈0.5 SD greater than those of infants fed control formula, corresponding to ≈10% of the population variation in BMC. Such a difference could be biologically important in population terms if it persists beyond the study period. We were unable to detect a difference in radial BMC using SPA. Because most mineral accretion during the first year of life occurs in the skull and spine, it may be that differences between groups in forearm bone mineralization were too small to detect.

The influence of the position of palmitate in triacylglycerols has been investigated extensively in animals and humans with conflicting results (2, 4, 5, 11–15). In recent years, this issue has been

TABLE 5
Stool fatty acid composition according to feeding group

	Control formula (*n* = 21)	High–*sn*-2 formula (*n* = 20)	Breast-fed (*n* = 22)	95% CI of control compared with high–*sn*-2 formula	95% CI of breast-fed compared with high–*sn*-2 formula
	% by dry wt of total fatty acids				
Nonsoap saturated fatty acids					
12:0	0.20 ± 0.10[1]	0.15 ± 0.09	0.03 ± 0.08	−0.2, 0.3	−0.3, 0.06
14:0	0.21 ± 0.11	0.17 ± 0.10	0.08 ± 0.02	−0.2, 0.3	−0.3, 0.1
16:0	1.37 ± 0.69	1.32 ± 0.76	0.87 ± 0.19	−2.0, 2.1	−2.0, 1.1
18:0	0.32 ± 0.15	0.38 ± 0.16	0.73 ± 0.20	−0.5, 0.4	−0.2, 0.9
20:0	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	−0.04, 0.04	−0.04, 0.03
Total nonsoap fatty acids	5.51 ± 2.71	6.25 ± 3.27	4.81 ± 0.99	−9.3, 7.8	−8.1, 5.2
Soap fatty acids					
12:0	0.79 ± 0.08	0.62 ± 0.07	0.09 ± 0.02	−0.06, 0.4	−0.4, −0.7[2]
14:0	1.91 ± 0.16	0.93 ± 0.10	0.36 ± 0.05	0.6, 1.4[2]	−0.3, −0.8[2]
16:0	18.37 ± 1.47	9.29 ± 1.01	4.45 ± 0.48	5.4, 12.7[2]	−2.6, −7.0[2]
18:0	3.69 ± 0.29	2.40 ± 0.23	4.91 ± 0.40	0.5, 2.0[2]	1.6, 3.5[2]
20:0	0.23 ± 0.02	0.16 ± 0.02	0.12 ± 0.01	0.01, 0.2[3]	−0.008, −0.08[3]
Total 18:2	0.92 ± 0.28	1.20 ± 0.41	0.73 ± 0.12	−1.3, 0.7	−1.3, 0.4
Total 18:3	0.09 ± 0.03	0.09 ± 0.04	0.02 ± 0.01	−0.1, 0.1	−0.1, 0.002
Total soap fatty acids	29.51 ± 2.34	18.64 ± 1.72	13.17 ± 1.23	5.0, 16.8[2]	−1.2, −9.7[3]
Total fatty acids[4]	34.88 ± 2.64	24.82 ± 2.84	17.93 ± 1.65	17.9, 22.4[3]	−0.4, −13.4[3]

[1] $\bar{x}$ ± SE.
[2] $P \leq 0.001$.
[3] $P \leq 0.05$.
[4] Includes fatty acids not listed in the table.

TABLE 6
Comparison of anthropometric data according to feeding group at 12 wk of age[1]

	High–*sn*-2 formula (n = 75)	Control formula (n = 76)	Breast-fed (n = 109)
Weight (kg)	6.03 ± 0.64	6.12 ± 0.72	5.95 ± 0.63
Length (cm)	61.2 ± 2.4	61.7 ± 2.3	61.0 ± 2.1
Head circumference (cm)	40.6 ± 1.3	41.0 ± 1.3	40.7 ± 1.4
Midupper arm circumference (cm)	3.9 ± 0.9	3.8 ± 1.0	3.7 ± 1.1
Triceps skinfold thickness (mm)	8.7 ± 1.6	8.64 ± 1.5	8.35 ± 1.6
Subscapular skinfold thickness (mm)	7.53 ± 1.6	7.12 ± 1.6	6.8 ± 1.2[2]

[1] $\bar{x}$ ± SD. Data for infants who completed 12 wk of the study formula.
[2] Significantly different from formula groups, P = 0.004 (ANOVA).

examined by using synthetic triacylglycerols. In one small randomized crossover study, preterm infants fed a high–*sn*-2 formula had better absorption of myristic, palmitic, and stearic acids with lower stool calcium and higher urinary calcium than did those fed a standard preterm infant formula (13), although a similar study by Verkade et al (12) found no such effect. More recently, we reported increased fatty acid absorption (14), decreased stool calcium soap formation, and increased calcium absorption in preterm infants fed a high–*sn*-2 formula. In term infants, the addition of synthetic triacylglycerol to a formula resulted in reduced fatty acid and calcium excretion, and softer, less-formed stools (15).

Infants who received the high–*sn*-2 formula in the current study had reduced amounts of 14-, 18-, and 20-carbon fatty acid soaps, as well as palmitate soaps in their stools. This was noted previously (2, 18). The formation of palmitate soaps may reduce absorptive capacity in the gut by binding bile salts or by acting as a solvent for other fatty acids. Whatever the mechanism, prevention of palmitate soap formation is associated with better absorption of other saturated fatty acids. The high–*sn*-2 formula-fed group had stool soap fatty acid concentrations intermediate between those of breast-fed infants and those of infants fed the control formula, consistent with the fact that the percentage of *sn*-2 palmitate in the experimental formula, although higher than that in the control formula, was not as high as that in human milk (50% compared with 70%). This is also consistent with results of a previous study in term infants that used formulas with 12.6%, 40%, or 66% palmitic acid in the *sn*-2 position in which palmitic acid absorption increased in proportion to the percentage of *sn*-2 palmitate (15).

We showed previously that calcium fatty acid soaps are a major factor in determining stool hardness (8) and hypothesized that the high–*sn*-2 formula would be associated with less troublesome stool hardness. Although we found the expected reduction in stool hardness, paradoxically, this was not accompanied by reduced parental concern. Mothers of infants receiving high–*sn*-2 formula were concerned about their infants having runny stools and were more concerned about runny stools than were breast-feeding mothers, despite a much higher proportion of such stools in breast-fed infants. This may reflect maternal expectations about the consistency of their infants' stools, and this issue would obviously need to be addressed if synthetic triacylglycerol were added to infant formula in the future.

Although infants receiving high–*sn*-2 formula had softer stools, stool volume did not increase to that seen in breast-fed infants. This was not a balance study and it is impossible to say whether high–*sn*-2 formula increased the water content of the infants' stools. However, Carnielli et al (15) found that term infants fed a high–*sn*-2 formula had reduced stool hardness with lower stool volume, but no change in water content. This might be considered advantageous to mothers feeding formula because a potential increased volume of stools would be associated with an increase in diaper usage and consequent cost.

The high–*sn*-2 formula was well tolerated, with no significant effect on growth. Although it had been questioned previously whether the rearrangement of fatty acids in the experimental formula fat blend might reduce essential fatty acid absorption, we found no evidence of this; the proportions of linoleic (18:2n−6) and linolenic (18:3n−3) acids in the stools were the same for both formula groups.

In summary, as hypothesized, addition of a new fat blend containing synthetic triacylglycerol with palmitate in the *sn*-2 position to an infant formula resulted in significantly higher whole-body bone mass at 12 wk of age, in reduced fecal excretion of soap fatty acids, and in softer stools. These data raise the question of whether infant formulas should contain such synthetic triacylglycerols. Although their incorporation in formula resulted in softer stools, closer to those of breast-fed infants, this actually increased the concerns of parents about stool softness. Nevertheless, the more important biological effects on bone mass could have clinical relevance, particularly if these effects persist beyond the trial period.

A potential alternative strategy used in some American and European formulas for reducing stool fatty acid soap formation and enhancing calcium absorption is to reduce the palmitate content of the formula to amounts much lower than those found in breast milk. This has been reported to increase fat and calcium absorption (19). Clearly, further work is needed to explore the biological importance, if any, in both healthy and high-risk infants, of dietary palmitate, given that it is the predominant fatty acid in breast milk. If, in the absence of such data, an empirical objective is to mimic in formula the fatty acid profile of breast milk, use of synthetic triacylglycerol would appear to be a valid approach. Regardless of these considerations, our study illustrates the important general point that subtle differences in formula composition may have a significant effect on the physiologic response of infants. This emphasizes the need for research on the efficacy and safety of new formulations of breast milk substitutes even when these conform to international guidelines for overall nutrient composition.

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