Randomized Trial of Varying Mineral Intake on Total Body Bone Mineral Accretion During the First Year of Life

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ABSTRACT. *Objective.* **The effect of varying mineral intakes on total body bone mass accretion during the first year of life in healthy full-term infants is unknown. The purpose of this study was to determine whether total body bone mass accretion during the first year of life was influenced by the calcium and phosphorus intake of an infant and whether early differences in bone accretion persist through 1 year of age.**

Design. **This prospective, randomized trial was conducted in two phases. In phase I, 67 infants were randomized within the first 2 weeks of life into either a low (439 mg of calcium per liter and 240 mg of phosphorus per liter) or moderate (510 mg of calcium per liter and 390 mg of phosphorus per liter) mineral-containing formula feeding group. An additional group of 34 human milk–fed (low mineral) infants also was enrolled. Phase II involved an additional randomization of all infants at 6 months of age into moderate-mineral formula (see above), high-mineral formula (1350 mg of calcium per liter and 900 mg of phosphorus per liter), or cow milk (1230 mg of calcium per liter and 960 mg of phosphorus per liter) feeding group. Anthropometric measurements, nutrient intake, and total body bone mineral content (BMC) by dual-energy *x*-ray absorptiometry were measured at 1, 3, 6, 9, and 12 months.**

Results. **During the first 6 months, the moderate-mineral group had a greater increase in weight (3.42 ± 0.62 kg) compared with the human milk group (2.93 ± 0.56 kg); the low-mineral group (3.19 ± 0.62 kg) was intermediate. Bone mass accretion differed in a similar direction, with the moderate-mineral feeding group having a greater increase than the human milk group and the low-mineral group being intermediate of the two. Including weight, length, and bone area as covariates, both the low-mineral formula– and human milk–fed groups had similar BMC, which was lower than that of the moderate-mineral group at 3 and 6 months of age. Adjusted mean BMC values for the moderate-mineral formula–fed group compared with the low-mineral formula– and human milk–fed groups were 127.8 ± 1.5 (SEM) g vs 119.2 ± 1.5 and 122.1 ± 1.4 g, respectively, at 3 months of age and 168.7 ± 2.5 g vs 157.6 ± 2.5 and 158.7 ± 2.4 g, respectively, at 6 months of age. The BMC at 6 months of age among the formula-fed infants was correlated with both average dietary phosphorus intake ($r = .592$) and average daily calcium intake ($r = .620$) during the first 6 months. The relationships between BMC and these minerals remained significant even after controlling for caloric intake. It was not possible to determine the independent effects of dietary calcium and phosphorus on BMC because of the strong correlation of these minerals with each other. Despite significant differences in both calcium and phosphorus intakes during the second 6 months of life, there were no differences in growth parameters or bone mass accretion. Means for BMC, adjusted for body weight, length, and bone area, were not significantly different among feeding groups at either 9 or 12 months of age. Adjusted means were 199 ± 2 (SEM) and 237 ± 3 g at 9 and 12 months of age for infants receiving moderate-mineral formula; 198 ± 2 and 236 ± 3 g at 9 and 12 months of age for infants receiving the high-mineral formulas and 202 ± 5 and 233 ± 5 g at 9 and 12 months of age for infants receiving cow milk. The gain in bone mass during the second 6 months differed by the first 6-month feeding group; mean changes in BMC between 6 and 12 months, adjusted for changes in weight, length, and bone area, were greater in human milk–fed infants than in either the low- or moderate-mineral–containing formula groups: 81 ± 16 g in human milk–fed infants and 73 ± 15 and 71 ± 15 g in the low- and moderate-mineral formula groups, respectively. Infants fed whole cow milk during the second 6 months were excluded from this analysis because of the small number of infants completing the study. By 12 months of age there were no differences in BMC in either the early or late feeding groups.**

Conclusion. **These results indicate that during the first 6 months, bone mass accretion is less in infants fed human or low-mineral formula compared with infants fed moderate-mineral formula. Infants fed human milk during the first 6 months had greater bone mass accretion during the second 6 months compared with formula-fed infants. By 12 months of age there were no differences in bone mass among the different feeding groups.** ***Pediatrics*** **1997;99(6). URL: http://www.pediatrics.org/cgi/content/full/99/6/e12;** ***bone, growth, infant feeding.***

ABBREVIATIONS. BMC, bone mineral content; ANOVA, analysis of variance.

Unlike most other mammalian milk, human milk is exceptionally low in phosphorus content. In infants, there are three reasons to suggest utilization of formulas that provide low but adequate phosphorus intake and a balanced ratio of calcium to phosphorus: (1) a low intestinal phosphorus concentration is an essential condition for generating an acid pH of the feces, which inhibits the growth of potentially pathogenic organisms; (2) the immature infantile kidney cannot readily excrete a surplus of phosphorus, and higher phosphorus intake during the neonatal period is a significant risk factor for hypocal-

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Received for publication Jun 11, 1996; accepted Jan 29, 1997.
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cemic tetany in both preterm[1] and full-term infants[2–7]; and (3) high phosphorus and calcium intake in the newborn can result in metabolic acidosis secondary to a decreased renal capacity for acid excretion. It is thought that all three pathophysiologic mechanisms were effective in the biochemical evolution of humans, selecting women with low phosphorus milk and infants with a constantly high intestinal absorption rate of phosphorus.[8]

In comparison with human milk, the standard infant formulas prepared from cow milk have relatively high phosphorus and calcium concentrations and a relatively low calcium-to-phosphorus ratio. Studies have shown lower bone mineral content (BMC) of the radius throughout the first 6 months of life of infants fed human milk compared with cow milk formula.[9,10] No studies have been reported on the effect of varying mineral intakes on total body bone mass accretion during the first year of life, which should be a better indicator of mineral nutriture. In addition, no studies have been designed in such a way as to determine whether early feeding during the first 6 months of life has a persistent effect on differences in bone mass accretion.

In the current study, we hypothesized that during the first year of life a higher–mineral content formula would result in higher total body bone mass accretion. We used a factorial design to determine whether bone mass accretion during the first and second 6 months of life was influenced by the mineral intake of commercially available formula during those periods and whether bone mass accretion during the second 6 months differed according to the mineral intake during the first 6 months.

METHODS

The study was designed so that an estimated 90 white infants would complete the study. The study was designed as two phases. In phase I, a randomization schedule, stratified by gender, was prepared for enrollment of formula-fed infants into one of two initial formula feeding groups: one that received a low-mineral formula (Good Start; Carnation Nutritional Products, Glendale, CA) or one that received a moderate-mineral formula (Similac; Ross Laboratories, Columbus, OH). Thirty-four infants whose mothers elected to breastfeed for approximately 6 months were enrolled in a human milk group; supplemental formula feedings were allowed but not encouraged. The supplemental formula supplied to these infants was the low-mineral formula. Infants were enrolled shortly after birth, and use of the study formula was begun preferably at discharge but no later than 2 weeks of age.

In phase II, a second randomization schedule was used to determine the type of feeding from 6 to 12 months of age. All formula- and human milk–fed infants were randomized, after stratifying by initial feeding assignment to receive a moderate-mineral cow milk–based formula (Similac), a high-mineral cow milk–based formula (Carnation Follow-Up formula), or whole cow milk. A whole–cow milk feeding group was originally included to investigate the effect of high mineral and high protein concentrations on bone mass accretion during the second 6 months of life. Mineral and protein contents of the different feedings are given in Table 1. Because of concerns regarding low iron intake for infants receiving whole cow milk,[11] all mothers of cow milk–fed infants were supplied with iron supplements (Fer-In-Sol; Mead Johnson Nutritionals, Evansville, IN). If there were physician concerns about the infant being randomized to cow milk at 6 months of age, the infant was rerandomized to either the moderate- or high-mineral formula. Human milk–fed infants also were randomized to these feeding groups, with five infants still receiving some human milk at 12 months of age.

Mothers were recruited from private obstetric practices and maternity wards in area hospitals and by referrals from other study participants. Infants who met the following criteria were eligible for the study: (1) full-term infants (38 to 42 weeks of gestation based on last menstrual period); (2) birth weight, length, and head circumference above the 10th percentiles, as established by National Center for Health Statistics data[12]; (3) apparent good health without evidence of cardiac, respiratory, gastrointestinal, or other systemic disease; (4) no maternal medical history of diabetes, use of antihypertensives, anticonvulsants, anticoagulants, steroids, or ritodrine, and/or a condition with proven adverse effects on the fetus; and (5) a 1-minute Apgar score of 7 or greater and a 5-minute Apgar score of 8 or greater. These exclusions are factors that may influence normal growth or calcium homeostasis in the infant.[13,14]

Anthropometric measurements, nutrient intake, and total body BMC were evaluated at 1, 3, 6, 9, and 12 months of age. The weight of the infant was measured with a digital scale, and crown-to-heel length was measured with a length board. Three-day diet records on the infants were obtained during the week preceding each visit, and nutrient content was computed using the Minnesota Nutrition Data System (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN).

Total body BMC was measured using dual-energy x-ray, absorptiometry (QDR1000W; Hologic, Inc, Waltham, MA). The Hologic system was operated in the single-beam mode and uses fast–kilovolt (peak) switching of a radiographic tube to generate both high- and low-energy photon beams (140 and 70 kV[p], respectively). The beam is transmitted in a rectilinear fashion throughout the body, and a detector measures the transmitted photon intensity on a pixel-by-pixel basis, with each pixel measuring 2 × 5 mm. Data from a rotating reference wheel containing known amounts of bone- and soft tissue–equivalent materials is used for continuous internal calibration during the scan. The relative attenuation of the two photon beams can be related to the mass of bone mineral and soft tissue. All infants were scanned wearing light clothing and after being placed on an "egg crate" foam pad restraint system. The Hologic pediatric software (version 5.56) was used for the analysis of the scan results. Our coefficient of variation, determined from duplicate scans on 17 infants 3 to 17 months of age, is 4.5% for total body BMC. Radiation exposure from a total body scan is less than 0.5 mrem per scan, well within acceptable ranges for use in pediatric populations.

TABLE 1. Mineral and Protein Content of Different Feeding Regimens*

	Ca, mg/L	P, mg/L	Ca:P Ratio	Protein, g/L
Feeding groups during the first 6 mo				
Human milk	300	150	2.0	10.5
Low mineral	430	240	1.8	16.2
Moderate mineral	510	390	1.3	14.5
Feeding groups during the second 6 mo				
Moderate mineral	510	390	1.3	14.5
High mineral	1350	900	1.5	19.8
Cow milk	1230	960	1.3	33.9

* Based on published data from Ross Products Division, Abbott Laboratories, A7368, September 1995, and Carnation Nutritional Products, information FF 150-0041, 1994. Conversion from milligrams of calcium per day to millimoles of calcium per day is 0.02495; conversion from milligrams of phosphorus per day to millimoles of phosphorus per day is 0.03229.

TABLE 2. Numbers of Infants Completing the Study

Mineral Intake, 6–12 mo (Phase II)	Mineral Intake for First 6 mo (Phase I)			Phase II Total
	Human Milk	Low	Moderate	
Moderate	14	12	12	38
High	11	14	14	39
Cow milk	5	3	2	10
Withdrew after 6 mo	1	1	3	. . .
Phase I total	31	30	31	. . .

A total of 101 infants were enrolled; 14 infants were withdrawn from the study, and 87 completed the study through 12 months of age. Nine infants were withdrawn before 6 months of age (3 in the human milk, 2 in the low-mineral, and 4 in the moderate-mineral feeding groups), and 5 withdrew between 6 and 12 months of age (1 in the moderate-mineral formula, 2 in the high-mineral formula, and 2 in the cow milk feeding groups). Eight of the 14 infants were withdrawn because of recommended changes in feeding. Thirty-one human milk–fed infants completed the study through 6 months of age. However, supplemental feedings with low-mineral formula were begun before 6 months of age in 27 of the 31 infants. Thirty and 31 infants in the low- and moderate-mineral formula groups, respectively, completed the study through 6 months of age.

The final numbers of infants completing the study are given in Table 2 by their final feeding group assignments. Seventeen of the infants initially in the cow milk group were rerandomized to formula groups because of parental or physician concerns (8 to moderate- and 9 to high-mineral formula). Therefore, a total of 38 and 39 infants received the moderate- and high-mineral formula, respectively, and only 10 infants remained in the cow milk group through 12 months. There were no differences in any of the anthropometric measurements at 6 or 9 months of age between infants who were rerandomized and the 10 remaining infants in the cow milk feeding group. Analyses were performed using the final group feeding assignment.

Data analyses were conducted to determine the effects of mineral intake during the first 6 and second 6 months on bone mass accretion during those intervals. Additional analyses were conducted to determine the effect of early diet (first 6 months) on anthropometric measurements and bone mass accretion during the second 6 months. Analysis of variance (ANOVA) was used to determine group differences in baseline characteristics or changes in anthropometric and bone mass measurements, and the Tukey-Kramer honest significant difference test was used to compare individual means if the overall ANOVA was significant at $P < .05$ (JMP; SAS Institute, Inc, Cary, NC). Repeated-measures ANOVA was used to determine whether there were significant group differences and whether changes over time differed among dietary groups (time by group interaction). Approval was obtained from

TABLE 3. Characteristics of Infants Completing the Study Through 6 Months of Age

Characteristic	Human Milk	Low Mineral	Moderate Mineral	*P*
No. of infants	31	30	31	
M/F	10/21	13/17	16/15	NS*
Age, wk				
1 mo	4.7 ± 0.5†	4.8 ± 0.5	4.6 ± 0.5	NS
3 mo	13.3 ± 0.8	13.6 ± 0.6	13.3 ± 0.7	NS
6 mo	26.3 ± 0.6	26.5 ± 0.8	26.3 ± 0.6	NS
Weight, kg				
1 mo	4.51 ± 0.42	4.33 ± 0.59	4.39 ± 0.54	NS
3 mo	5.94 ± 0.54	5.96 ± 0.83	6.19 ± 0.74	NS
6 mo	7.46 ± 0.55	7.54 ± 0.97	7.82 ± 0.87	NS
Length, cm				
1 mo	55.2 ± 1.7	54.0 ± 2.4	54.7 ± 2.5	NS
3 mo	60.2 ± 2.4	60.1 ± 3.2	59.6 ± 2.7	NS
6 mo	66.3 ± 2.2	66.6 ± 2.6	66.5 ± 2.5	NS
Bone mineral content, g				
1 mo	96.2 ± 10.9‡	85.7 ± 14.7‡	93.6 ± 16.2	.02
3 mo	120.7 ± 14.1‡	115.7 ± 18.7[b]§	132.5 ± 17.6[ab]‡§	<.001
6 mo	155.4 ± 17.9‡	154.1 ± 21.5[b]§	175.6 ± 30.9‡§	<.001
Energy intake, kcal/d‖				
1 mo		457 ± 86	454 ± 79	NS
3 mo		548 ± 123	539 ± 102	NS
6 mo		631 ± 116	642 ± 112	NS
Protein intake, mg/d				
1 mo		10.9 ± 2.1	10.1 ± 1.7	.08
3 mo		13.1 ± 3.0	11.9 ± 2.4	.07
6 mo		14.3 ± 2.6	14.0 ± 2.6	NS
Calcium intake, mg/d¶				
1 mo		306 ± 58[a]‡	353 ± 60[a]‡	.003
3 mo		371 ± 82[a]‡	417 ± 83[a]‡	.03
6 mo		429 ± 87[a]‡	504 ± 111[a]‡	.006
Phosphorus intake, mg/d#				
1 mo		165 ± 32[a]‡	263 ± 45[a]‡	<.001
3 mo		203 ± 45[a]‡	310 ± 62[a]‡	<.001
6 mo		269 ± 77[a]‡	403 ± 100[a]‡	<.001

* NS indicates not significant at $P > .20$. *P* values less than .20 are provided.
† Data are expressed as mean ± SD.
‡§ Feeding groups with the same symbols are significantly different from each other.
‖ Conversion from calories per day to joules per day is 4.183.
¶ Conversion from milligrams of calcium per day to millimoles of calcium per day is 0.02495.
\# Conversion from milligrams of phosphorus per day to millimoles of phosphorus per day is 0.03229.

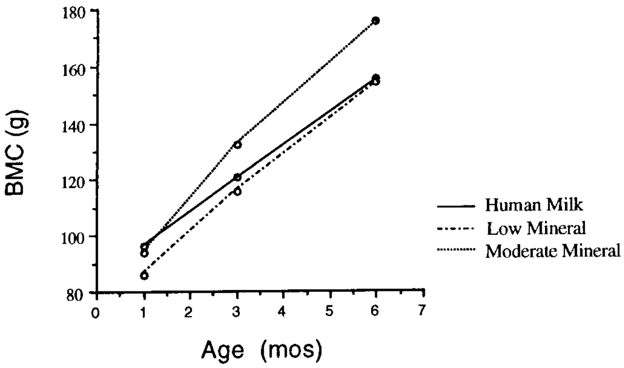

Fig 1. The mean total body bone mineral content (BMC) at 1, 3, and 6 months of age in infants fed human milk, low-mineral formula, or high-mineral formula. Similar differences were observed after adjusting for weight, height, and bone area. Infants fed moderate-mineral formula had significantly greater bone mass at 3 and 6 months of age compared with infants fed human milk or low-mineral formula ($P < .001$ at both ages).

the Children's Hospital Medical Center Institutional Review Board, and written informed consent was obtained from parents.

RESULTS

Phase I: First 6 Months

Mean anthropometric measurements, bone data, and nutrient intake for each age and formula feeding group are given in Table 3 and Fig 1. Both calcium and phosphorus intakes differed between formula groups. There were significant differences in the changes in weight and bone mass accretion among feeding groups (Table 4). The moderate-mineral group had a greater increase in weight compared with the human milk group, and the low-mineral group was intermediate. Bone mass accretion differed in a similar direction, with the moderate-mineral feeding group having a greater increase than the human milk group and the low-mineral group being intermediate of the two. Group differences in the change in length was of borderline significance. Similar results were observed when the repeated-measures ANOVA technique was used to compare changes over time.

Because BMC is highly correlated with body size, additional analyses were performed to determine whether group differences in bone mass remained significant when weight, length, and bone area were included as covariates.[15] When including these variables in the regression models, both the low-mineral formula– and human milk–fed groups had similar BMC, which was lower than the that in the moderate-mineral group at 3 and 6 months of age. Adjusted mean BMC values for the moderate-mineral formula group compared with the low-mineral– and human milk–fed groups were 127.8 ± 1.5 (SEM) g vs 119.2 ± 1.5 and 122.1 ± 1.4 g, respectively, at 3 months of age (ANOVA, $P < .001$) and 168.7 ± 2.5 g vs 157.6 ± 2.5 and 158.7 ± 2.4 g, respectively, at 6 months of age (ANOVA, $P < .001$). Although there were slight gender differences in BMC at 3 and 6 months of age, with male infants having higher BMC than female infants, these differences were explained by gender differences in body weight. Therefore, it was not necessary to include gender as a covariate.

To determine whether among human milk–fed infants supplemental feedings had an effect on BMC, the relationships between BMC and the ounces of formula consumed were investigated at 3 and 6 months of age. At 1 month of age, all but three infants were exclusively fed human milk. The mean ounces of formula consumed among the infants fed human milk were 16 ± 14 (SD) and 23 ± 14 oz/d at 3 and 6 months, respectively. Weight, length, and BMC at any age were not associated with ounces of formula consumed. In addition, there were no significant differences in weight, length, and BMC at 3 or 6 months of age between human milk–fed infants receiving less than 4 oz/d and those receiving more.

BMC at 6 months of age, among the formula-fed infants, was correlated with both average dietary phosphorus intake ($r = .592$; $P < .001$) and the average dietary calcium intake ($r = .620$; $P < .001$) during the first 6 months. The relationships between BMC and these minerals remained significant even after controlling for caloric intake ($P = .004$ for phosphorus; $P = .02$ for calcium). It was not possible to determine the independent effects of dietary calcium and phosphorus on BMC because of the strong correlation of these minerals with each other ($r = .871$; $P < .001$).

Phase II: Second 6 Months

The baseline characteristics at 6 months of age for infants completing 12 months are given in Table 5. Despite significant differences in both calcium and phosphorus intakes during the second 6 months of life, there were no differences in growth parameters or bone mass accretion. Means for BMC, adjusted for body weight, length, and bone area, were not significantly different among feeding groups at either 9 ($P = .23$) or 12 ($P = .46$) months of age. Adjusted means were 199 ± 2 (SEM) and 237 ± 3 g at 9 and 12 months of age for infants receiving the moderate-mineral formula, 198 ± 2 and 236 ± 3 g at 9 and 12 months of age for infants receiving the high-mineral formula, and 202 ± 5 and 233 ± 5 g at 9 and 12 months of age for infants receiving cow milk. The increases between 6 and 12 months of age in weight,

TABLE 4. Absolute Changes (Mean ± SD) in Anthropometric Measurements and Bone Mass Between 1 and 6 Months of Age by Feeding Group During the First 6 Months of Age

Measurement	Human Milk	Low Mineral	Moderate Mineral	P*
Weight, kg	2.93 ± 0.56†	3.19 ± 0.62	3.42 ± 0.62†	.007
Length, cm	11.1 ± 1.7†	12.5 ± 1.9†	11.8 ± 2.9	.05
Bone mineral contents, g	59.2 ± 17.1†	65.6 ± 17.5‡	81.7 ± 25.4†‡	<.001

* *P* values are for group differences.
†‡ Means with the same symbols on each row are different at $P < .05$.

TABLE 5. Characteristics of Infants After Randomization at 6 Months of Age (Includes Only Infants Completing 12 Months)

Characteristic	Moderate Mineral	High Mineral	Cow Milk	*P*
No. of infants	38	39	10	
M/F	16/22	17/22	4/6	NS*
Age, wk				
6 mo	26.3 ± 0.7†	26.3 ± 0.6	26.6 ± 0.5	NS
9 mo	39.5 ± 0.8	39.3 ± 0.9	39.9 ± 0.9	NS
12 mo	52.6 ± 0.9	52.7 ± 1.1	53.0 ± 1.0	NS
Weight, kg				
6 mo	7.65 ± 0.80	7.58 ± 0.93	7.58 ± 0.66	NS
9 mo	8.86 ± 0.85	8.79 ± 1.17	8.66 ± 0.82	NS
12 mo	9.67 ± 0.99	9.77 ± 1.36	9.67 ± 0.86	NS
Length, cm				
6 mo	66.6 ± 2.8	66.5 ± 2.1	65.9 ± 2.2	NS
9 mo	71.1 ± 2.6	71.2 ± 2.4	69.9 ± 2.4	NS
12 mo	75.0 ± 2.9	75.1 ± 2.5	74.6 ± 2.7	NS
Bone mineral content, g				
6 mo	160.8 ± 20.8	164.2 ± 32.2	157.9 ± 19.2	NS
9 mo	200.3 ± 25.1	195.7 ± 32.0	203.3 ± 28.2	NS
12 mo	234.3 ± 28.3	239.7 ± 42.9	229.6 ± 29.8	NS
Energy intake, kcal/d‡				
6 mo	646 ± 152	625 ± 117	559 ± 183	NS
9 mo	801 ± 192	826 ± 175	733 ± 73	NS
12 mo	942 ± 282	965 ± 261	973 ± 359	NS
Protein intake, mg/d				
6 mo	14.1 ± 3.5	14.2 ± 2.8	12.1 ± 3.8	NS
9 mo	18.4 ± 5.1§‖	24.3 ± 6.7‖	29.0 ± 4.3§	<.001
12 mo	27.5 ± 12.8§	31.9 ± 9.7	38.8 ± 14.9§	.04
Calcium intake, mg/d¶				
6 mo	462 ± 122	443 ± 109	378 ± 119	.16
9 mo	555 ± 154‖	868 ± 199§	922 ± 182‖	<.001
12 mo	588 ± 287‖	813 ± 196§	881 ± 321‖	.001
Phosphorus intake, mg/d#				
6 mo	324 ± 114	307 ± 99	264 ± 118	NS
9 mo	512 ± 185§‖	662 ± 162§	776 ± 124‖	<.001
12 mo	606 ± 287§	727 ± 187	874 ± 305§	.01

* NS indicates not significant at $P > .20$. *P* values less than .20 are provided.
† Data are expressed as mean ± SD.
‡ Conversion from calories per day to joules per day is 4.183.
§‖ Feeding groups with the same symbols are significantly different from each other.
¶ Conversion from milligrams of calcium per day to millimoles of calcium per day is 0.02495.
\# Conversion from milligrams of phosphorus per day to millimoles of phosphorus per day is 0.03229.

TABLE 6. Changes (Mean ± SD) in Anthropometric Measurements and Bone Mass Between 6 and 12 Months of Age by Feeding Group During the Second 6 Months of Age

Measurement	Moderate Mineral	High Mineral	Cow Milk	*P*
Weight, kg	2.03 ± 0.49	2.23 ± 0.61	2.09 ± 0.50	.27
Length, cm	8.4 ± 1.8	8.6 ± 1.6	8.6 ± 1.3	.87
Bone mineral content, g	72.9 ± 21.1	75.9 ± 24.9	71.7 ± 38.1	.84

* *P* values are for group differences.

length, bone mass, and bone mass per kilogram of body weight are given in Table 6.

Although the increases in anthropometric measurements between 6 and 12 months of age were not related to the second 6-month feeding group, there were significant differences during the second 6 months by the first 6-month feeding groups. Infants who received human milk during the first 6 months gained slightly less weight during the second 6 months than infants fed either low- or moderate-mineral formula (1.92 ± 0.45, 2.24 ± 0.55, and 2.21 ± 0.60 g, respectively; $P = .05$). Mean changes in BMC between 6 and 12 months, adjusted for changes in weight, length, and bone area, differed significantly among the early feeding groups, with human milk–fed infants having a greater gain in BMC than either the low- or moderate-mineral formula groups ($P = .04$): 81 ± 16 g in human milk–fed infants and 73 ± 15 and 71 ± 15 g in the low- and moderate-mineral formula groups, respectively. Infants fed whole cow milk during the second 6 months were excluded from this analysis because of the small number of infants completing the study. By 12 months of age there were no differences in BMC in either the early or late feeding groups (Fig 2).

DISCUSSION

The feeding of high-phosphate–containing formula and formulas with a relatively low calcium-to-phosphorus ratio has resulted in low serum calcium in both preterm and full-term infants.[1–7,16] The effect is transient and occurs during the first weeks of life. The concern of increased risk of neonatal hypocalcemia has led infant formula companies to reduce the

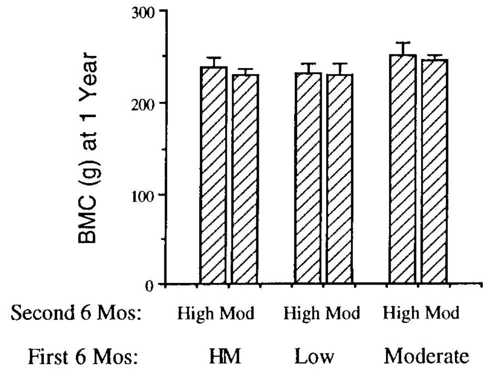

Fig 2. The mean total body bone mineral content (BMC) at 12 months of age was similar among the different feeding groups (P = .22 and .56 for early and late feeding groups). HM indicates human milk.

phosphorus content of the formula and to try to provide a formula with a calcium-to-phosphorus ratio more similar to that of human milk. However, beyond the neonatal period a higher phosphorus concentration may actually lead to increased bone mineralization as phosphorus and calcium are deposited onto bone matrix. A greater increase in BMC of the radius has been previously reported in cow milk–based formula–fed infants compared with human milk–fed infants, and it has been speculated that the low phosphorus content of human milk is responsible for the lower BMC.[9,17–19] There have been no studies reported that have examined the longitudinal effects on total body bone mass accretion in healthy, full-term infants.

We found that by 1 month of age there already were differences among the feeding groups. These findings indicate either a dramatic effect of early feeding on bone metabolism (because the infants have been consuming the formula for only 2 to 4 weeks) or baseline differences between infants fed human milk or formula. Because the early differences at 1 month of age may be spurious, the changes in anthropometric and bone measurements from baseline (1 month of age) among the different diet groups were investigated. Differences in total body bone mass accretion among feeding groups persisted, even after statistically adjusting for body size, as suggested by others.[15]

We observed a significant effect of infant mineral intake on bone mass accretion during the first 6 months of life, but this effect was not evident during the second 6 months. Despite a significant difference in mineral content of formulas consumed during the second 6 months, bone mass accretion did not differ. It is possible that the mineral content of both the moderate- and high-mineral formulas were high enough to have reached a threshold effect of these minerals on bone mass accretion already. A difference in the response during the second 6 months might possibly have been observed had a lower-mineral formula been used. However, the formulas used represent commercially available formulas that are marketed to the 6- to 12-month-old infant.

The design of the trial enabled us to evaluate short- and long-term effects of diet during the first 6 months of life on changes in bone mass accretion throughout the first year. We were able to determine whether bone mass accretion during the second 6 months differed according to the mineral intake during the first 6 months. A greater bone mass accretion was demonstrated during the second 6 months of life among infants who received human milk early in life compared with infants who received either low- or moderate-mineral formula. This difference seems to indicate, using the human milk–fed infant as the standard of reference, that a slowdown occurs during the second 6 months among infants fed formula in the first 6 months of life. This slowdown resulted in similar bone mass at 1 year of age among the different feeding groups.

The results of a randomized trial of calcium supplementation in young children found that supplementation increased bone mass accretion among prepubertal children.[20] However, 1 year after cessation of the trial, bone mass measurements were similar among both groups.[20] Our findings, coupled with those of other investigators, indicate that the effect of mineral intake on bone mass accretion during growth seems to be transient and not long term.

In conclusion, we found that the mineral intake of an infant's diet is significantly associated with bone mass accretion during the first 6 months of life. In addition, infants fed human milk during the first 6 months of life had a greater gain in bone mass during the second 6 months of life when they were provided a moderate- to high-mineral formula than infants previously fed low- or moderate-mineral formula. These early effects of diet on bone mass accretion resulted in similar total body bone mass at 1 year of age.

ACKNOWLEDGMENTS

This work was supported in part by funds from Carnation Nutritional Products Division and General Clinical Research Center grant M01RR08084 from the National Institutes of Health.

REFERENCES

1. Tsang RC, Light IJ, Sutherland JM, et al. *J Pediatr.* 1973;82:423–429
2. Bakwin H. Tetany in newborn infants. *J Pediatr.* 1939;14:1–10
3. Bancroft JD. Late-onset neonatal hypocalcemic tetany. *Am J Dis Child.* 1986;140:92. Letter
4. Cunningham AS, Venkataraman PS. Persistent secondary hyperparathyroidism in infants fed humanized cow milk formula. *Am J Dis Child.* 1985;139:1182. Letter
5. Gardner LI, MacLachlan EA, Pick W, et al. Etiologic factors in tetany of newly born infants. *Pediatrics.* 1950;5:228–240
6. Oppe TE, Redstone D. Calcium and phosphorus levels in healthy newborn infants given various types of milk. *Lancet.* 1968;1:1045–1048
7. Venkataraman PS, Tsang RC, Greer FR, et al. Late infantile tetany and secondary hyperparathyroidism in infants fed humanized cow milk formula. *Am J Dis Child.* 1985;139:664–668
8. Manz F. Why is the phosphorus content of human milk exceptionally low? *Monatsschr Kinderheilkd.* 1992;140:S35-S39
9. Greer F, Marshall S. Bone mineral content, serum vitamin D metabolite concentrations, and ultraviolet B light exposure in infants fed human milk with and without vitamin D2 supplements. *J Pediatr.* 1989;114: 204–212
10. Greer FR, McCormick A. Bone growth with low bone mineral content in very low birth weight premature infants. *Pediatr Res.* 1986;20:925–928
11. Committee on Nutrition, American Academy of Pediatrics. The use of whole cow milk feeding. *J Dent Child.* 1985;5:384–387
12. Johnson TR, Moore WM, Jeffries JE. *Children Are Different: Developmental Physiology.* 2nd ed. Columbus, OH: Ross Laboratories; 1978
13. Mimouni F, Koo WWK. Neonatal mineral metabolism. In: Tsang RC, ed.

Calcium and Magnesium Metabolism in Early Life. Boca Raton, FL: CRC Press; 1995:71–90

14. Koo WWK, Mimouni FM. Calcium and magnesium metabolism in preterm infants. In: Tsang RC, ed. *Calcium and Magnesium Metabolism in Early Life.* Boca Raton, FL: CRC Press; 1995:55–70
15. Prentice A, Parsons T, Cole T. Uncritical use of bone mineral density in absorptiometry may lead to size related artifacts in the identification of bone mineral determinants. *Am J Clin Nutr.* 1994;60:837–842
16. Specker BL, Tsang RC, Ho ML, Landi TM, Gratton TL. Low serum calcium and high parathyroid hormone levels in neonates fed "humanized" cow's milk-based formula. *Am J Dis Child.* 1991;145: 941–945
17. Hillman LS. Bone mineral content in term infants fed human milk, cow milk-based formula, or soy-based formula. *J Pediatr.* 1988;113:208–212
18. Hillman LS, Chow W, Salmons SS, et al. Vitamin D metabolism, mineral homeostasis, and bone mineralization in term infants fed human milk, cow milk based formula or soy based formula. *J Pediatr.* 1988;112: 864–874
19. Chan G, Mileur L, Hansen J. Effects of increased calcium and phosphorus formulas and human milk on bone mineralization in preterm infants. *J Pediatr Gastroenterol Nutr.* 1986;5:444–449
20. Slemenda CW, Reister TK, Peacock M, Johnston CC. Bone growth in children following cessation of calcium supplementation. *J Bone Miner Res.* 1993;8:S154. Abstract

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PEDIATRICS
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

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