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Bone Mineralization Outcomes in Human Milk-Fed Preterm Infants

RICHARD J. SCHANLER, PAMELA A. BURNS, STEVEN A. ABRAMS, AND CUTBERTO GARZA

United States Department of Agriculture/Agricultural Research Service Children's Nutrition Research Center and Section of Neonatology, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030; and Division of Nutritional Sciences, Cornell University, Ithaca, New York 14853

ABSTRACT. We evaluated bone mineralization by single photon absorptiometry at 2 y in a cohort of preterm infants studied since birth. Infants were fed human milk fortified with Ca [to achieve 80 mg/dL (19.96 mmol/L)] and P [40 mg/dL (12.91 mmol/L)] from wk 2 through 8 after birth. After hospital discharge, infants were divided into two groups (HM and F) determined by the timing of the introduction of cow milk-based formula. Mid-radius bone mineral content (BMC) was assessed in 10 infants who were breast-fed (HM) for a minimum of 2 mo after hospital discharge and 11 who were bottle-fed (F). The mean duration of human milk-feeding differed by design between HM and F groups (31 ± 15 *versus* 11 ± 3 wk, respectively). Although we had observed previously that group F had significantly greater BMC values at 16, 25, and 52 wk compared with values in group HM, we found similarities in BMC values (180 ± 30 mg/cm) between groups at 2 y. The 2-y cohort comprised healthy infants and the groups had similar birth weights, lengths of gestation, and values for weight (10.8 ± 1.1 kg), length (82 ± 2 cm), and bone width (7.8 ± 1.1 mm). Follow-up outcomes at 2 y in preterm infants fed fortified human milk in hospital suggest that if they continue to receive human milk after hospital discharge, radius BMC will "catch-up" to that of similar infants given formula in the posthospitalization period. (*Pediatr Res* 31: 583–586, 1992)

Abbreviations

BMC, bone mineral content
HM, human milk-fed infants
F, commercial formula-fed infants
NCHS, National Center for Health Statistics

To provide appropriate mineral homeostasis in preterm infants, in-hospital feeding regimens often dictate that human milk be fortified with Ca and P (1). After hospitalization, however, some preterm infants receive unfortified human milk via breast-feeding, whereas others receive commercial formula. In previous publications, we have evaluated bone mineralization in two groups of preterm infants whose diets were changed from fortified human milk feedings in hospital to either exclusive breast-feeding or commercial formula-feeding after hospital discharge (2, 3). Body lengths and weights in the two groups were similar at hospital discharge (approximately 10 wk) and throughout visits during the 1st y after birth (2, 3). We found differences, however, in mid-radius BMC at 16, 25, and 52 wk postnatally. Although indices of bone mineralization were similar among the infants at discharge, the group who continued to be breast-fed after discharge had significantly lower values throughout the 1st y. The formula-fed infants, however, attained bone mineralization indices equivalent to those of full-term infants by 25 wk (6 mo after birth).

Our concern that the BMC of human milk-fed preterm infants at 1 y remained well below that of full-term infants prompted a further evaluation. Therefore, we extended the follow-up of our cohort of preterm infants to their 2nd birthday. In this study, we tested the hypothesis that BMC in human milk-fed preterm infants remains lower than that of similar formula-fed preterm infants at their 2nd birthday.

MATERIALS AND METHODS

The subject population consisted of a cohort of healthy preterm infants enrolled in a study of the efficacy of feeding fortified human milk during their hospitalization (4, 5). While in the hospital, the infants were fed their mothers' milk combined with available mineral-protein mixtures (4, 5). Fortification of each mother's milk was continued until her infant achieved a body weight of 1.8 kg, at which time unfortified human milk feedings were begun. Before hospital discharge between 8 and 10 wk after birth, lactation counselors discussed future plans for breast-feeding with the parents. Approximately 50% of the parents indicated that for a variety of reasons (*e.g.* return to work, never intended to breast-feed) they would switch their infants to cow milk-based formula soon after hospital discharge. From the initial cohort of infants, two groups emerged for study after hospital discharge: one (HM) for whom breast-feeding would continue, and one (F) to whom cow milk-based formula would be fed. Follow-up visits have been reported for 10, 16, 25, and 52 wk after birth (2, 3). Those infants still residing in the south Texas region were brought back for follow-up measurements at their 2nd birthday. Of the original cohort of 33 infants, 21 were located and were available for study.

Body length was measured with a pediatric length board (Stadiometer; Holtain Ltd., Crymych, UK); body weight was measured with an electronic scale (Sartorius; Brinkman Instruments, Westbury, NY). The heights of both parents, reported by history, were averaged as "mid-parent" height. BMC of the left mid-radius was measured by single photon absorptiometry using the SP-2 scanner from Lunar Radiation Corporation (Madison, WI). To account partially for differences in bone growth between subjects, density, or BMC per unit bone width, was estimated.

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Correspondence: Richard J. Schanler, M.D., Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, 1100 Bates St., Houston, TX 77030.
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Data were analyzed by *t* test, χ^2 and Fisher exact analyses, and multiple linear regression. Data are expressed as the mean ± SD.

RESULTS

Table 1 depicts the characteristics of the study population. By design, the duration of human milk-feeding (defined as >75% of milk intake) was greater in group HM (median 26 wk, range 20 to 72 wk) than in group F (median 11 wk, range 6 to 16 wk). Subsequently, infants were fed a variety of cow milk-based formulas; whole milk and solid foods were introduced at the discretion of the parents and pediatricians caring for the children. There were no differences between groups in birth weight, length of gestation, or racial distribution; gender distribution differed between groups ($p = 0.04$). When used as covariates in subsequent analyses, neither gender nor race altered the interpretation of the results.

At the 2-y visit (mean age 24.4 mo), similarities between groups were observed for ages corrected for prematurity (21 mo), body weights, lengths, and head circumferences (48 ± 1 cm). The average growth measurements were in the 20 to 30 percentile ranges on National Center For Health Statistics grids and did not differ significantly from the corresponding height for age and weight for age percentiles and Z-scores at 1 y (paired *t* test for each, $p > 0.2$). A similar number of children were below the 10th percentile in each group.

Significant differences between groups in bone mineralization indices at 1 y were no longer apparent at 2 y (Table 2). The rate of change in BMC during the 2nd y did not differ significantly between groups (Fig. 1). Overall, the rate of change during the 2nd y was significantly lower than the rate during the last 6 mo of the 1st y ($p < 0.001$). The rates of change in BMC in the 2nd y were 77% (group HM) and 48% (group F) of the BMC values in the last 6 mo of the 1st y ($p = 0.3$). The increment in bone width from 1 to 2 y, however, was significantly greater in group HM (1.2 ± 0.8 mm) than in group F (0.4 ± 0.7 mm) ($p = 0.05$).

The patterns of mineralization at 1 y (BMC 107 ± 20 *versus* 139 ± 32 mg/cm, bone width 6.3 ± 0.8 *versus* 7.0 ± 1.0 mm, in groups HM *versus* F) among the nine infants that we were unable to locate for the 2-y follow-up were similar to the overall population at 1 y.

BMC values of study preterm infants were compared with published BMC values from full-term infants at 1 and 2 y (6–10). When fed cow milk-based formula or mixed diets, the average radius BMC values for full-term infants were reported as 132 to 150 mg/cm at 1 y and 182 mg/cm at 2 y (7, 9, 10). Average BMC values in our preterm infants in group F at 1 and 2 y were similar to the published estimates from full-term infants. BMC values in group HM, however, were similar to full-term infants only at 2 y.

We examined several factors considered to affect or be associated with BMC (Table 3). Results of observations at 1 y are included for comparison with observation at 2 y. Although the relationships appeared stronger at 1 y than at 2 y, the findings were consistent in both years. We observed no relationships between BMC and gender, racial distribution, mid-parental height, or season of measurement. Significant relationships were detected between BMC and body length ($p = 0.02$). Paternal height and child's height at 2 y also were correlated significantly ($r = 0.54$, $p < 0.01$).

DISCUSSION

Two groups of preterm infants were fed fortified human milk during their hospitalization. After hospital discharge, the rate of bone mineralization was lower in human milk-fed compared with formula-fed infants. The differences in bone mineralization persisted to 1 y. Data from the present study, however, suggest that "catch-up" bone mineralization is attained by the 2nd birthday.

We have reported that high Ca and P intakes from fortified human milk during the early months of hospitalization of preterm infants improve mineral retention and normalize serum mineral status indices. We have also suggested that mineral bioavailability be considered when attempts are made to augment Ca and P retentions, because such efforts have not yet achieved net retentions that equal intrauterine Ca and P accretions (2–5, 11). Therefore, at hospital discharge, it is not surprising that the mid-radius BMC values of preterm infants are well below those reported for full-term infants (2). The greater BMC values after hospital discharge in our formula-fed infants compared with human milk-fed infants suggest that the failure to match intrauterine Ca and P accretion in the early months after birth is critical. Our data further suggest that if the quantity of dietary Ca and P cannot be augmented adequately in fortification mixtures during hospitalization, infants should be monitored after discharge for evidence of mineral insufficiency. If such evidence exists (*e.g.* elevated serum alkaline phosphatase activity, low serum phosphorus concentration, and low BMC), then the du-

Table 1. *Characteristics of 21 study children*

Subject characteristics	Study group HM ($n = 10$)	Study group F ($n = 11$)
Duration of human milk (wk)	31 ± 15*†	11 ± 3
Birth weight (kg)	1.1 ± 0.1	1.1 ± 0.2
Length of gestation (wk)	28 ± 0.7	29 ± 1.1
Gender (boy/girl)‡	9/1	5/6
Race (Caucasian/black/Hispanic/Asian)	5/3/1/1	10/1/0/0
Age at follow-up (wk)	105 ± 2	105 ± 2
Corrected age (mo)	21.4 ± 0.6	21.6 ± 0.6
Body weight (kg)	10.6 ± 1.2	10.9 ± 1.1
Recumbent length (cm)	83 ± 3	82 ± 2
Weight for age percentile§	19 ± 17	29 ± 27
Height for age percentile§	30 ± 29	25 ± 18

* Mean ± SD.

† $p < 0.001$ by *t* test.

‡ $p = 0.04$ by Fisher exact test.

§ National Center for Health Statistics standards.

Table 2. *Bone mineralization of radius in study children*

Bone index	Study groups HM	F	95% CI*
At 1 y			
Subjects (*n*)	13	17	
Bone mineral content (mg/cm)†	114 ± 22‡	137 ± 26	(5, 41)
Bone width (mm)§	6.6 ± 0.8	7.2 ± 0.8	(−0.01, 1.3)
Bone density (mg/cm²)	177 ± 46	190 ± 26	(−17, 43)
At 2 y			
Subjects (*n*)	10	11	
Bone mineral content (mg/cm)	177 ± 35	182 ± 27	(−34, 24)
Bone width (mm)	7.8 ± 1.2	7.7 ± 1.1	(−0.9, 1.2)
Bone density (mg/cm²)	227 ± 34	238 ± 24	(−38, 17)

* 95% CI, confidence interval of differences.
† $p = 0.016$.
‡ Mean ± SD.
§ $p = 0.052$.

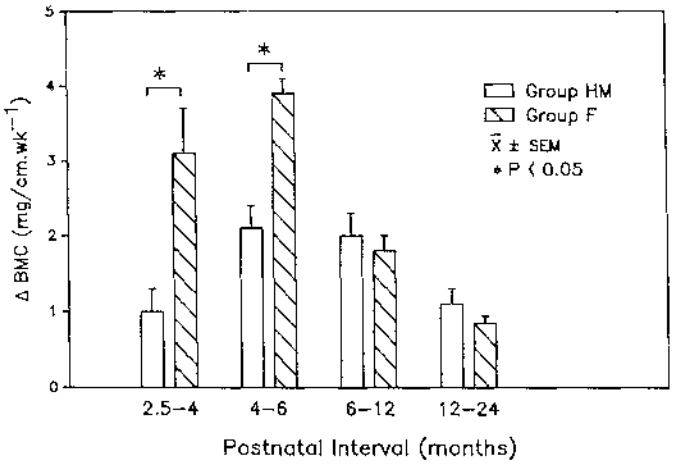

Fig. 1. The change in mid-radius BMC of preterm infants during selected intervals in the first 2 y after birth.

ration of fortified human milk feedings may need to be extended into the posthospitalization period.

We found no change in weight and height percentiles on the National Center for Health Statistics grids for the study infants between the 1st- and 2nd-y visits. The bone mineralization indices in group HM, however, approached those of the full-term infant only at the 2nd y. These data suggest that skeletal mineralization lags behind somatic growth. Other investigators, however, have reported catch-up bone mineralization before catch-up growth (12). We observed a relationship between body length and BMC, which suggested that somatic growth and BMC are interdependent. Lucas *et al.* (13) have demonstrated that poorer growth and bone mineralization are long-term outcomes of extreme dietary inadequacies in the early postnatal period. Because human milk was fortified early in the neonatal period, thus avoiding extreme deficiencies in dietary Ca and P, we encountered no growth faltering among infants in our studies.

Data from the present study were compared with published BMC data of the same bone region measured by similar instrumentation. We found that BMC in our study population at 2 y was similar to that of full-term infants who had received a mixed diet (6–8). BMC in group F at both 6 mo and 1 y was similar to that of full-term infants fed either commercial formula or a mixed diet (6–10). One study (9) reported no statistically significant differences in bone mineralization between full-term infants fed human milk and those fed formula; there was, however, greater scatter in the data obtained from human milk-fed infants. Other reports, using different measurement sites and instrumentation, also reported no differences between full-term infants fed human milk and those fed formula at 1 y (14, 15). If full-term infants accumulate bone minerals at a similar rate whether the infants are fed human milk or formula, then why do preterm infants fed human milk after hospital discharge accumulate bone minerals at a slower rate than preterm infants fed formula? We suggest that the amounts of Ca and P accumulated by preterm infants fed fortified human milk while in the hospital are limited by the quantities supplied in the milk; this limitation is not corrected by the subsequent feeding of unfortified human milk after hospital discharge.

The implications of catch-up bone mineralization for the long-term health of our study population are not entirely clear. Although we studied only 21 healthy preterm infants, the 95% confidence interval for the differences in BMC (Table 2) suggested that biologically important differences (greater than 1 SD from the mean) were not missed. The risk imposed by low BMC values has yet to be determined, especially for those infants whose health status is compromised. Inadequate growth may be one of the detrimental outcomes of feeding preterm infants unfortified human milk; rickets, bone deformity, and fractures also have been reported (16). In addition, respiratory function may be affected by bone mineral deficits (17). Poor thoracic mineralization may affect chest wall compliance, promote atelectasis, and facilitate the development of chronically impaired respiratory function. Whether these potential risks arise as a consequence of feeding unfortified human milk has yet to be verified.

We found no significant relationships between BMC and gender or BMC and parental stature as reported in other studies (6, 7). We found no apparent effect on BMC at 2 y in preterm infants who were fed human milk. Because the rate of catch-up

Table 3. *Relationships between BMC and associated factors in study population at age 2 compared with age 1*

Factor	1 y *r*	1 y 95% CI*	2 y *r*	2 y 95% CI
Body length	0.55†	(0.23, 0.76)	0.49‡	(0.07, 0.76)
Body weight	0.5†	(0.26, 0.77)	0.38	(−0.06, 0.70)
Sex (boy/girl)	0.23	(−0.14, 0.55)	0.20	(−0.58, 0.25)
Race (Caucasian/non-Caucasian)	−0.10	(−0.45, 0.27)	0.20	(−0.25, 0.58)
Duration of human milk	−0.48†	(−0.72, −0.14)	0.09	(−0.36, 0.50)
Previous BMC§	0.61†	(0.32, 0.80)	0.40	(−0.03, 0.71)
Serum alkaline phosphatase activity	−0.46	(−0.79, 0.07)	0.39	(−0.37, 0.84)
Mid-parent height	0.16	(−0.40, 0.64)	−0.18	(−0.65, 0.39)

* CI, confidence interval of correlation coefficient.
† $p < 0.01$ by linear regression.
‡ $p = 0.02$ by linear regression.
§ Previous BMC refers to the measurement at 1 y for the 2-y correlation and measurement at 6 mo for the 1-y correlation.

bone mineralization in the human milk-fed group appeared to increase between 1 and 2 y, preterm infants are obviously capable of responding to as yet unidentified factors to achieve normalization of BMC. Furthermore, differences in the rate of mineralization between 1 and 2 y suggest the possibility of continued differences between groups. Further study of this cohort may be warranted.

Our data have demonstrated that bone mineralization in preterm infants fed human milk catches up by their 2nd birthday to that of preterm infants fed formula. We emphasize that this follow-up study evaluated infants who received mineral-fortified human milk as part of their early nutritional management. We are concerned that without intensive nutritional support during their early months catch-up BMC in these infants might not have occurred at 2 y. We further speculate that additional or continued mineral fortification of human milk fed to preterm infants after hospital discharge might lead to earlier catch-up in BMC. These possibilities warrant further investigation.

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