Osteopenia Versus Rickets in Premature Infants

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DEFINITION

Metabolic bone disease is a well recognized problem in premature infants. It has been called “osteopenia, osteoporosis, hypomineralization, undermineralization, demineralization, or rickets of prematurity.” As stressed by Brooke and Lucas (1), no term is ideal for a condition that encompasses a variety of disturbances ranging from mild undermineralization to frank radiological rickets with fractures. Although, the border between the two conditions may be blurred, osteopenia should be used to define a mild to severe degree of hypomineralization of the skeleton when compared to fetal accretion rate whereas rickets should be reserved for cases in which there are definite radiological features of rickets in metaphyses of long bones with or without fractures.

INCIDENCE

The reported incidence of metabolic bone disease in premature infants varies widely from center to center. This is mainly due to the lack of general agreement on diagnosis criteria and the heterogeneity of studied populations. Callenbach et al. (2) reported that the incidence of rickets was 32% in a retrospective review of 125 consecutive premature infants with birthweights below 1500 g in whom serial radiographic and biochemical data were obtained. In preterm infants with a birthweight below 1000 g, Hillman et al. (3) and Lyon et al. (4) found osteopenia in 92% and 75% of survivors, respectively. Osteopenia or overt rickets appears to be more common in premature infants fed breast milk or soy formulas than in those fed standard infant formulas or preterm formulas (5). Most studies show that severe metabolic bone disease is consistently associated with a very low birthweight (less than 1000 g) or severe immaturity (less than 28 weeks of gestation) and that premature infants who developed rickets were simply “sicker” than those who did not.
DIAGNOSIS

Diagnosis of inadequate bone mineralization in premature infants has been based on different criteria including clinical signs, radiological observations, bone mineral content, biochemical markers, mineral balance studies, and postmortem analysis of bone structure and mineral composition.

The clinical signs such as craniotabes, rachitic rosary, respiratory distress, and rib fractures are unreliable and very much delayed in premature infants (4).

The radiologic characteristics of metabolic bone disease in premature infants are dependent on the severity and duration of the impaired mineralization. In 1982, Koo et al. (6) proposed a useful and simple grading of radiologic changes observed on single view radiographs of wrists and ankles taken at 5 and 10 weeks postnatally. Using those definitions, Lyon et al. (4) found 75% of radiological abnormalities among 48 premature infants with a birthweight below 1000 g. Radiological densitometry is imprecise and depends on interpretative skill since minor radiological signs of osteopenia represent major bone undermineralization. However, dual energy x-ray densitometry was recently used to follow postnatal changes in the bone mineral content in low birthweight infants. The approach appears both accurate and precise and can be used in infants undergoing intensive care (7).

Dual photon absorptiometry and quantitative computed tomography which are widely used in adults are not applicable to the measurements of infant's bone. However, single photon absorptiometry has been modified for quantification and the sequential evaluation of bone mineral content in preterm infants. Disagreements still exist among investigators about which machine to use, which bone and which site on the bone to measure, which age-specific standard curves to refer to and how to express the results. The radius shaft has been used for most research on infants. However, the difficulty of measuring bone mineral content on poorly mineralized radii of low birthweight infants has led a few investigators to measure the shaft of the humerus (8). A single measure in a single patient does not allow an accurate diagnosis of bone mineralization because of large biologic variations. Most studies involving serial measurements of bone mineral content show that postnatal skeletal mineralization of preterm infants lags behind intra-uterine bone mineralization among diverse groups of infants fed human milk, currently available proprietary formulas, or parenteral nutrition (8).

Ultrasound has also been used to measure bone density in neonates but the precision must be improved before this new method could prove useful (9).

Biochemical markers such as plasma calcium and inorganic phosphate concentrations, bone-gla-protein (osteocalcin), and serum alkaline phosphatase (AP) activity have been used for screening of rickets in preterm infants. The interpretation of AP levels may be equivocal. Kulkarni et al. (10) concluded from a study in 22 premature infants with rickets that AP levels are good indicators of rickets. Similarly, Glass et al. (11) and Callenbach et al. (2) consider that high AP levels (above 750 or 1000 IU/l, respectively) are related to severe osteopenia and may precede the radiological signs of rickets by 2 to 4 weeks. From a large multicenter trial involving
857 preterm infants randomly assigned to various feeding regimens, Lucas et al. (12) found that AP values of over 1200 IU/l correlated epidemiologically with x-ray evidence of bone disease. However, they reported that 66% of human-milk-fed infants under 1200 g at birth had AP levels of over 1000 IU/l yet less than 2% of these had overt clinical manifestations of bone disease. Other authors found poor correlation between the AP levels and the degree of undermineralization. Lindroth et al. (13) showed that AP levels in 12 premature infants developing rickets were in no case higher than in those without rickets. Lyon et al. (4) observed in 97 preterm infants with birthweights of 1000 g or less that peak values of serum AP higher than 1000 IU/l are associated with radiological signs of rickets in some premature infants but most of them with rickets have peak serum AP levels which do not differ from that observed in the group with normal bones.

Serum levels of osteocalcin or bone-gla-protein (BGP) which is a vitamin K-dependent non-collagenous bone protein synthesized by osteoblasts, may correlate with bone mineralization. In an unpublished longitudinal study carried out in 14 very-low-birthweight premature infants fed fortified human milk or preterm formulas, we found that serum BGP levels increased with postnatal age. There was also a significant positive correlation between BGP levels and AP activity, but both values were a poor indicator of radiological bone status. Pettifor et al. (14) and Salle et al. (15) made similar observations.

In conclusion, AP or BGP values may provide confirmatory evidence in premature infants with radiological signs of severe bone disease. However, the overlap with values from infants with radiologically normal bones is such that its diagnostic value is limited. Skeletal AP is localized on the membrane of osteoblast and its activity is proportional to the rate of bone formation. As a result, serum AP or BGP values reflect probably more bone turnover and remodeling than bone hypomineralization.

PATHOPHYSIOLOGY

The pathogenesis of skeletal lesions in premature infants is usually multifactorial. However, inadequate intakes of calcium and/or phosphorus is the main factor although poor vitamin D status, intestinal malabsorption, copper deficiency, increased acid load, and chronic diuretic therapy have also been implicated.

Rapid mineralization occurs during the last trimester of intra-uterine life. From the chemical analyses of human fetuses it can be calculated that, between 26 and 36 weeks of gestation, the mean intra-uterine accumulation of calcium and phosphorus is about 130 and 75 mg/kg per day, respectively (16,17).

Human milk and most standard infant formulas do not contain enough calcium and phosphorus to allow preterm infants to accumulate these elements at the intra-uterine rate, even if all the calcium and phosphorus were absorbed and retained (18,19).

Mature human milk contains 24–34 mg of calcium and 11–16 mg of phosphorus per 100 ml. These figures do not differ materially in milk from mothers of preterm
infants (20). Metabolic balance studies in preterm infants fed human milk show that net intestinal absorption of calcium is in the range of 60–80% whereas that of phosphorus is about 90%. Urinary excretion of phosphorus is very low (less than 1 mg/kg/day) but that of calcium is not, being often greater than 10 mg/kg/day (18,21). As a result preterm infants fed 150 to 200 ml of human milk per kg per day accumulate only 25–30 mg of calcium as well as 20–25 mg of phosphorus per kg per day, which corresponds to 15–20% of the calcium and 30–35% of the phosphorus accumulation by the fetus in utero. The problem is that in human-milk-fed preterm infants, the phosphate supply is especially limiting so that the low intake of phosphorus cannot meet the demand of rapid soft tissue and skeletal growth. In these conditions, a phosphorus-deficiency syndrome develops which is characterized by hypophosphatemia, normal or high serum calcium, hypercalciuria, absent urinary phosphorus, radiological signs of rickets, and elevated plasma alkaline phosphatase activity. When phosphorus intake is inadequate, plasma reserves fall and phosphorus is withdrawn from the skeleton. Calcium cannot be used for bone growth in the absence of phosphorus and is “wasted” in the urine with a tendency to hypercalcemia (22). Thus, the high urinary calcium excretion which does not imply a defective renal handling of the calcium or of phosphorus, leads to clinical bone disease with the radiological patterns and histologic features of a rachitic process since phosphorus deficiency is able to impair both bone matrix formation and mineralization. It is essentially a direct effect independent of either vitamin D metabolites or parathyroid hormone. Resolution of rickets and correction of hypophosphatemia and hypercalciuria with resultant increase in calcium retention has been demonstrated in preterm infants fed human milk supplemented with phosphate alone (21). However, significant improvement in calcium and phosphorus retention and in bone mineral content is only observed in premature infants fed human milk supplemented with both calcium and phosphorus in an appropriate ratio ranging from 1.5:1.0 to 2.0:1.0 (19,23,24).

Mineral content of most infant formulas is higher than that of human milk. Metabolic balance studies have shown that phosphorus is usually well absorbed, net absorption ranging from 75–95% (18) lower phosphorus absorption rates have however been observed in preterm infants fed soy-based formulas or with whey predominant cow’s milk based preterm formulas (whey:casein ratio 60:40) containing tricalcium phosphate (5). In formula-fed premature infants, the net absorption of calcium is generally lower than with human milk, and as little as a 10% net absorption may occur (5,16,18).

In our experience, calcium absorption rate is influenced by a number of factors including gestational and postnatal ages, vitamin D status, calcium and phosphate salt bioavailability, quantity and quality of fat in the diet, lactose intake, and intestinal loss of endogenous calcium.

Supplementation of infant formulas with calcium salts is generally associated with increased calcium retention. However, this improvement may be overestimated. For example, it has been claimed that oral calcium supplements can result in calcium retention similar to the intra-uterine rate. There has however been some concern
with the interpretation of these balance data since phosphate retention was not affected by the supplementation and an apparent Ca:P retention ratio as high as 5:1 has been reported (25). This strongly suggests that sedimentation of calcium salts had occurred in the bottle so that the babies received less calcium than was thought.

More recently, infant formulas have been designed specifically for preterm infants. They usually contain greater amounts of minerals than standard infant formulas and also easily absorbed fat such as medium-chain triglycerides. Balance studies with these formulas have demonstrated increased calcium and phosphorus retention, and some groups have claimed in utero accretion rates for calcium retention and bone mineralization but those results may be erroneously high in view of the heavy precipitation of calcium and phosphorus in some of these formulas (5). In a recent study, Horsman et al. (26) showed, by measuring bone–mineral content of the forearm that mineral supplementation can reduce but not cure osteopenia of prematurity.

ROLE OF VITAMIN D

Current evidence suggests that in most cases bone disease is caused by a mineral rather than a vitamin D deficiency (27,28). However, we have clearly demonstrated that net absorption of calcium in human-milk-fed as well as formula-fed premature infants is dependent on their vitamin D status (18). Premature infants may have greater requirements for vitamin D than infants born at term. They have lower stores at birth and impaired absorption due to low intestinal bile salt concentration. Although Glorieux et al. (29) have shown that the vitamin D activation pathway is operational in premature infants, it may not be fully developed in very early gestation. The dose normally recommended for full term infants, 400 IU/day (10 µg), is probably insufficient to maintain or to reach adequate 25-hydroxyvitamin D (25(OH)D) plasma concentration in premature infants from mothers with borderline vitamin D status during pregnancy, a situation which is common in North European countries where milk is not routinely enriched with vitamin D. Even premature infants born from mothers living in North America, who generally have a far better vitamin D status at birth, require doses up to 800–2000 IU/day to get adequate production of 1,25-dihydroxyvitamin D, the major metabolite involved in intestinal calcium absorption. Hillman et al. (30) found that 55% of a group of preterm infants failed to increase their serum 25(OH)D levels when supplemented with 400 IU of vitamin D and they suggest the administration of 800 IU of vitamin D per day. The use of small doses of 25(OH)D rather than vitamin D may seem logical since in very small preterm infants absorption of vitamin D may be impaired by small bile pool size. The administration of 25(OH)D during the first two days of life at a dose of 10 µg/day has been shown to increase rapidly 25(OH)D plasma levels (18). However, its administration at higher doses and for longer periods of time may be dangerous since it can lead to accumulation and toxicity due to the long half-life of this compound. In addition, in the experience of Hillman et al. (30) the administration of 25(OH)D in contrast to 800 IU of vitamin D does not improve bone mineralization.
On the other hand, the administration of active metabolites of vitamin D and especially the more potent ones (1α-hydroxy- and dihydroxy-derivatives) is potentially dangerous since they increase serum and urine calcium concentrations and urinary excretion of hydroxyproline secondary to increased bone resorption (31).

CONCLUSION

Metabolic bone disease is extremely common in very low birthweight premature infants. While various incidence figures have been reported, it should be emphasized that some degree of hypo-mineralization is an inevitable consequence of feeding with human milk and indeed many available formulas.

Such bone disease has been investigated by x-rays, single-photon absorptiometry, and sequential monitoring of plasma alkaline phosphatase activity and phosphorus level.

Evidence suggests that in most cases, osteopenia is caused by a deficiency of mineral substrate rather than vitamin D. With the use of preterm formulas or human milk fortifiers, overt clinical rickets with fractures becomes relatively uncommon; the vast majority of cases are subclinical. Attempts at preventing or treating metabolic bone disease of premature infants with massive dose of vitamin D or its potent metabolites were usually futile in the absence of mineral supplementation. Nevertheless, even if the mineral intake is sufficient, poor vitamin D status may be a contributory factor by impairing net intestinal mineral absorption.

Human-milk-fed infants should be supplemented at least with phosphorus to avoid a severe metabolic bone disease related to a phosphorus-deficiency syndrome. At the bedside, a plasma concentration of phosphorus below 4.6 mg/l or 1.5 mmol/l with either a urinary excretion of calcium greater than 6 mg/kg per day, or a urinary calcium to creatinine ratio (mg/mg) greater than 0.8 or a urinary Ca:P ratio greater than 1 are good indicators of the need for phosphorus supplementation. These infants should not be supplemented with calcium alone since they already have significant calciuria, and further addition of calcium without phosphorus may lead to hypercalcemia and renal calcifications. By contrast, addition of both calcium and phosphorus in human milk is feasible and improves calcium and phosphorus retention as well as bone mineralization. The use of calcium and phosphorus enriched preterm formulas results also in considerable reduction in the incidence of metabolic bone disease.

One important aspect lies with the possible long term significance of such metabolic bone disease. Lucas et al. (12) have shown that high plasma alkaline phosphatase activity is associated with a deficit in body length of approximately 1.6 cm at 18 months post-term, even after adjusting for others factors influencing length. These findings suggest that even silent bone disease may have persisting consequences and warrants active preventive measures. In connection with that, recent data (26,32) suggest that very-low-birthweight premature infants would benefit from increased mineral intake following hospital discharge.
REFERENCES

DISCUSSION

Dr. Marx: Very premature infants may have extremely high needs for calcium and phosphorus. Since available formulas are nearly supersaturated with calcium and phosphorus, is there any role for intravenous alimentation?

Dr. Senterre: It is difficult to provide large amounts of calcium and phosphate by infusion because of the solubility problem. It is possible to give a mixture of aminoacids containing 50 mg of calcium and 45 mg of phosphate per dl. To keep it stable and without precipitation the pH in the solution has to be low. If you add bicarbonate, everything is precipitated. We only use these infusions on babies on total parenteral nutrition for a long time. They are the sickest babies and it is difficult to provide enough minerals to achieve a calcium and phosphate intra-uterine accretion rate. They, in fact, accumulate less minerals than those fed preterm infant formula or human milk supplemented with minerals.

Dr. Holick: Because of apparent higher requirements for vitamin D, it has been suggested that, at least in the premature infants, there was a partial inability of the liver to convert vitamin D to 25(OH)D. Do you have evidence for that, when measuring 25(OH)D levels?

Dr. Senterre: There are two points to consider. The first is vitamin D absorption itself, which is probably less efficient than later in life because of the fat malabsorption in the premature infant. The other point, as you mentioned, is the liver capacity to 25 hydroxylate vitamin D. On theoretical grounds there may be a measurable difference. I don't think it really matters in practical terms.

Dr. Markestad: We have found that premature infants need less vitamin D than what you suggest. A daily intake of 500 IU per day was sufficient to rapidly attain high 25(OH)D levels (1). After a few weeks the 25(OH)D concentrations reached a plateau in the high normal range whether the baby started out with high or low 25(OH)D values indicating that the same regulatory mechanisms as in older individuals are operating even in the small preterm infant. Higher vitamin D doses may be unnecessary, and I would be worried about vitamin D toxicity from giving around 2000 IU per day for weeks. We have confirmed earlier findings by Glorieux et al. (2) that even small preterm infants have a normal regulation of 1,25(OH)2D synthesis. Within 3 days of birth the serum concentration is increased, probably in response to the postnatal drop in serum Ca, and within 2–3 weeks they have levels which are 2–3 times higher than those of adults (3). Our studies show that small preterm babies have an appropriate ability to absorb and metabolize vitamin D in a regulated manner in response to dietary supply of minerals.

Dr. Senterre: Looking at 25(OH) levels, it is clear that the situation is different in your country and mine, you have values which are well over 10 ng/ml, while most of our babies
show levels below 8 ng/ml, that correspond to vitamin D deficiency. We thus need to correct
that deficiency rapidly to allow efficient intestinal absorption of calcium and phosphorus but
I agree that once 25(OH) levels are over 20 ng/ml, a daily intake of 500 IU per day is sufficient.

Dr. Guesry: You stated that it is good to add sodium phosphate to human milk and it
would be even better to add also calcium gluconate. If you mix in the same feeding syringe
sodium phosphate and calcium gluconate, you obtain calcium phosphate which is remaining
in the syringe, as published by E. Ziegler and Sam Fomon. On the other hand, if you inject
calcium gluconate by bolus, you increase the risk of ulceronecrotizing enterocolitis. So what
is your recipe for adding calcium gluconate and sodium phosphate to human milk for these
very-low-birthweight infants?

Dr. Senterre: Through trials and errors we found that it was best to add first sodium
phosphate into the milk, then shake well, wait five minutes, and add the calcium gluconate.
You may then let the milk sit overnight, the mixture is stable (see chapter reference 19).

Dr. David: To explain the discrepancies between Dr. Markestad findings and your findings,
it is possible that according to the different forms of vitamin D preparation, either alcoholic,
aqueous, or lipid solutions, the absorption of vitamin D might be modified therefore giving
different results. Could you tell us which kind of vitamin D preparation you used?

Dr. Senterre: It is known that lipid-soluble vitamins, in lipid solution, are poorly absorbed.
We found that when they are dispersed (miscellization) in aqueous solution absorption is
improved. We also obtained rapid increase in plasma 25(OH)D levels with oral administration
of 10 μg of 25-hydroxy D which is a more polar metabolite, much better absorbed in the
intestinal tract (see chapter reference 18).

Dr. Marx: These formulas intended for premature infants can be difficult to prepare. As
they approach saturation for various components, unpredictable effects could arise from other
components not thought to be important. This might account in part for apparently conflicting
results from centers using formulas believed to have similar compositions.

Dr. Markestad: Concerning mineral requirements of preterm infants we know that all
preterm infants fed breast milk or a standard commercial formula become osteopenic. The
question is really—how important is it? In our unit we have provided sufficient Ca and P to
avoid clinical and biochemical rickets, but not attempted to provide a mineral supply any-
where near expected intra-uterine accretion rates. A high mineral supplementation is not
without hazards. It may lead to life threatening intestinal obstruction and to a lower dietary
fat absorption in these often calorie deprived infants.

Dr. Senterre: I agree completely. However, it is very important to avoid the phosphorus
deficiency syndrome and, thus, to supplement human milk with phosphate.

Dr. Beauvais: The pathology of premature babies has changed a lot these last years, and
we treated more and more babies who present with bronchopulmonary dysplasia. What is the conse-
quence of this new pathology on bone metabolism?

Dr. Senterre: To answer your question would require a well controlled study. This is
difficult because several problems are concomitant in those very sick babies. What is clear
is that they have chronic acidosis and are hypercalciuric. The latter may very well be related
to an increase in bone resorption induced by acidosis.

Dr. Markestad: We have looked at serum vitamin D metabolites in two infants with bron-
chopulmonary dysplasia and found a normal pattern. As you suggested they may excrete an
excess of Ca because of a chronic acidic state. They also tend to be treated with a lot of
diuretics which cause a large renal loss of Ca.
REFERENCES

