

Breast-feeding and formula feeding in healthy term infants and bone health at age 10 years

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Abstract

Few studies have investigated the effects of infant nutrition on later bone health in term infants, although low sn-2 palmitate in infant formulas has been shown to result in the formation of stool fatty acid soaps, reduced Ca absorption and lower bone mass in the short term. To investigate the effect of (1) breast-feeding (BF) and (2) the type of infant formula (standard fat blend *v.* high-sn-2 fat blend) on bone mass at age 10 years, anthropometry and bone mass (from dual-energy X-ray absorptiometry (GE Lunar Prodigy); lumbar spine (LS) and total body less head; adjusted for size (bone mineral apparent density standard deviation score (SDS) and regression)) were measured in 10-year-old subjects born at term and either breast-fed (*n* 34) or randomised to a standard control formula (*n* 27) or a high-sn-2 palmitate formula (*n* 30) for the first 12 weeks of life. At follow-up, previously BF children were older but lighter (by 0.5 SDS, *P*=0.03) than formula-fed children with a lower LS bone mineral density SDS (by 0.44, *P*=0.03), but size-adjusted bone mass did not differ. There were no significant differences in bone mass between the formula-fed groups. These findings suggest that there is no significant effect of BF or high-sn-2 infant formula on size-adjusted bone mass in mid-childhood, and that the effects of infant nutrition on bone mass previously reported may be confined to the short term. A larger study would be required to exclude smaller effects.

Key words: Bone mass: Term infants: Breast-feeding: Infant formula: Synthetic TAG: Randomised trials

Osteoporosis is a major cause of morbidity and mortality worldwide, principally due to its association with fractures⁽¹⁾. Evidence that factors operating early in life may programme later bone mass and geometry and, perhaps, later osteoporosis risk has led to an increased focus on measures that may optimise the accretion of bone mass during fetal life, infancy and childhood⁽²⁾. Little research has focused specifically on the effect of infant diet, particularly in healthy term infants. In one prospective cohort study, higher bone mass was found at age 8 years in children previously breast-fed compared with those who had been formula-fed⁽³⁾. More recently, we reported an association between neonatal intake of human milk in preterm infants and whole-body bone mass in early adult life⁽⁴⁾.

Although the possibility of residual confounding remains in these observational studies, beneficial effects of breast milk on bone could result from one of the many growth factors or hormones present in human milk, or from differences in macro- or micronutrient content and bioavailability between human milk and infant formulas. Another plausible mechanism relates to structural differences in TAG in breast milk

and infant formula. Palmitate constitutes about one-quarter of the fatty acids in human milk, with 70% in the sn-2 (middle) position on the glycerol backbone. Most modern formulas contain a mixture of animal and vegetable oils, adjusted to mimic the pattern of fatty acid saturation and chain lengths found in breast milk. However, such fat mixtures have a reduced content of fatty acids esterified to glycerol in the sn-2 position and an increase in those esterified in the sn-1 and sn-3 positions. The latter undergo hydrolysis in the gut, releasing palmitic acid, which is poorly absorbed and tends to form Ca soaps; this may be partly responsible for the harder stools seen in formula-fed infants, and could influence Ca absorption.

To address this problem in formulas, two approaches have been used: reducing the palmitate content, or using a modified synthetic fat blend ('Betapol') with a high proportion of fatty acids esterified in the sn-2 position to mimic that found in human milk. A recent review identified nine publications in which the use of 'standard' preterm or term infant formulas was compared with either low-palmitate formulas or formulas containing a modified 'high-sn-2' TAG⁽⁵⁾. Standardised results

Abbreviations: BA, bone area; BF, breast-feeding; BMAD, bone mineral apparent density; BMC, bone mineral content; BMD, bone mineral density; LS, lumbar spine; SDS, standard deviation score.

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from these studies were consistently positive in favour of the low/synthetic palmitate groups with respect to intestinal fractional absorption of fat, palmitic acid and Ca. Total body bone mass was also significantly higher in two studies^(6,7), including our own randomised trial using a formula containing a high-sn-2 fat blend, in which whole-body bone mass at 12 weeks of age was similar in the intervention group to that of the breast-fed reference group⁽⁷⁾. These results suggest that increased Ca absorption results in measurable biological effects in the short term. However, it is not known whether such benefits persist, resulting in long-term improvements in bone mass which could have implications for later osteoporosis risk.

To investigate whether the type of infant feeding influences later bone mass, we performed a 10-year follow-up of subjects born at term and either breast-fed or randomised to use a standard formula or an experimental high-sn-2 infant formula for the first 12 weeks of life.

Methods

Original randomised trial: subjects and trial design

Full details of the original trial have been given elsewhere⁽⁷⁾; a summary is provided here. A total of 203 formula-feeding infants were recruited from the Rosie Maternity Hospital in Cambridge, UK. Subjects were term infants (≥ 37 weeks of gestation) with birth weight > 5 th percentile. They were enrolled within the first 8 d, after the mother had decided on her chosen mode of feeding and had started either breast- or formula feeding, and randomised to receive one of two formulas using a double-blind random permuted block allocation with dietary assignments identified by a barcode. A reference group of 120 term breast-fed infants with birth weights > 5 th percentile were also recruited. The mothers of these infants were approached soon after birth and re-contacted at 10 weeks; if still being exclusively breast-fed, they were invited to join the study. Subjects completed the study at 12 weeks. All infants completed the study at 12 weeks. Ethics approval was obtained from the Cambridge Health Authority and local Medical Research Council ethics committees. Parents gave written informed consent.

Trial diets

Both formulas were supplied by Nutricia and had similar concentrations of palmitate; however, the experimental formula with synthetic TAG ('Betapol', high-sn-2 formula; Lodders Crokiaan b.v) contained 50% of palmitate in the sn-2 position compared with 12% in the control formula. The Betapol fat blend was prepared by enzymatic interesterification of fractionated palm oil with fatty acids derived from high-oleic sunflower oil. Formula packaging was identical except for differences in the barcodes; investigators and parents were blinded to the dietary allocation.

Main outcomes in the initial trial were bone mineral content (BMC) at 12 weeks of age measured by single-photon

absorptiometry and dual-energy X-ray absorptiometry, and stool characteristics.

Follow-up at age 10 years

All subjects from the original cohort who could be traced were invited to participate in the follow-up study. Those willing to do so attended a research clinic in Cambridge (Wellcome Trust Clinical Research Facility; n 57) or London (Institute of Child Health; n 31). Written informed consent was obtained from the parent or guardian and assent from the child. The study was conducted according to the guidelines laid down by the Declaration of Helsinki, and all procedures involving human subjects were approved by the Eastern Multi-Centre Research Ethics Committee.

Weight was measured using digital scales and height using a wall-mounted stadiometer. Weight and height standard deviation scores (SDS) were calculated using UK population reference data. A FFQ quantified current Ca intake (Calquest⁽⁸⁾); a simple questionnaire determined hours of weight-bearing activity/week; and parents rated the child's activity level compared with his peers (rated 1–5: 1 = much less active; 5 = much more active). A general medical and fracture history was taken. Maternal and paternal height was reported by the mother.

Bone densitometry

Dual-energy X-ray absorptiometry (software version 8.1; Lunar Prodigy) was used to measure BMC, bone area (BA) and bone mineral density (BMD) at the lumbar spine (LS, L2–L4) and whole body. Subjects wore light indoor clothing after removing metal objects. Total radiation exposure was below daily background levels (approximately $7 \mu\text{Sv/d}$ in the UK). As recommended by the International Society for Clinical Densitometry⁽⁹⁾, we used 'total body less head' values for whole-body scans. The same make and model of the dual-energy X-ray absorptiometry scanner was used in both study sites and machines were cross-calibrated using the European Spine Phantom.

Statistics

In view of the significant cohort attrition, anthropometric and bone densitometry data were compared between the breast-fed reference group and the formula-fed groups using ANOVA or χ^2 as appropriate with variables transformed where necessary to ensure a normal distribution. Where a significant between-group effect was identified using ANOVA, *post hoc* pairwise comparisons were performed using the Bonferroni test. We adopted this approach rather than comparing previously randomised groups using the *t* test as our primary analysis, in view of the significant cohort attrition experienced (follow-up rate $\leq 30\%$). Bone mass was adjusted for size in three ways: (1) bone mineral apparent density (BMAD) of the LS, calculated as $\text{BMC}/\text{BA}^{1.5}$. BMAD SDS were calculated for age, sex and ethnic group using UK machine-specific reference data⁽¹⁰⁾; (2) for whole-body

bone mass, a two-stage procedure was used. The indices lean/height^{1.5} and BMC/lean^{1.2} were calculated using the power relationships required to remove any residual association with height determined using log–log regression; (3) multiple regression was used first to examine the effect of infant diet on later bone mass at skeletal sites after adjusting for age, sex, pubertal stage and body size (weight and height), and, second, to adjust for potential confounding factors, including current physical activity and Ca intake.

Results

At follow-up, thirty-four breast-fed subjects and fifty-seven formula-fed subjects were seen (thirty previously randomised to the high-sn-2 formula and twenty-seven to the control formula). The overall follow-up rate was 28, 30 and 26% of subjects enrolled for the breast-fed, high-sn-2 and control groups, respectively, with corresponding rates of 31, 35 and 31% for infants seen at 12 weeks of age (Fig. 1). Reasons for non-participation for the remaining subjects were as follows: declined to take part (*n* 18); did not reply to two invitation letters (*n* 139); agreed to take part but did not attend appointments (*n* 24); moved abroad (*n* 10); general practitioner advised contact would not be appropriate (*n* 6); not traceable (*n* 35). There were no significant differences in baseline characteristics or outcome measures at 12-week follow-up between the formula-fed or breast-fed subjects seen or not seen at the 10-year follow-up (Table 1). At follow-up, one child from the breast-fed group, four from the high-sn-2 group and two from the control group were reported to have asthma treated with inhaled bronchodilators; four of these children (one breast-fed, two high sn-2 and one control) also used inhaled corticosteroids. Also, eight previously breast-fed children and three formula-fed children (one high-sn-2 and two controls) were reported to be taking a multivitamin supplement including vitamin D. Estimated daily Ca intake and time spent in vigorous

physical activity were not significantly different between the groups. A significantly greater proportion of the breast-fed group came from higher socio-economic backgrounds.

The breast-fed group was significantly older when seen for follow-up compared with both formula-fed groups (*post hoc* $P=0.005$ for breast-feeding (BF) *v.* high sn-2; $P\leq 0.001$ for BF *v.* control) and a lower proportion were pre-pubertal (32 *v.* 60% for the high sn-2 group and 67% for the control group; $P=0.02$). However, the control formula subjects had significantly higher weight SDS than the breast-fed subjects (*post hoc* $P=0.041$), with intermediate values for the high-sn-2 group (*post hoc* $P=0.6$ *v.* BF group). Although there was a trend towards higher LS BMD SDS in the high-sn-2 group compared with the breast-fed reference group (*post hoc* $P=0.06$), the BMAD SDS did not differ significantly between the breast-fed and formula-fed groups (Table 2). Furthermore, in models with adjustment for body size (weight and height), sex, pubertal status (pre-pubertal *v.* pubertal) and BA, there were no significant differences in bone mass at any skeletal site between the dietary groups, nor any significant interaction between sex and milk group or pubertal status and milk group. Significant predictors in these models were BA for LS BMC, and BA, height and sex for total body BMC. There were no significant differences between the groups in the number of children who reported a previous fracture, nor in estimated current daily Ca intake.

Comparing data for the pooled formula-fed groups and the breast-fed group (Table 3), breast-fed children were significantly older with a significantly higher proportion in pubertal stage 2 or above (68 *v.* 37%), but they were also significantly lighter (by 0.5 SDS, $P=0.03$) with significantly lower LS BMD SDS (by 0.44, $P=0.03$). However, size-adjusted LS bone mass (BMAD SDS) was not significantly different between the groups, and after adjusting for bone and body size and sex, there were no differences in whole-body or LS bone mass between the groups.

Discussion

We previously reported that healthy breast-fed infants had bone mass similar to that of infants who received a high-sn-2 fat blend infant formula, and higher than that of infants fed a standard term formula⁽⁷⁾. At follow-up, the breast-fed reference group was significantly older and lighter, with a trend towards lower LS BMD *z*-scores. However, there were no significant differences in bone mass between the formula-fed and breast-fed subjects after adjusting for size. Only two previous studies have reported on the long-term effects of BF on the offspring's bone health. Jones *et al.*⁽³⁾ studied 330 8-year-old children from a Tasmanian cohort and reported that those who were born at term and who had been breast-fed had significantly higher BMD at the femoral neck (SD +0.26), LS (SD +0.34) and whole body (SD +0.41) compared with those not breast-fed. This effect remained after adjusting for size, lifestyle and socio-economic factors, but was only seen in those breast-fed for more than 3 months. There was also some evidence for a 'dose–response' effect in that BMD was highest in subjects reported to

Randomised trial			
	Betapol	Control	Breast-fed
Randomised	100	103	
Enrolled			120
Seen at 12 weeks	86	87	109
DXA scan	42	40	69
Seen at 10 years	30	27	34
Male			
<i>n</i>	17	14	16
%	57	52	47
Percentage of those recruited	30	26	28
Percentage of those seen at 12 weeks	35	31	31

Fig. 1. Study flow chart. DXA, dual-energy X-ray absorptiometry.



Table 1. Baseline characteristics for the formula-fed and breast-fed subjects seen v. not seen at 10 years (Mean values and standard deviations)

	Breast-fed infants				Formula-fed infants			
	Seen (<i>n</i> 34)		Not seen (<i>n</i> 86)		Seen (<i>n</i> 57)		Not seen (<i>n</i> 14)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Birth weight (g)	3507	408	3475	391	3547	421	3512	449
Gestational age (weeks)	39.9	1.1	39.9	1.4	39.9	1.2	40.0	1.4
Age at enrolment or randomisation (d)	70	5	70	4	6.1	1.9	5.9	1.8
Maternal characteristics								
Age (years)	30.7	3.8	32.0	4.7	28.1	4.7	26.8	5.6
% social class 1 or 2	85		76		25		21	
At randomisation								
Weight (kg)					3.50	0.42	3.47	0.46
Length (cm)					51.3	1.5	51.3	2.2
Head circumference (cm)					35.2	1.2	35.2	1.4
At 12 weeks								
Weight (kg)	5.94	0.67	5.96	0.62	6.15	0.70	6.02	0.67
Length (cm)	60.8	2.4	61.1	2.0	61.5	2.2	61.3	2.4
Head circumference (cm)	40.8	1.1	40.6	1.6	40.9	1.2	40.7	1.3
DXA at 12 weeks								
<i>n</i>	21		48		27		55	
Whole-body BMC (g)	130.7	18.4	126.8	20.6	129.2	20.9	124.1	20.8
Whole-body BA (cm ²)	521.1	53.8	519.3	46.7	535.5	65.0	510.5	68.1
Whole-body BMD (g/cm ²)	0.250	0.019	0.243	0.023	0.241	0.022	0.240	0.023

DXA, dual-energy X-ray absorptiometry; BMC, bone mineral content; BA, bone area; BMD, bone mineral density.

breast-feed more frequently at night at age 3 months. Interestingly, the effect was not observed in preterm subjects from the same cohort. In contrast, we recently reported higher whole-body BA and BMC in young adults who were born preterm and received human milk, with an apparent 'dose-response' effect relating the proportion of human milk in the diet during the neonatal period to later bone outcomes⁽⁴⁾. This effect was seen despite the inadequate nutrient content of human milk for preterm infants – in particular, the low Ca and phosphate content and the lack of nutrient or mineral supplementation of these infants. This effect was not observed at the 10-year follow-up of the same cohort. The reason for the discrepancy between the previous findings of a positive longer-term effect of BF on bone mass in term infants and those from the present study is not clear but could relate to the duration or 'dose' of BF. We do not have reliable data on the duration or exclusivity of BF for the present subjects. To be enrolled in the breast-fed reference group, infants had to be exclusively breast-fed at 10 weeks of age; however, given BF practices in the UK at the time, it is unlikely that exclusive or predominant BF was continued much beyond 3 months in most of the subjects. Another possibility is that the lack of an observed effect of BF in the present cohort reflects the small sample size; this seems less plausible as an explanation given that, if anything, the bone mass was lower in the breast-fed group.

In our original publication from this randomised trial, we also reported that use of a term infant formula containing a modified fat blend with a higher proportion of palmitate in the sn-2 position as found in breast milk, designed to improve Ca absorption, resulted in higher whole-body bone mass at the time of the intervention⁽⁷⁾. Our hypothesis that this

beneficial effect would persist beyond the intervention period was not confirmed by the present follow-up study; we found no evidence of a persisting effect on bone mass in mid-childhood. Children previously assigned to the high-sn-2 formula had measures of bone mass that were slightly higher than children from the control group, with a difference of 0.22 SDS for LS BMD and 0.37 SDS for LS BMAD. Children from this group were also slightly smaller than controls, both in infancy and at follow-up; nevertheless, bone mass adjusted for size using three different methods was not significantly different between the groups. It is important to note that, although the present study was a follow-up of a randomised trial, and the primary analysis would therefore normally be a comparison of randomised groups, in view of the significant attrition experienced (less than 30% follow-up overall) which may to some extent invalidate some of the benefits of random assignment⁽¹¹⁾, we adopted a conservative approach and examined the associations between early diet and later bone outcomes in a three-way ANOVA with subsequent *post hoc* pairwise comparisons performed only if a significant main effect was identified.

Although possibly a reflection of our relatively small sample size, given the results of trials of Ca or mineral supplementation during infancy and childhood, the absence of a sizeable long-term effect of the high-sn-2 formula on bone mass is perhaps not surprising. In a randomised trial comparing infant formulas with varying Ca and P contents in healthy term infants, together with a breast-fed reference group, Specker *et al.*⁽¹²⁾ reported transient effects during the first 6 months, with higher bone mineralisation in infants consuming a higher mineral formula; however, by 12 months of age, there were no differences in bone mass among the different

Table 2. Anthropometry and bone densitometry data at baseline, during infancy and at 10-year follow-up according to infant diet group (Mean values and standard deviations)

	Betapol (n 30)		Control (n 27)		Breast-fed (n 34)		P (ANOVA or non-parametric equivalent)
	Mean	SD	Mean	SD	Mean	SD	
Birth weight (g)	3446	395	3659	428	3507	408	0.1
Gestational age (weeks)	39.9	1.4	40.0	1.0	39.9	1.1	0.9
Age at randomisation (d)	6.5	1.7	5.7	2.1	–	–	
Weight at randomisation (kg)	3.41	0.40	3.60	0.43	–	–	
Weight SDS at randomisation	–0.51	0.85	–0.07	0.89	–	–	
Length at randomisation (cm)	51.1	1.4	51.4	1.7	–	–	
Length SDS at randomisation	–0.13	0.72	0.09	0.76	–	–	
12 weeks							
Weight (kg)	6.13	0.63	6.17	0.79	5.94	0.67	0.4
Weight SDS	0.24	0.84	0.34	1.0	0.053	0.91	0.5
Length (cm)	61.1	2.0	62.0	2.3	60.8	2.4	0.1
Length SDS	0.47	0.82	0.97	1.1	0.46	1.0	0.09
Whole body BMC (g)	129.3	16.6	129.1	26.1	130.7	18.4	1.0
Whole body BA (cm ²)	522.8	57.3	551.3	72.9	521.1	53.8	0.4
Whole body BMD (g/cm ²)	0.248	0.019	0.233	0.024	0.250	0.019	0.06
10 years							
Age (years)	10.81	0.61	10.51	0.54	11.22	0.36	<0.001*
Male							
n	17		14		16		
%	57		52		47		
Social code 1 or 2							0.003 (χ ²)
n	10		7		22		
%	35		26		67		
Maternal height (cm)	163.7	5.9	162.0	6.6	165.1	7.69	0.2
Paternal height (cm)	176.5	8.7	179.9	8.2	178.3	8.0	0.3
Pre-pubertal stage (Tanner = 1)							0.02 (χ ²)
n	18		18		11		
%	60		67		32		
Reached menarche (girls)							
n	0		1		2		
%	0		11		11		
Daily Ca intake (mg)	1058	488	1062	482	918	447	0.4
Vigorous activity (h/week)							0.08
Median	12.5		7.5		7		
25th–75th percentile	5–17.5		5–22.5		5–11.3		
Previous fracture	8	27	3	11	5	15	
Weight (kg)	40.48	9.34	42.48	12.3	39.52	8.59	0.5
Weight SDS	0.58	1.07	0.92	1.17	0.22	1.08	0.046†
Height (cm)	145.4	7.1	145.6	8.1	146.5	7.3	0.8
Height SDS	0.42	0.9	0.61	1.1	0.28	1.03	0.4
Lumbar spine (L2–L4)							
BMC (g)	24.38	5.15	22.54	4.52	23.12	4.46	0.3
BA (cm ²)	29.74	3.65	28.75	3.87	29.29	3.27	0.6
BMD (g/cm ²)	0.815	0.104	0.780	0.077	0.784	0.085	0.3
BMD SDS	0.01	1.01	–0.21	0.88	–0.53	0.85	0.06‡
BMAD SDS	–0.016	1.37	–0.39	0.88	–0.51	0.91	0.2
Whole body (TBLH)							
BMC (g)	1097	231	1049	264	1062	213	0.7
BA (cm ²)	1305	196	1271	216	1287	188	0.8
BMD (g/cm ²)	0.939	0.067	0.929	0.058	0.935	0.041	0.8
BMD SDS	0.40	0.86	0.39	0.76	0.170	0.60	0.4
DXA lean mass (kg)	27.31	3.53	27.78	5.3	27.82	3.92	0.9
DXA LMI (kg/m ²)	11.1	0.9	11.4	1.3	11.0	0.7	0.4
BMC/FFM index	0.021	0.003	0.019	0.003	0.020	0.002	0.1

SDS, standard deviation score; BMC, bone mineral content; BA, bone area; BMD, bone mineral density; BMAD, bone mineral apparent density; TBLH, total body less head; DXA, dual-energy X-ray absorptiometry; LMI, lean mass index; FFM, fat-free mass.
 * Post hoc Bonferroni: breast-fed v. Betapol, P=0.005; breast-fed v. control, P<0.001; Betapol v. control, P=0.09.
 † Post hoc Bonferroni: breast-fed v. Betapol, P=0.6; breast-fed v. control, P=0.04; Betapol v. control, P=0.7.
 ‡ Post hoc Bonferroni: breast-fed v. Betapol, P=0.06; breast-fed v. control, P=0.5; Betapol v. control, P=1.0.

feeding groups. It is not clear whether a transient increase in bone mineralisation, such as that reported by Specker, and during the intervention phase of the present trial, would have any short-term benefit for a healthy term infant with low fracture risk. Winsenberg *et al.*⁽¹³⁾ performed a

meta-analysis of nineteen studies of Ca supplementation in children aged 3 years and above, with bone outcomes measured after at least 6 months of follow-up. Ca supplementation had no effect on LS or hip BMD. A small and transient effect was observed for whole-body BMC, and this has been

Table 3. Anthropometry and bone measurements for combined formula-fed subjects v. breast-fed subjects (Mean values and standard deviations)

	Formula-fed (n 57)		Breast-fed (n 34)		Difference	P
	Mean	SD	Mean	SD		
Age (years)	10.67	0.59	11.22	0.36	-0.55 (-0.75 to -0.35)	<0.001
Weight SDS	0.75	1.13	0.22	1.08	0.53 (0.05 to 1.00)	0.03
Height SDS	0.51	0.98	0.28	1.03	0.23 (-0.20 to 0.66)	0.3
Maternal height (cm)	162.9	0.22	165.1	7.7	-2.1 (-5.1 to 0.8)	0.2
Paternal height (cm)	178.1	8.6	178.3	8.0	-0.18 (-3.8 to 3.5)	0.9
Lumbar spine (L2-L4)						
BMC (g)	23.51	4.91	23.12	4.46	0.39 (-1.65 to 2.43)	0.7
Area (cm ²)	29.27	3.76	29.29	3.27	-0.019 (-1.56 to 1.52)	1.0
BMD (g/cm ²)	0.798	0.094	0.784	0.085	0.014 (-0.025 to 0.052)	0.5
BMD SDS	-0.095	0.95	-0.53	0.85	0.44 (0.045 to 0.83)	0.03
BMAD SDS	-0.19	1.2	-0.51	0.91	0.32 (-0.15 to 0.78)	0.2
Whole body						
BMC (g)	1413	266	1411	216	1.27 (-106 to 108)	1.0
Area (cm ²)	1504	207	1506	192	-2.6 (-90 to 84)	1.0
BMD (g/cm ²)	0.934	0.062	0.935	0.041	-0.0008 (-0.02 to 0.02)	0.9
BMD SDS	0.39	0.81	0.17	0.60	0.22 (-0.09 to 0.54)	0.2

SDS, standard deviation score; BMC, bone mineral content; BMD, bone mineral density; BMAD, bone mineral apparent density.

postulated to result from a temporary reduction in bone remodelling. A small persisting effect was seen at the upper limb; however, this was considered to be unlikely to reduce the risk of fracture either in childhood or later life, and therefore of little public health significance.

Conclusion

In the present cohort of healthy term-born subjects, we found no difference in childhood bone mass between subjects who had been breast-fed or formula-fed, and no persisting beneficial effect of an infant formula with a high-sn-2-modified fat blend, designed to increase Ca absorption. The present finding is consistent with trials of Ca or mineral supplementation during infancy and childhood, and suggests that the major beneficial effect of the addition of this modified fat blend to infant formulas may be the observed alteration in stool characteristics seen during infancy. However, both findings must be interpreted in the context of the relatively small sample size, which means that we cannot exclude a difference of <0.7 SD between the groups. A larger study would be required to exclude smaller, but potentially biologically relevant effects.

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References

1. Torgerson DJ, Iglesias CP & Reid DM (2001) The economics of fracture prevention. In *The Effective Management of Osteoporosis*, pp. 111-121 [DH Barlow, RM Francis and A Miles, editors]. Farringdon: Aesculapius Medical Press.
2. Fewtrell MS (2006) Osteoporosis; is primary prevention possible? In *Primary Prevention by Nutrition Intervention in Infancy and Childhood*. Nestlé Nutrition Workshop Series Pediatr Program 57, pp. 135-152 [A Lucas and HA Sampson, editors]. Basel: Karger.
3. Jones G, Riley M & Dwyer T (2000) Breastfeeding in early life and bone mass in prepubertal children: a longitudinal study. *Osteoporos Int* **11**, 146-152.
4. Fewtrell MS, Williams JE, Singhal A, *et al.* (2009) Early diet and peak bone mass: 20 year follow-up of a randomised trial of early diet in infants born preterm. *Bone* **45**, 142-149.
5. Koo WKK, Hockman EM & Dow M (2006) Palm olein in the fat blend of infant formulas: effect on the intestinal absorption of calcium and fat, and bone mineralisation. *J Am Coll Nutr* **25**, 117-122.
6. Koo WKK, Hammami M, Margeson DP, *et al.* (2003) Reduced bone mineralisation in infants fed palm olein



- containing formula: a randomised, double blinded, prospective trial. *Pediatrics* **111**, 1017–1023.
7. Kennedy K, Fewtrell MS, Morley R, *et al.* (1999) Double-blind randomised trial of a synthetic triglyceride (Betapol) in formula fed term infants: effects on stool biochemistry, stool characteristics and bone mineralisation. *Am J Clin Nutr* **70**, 920–927.
 8. Nelson M, Hague GF, Cooper C, *et al.* (1988) Calcium intake in the elderly: validation of a dietary questionnaire. *J Hum Nutr Dietetics* **1**, 115–127.
 9. Bishop N, Braillon P, Burnham J, *et al.* (2008) Dual-energy X-ray absorptiometry assessment in children and adolescents with diseases that may affect the skeleton: the 2007 ISCD Pediatric Official Positions. *J Clin Densitom* **11**, 29–42.
 10. Crabtree NJ, Oldroyd B, Truscott JG, *et al.* (2004) UK Paediatric reference data (GE Lunar Prodigy). *Osteoporos Int* **S2**, S6.
 11. Fewtrell MS, Kennedy K, Singhal A, *et al.* (2008) How much loss to follow-up is acceptable in long-term randomised trials and prospective studies? *Arch Dis Child* **93**, 458–461.
 12. Specker BL, Beck A, Kalkwarf H, *et al.* (1997) Randomised trial of varying mineral intake on total body bone mineral accretion during the first year of life. *Pediatrics* **99**, e12.
 13. Winsenberg TM, Shaw KA, Fryer J *et al.* (2006) Calcium supplementation for improving bone mineral density in children. *The Cochrane Database of Systematic Reviews* 2006, issue 2, CD005119.