Critical analysis of pathophysiological, diagnostic and therapeutic aspects of metabolic bone disease in very low birth weight infants

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Abstract

Objective: to perform a systematic review on the pathophysiology, diagnosis and approach of metabolic bone disease in very low birth weight infants.

Sources: literature review of articles published in Medline within the last twenty years.

Summary of the findings: the higher survival of very low birth weight infants was concurrent with the increased incidence of metabolic bone disease. The process of bone mineral acquisition suffers some alterations during the neonatal period, including low bone mineral content at birth, insufficient mineral supply in the neonatal period, and regulatory disorders, which may compromise growth and development on the long run. The diagnosis is based on the association of risk factors, and biochemical and radiological alterations. The early intervention in the neonatal period prevents the development of severe metabolic bone disease, reducing complications during the first year of life.

Conclusions: the early diagnosis of metabolic bone disease allows for early intervention, thus preventing complications that may originate from the alterations in bone mineral acquisition.


Introduction

Osteopenia of prematurity in very low birth weight infants was first reported in the literature in the study of Ylpo et al.1 Vitamin D deficiency was considered an important etiological factor, which motivated its use as a nutritional supplement. As a consequence, a decrease in vitamin D deficiency was observed in the following decade.

After this period, until 1943, few studies were carried out on the presence of osteopenia of prematurity. In 1943 Benjamin et al.,2 while carrying out metabolic balance analysis in preterm newborn infants (PNB), concluded that maternal milk as the only source of nutrition was insufficient in terms of minerals, thus favoring alterations in bone mineralization. In corroboration to these findings, Von Sidow et al.3 described osteopenia of prematurity in newborns (NB) on exclusive breastfeeding and without phosphorus (P) supplementation.

During the 1960s, studies started to underscore the need for mineral supplementation, especially of P, in order to
ensure appropriate bone mineralization rate (similar to that in intrauterine life) in PNB and during the first weeks of life.

Shaw et al. described a series of minimal nutritional requirements of very low birth weight NB (VLBW-NB). These requirements were aimed at allowing for a rate of growth similar to that of intrauterine life. At the same time, other authors have underscored the importance of breastfeeding as a nutritional source for PNB. In 1978, Forbes et al. questioned whether the amounts of calcium and phosphorus in human milk were sufficient for a bone mineralization rate similar to that of intrauterine life and for this group of NB.

Considering that calcium (Ca) and phosphorus (P) are the most abundant ions in the organism, and are predominantly distributed in the bone, their homeostasis is essential for a favorable medium for the mineralization process. An adequate bone Ca:P ratio favors the formation of hydroxyapatite and, consequently, a more satisfactory mineralization. Consequently, the amounts of Ca and P received in both intrauterine life and the neonatal period exert a significant influence on the homeostasis of this process. This influence occurs in addition to the effect of various hormones such as parathyroid hormone (PTH), 1,25-dihydroxyvitamin D [1,25 (OH)2 D], calcitonin, parathyroid hormone-related protein (PTHrP), and other hormones.

The greater accretion of minerals in intrauterine life occurs during the third trimester of gestation, and it peaks at 34 weeks. Consequently, for PNB the amount of Ca and P received during the neonatal period is fundamental, since these babies were deprived of the stage of greater accretion of minerals during gestation.

Initially, the alterations in mineralization observed in VLBW newborns were called osteopenia of prematurity. This term was employed in an attempt to characterize a situation of bone hypomineralization during the evolution of these NB in the neonatal period. In other studies, this condition was called rickets of prematurity, due to the X-ray alterations of rickets with the term used. This term comprehends slight bone hypomineralization and X-ray alterations of rickets with spontaneous fractures; thus, it includes both previously described abnormalities. Moreover, the term rickets of prematurity can lead to a misinterpretation in that it deals with a status of vitamin D deficiency. In 1985, it was determined that metabolic bone disease (MBD) be the term used. This term comprehends slight bone hypomineralization and X-ray alterations of rickets with spontaneous fractures; thus, it includes both previously described abnormalities. Moreover, the term rickets of prematurity can lead to a misinterpretation in that it deals with a status of vitamin D deficiency - which is not related to the physiopathology of MBD.

Improved survival of PNB lead to an increase in incidence of MBD. In this sense, there has been an increased interest in elucidating the physiopathology of MBD and in defining a scheme for adequate prevention and treatment of this metabolic abnormality. In addition, the factors that govern bone mineral acquisition have been given more attention in view of normal growth, especially in the sense of increasing peak bone mass as a strategy for the reduction of fractures in adult life.

The greatest challenge in the handling of MBD is the absence of well-defined biochemical and/or radiological abnormalities that appear in early stages as indicators of mineral deficiency. Numerous studies have tried to define laboratory data as indicators of Ca and P deficiency, but none of these attempts were successful. Thus, the definition of parameters for early diagnosis of MBD is still to be done in order to allow for early detection and intervention. Moreover, these parameters would also allow for prevention of a severe clinical status that may compromise long-term growth of PNBs.

**Intrauterine metabolism of minerals**

Calcium is the most abundant cation in the organism. Ninety-eight percent of bodily Ca is found in the bones, thus constituting one of its main inorganic compounds.

Phosphorus is the second most abundant ion in the organism. It is mainly distributed in the bone, in which 80% is found in the skeleton and 9% in skeletal muscle. The remaining P is distributed in lipids of the cellular membrane, which are composed of high energy (adenosine triphosphate - ATP), intracellular proteins for signal translation, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA).

During all stages of growth, bone mineral acquisition is dependent on delivery of adequate mineral amounts of vitamin D. Moreover, it is also dependent on perfect hormone control that favors mineralization and limits bone mobilization, thus promoting an increase in bone mineral content.

During intrauterine life, Ca is actively transported against a concentration gradient in the direction mother-to-fetus at the placenta. The umbilical cord levels are more elevated than those of the mother.

Serum P levels of the mother tend to lower during gestation as a result of the increased requirements of the fetus. Concentration of P in the fetus during the third trimester of gestation is greater than that of the mother. The placental transfer of P is an active process against a concentration gradient; it uses the sodium (Na) gradient as a source of energy with coupled transport of P.

Intrauterine accretion of minerals occurs after the 24th week of gestation until the end of the term. There is a constant ratio of Ca:P, which is ideal at 2:1 at bone level and at 1.7:1 in the body. Maximum peak accretion of minerals was reported between 34 and 36 weeks of gestation; during this stage, different authors have reported an accretion of Ca of approximately 100-120 mg/kg/day, and of P of 60-75 mg/kg/day.

During gestation, there is an adaptation in the metabolism of the mother, which favors an increase in intestine absorption of Ca and in bone reabsorption in order to fulfill fetal requirements.
These activities are a result of an increase in circulating levels of 1.25 (OH)₂D, which favors bone absorption and mobilization. The described alterations do not depend on maternal reserves nor on vitamin D supplementation.

Various hormone factors favor the process of intrauterine mineralization: the parathyroid hormone-related protein, which plays an important role in maintaining the transplacental gradient of Ca; the production of 1.25 (OH)₂D in the placenta, which can be involved in regulating the production of Ca carrier proteins; the low PTH concentrations, which limit the mobilization of bone minerals; the presence of elevated concentrations of calcitonin in the fetal period, during which it plays its most important physiological role favoring mineral deposition; the release of insulin-like growth factors (IGF-I), which stimulate bone growth and increase mineralization; the high levels circulating estrogen in maternal blood, which favor mineralization. The result of the association of these factors is an increase in bone formation and decrease in reabsorption, which favors a rapid gain of minerals during the third trimester of gestation (during which 80% of bone mineralization takes place).⁷,¹⁴-¹⁸

Regulation of metabolism of minerals

Many hormones are involved in the regulation of the metabolism of minerals.

The vitamin D, through the formation of active compounds, acts on maintaining serum Ca levels, thus stimulating intestinal absorption of the vitamin through activation of local enzymes (Ca/Mg ATPase and Ca/Na ATPase); on promoting bone mobilization, on increasing Ca renal reabsorption acting together with PTH. The active compound of vitamin D (1.25 (OH)₂D) leads to an increase in intestinal absorption of P through an active process and through favoring its uptake process. In addition, at the bone level, the active compound stimulates the formation of matrix proteins, thus favoring the process of mineralization.⁸-¹⁰,¹²

The secretion of the parathyroid hormone, a hormone produced in the parathyroid gland, is inversely related to the serum concentrations of Ca and Mg.⁹ The PTH increases activity and number of osteoclasts through the release of osteoblast factors; enhances tubular reabsorption of Ca and reduces reabsorption of P; elevates the activity of 25 (OH) D-1 alpha-hydroxilase leading to the formation of 1,25 (OH)₂ D; all of these actions are aimed at restoring the values of calcemia.⁷,⁹,¹²

During fetal life, the synthesis and secretion of PTH are probably suppressed by the high serum concentrations of Ca of this period.⁷,¹⁹

The parathyroid hormone-related protein (PTHrP) is involved in the metabolism of minerals and its specific functions in mineral homeostasis are still not well-understood. The PTHrP is found in the placenta and in fetal tissue around 8 to 12 weeks of gestation; this suggests an important role in the process of bone mineralization.⁸,⁹ PTHrP has functions similar to those of PTH in both bone absorption and mineralization, renal P excretion, Ca reabsorption, and production of calcitriol.

Calcitonin is a peptide produced in the parafollicular cells (C cells) of the thyroid. It acts on a receptor connected to the G protein inhibiting the absorption of osteoclasts (which is induced by PTH) and the maturing of precursor cells in mature osteoclasts, thus reducing the mobilization of bone Ca and P.⁷

The action of calcitonin leads to a decrease in tubular reabsorption of Ca, Mg, P, and Na increasing free water clearance. It is possible that the bioactivity of calcitonin modulates the action of PTH in target organs.⁹

During fetal life, it is possible to observe an increase in calcitonin levels, which block the effects of PTH and PTHrP in osteoclasts. These levels are higher than those of adult life. This suggests that the most important physiological role of calcitonin is in mineralization during intrauterine life.⁷,¹²

Metabolism of minerals during the neonatal period

Reported serum Ca concentrations during the neonatal period are of 3 mmol/l in the umbilical cord. These levels are higher than those reported for the mother. However, after birth, the serum Ca concentrations decrease and reach the nadir during the first 48 hours of life. After this period, they increase to levels higher than those of adult life, which last throughout childhood in order to promote a positive balance of Ca and with the objective of attaining normal skeleton development and growth.²⁰

Intestinal absorption is the determining factor of the supplying of minerals. Ca is absorbed in the duodenum by two different processes: an active process with saturable absorption and dependent on adequate levels of 1,25 (OH)₂D; and a passive process dependent on the concentration gradient between the intestinal lumen and serum concentration.²¹,²²

The absorption varies from 30 to 65%; it nears 65% when the baby is being breastfed and remains around 40% when administration of special formulas for preterm newborns.¹⁰,¹⁷ Absorption is influenced by protein-mineral interaction, bioavailability of salts used in the diet, absolute amount of Ca in diet, Ca:P ratio in the milk used, and presence of carbohydrates - which favor absorption. Moreover, absorption is markedly influenced by the action of 1,25 (OH)₂ D, which increases absorption from 35 to 68%.¹⁰,²³

Rowe et al.²³ showed a Ca absorption rate of approximately 74% and retention of approximately 70%. Thus, it is important to evaluate the salts used in diet once their insolvibility leads to lower retention and compromises the process of mineralization.⁷,¹⁰,¹⁷
At the renal level, Ca in glomerular filtrate corresponds to 60% of the plasmatic concentration. Reabsorption occurs mainly at the proximal tubule (50%); renal excretion corresponds to only 1 to 2% of the filtered Ca.10,24

The serum concentration of phosphorus in the umbilical cord is of 1.8 to 2.3 mmol/l. This concentration increases during the first 48 hours independently of intestinal absorption. This increase is possibly influenced by low renal excretion of this ion during the neonatal period. Serum levels remain elevated throughout childhood, which suggests a correlation with skeletal growth rate. Normal values are 2.3 ± 0.2 mmol/l.9

The absorption of P occurs mainly in the duodenum and in the jejunum. It occurs by simple, facilitated diffusion (80-95%) and it is dependent on both intestinal concentration, according to P delivered in the diet and in active sodium transport, and on the action of 1,25 vitamin D. The earlier is the most important determinant mechanism of the absorption of P.10,25

Absorption of P can be as high as 90%, as is the case of breastfeeding. Absorption of P is considered optimal except with soy milk, in which case the presence of phytates compromises absorption because it forms complexes with P.8,17,21,22 The absorption and retention rates for P observed in the study by Rowe et al.23 were, respectively, of 76 and 74%.

Regulating of absorption of P is carried out basically by the kidney, in which case there can be an important tubular reabsorption with urinary P levels that are practically undetectable.7,24,25 At the kidney level, 80% of filtered P is reabsorbed at the proximal tubule, 10% in the distal tubule, and 10% is excreted in urine.

Due to the importance of the diet as the source of Ca and P in the neonatal period, strategies for delivery of P to newborns at risk for MBD should consider the amounts of P in different types of milk. Moreover, these strategies should consider the probable absorption rates of Ca and P.

Regulating mineral homeostasis

At birth, there is an increase in release of PTH during the first 48 hours of life, probably as a response to the fall in serum levels of Ca. As the calcemia starts to normalize, there is a reduction in concentrations of PTH, which near the adult values around the first week of life.7,15

In the NB, the levels of 1,25 (OH)2 D increase during the first 24 hours of life. Next, these levels vary according to the delivery of Ca and P in the diet, probably as a result of the need for greater intestinal absorption of these elements in order to maintain mineral homeostasis.7,9,17

Special attention should be given to the delivery of vitamin D and sun exposure during this period, considering that these are the two main sources of the metabolite. It is also important to underscore that maternal milk delivers amounts of vitamin D that are not sufficient to maintain adequate serum levels.10

Serum concentrations of calcitonin are greater in umbilical cord blood than in maternal blood. There is a marked increase in this concentration during the first days of life for values five- to tenfold higher than those of adults independently of the serum concentrations of Ca. The role of calcitonin during the neonatal period is still not well-understood.9 Apparently, the increase in concentration is stimulated by the action of the catecholamines and glucagon, which are released during birth.7,15

Providing Ca and P in the neonatal period

After birth, the main nutritional source is maternal milk. The milk is responsible for satisfying the needs of the newborn and for promoting better adaptation to the neonatal period.

Nutritional handling of VLBW newborns is still controversial since the basic requirements of these babies are not well-established. The delivery of nutrients is aimed at achieving the same rate of growth of intrauterine life according to the postconceptional gestational age. This rate is based on increase in weight and height and on the values of retention of nutrients and minerals.26

The milk of mothers of term newborns has an average Ca content of 340 mg/l and P content of 140 mg/l. These values present slight variations during the different stages of lactation.27 The ration of CA:P in maternal milk is of 1.8:1 to 2.2:1.

In case cow milk is used in replacement of maternal milk, it is important to consider that despite the greater mineral content of the earlier, most of it is excreted as calcium palmitate resulting, thus, in lower absorption.28

The use of cow milk as a nutritional source for VLBW newborns requires special attention as to what concerns its mineral content. The mineral concentrations found in the maternal milk vary from 20 to 25 mg/dl for Ca and from 10 to 15 mg/dl for P. The ratio of Ca:P is of 1.8 to 2.2:1.7,10 Consequently, for an enteral delivery of 200 ml/kg/day and considering an absorption rate of 60% for Ca and of 90% for P, the maternal milk provides approximately 60 mg/kg/day of Ca and 30 mg/kg/day of P.14,17,29 These rates are much lower than those predicted during the third trimester of gestation, which is the period of greater mineral acquisition for the baby.

In order to improve the delivery of minerals to exclusive breastfeeding, VLBW newborns supplementation of human milk in the form of powder or liquid with added minerals, calories, and proteins. The fortified human milk allows for an accretion more similar to that of intrauterine life.25,30,31 As a result, it is possible to deliver approximately 60 mg/dl of Ca and 33 mg/dl of P.29
The Ca and P solutions, used as a nutritional supplement, are an alternative for a more adequate delivery of minerals. This allows for the correction of abnormalities in mineralization in the absence of the option of fortified human milk.32

Another alternative for providing the nutritional requirements of preterm newborns is through the use of specially designed formulas. These formulas provide approximately 70 to 140 mg/dl of Ca and 40 to 70 mg/dl of P. These values near those of intraterine life.10 Consequently, it is possible to observe a variation in delivery of Ca of 110 to 216 mg/kg and of P of 76 to 108 mg/kg for an enteral feeding of 200 ml/kg/day.14

The activity of vitamin D in human milk is insufficient for maintaining adequate serum levels of 25 (OH)D; thus it is recommended that 400 IU/day of vitamin D be used as a nutritional supplement.19

Metabolic bone disease

The term metabolic bone disease (MBD) refers to abnormalities in bone mineralization of VLBW newborns in comparison to normal fetal skeleton and as a result of insufficient delivery of minerals during the neonatal period.15,17

The MBD does not have a characteristic clinical presentation. It can develop hindering longitudinal growth; maintaining head circumference; and presenting severe rickets with craniotabes, rachitic rosary, and epiphysial dysplasia in the long bones (especially the wrists) in some cases developing with spontaneous fractures.16,33 These newborns may present clinical status of delayed-onset respiratory distress due to the lack of thoracic cavity support and with the development of atelectasis and the deterioration of bronchopulmonary dysplasia.15 Clinical manifestations of the disease appear between the 6th and the 12th weeks of life.14

Currently, it is understood that MBD affects all risk-group newborns in its lighter form, which is called osteopenia.17,34 This develops to more severe forms of the disease in approximately 30% of these newborns.16 Others have reported an approximate incidence of 55% for newborns with less than 1,000 grams and of 23% for those with less than 1,500 grams; fractures were reported in 24% of cases.14,17 The incidence of MBD according to the delivery of nutrients is of 40% in breastfed, preterm newborns; 20% in partially breastfed preterm newborns; and 16% in special formula-fed preterm newborns.35

It is important that this disease that affects VLBW newborns be understood due to the alterations in growth with the development of the disease. Many studies have shown a reduction in bone mineral content in exclusively breastfed, VLBW preterm infants in comparison to preterm infants fed with a nutrient enriched formula during the first year of life.36,37 Schanler et al.,38 carried out a 2-year follow-up of preterm newborns after hospital discharge. The authors observed that the differences observed in the first year of life were not observable at the end of the second year. The bone mineral content (BMC) caught up to that of term newborns with the same postconceptional age. However, James et al.39 carried out a comparative study with full term and preterm infants at a postconceptional age of 40 weeks. Results indicated that the latter presented a significantly lower height, weight, and BMC even with the administration of an adequate diet for preterm newborns.

Lucas et al.40 observed that preterm newborns who were given nutrient-enriched milk formula presented significant increases in linear growth and weight gain than those who were given standard term formula. This is probably a result of a more adequate nutritional and mineral content in the enriched milk.

These differences become less significant as other types of foods become the main nutritional source of infants. This occurs also as their growth rate decreases and, consequently, so do their requirements of Ca and P.34,41

Associated risk factors

The following conditions during the neonatal period favor the development of MBD12,14,16,17,25:

– All newborns with birth weight less than 1,500 g, especially those smaller than 1,000 g are susceptible to the development of MBD. This predisposition is more important in the case of newborns with lower birth weights, who have higher growth and mineralization rate requirements to be met in order to mirror intraterine life. In this sense, these infants are more at risk for being affected by postnatal mineral and nutritional deficiency;

– Newborns with gestational age less than 32 weeks, especially because they present lower mineral reserves at birth. This is a result of these infants being deprived of the stage of greater accretion of minerals, which occurs during the third trimester of gestation;

– Prolonged use of parenteral nutrition, which delivers less Ca and P in comparison to intraterine life. Moreover, there is the possibility of aluminum contamination and its toxic effects that inhibit bone mineralization. Another limiting factor of the delivery of minerals in prolonged use of parenteral nutrition is the volume administered to each newborn; small volumes limit the amount of minerals due to the risk of precipitation in the solution. The solubility of the solution depends on the salts used, duration of storage, room temperature, pH, amino acid concentration, and dextrose;

– Treatment with diuretics, which leads to renal loss of Ca due to inhibition of the absorption of electrolytes in the ascending limb of the loop of Henle. Calcium will be proportional to renal loss of Na;

– Situations that lead to a delay in the start of enteral nutrition, either due to obstacles to its introduction, to
limitation of the volume used (as in cases of respiratory failure, bronchopulmonary dysplasia, patent ductus arteriosus, necrotizing enterocolitis, and so on;  
- Low delivery of minerals in the diet, either due to insufficient content in milk or insufficient volume of milk;  
- Bronchopulmonary dysplasia, which presents various associated risk factors for the development of MBD, including deficient enteral delivery of minerals (due to the limitation in volume administered); use of diuretics (leading to renal loss of Ca); use of corticoids (with the reduction in bone mineral content due to reduction in absorption or renal loss); sedation and immobilization (can lead to loss of bone mass);  
- The use of corticosteroids, which is associated with a reduction of intestinal absorption of Ca, renal loss of Ca, and reduction in BMC.

**Physiopathology**

One of the factors that certainly contribute to the development of MBD is the fact that preterm newborns present low mineral reserves at birth (80% of bone mineral accretion occurs during the third trimester of gestation). To attain the same rate of intrauterine mineral accretion in these newborns is an important objective for the prevention of MBD, as long as the rate is attained without producing undesirable metabolic effects.  

Preterm newborns with exclusive breastfeeding develop a phosphorus deficiency syndrome. The reduction in the delivery of this mineral stimulates an increase in the production of 1,25 (OH)2D with a resulting increase in intestinal absorption of Ca and P. Moreover, the release of PTH is inhibited, which leads to a reduction in renal loss of Ca and P, and, consequently, to hypercalciuria. This inhibition of the release of PTH can provide a protective effect as to what concerns bone mobilization. The effect of 1,25 (OH)2D, however, will remain, stimulating the action of osteoblasts and leading to mobilization of Ca and P through activation of osteoclasts. The continuation of deficient delivery of minerals will result in an increasing mobilization, with a consequent deterioration of bone loss. In this situation, despite a concomitant deficiency of Ca due to low nutritional content of the mineral, it is possible to observe a significant renal loss of the ion due to the absence of bone deposition, which is the result of an inadequate Ca:P ratio.  

The studies developed by Koo et al. have shown that serum levels of 25 (OH)D were normal with the supplementation of 400 IU/day of vitamin D. This corroborates the hypothesis that the deficiency of vitamin D is not a determinant factor for MBD. In turn, the finding of elevated levels of 1,25 (OH)2D is suggestive of mineral deficiency. The increase in production of this metabolite is an attempt to intensify intestinal absorption of Ca and P with the objective of restoring normal bodily content.

Other studies have suggested that MBD is a state of increase bone turnover. The presence of elevated serum alkaline phosphatase (indicating osteoblastic activation) and high urinary hydroxyproline (a marker of bone mineralization) indicated increased bone turnover.  

Seemingly, local alterations related to the release of prostaglandins and PTH-related peptide can be involved in this process of bone reabsorption.  

Studies with radioisotopes showed that urinary Ca in preterm newborns have their origin in both the diet and bone tissue. Moreover, the hypercalciuria observed in preterms, even with adequate mineral supplementation, with good retention rates, suggests the presence of high bone turnover in these newborns.  

Other authors have described the occurrence of MBD in newborns who received “preterm” human fortified milk. Apparently, the use of special formulas improves growth and BMC with the increase in delivery of Ca, P, and proteins; this is achieved, however, without eliminating relative deficiency of P, indicated by a BMC lower than expected considering a similar period of intrauterine life.  

Studies evaluating bone mineral accretion of preterm infants indicated rapid accretion of minerals between 40 and 60 weeks of postconceptional age, which at times was fivefold higher than in term infants, comparatively.  

Considering these findings, studies have raised the hypothesis that there is a phase of rapid mineral accretion starting at 40 weeks’ postconception in preterm infants that substantially reduces the perinatal mineralization deficit.

**Diagnosis**

There is no defined diagnostic method for MBD. Compromise of the clinical status appears late and, in certain situations, the diagnosis is not obtained.  

It is still necessary to develop precise diagnostic methods for this disease in order to reduce the number of complications affecting these newborns, who are already affected by a series of complications during the neonatal period due to their prematurity.

**Biochemical alterations**

Biochemical dosing has been carried out in preterm newborns for early detection of mineral deficiency. These alterations, which precede the appearance of variations in bone densitometry and radiological findings, are observed during the third week of life.  

The presence of serum levels of P lower than 3.6 mg/dl in newborns on exclusive maternal breastfeeding is suggestive of the deficiency of this mineral; this indicates the need for monitoring these infants for the development of MBD.  

Studies have shown that newborns being fed exclusively their mother’s milk presented lower levels of P than those receiving special formulas or mineral
supplementation. Newborns fed with preterm formulas can present associated MBD when their dosage of Ca is normal or low and of P is lower than 5.7 mg/dl. Some authors have already described these alterations with weeks of life.

Supplementation of vitamin D at 400 to 800 IU/day allows for normal serum levels of 25 (OH)D. This would be enough to maintain a normal status of vitamin D in the organism. The dosages of 1.25 (OH)2D, in turn, were elevated.

The activity of alkaline phosphatase involves predominantly the bone isoenzyme, and only 10% involves the intestinal isoenzyme. It is a phosphotranspherase located on the bleb of the matrix of osteoblasts, and it is responsible for the transfer of phosphate residues into the blebs, where the process of crystallization on the connection with Ca is started. With the progress of crystallization, there is a rupture of the blebs with an outflow of alkaline phosphatase to the circulation. In the presence of mineral deficiency, there is an increase in synthesis of alkaline phosphatase without the mineralization being processed. This leads to an increase in serum levels of alkaline phosphatase.

The study carried out by Lucas et al. showed that breast-fed newborns with lower concentrations of Ca and P presented higher levels of alkaline phosphatase. The increase in alkaline phosphatase occurred in the first three weeks of life, with peak values between the fifth and sixth week. These values remained higher for a longer period of time in newborns with MBD alterations.

Despite the fact that these studies hint at the importance of the measurement of alkaline phosphatase for the diagnosis of MBD (alkaline phosphatase values sixfold higher than the upper limit indicate the need for investigation of MBD), this measurement does not, however, give a final diagnosis of the disease; other laboratory findings are required for the final diagnosis. Alkaline phosphatase values do not allow for final diagnosis of MBD because they can change, especially in preterm newborns due to the high bone turnover that is characteristics to these patients and that can be related to the higher growth rates.

In newborns with MBD, it is possible to observe important hypophosphaturia for almost 100% of tubular reabsorption of P, whereas the expected excretion of P should be of approximately 2.0 to 2.3 mmol/l for the preterm infant.

The measurement of fractional excretion of P was (FeP) was reported with a reduction of 20 to 3% during the first week of life. FeP values reached minimum values after three months of age. Moreover, others have observed an increased fractional excretion of Ca in cases of P deficiency. The values observed were higher than 4 mg/kg/day with previous reports of values as high as 35 mg/kg/day. The literature indicates that beginning of mineral supplementation lead to phosphaturia, which was previously absent, and to reduction of calciuria to values of approximately 6 mg/kg/day.

Measurements of the Ca/Creatinine relation showed values of 1 to 1.7 mmol/l in newborns being fed their mother’s milk; moreover, there were results greater than 0.6 in the presence of MBD due to the increase in Ca excretion.

Some authors have advocated the monitoring of urinary Ca and P as a means to evaluate the presence of bone mineralization. During an early stage, in which the delivery of minerals is limited, the authors observed elevated calciuria with practically no excretion of phosphorus (less than 1 mg/kg/day). As the delivery of minerals improved, it was possible to observe urinary excretion of phosphorus and reduction in excretion of calcium, which indicated an increase in bone mineral accretion. In this sense, urinary excretion of Ca and P could be used as a parameter for assessment of delivery of minerals and catch up mineralization in the monitoring of MBD. Mancini et al. carried out a study with the objective of identifying early markers of mineral deficiency. The authors showed that values of calciuria at timed 6-hour collections are higher in newborns who developed MBD; these values were always together with cases of hypophosphaturia. The authors collected samples between the third and fifth weeks of life and underscored the importance of monitoring calciuria for the diagnosis of mineral deficiency.

Based on these studies, at the Maternity of the Pediatrics Department at the Hospital de Clínicas of the FMUSP, the recommendations are to monitor calciuria and phosphaturia at timed 6-hour collections and between the third and fourth weeks of life. These procedures are used for early detection of phosphorus deficiency. The objective of these sample collections are to adjust the delivery of minerals in cases of elevated urinary excretion of Ca (greater than 4 mg/kg/day) associated with reduced excretion of P (less than 1 mg/kg/day) (Figure 1). It is understood that increased Ca excretion together with hypophosphaturia is suggestive of inadequate delivery of minerals to the patient. In this case, it is necessary to reevaluate enteral feeding not only of Ca and P, but also of energy and proteins.

**Radiological alterations**

Abnormalities in radiological examinations are a result of an alteration in the process of bone remodeling. These alterations are a consequence of inadequate intake of minerals, which leads to a deficiency in mineralization and occur in the presence of an increase in the formation of the bone matrix.

Radiological alterations have been reported in 64% of newborns with birth weight less than 1,000 grams. These alterations occur only in cases of important bone mobilization, with a 30 to 40% reduction in bone mineral content. In general, the modifications are detected after 6 weeks of life and indicate an abnormal process of remodeling with an increase of the bone matrix. This occurs without concomitant mineral accretion and are usually detected later in the infant’s life.
Koo’s score is used for the description of these radiological alterations:

- **Grade 1**: presence of bone rarefaction;
- **Grade 2**: presence of bone rarefaction associated with metaphysial alterations, shadows, and subperiosteal bone formations;
- **Grade 3**: associated with the presence of spontaneous fractures.

**Bone densitometry alterations**

The measurement of BMC using densitometry is being developed in an attempt to monitor the speed of mineral accretion in newborns. This measurement represents the amount of hydroxypatite per centimeter of bone.

The measurement of BMC using single photon absorption (g/cm) was initially described as an efficient and rapid method for verifying sequential changes in BMC on the same location. Normally, in this technique, radium is used for the measurement. However, the dosage of one single location compromises precision and reproducibility of the method; in which case whole body methods are preferable. Studies have shown that single photon absorption does not affect bone metabolism as a whole since it uses one single location. Moreover, the measurement is carried out on the cortical bone, thus not including the metaphysial regions with elevated cellular activity.

Bone width (BW) can also measured in order to establish its relation with BMC. Studies have shown a reduction of BMC in preterm infants when compared to values of intrauterine life. The ratio BMC:BW decreased due to the increase in bone matrix formation during a stage of rapid linear growth associated with mineralization limited by low mineral content in diet.

The use of two-photon bone densitometry (g/cm² of the scanned area) presents better precision and uses whole-body measurements. It is not affected by adjacent tissues since it uses the spinal column and hips. This technique differentiates bones from soft tissue and air, thus reducing their interference in the measurements. Two-photon densitometry requires an extended period of time to be carried out. In this sense, the newborn has to be sedated. This aspect of the technique is found to limit its use.

Quantitative CT scans allow for the evaluation of mineral density of the lumbar spine. These measurements are tridimensional. CT scans are, however, affected by the amounts of fat in which case the trabecular bone density is underestimated. The greatest disadvantage of this method is the high dosage of radiation.

The latest technology used is that dual energy X-ray absorptiometry (DEXA) measurements of bone density, which is based on densitometry by two-photon absorption associated with X-rays. This allows for better precision and reduced duration of exposure. This method still need to be approved for pediatric patients.

The study carried out by Minton et al. showed that preterm newborns require a period of approximately 12 weeks to achieve mineral accretion similar to that of 5 weeks of intrauterine life; the authors also observed that the periods with greater mineral accretion correspond to those of improvement in enteral feeding of minerals.

Other studies have shown that newborn infants who were fed fortified or preterm formulas presented greater BMC than those who were fed exclusively their mother’s milk. The reported BMC of these infants is still lower than that of intrauterine life.

**Recommendations**

All preterm newborn infants with risk for developing MBD should be investigated. Early detection of mineral deficiency would allow for prevention of the more severe forms of the disease.

At the Maternity of the HC-FMUSP, it is recommended that monitoring begin between the third and fourth week of life with timed 6-hour urine sample collections. These samples should be used to detect mineral excretion suggestive of mineral deficiency, in other words, to detect significant hypophosphaturia (less than 1 mg/kg/day) and increases in urinary excretion of Ca (greater than 4 mg/kg/day).

In the presence of hypophosphaturia and hypercalciuria, administration of Ca and P supplementation is recommended.
Despite the controversy in recommendations for amounts of Ca and P (with reported values ranging from 77 to 231 mg/kg/day of Ca and 55 to 148 mg/kg/day of P), the values of 200 and 100 mg/kg/day for Ca and P, respectively, have been sufficient for maintaining a mineralization curve similar to that of intrauterine life. Some studies have shown that 150 and 77 mg/kg/day of Ca and P, respectively, for a 2:1 ratio are sufficient; these values allow for avoiding side-effects of high dosages of these minerals, such as nephrocalcinosis, extraskeletal calcifications, and excessive accumulation of Ca and P in bone.

In face of the requirements of minerals for the prevention of MBD, it is recommended that newborns on exclusive breastfeeding be given mineral supplementation through fortified human milk or formulas of Ca and P. After the beginning of Ca and P supplementation, a reduction on urinary excretion of Ca should be expected. This reduction should occur together with normalization of urinary levels of P (greater than 1 mg/kg/day).

The duration of mineral supplementation is still a controversial matter. It is known that newborns who are fed preterm formulas presented bone mineralization at six months of age comparable to that of term newborn infants. In turn, preterm newborn infants who are fed their mother’s milk do not attain comparable bone mineralization until they are 2 years old. Studies have also reported important differences in BMC at 3 and 9 months of age in newborn infants who were fed mineral supplementation.

At our services, thus, we recommend mineral supplementation until 40 weeks of corrected age, either using fortified human milk or preterm formulas in case it is not possible to feed maternal milk.

The study carried out by Cooke et al. shows that there are differences in rate of growth after hospital discharge between infants who were fed conventional formulas and preterm formulas. This suggests the importance of nutrition after discharge for faster catch-up. Schanler et al. observed that BMC values for preterm newborn infants at one year of age caught up to those of term infants when commercial formulas were used; in turn, infants who were breastfed and only fed supplementation during hospital stay caught up only at two years of age.

Some authors recommend the use of mineral supplementation up to 3 months of life, in an attempt to include the stage of greater risk for fractures in these newborn infants.

In addition to mineral supplementation, it is also recommended that vitamin D be administered at 400 IU/day during the first year of life.

The increase in survival of very low birth weight newborns has stimulated the understanding of MBD that affects these newborns. Studies carried out on MBD indicate that the compromising of bone mineralization can affect older ages. This underscores the importance of the diagnosis and intervention as early as possible, with the objective of preventing long-term repercussions of MBD.

References


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