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Bone mineralization status measured by dual energy radiographic densitometry in preterm infants fed commercial formulas

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Abstract

We have studied the effect of two preterm commercial infant formulas with different calcium and phosphorus contents on the mineral balance and bone mineralization of 30 preterm infants at 1 month of age. Bone mineralization was measured by dual energy X-ray densitometry. The formula supplying a higher content of calcium and phosphorus promoted higher mineral retention ($P<0.01$) as well as higher bone mineral content (1.556 vs. 1.073 g, $P<0.01$) and bone mineral density (0.458 vs. 0.424 g/cm^2, $P<0.05$), approaching values of the control group, which comprised a cohort of 15 preterm newborns whose gestational age was 4 weeks older than the subjects selected to be fed with the formulas. The intake of calcium correlated with retention ($r=0.69$); the phosphorus intake also correlated with phosphorus retention ($r=0.95$). Intakes of calcium and phosphorus correlated with the bone mineral content ($r=0.65$) and with bone mineral density ($r=0.49$). We conclude that formulas for preterm infants should not have a calcium content lower than 120 mg/100 kcal and should have a calcium/phosphorus ratio of about 2 to promote adequate bone mineralization.

Keywords: Bone mineralization; Calcium; Dual energy X-ray densitometry; Phosphorus; Preterm infant

1. Introduction

Osteopenia as a consequence of poor bone mineralization is a well recognized

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problem in the preterm infant [13,25]. Relatively high levels of calcium and phosphorus in low birth weight infant formulas and supplementation of human milk with those minerals ameliorate the mineral balance and prevent the appearance of osteopenia [6,13,24]. Daily intakes of 200–250 mg/kg of calcium and 110–125 mg/kg of phosphorus have been reported to promote a bone mineral accretion similar to that obtained during fetal life [25]. However, the intake of calcium and phosphorus is lower in neonates fed on formulas with a low content of these minerals in relation to the estimated needs of the fetus under reference [30].

The aim of this study was to compare the mineral balance and the bone mineralization status of two groups of preterm infants fed infant formulas differing in their calcium and phosphorus contents at 1 month of life. The bone mineral mass and density were estimated in the femur using dual energy X-ray densitometry.

2. Materials and methods

2.1. Patients

Forty-five preterm newborns were admitted to the study; within this group, 30 were included for mineral balance and bone mineralization at 30 days of life and 15, whose gestational age was 4 weeks older on average (Table 1), were considered a control group since their intrauterine growth was considered normal. In the latter group bone mineralization was measured at the first day of postnatal life. All neonates had adequate birth length and weight, presented a good nutritional status in relation to their gestational age according to the standard of our population [16,18], and were free of neonatal disease. The study was approved by the Ethics Committee of the University Hospital and informed consent was obtained from all parents.

The group was divided into two subgroups of 15 neonates, each of which was fed with one of two low birth weight infant milk formulas (Puleva, Spain). Both formulas

Table 1
General characteristics of the preterm infants

	Group I ($n=15$)	Group II ($n=15$)	Control ($n=15$)
Gestational age (weeks)	31.13±1.55	31.26±1.53	35.12±0.22
Male/female ratio	8/7	7/8	9/6
Birth weight (g)	1894±117	1898±131	2173±230
Weight at 30 days (g)	2152±128	2155±131	
Birth length (cm)	43.5±1.26	43.42±1.45	46.2±1.8
Length at 30 days (cm)	45.5±1.03	45.7±1.24	

Results are expressed as mean±SD.

supplied similar levels of energy and nitrogen but differed in their content of calcium and phosphorus per 100 kcal: Group I received 120 mg of calcium and 75 mg of phosphorus; Group II, 100 mg of calcium and 64 mg of phosphorus. The groups had a similar gestational age, birth length, and weight (Table 1). All newborns received 800–1000 IU/day of vitamin D during the first month of age.

2.2. Methods

Mineral balance studies were carried out as previously described [4,5,10]. The urine was collected in autoadhesive plastic bags and for feces a plastic swathe attached to the waist of the infant was used. The volume of urine was measured in a 1 ml precision volumeter and feces were accurately weighed in a precision scale (Metter H-20-T, Curtin Matherson Scientific, Maryland, MO, USA).

Each output of feces and urine was collected and stored at 4°C in a sterile container for the duration of the balance period (72 h); total excreta were then frozen at −40°C until analysis. The intake of formula was calculated by weighing milk bottles before and after feeding and quantifying all losses derived from vomit and regurgitation; the intake of calcium and phosphorus was calculated from the content of these minerals in the formulas and the milk intake.

Calcium and phosphorus in formulas, feces and urine were determined after ashing with nitric acid using a 23 ml Teflon Parr pump and a Moulinex microwave oven at maximum power for 30 s according to the technique described by Lachica [14]. Feces were previously homogenized in 10 MΩ deionized water (1:5 w/w) using a sterile plastic bag and a Stomacher homogenizer. Calcium was determined by flame atomic absorption spectrometry using a Perkin-Elmer AAS model 238 [14] and phosphorus was analysed spectrophotometrically in a UV/Vias Lambda 2 Perkin-Elmer spectrophotometer [9].

Bone mineral content (BMC) and bone mineral density (BMD) were measured by dual energy X-ray densitometry (DEXA) using a Hologic QDR-1000™ X-ray bone densitometer (Hologic Inc., USA) which utilizes quantitative digital radiography to increase the precision and the accuracy of the determinations and to shorten exploration time (3–6 min). The radiation dose was very low (<1 mrem), and DEXA appears to be an excellent and safe tool for pediatric bone mineral measurements [3,17,23,27]. The measurements were performed on the left femur and the analysed area included head, neck, major and minor trocanter, and the intertrocanter region. The information obtained by the densitometer was computerized and BMC expressed in grams of calcium hydroxyapatite, and BMD given in grams of calcium hydroxyapatite/cm^2, were calculated. Both BMC and BMD estimations correspond to the sum of those parameters determined in the neck, trocanter and intertrocanter regions. The coefficient of variation of this technique after repositioning of the neonate is $<3\%$.

The results obtained were analysed by a paired *t*-test for the intervention groups and impaired *t*-test for comparisons between the control group and the intervention group. Linear regression analysis and the correlation coefficient were used to evaluate the relationship between the studied parameters.

3. Results

The increases in weight and length were similar in the two study groups; the weight and length gain averages were 8.61 g per day and 2.10 cm in 30 days for the first group and 8.57 g per day and 2.28 cm in 30 days for the second one (Table 1).

Table 2 shows the results of the calcium and phosphorus balance studies. Absolute values of retention of calcium and phosphorus were significantly higher ($P < 0.01$) in the group of neonates which received the formula with the higher content of those mineral elements. It is unlikely that these differences were influenced by the Ca/P ratio because the two formulas had similar values (1.6 in group I vs. 1.53 in group II). The intake (x) of calcium and phosphorus was the main factor among those tested which determined their retention (y); calcium intake directly correlated with its retention ($r = 0.69$, $P < 0.01$; $y = 1.13x - 198.07$), and this was also true for phosphorus ($r = 0.95$, $P < 0.01$; $y = 1.03x - 35.63$).

The fecal and urinary losses of calcium and phosphorus were not significantly different in the considered groups. No correlation could be found between dietary mineral intake and urinary and fecal calcium and phosphorus losses.

The group of preterm neonates receiving the formula with the higher content of calcium and phosphorus presented a better bone mineralization status at 1 month of postnatal age, as evidenced by higher BMC ($P < 0.01$) and BMD ($P < 0.05$) values (Table 3). The BMC of Group I did not differ from that of the control, but BMC for Group II was significantly lower ($P < 0.01$); however, both study groups had a lower BMD than that of the control group, although the significance of the difference was higher for Group II ($P < 0.01$) than for Group I ($P < 0.05$). There was a direct linear correlation between calcium and phosphorus intake and BMC and BMD (Table 4).

Table 2
Calcium and phosphorus balances in preterm infants during 30 days of life

	Group I	Group II
Calcium intake (mg/kg/day)	148.66±8.67	121.89±11.07[a]
Phosphorus intake (mg/kg/day)	92.76±5.41	79.60±7.23[a]
Calcium losses (mg/kg/day)		
Urine	3.71±2.83	4.46±4.15
Feces	47.30±16.88	42.45±26.61
Phosphorus losses (mg/kg/day)		
Urine	2.63±2.93	2.11±1.28
Feces	3.52±1.93	3.50±2.63
Calcium retention (mg/kg/day)	97.64±19.93	74.96±32.73[a]
Phosphorus retention (mg/kg/day)	86.61±6.42	73.97±8.71[a]

[a] $P < 0.01$.

Table 3
Bone mineralization in preterm infants fed infant milk formulas with different calcium and phosphorus levels

	Group I ($n=15$)	Group II ($n=15$)	Control ($n=15$)
BMC (g)	1.556±0.237	1.073±0.341[b]	1.601±0.340[c]
BMD (g/cm^2)	0.458±0.023	0.424±0.047[a]	0.463±0.035[c,d]

Results are expressed as mean±SD.
[a]$P<0.05$; [b]$P<0.01$ vs. Group I; [c]$P<0.01$ vs. Group II; [d]$P<0.05$ vs. Group I.
BMC, bone mineral content; BMD, bone mineral density.

4. Discussion

All neonates in this work exhibited a growth rate which resembled that of the control group, with higher BMC and BMD in the group of neonates which received higher quantities of calcium and phosphorus. These infants present values of BMC and BMD close to those obtained during intrauterine life, although mineral retention was approximately 66% of that estimated for the fetus. These data suggest that preterm infants do not need calcium and phosphorus supplies much greater than those provided by the formula I used in this study and that the ESPGAN recommendations for preterm infants regarding calcium and phosphorus intakes [8] are adequate for these neonates.

The discrepancy between the results obtained in this study and those reported in the literature related to bone mineralization might be due to the methodology used to determine that parameter. Dual energy X-ray bone densitometry permits a better estimation of the axial skeleton mineralization than that obtained by monophotonic absorptiometry [17,23]. In fact, it has been proposed that this latter technique should be applied to the humerus more than to the radius since by using the latter bone the determination of bone mineralization presents a higher error [26]. Biphotonic absorptiometry permits the estimation of the bone mineral mass at the axial skeleton level and is more precise and safer than monophotonic absorptiometry [21], although its use during the first year of life should be limited because of the duration of exploration and the need for patient collaboration.

The results obtained with dual energy X-ray densitometry are highly correlated

Table 4
Relation between bone mineral content (BMC) and bone mineral density (BMD) and calcium and phosphorus intake in preterm infants

	BMC (g) ($n=30$)	BMD (g/cm^2) ($n=30$)
Calcium intake (mg/day)	$r=0.65$[a]	$r=0.49$[a]
	$y = 0.344 + 0.004x$	$y = 0.365 + 0.0003x$
Phosphorus intake (mg/day)	$r=0.65$[a]	$r=0.48$[a]
	$y = 0.533 + 0.0003x$	$y = 0.38 + 0.0004x$

[a]$P<0.001$. BMC and BMD (y); calcium and phosphorus (x).

with those obtained by biphotonic absorptiometry both at vertebral ($r = 0.98$) and proximal femur levels ($r = 0.95$) [11] having the advantage of better image resolution and low radiation exposure. Lyon et al. [15] have determined bone mineralization in low birth weight infants using dual energy radiographic densitometry of the radius. Here, trabecular bone is included in the analysis, but because only one segment of bone is scanned, repeated measurements never describe exactly the same anatomical structure; precise repositioning is very difficult, and this is highly restrictive in longitudinal studies. In contrast, DEXA performs an integral measurement of the trabecular and cortical bone in the newborn lumbar vertebra and femur [3,17,23]. BMC and BMD of the lumbar spine and femur correlated positively with birth weight, body area, length, head circumference and gestational age [17,23].

The similarity of growth rates in the two groups of preterm infants considered in this study can by explained by the homogeneity of groups in terms of gestational age and birth weight as well as the resemblance of energy and nitrogen intakes. These data are in agreement with a previous report [28] which found that the weight gain of low birth weight infants fed milk formula can be predicted taking into account only the metabolizable energy intake. The weight and length gain in the studied neonates can be considered as adequate since they were only slightly lower than those achieved during the intrauterine life as estimated by somatometric data obtained in the control group.

The estimation of fetal body composition [29] has served as a reference for the calculation of nutrient requirements in the preterm infant. It has been demonstrated that higher intakes of calcium and phosphorus lead to a better retention of those minerals and to a bone mineralization status closer to that which could be obtained intrauterine [1,6,7,10,12,13,19,22,24]. Supplementation with calcium and phosphorus offers a simple and safe way of achieving the desired fetal bone mineral accretion rate in preterm infants; bone calcium deposit rates increased markedly and significantly as net calcium absorption and retention increased [2,20]. In our study the neonates in the two groups did not reach the estimated fetal calcium and phosphorus retentions, suggesting that, at least theoretically, the intakes were not sufficient. However, Ziegler considers that the calcium and phosphorus requirements of the preterm infant for adequate growth and bone mineralization are probably lower than those of the fetus [30].

We conclude that infant formulas for preterm infants should contain a minimum of 120 mg/100 kcal of calcium and the Ca/P ratio should be kept close to 2 to promote adequate bone mineral accretion and the bone turnover needed for growth.

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