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OSTEOPENIA OF PREMATURITY

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INTRODUCTION

Osteopenia of prematurity refers to the hypomineralized skeleton of the premature infant compared with that of the normal fetal skeleton resulting from the in utero accretion of minerals. It is best assessed with special roentgenologic techniques. In growing low birth-weight (LBW) infants (birth weight <1500 g and <32 weeks gestational age), it occurs almost without exception. This high incidence is not surprising considering that ~ 80% of fetal skeletal mineralization takes place during the last trimester of pregnancy. Thus, one would expect an increasing degree of osteopenia in premature infants with decreasing gestational age.

Unfortunately, in regard to the premature infant the terms osteopenia and rickets are often used synonymously. The term rickets should be reserved for specific histologic, radiographic, and physical findings that may be associated with several different diseases. Rickets is characterized by the accumulation of unmineralized osteoid, which interrupts the mineralization of the growth plate of bone. It is therefore a disease of growing children. A similar disease process, which occurs in the nongrowing bone of adults, is referred to as

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osteomalacia. However, rickets is found in infants with more severe degrees of osteopenia (see case 1 below). Fractures may also occur in osteopenic premature infants, with or without the radiologic features of rickets (see cases 2 and 3 below). The various clinical presentations of this metabolic bone disease in LBW premature infants are given in the following cases.

CLINICAL CASES

1. A four-month-old, breast-fed female infant was brought to the emergency room following an accident in which she had fallen from a sofa. The parents noted that her left forearm seemed tender. An X ray of the arm revealed cupping and flaring of the distal left radial and ulnar metaphases diagnostic of rickets but found no indication of trauma (see Figure 1). Past medical history revealed that the infant had been born at 27 weeks gestation weighing 970 g and had developed mild respiratory distress syndrome, thus requiring supplemental oxygen therapy for the first 10 days of life. At one week the infant began feedings of expressed mother's milk with a daily multivitamin supplement that included 400 IU of vitamin D. By two weeks, human milk intake averaged 165 ml/kg per day. During weeks four and five, a patent ductus arteriosus required treatment with fluid restriction (85 ml/kg per day of human milk) and intermittent diuretic therapy (2 mg/kg per day of furosemide). Between five and nine weeks, breast milk intake averaged 160 ml/kg per day. At nine weeks the infant was discharged from the hospital feeding directly from the breast. Supplemental vitamins were continued. Serum calcium, phosphorus, and alkaline phosphatase (AP) levels were not determined at this time.

In the emergency room the infant exhibited the following significant findings: weight 4026 g, marked swelling of the ribs at the costal-chondral junctions, soft and thin skull bones, and a slight swelling of both wrists. Laboratory studies included the following serum values: calcium, 10.2 mg/dl (normal 8.2–10.5 mg/dl); phosphorus, 2.8 mg/dl (normal >4.0 mg/dl); 25-hydroxyvitamin D (25-OHD), 40 ng/ml (normal 25–60 ng/ml); 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], 129 pg/ml (normal 17–44 pg/ml); and AP, 1020 IU/L (normal <500 IU/L). The infant was diagnosed with osteopenia of prematurity with rickets and was sent home with oral supplements of calcium and phosphorus. An evaluation two weeks later showed that all abnormal values obtained from the laboratory studies had returned to normal. Radiographic findings of rickets resolved within two months.

2. A 660-g male infant, the second of twins, was born at 25 weeks gestational age. Hospital course was complicated by severe respiratory distress syndrome, which progressed into chronic lung disease (bronchopulmonary dysplasia). The infant required supplemental oxygen therapy until 91 days of age. Lung disease was treated with a bronchodilator (aminophylline) and a

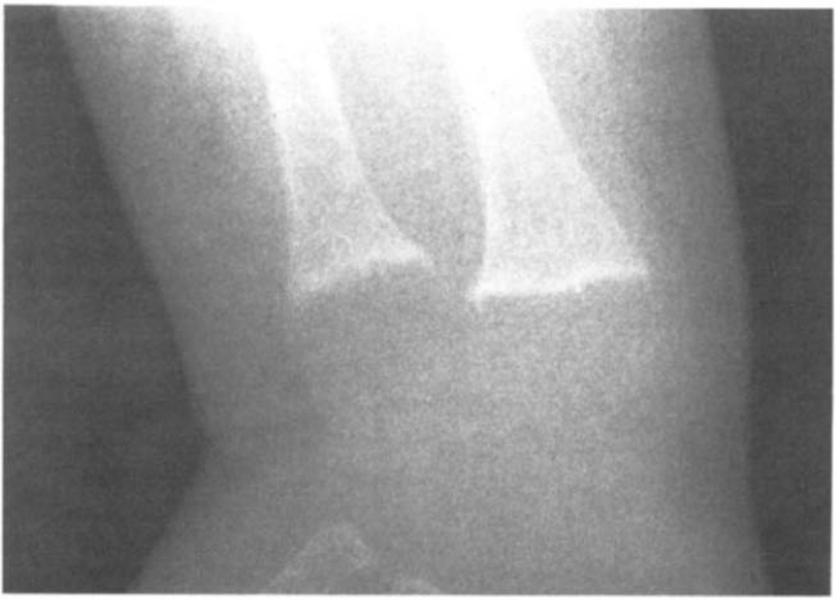

Figure 1 Wrist X ray from a four-month old premature infant fed exclusively human milk with vitamin supplementation. See case 1 in text.

twice daily diuretic (furosemide). Pulmonary fluid retention and several episodes of congestive heart failure required repeated restriction of oral fluids and modification of dietary intake. Nutritional status was a major concern throughout the hospitalization. Initial nutrition was parenteral hyperalimentation (amino acids, glucose, fat emulsion, minerals, and multivitamins, including 150 IU vitamin D per day). By day 27, oral feedings with a nasogastric tube were begun with a standard infant formula (20 kcal/oz). Full oral feedings (120 kcal/kg per day) were rapidly achieved over a three-day period, and hyperalimentation was discontinued. However, because of poor growth, feedings were changed to a 24-cal/oz formula on day 34. Medium-chain triglyceride oil (1 ml/feeding) was added a few days later to further maximize calories while minimizing fluid intake. Although a caloric intake of 110–120 kcal/kg per day was achieved, growth remained relatively poor. On day 85, a routine chest X ray revealed 11 healing rib fractures as well as severely demineralized bones (see Figure 2). Laboratory serum values included calcium at 9.8 mg/dl, phosphorus at 3.9 mg/dl, $1,25(OH)_2D$ at 205 pg/ml, and AP at 1222 IU/L. Diuretics were discontinued, and calcium and phosphate supplements were added to the formula. Over the next two months, no new rib fractures occurred, and bone mineralization improved. Serum values returned to normal.

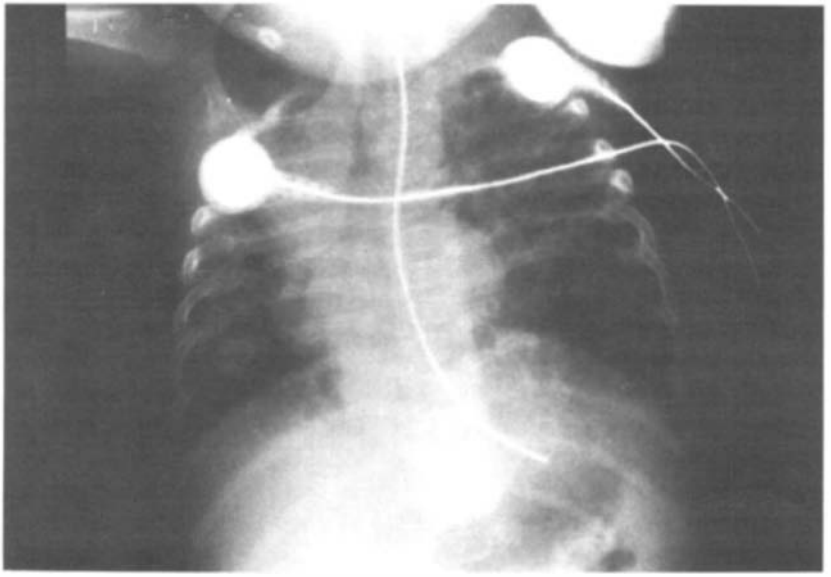

Figure 2 Rib fractures and hypomineralization in a three-month-old premature infant with bronchopulmonary dysplasia. See Case 2 in text.

3. A 860-g male infant was born at 27 weeks gestation. Hospital course was exacerbated by prolonged mechanical ventilation for respiratory distress syndrome complicated by a patent ductus arteriosus and congestive heart failure. Parenteral hyperalimentation and feedings of dilute human milk were begun during the first week of life, with a maximum caloric intake of 75 kcal/kg per day. Between weeks two and three, a severe episode of necrotizing enterocolitis occurred with intestinal perforation. Oral feedings were discontinued, and the infant was maintained on parenteral hyperalimentation. At nine weeks he underwent surgical ligation of the ductus arteriosus. A routine postoperative chest radiograph revealed multiple rib fractures. By 11 weeks the infant had been weaned off parenteral fluids to a formula feeding that supplied 104 kcal/kg per day. Weight was now 1700 g. At 13 weeks a left tibial fracture as well as a healing fracture of the right radius were diagnosed with X ray. At this time, serum calcium was 9.7 mg/dl, phosphorus 3.7 mg/dl, serum $1,25(OH)_2D$ 130 pg/ml, and AP 705 IU/L. Calcium and phosphate supplements were added to the formula feedings. Over the next six weeks all fractures healed and no new ones occurred.

INCIDENCE

As noted above, all infants born at <32 weeks gestation have some degree of hypomineralization during and subsequent to the prolonged period of hospitalization. Just as their growth rate lags behind the intrauterine rate, the skeleton is hypomineralized compared with the in utero rate of bone mineralization. The sicker and more premature infants normally have the most significant degree of osteopenia, and the frequency of rickets/fractures is in general inversely correlated with birth weight. Thus, the incidence of rickets or fractures in infants with a birth weight <1500 g reportedly ranges from 20–32% (8, 21, 33), increasing to 50–60% in infants with a birth weight <1000 g (37, 40, 42). The incidence of rickets or fractures is also increased in infants fed unsupplemented human milk or soy formulas (8, 26, 39).

PATHOPHYSIOLOGY

Data on the histopathology of osteopenia of prematurity are very limited. The histology has been described in only two reports (44, 27), which have emphasized the histologic differences between osteopenia of prematurity and vitamin D deficiency rickets: comparably decreased matrix formation and decreased osteoblastic activity in osteopenia of prematurity.

The etiology of this metabolic bone disease in premature infants is multifactorial (see Figure 3). As stated above, a major factor is preterm infants' lower stores of skeletal calcium and phosphorus at birth compared with term infants; approximately 80% of skeletal accretion of these minerals occurs during the last trimester of pregnancy. Even term infants may have decreased stores owing to maternal complications such as severe preeclampsia with fetal growth retardation and placental insufficiency. Fetal accretion of calcium has been estimated to increase from 130 mg/kg per day at 28 weeks gestation to as high as 150 mg/kg per day at 36 weeks gestation (57, 68). Meeting this intrauterine accretion rate of minerals has been one of the challenges of neonatology, and the failure to achieve skeletal retention of calcium and phosphorus equal to the intrauterine rate is the most important factor in the etiology of osteopenia. This problem is present even in the stable, relatively healthy, growing premature infant. For these infants (birth weight < 1500 g), mature human milk containing 25–35 mg/dl of calcium and 10–15 mg/dl of phosphorus simply does not contain enough minerals to support skeletal growth at the intrauterine rate, even assuming 100% retention. Preterm human milk does not contain appreciably more of these minerals.

However, meeting the intrauterine accretion rates of minerals extends beyond that of simple substrate supply. A number of formula products on the

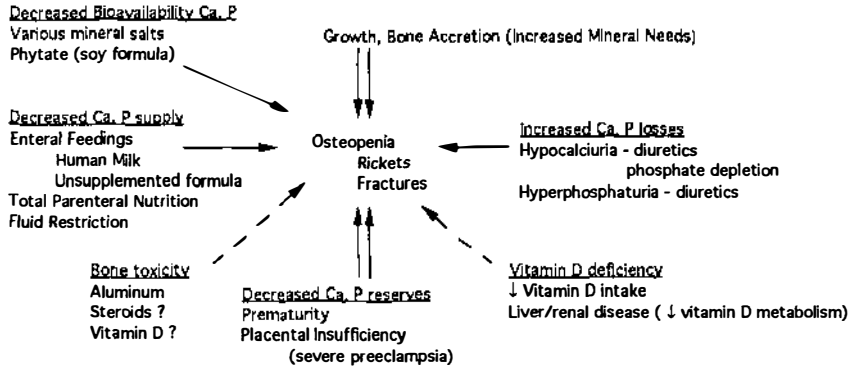

Figure 3 Schema showing factors in the etiology of osteopenia of prematurity.

market theoretically permit the premature infant to achieve an intake of calcium and phosphorus equal to that required to achieve the intrauterine rate of accretion. Some of these products are specially designed formulas with a high mineral content (up to 171 mg/dl of calcium and 85 mg/dl of phosphorus) for the very LBW infant. Other products are fortifiers that contain high levels of minerals and can be added to human milk fed to premature infants. Recent balance studies with these products have shown that retention of calcium ranges from 31–57%, although retention of phosphorus is higher (55–72%) (51, 52, 54). Thus, simply enhancing the mineral content of formulas or human milk does not result in the desired retention rate of minerals in premature infants. These products of high mineral content do not yield the direct relationship between mineral intake and mineral retention reported in previous studies with isolated increments of calcium and phosphorus in milk preparations for human infants (5, 16, 50). Bioavailability of the various mineral salts in these products is therefore a concern because in vivo mineral insolubility may limit mineral absorption and retention. The major calcium and phosphorus losses with these products are in the feces (54). Calcium absorption occurs both by active and passive transport, primarily in the duodenum (63). As stated above, calcium absorption in the premature infant is generally in the 30–60% range, as in term infants. Absorption from human milk may be somewhat higher (20). Phosphorus absorption occurs mainly in the jejunum by active and passive transport and is possibly as high as 90% in very LBW infants fed human milk (56). It remains high in most formulas with the exception of soy-based formulas since phosphate is bound by phytates present in soy protein. Many other factors affect calcium absorption in this population. These include gestational age, postconceptional age, quantity and quality of fat in the diet, lactose, endogenous (intestinal) loss of calcium, and vitamin D (63).

Vitamin D deficiency appears to be an unusual cause of bone disease in the premature infant. Vitamin D (parent compound) and its metabolite 25-OHD cross the human placenta to the fetus, and blood and maternal serum 25-OHD concentrations generally correlate well (17, 28, 55, 65, 67). In non-North American countries, where mothers do not receive vitamin D routinely during pregnancy, cord blood concentrations of vitamin D are lower. For $1,25(OH)_2D$, the most physiologically active vitamin D metabolite, cord blood and maternal values do not correlate well, which suggests that this metabolite does not readily cross the placenta (17, 55, 59, 67), although the human placenta may synthesize its own $1,25(OH)_2D$ (64, 66). Renal 1-alpha-hydroxylase, which is essential for $1,25(OH)_2D$ synthesis, appears to be functional in the human fetus immediately after birth (67). This finding may explain why $1,25(OH)_2D$ is almost always elevated in LBW infants with significant osteopenia. This elevation occurs despite little or no UV light exposure in the intensive care nursery, which implies that the fetus is dependent on the mother or on vitamin D supplements as a source of vitamin D. Finally, daily supplements of vitamin D of up to 2000 IU/day for six weeks did not affect the incidence of osteopenia in a group of 40 premature infants (mean birth weight ~ 1200 g) (21).

Total parenteral nutrition (TPN) is another risk factor for metabolic bone disease in premature infants. Not only is the delivery of calcium and phosphorus decreased compared with the intrauterine rate in these infants, but other factors in TPN, including vitamin D and aluminum (a contaminant), may be toxic to bones. TPN liver disease with cholestasis is another factor in bone disease in this population. The American Academy of Pediatrics Committee on Nutrition recommends a minimum of 30–40 mg/kg of elemental calcium and phosphorus in the TPN solutions for LBW infants, an amount well below the intrauterine accretion rate (AAP Committee on Nutrition, 1993). Thus standard TPN solutions for LBW infants have had low concentrations of calcium (20 mg/dl) and phosphorus (15.5 mg/dl). However, higher concentrations, namely 60 mg/dl of calcium and 46.5 mg/dl of phosphorus, can be achieved in such TPN solutions (34). Running these solutions at 120 ml/kg per day results in a much improved calcium and phosphorus delivery, although it is still less than the intrauterine rate. These higher concentrations are achieved by controlling a number of factors, including the solubility of calcium and phosphorus, length of storage, temperature, calcium salt used, order of mixing calcium and phosphorus, pH, and concentrations of amino acids and dextrose (19). However, for the extremely LBW infant requiring very small daily fluid volumes, such high amounts of calcium and phosphorus in TPN solutions still often result in precipitation of these minerals. Newer mineral preparations hold promise for increasing the calcium and phosphorus concentrations in TPN solutions for the very LBW infant, namely calcium-glycerophosphate and calcium-glucose-phosphate (18, 49).

Iatrogenic factors can result in osteopenia secondary to TPN therapy, including omission of calcium, phosphorus, or vitamin D from solutions. Even vitamin D itself has been implicated in metabolic bone disease in adults when added to TPN solutions to supply 500 IU on alternate days (58). Similarly, aluminum contamination of amino acid preparations and of mineral supplements used in TPN has been associated with metabolic bone disease in premature infants. However, the exact contribution of aluminum in TPN solutions to the bone pathology of LBW infants remains to be determined (31).

Several medications have been associated with increased calcium losses in premature infants. These include the diuretics furosemide and aldactone. Furosemide inhibits electrolyte reabsorption from the ascending loop of Henle, resulting in hypercalciuria proportional to the increased excretion of sodium. Its use as a diuretic agent in premature infants has also been associated with renal calcifications. Chlorthiazide theoretically decreases the renal excretion of calcium as a result of direct action on the distal tubule. However, chlorthiazide is almost always used in combination with aldactone for its K^+-sparing effect. In LBW infants, this combination results in hypercalciuria equal to that of furosemide (3).

Other drugs associated with bone disease in adults are corticosteroids. Though increasingly used in infants with severe bronchopulmonary dysplasia (or for prophylaxis of the disease), their effects on bone formation and bone reabsorption in this population particularly at risk for osteopenia have not been adequately studied.

DIAGNOSIS

In general, a physical examination is not very helpful in the diagnosis of osteopenia of prematurity unless the disease has progressed to an advanced state. In these cases, affected infants may experience tenderness at fracture sites in the long bones. In older patients, the rachitic rosary of the costochondral junction as well as craniotrabes of the skull may be present. Because these infants are non-weight bearing, the more obvious clinical signs of rickets are not evident.

In the vast majority of cases, the diagnosis is made from routine X rays, usually of the chest, where healing rib fractures or severe hypomineralization may be observed (see cases 1 and 2, Figure 2). In the more advanced form of the disease, standard radiographs of the wrists and knees may show the classic signs of rickets, but usually not before two months of age (See case 1, Figure 1). However, conventional radiologic methods cannot detect decreases in bone mineral content (BMC) until a 30–40% loss of bone mineral has occurred (25, 43). Moreover, quantitative morphometric and photodensitometric methods using standard radiographs can only detect bone loss within broad limits

(10–20% error) (25). Obviously, bone biopsy, tetracycline labeling, technetium scanning, and total skeletal biochemical analysis are not practical for serial determinations of BMC in infants. Thus, a number of other specialized radiographic techniques have been employed.

The first of these techniques to be used extensively in LBW infants was single photon absorptiometry (SPA). This method uses a low-energy ^{125}I source (20–200 mCi) that emits a well-collimated photon beam as it passes beneath the bone to be scanned. A scintillation detector moves simultaneously over the bone, measuring transmittance of the photon beam. Attenuation of this beam by bone is directly correlated with the BMC. In adults (43) and piglets (2), evidence indicates that BMC of the peripheral extremity as measured by SPA is significantly correlated with total body calcium content. This technique has proven accurate and reliable in preterm infants (23). As in adults, the bone of choice for examination is the radius. SPA studies of this bone at birth have been used to construct curves of intrauterine BMC (23, 24). Such curves can subsequently be applied in longitudinal studies to compare BMC in LBW infants with that of infants born closer to term. The unit of measurement is gram/centimeter or the amount of bone mineral in a 1-cm longitudinal segment of bone. Using this technology, many studies have documented delays in bone mineralization in premature infants compared with the intrauterine curve of mineral accretion (25, 26). The differences are most dramatic when premature infants are fed human milk vs formulas higher in mineral content (53). One limitation of this technique (probably related to the small segment of bone studied) is that changes in BMC measured by SPA occur very slowly. Thus in general, significant differences can first be detected after five to six weeks of serial measurements (25). Furthermore, no single value of BMC for any postconceptional age has been defined as diagnostic of osteopenia using this technique. Additionally, there is no correlation between BMC and the presence or absence of rickets and fractures in extremely LBW infants (birth weight <1000 g), although these findings are likely secondary to the very low BMC values measured in all of these infants (33).

Other techniques that have been used to measure BMC in premature infants include dual photon absorptiometry (DPA), dual energy X-ray absorptiometry (DEXA), quantitative computed tomography (QCT), and transmission ultrasound (10). DPA uses an isotope of gadolinium, which produces two photon peaks (44 and 100 KeV) with decay. However, the long scan times (20–50 minutes as opposed to 3–5 minutes for SPA) limit its usefulness in this population by increasing the possibility of movement errors. With QCT, the amount of radiation exposure is unacceptable, and little normative data exists for the preterm infant. Transmission ultrasound, while noninvasive and reproducible, lacks the necessary precision in the small bones of this population (10).

The newest of these techniques, DEXA, holds more promise for measuring BMC and assessing osteopenia in premature infants. DEXA uses two electronically generated X-ray beams of relatively high and low energy levels (70 and 40 KeV, for example) and, like DPA, can determine total body BMC as well as regional BMC with minimal tissue radiation (2 millirem). Image resolution (<1 mm), precision (~ 1%), and decreased scan time make DEXA a better technique than DPA. However, the scan time is still relatively lengthy (~ 15 min for total body BMC), and the equipment is too cumbersome to be used at the bedside for studies of sick premature infants. Only a few studies using this technique in premature infants have been published (6, 7, 61). Nevertheless, by measuring total body BMC, DEXA should theoretically be able to detect significant changes in BMC over much shorter time periods than with SPA. However, as with all of these techniques, DEXA is not widely available for use for diagnostic or prognostic purposes and thus remains a research tool.

A number of serum biochemical markers have been used to screen for osteopenia of prematurity. These include calcium, phosphorus, AP, parathyroid hormone (PTH), 25-OHD, $1,25(OH)_2D$, and osteocalcin (OC). Laboratory findings in osteopenia using these markers are summarized in Table 1. Serum calcium is typically normal, although levels may be elevated in cases of phosphate depletion with significant hypophosphatemia (39, 60). Serum phosphorus concentrations are usually "low normal" or low depending on the degree of phosphate depletion (14, 33). Serum AP concentrations in osteopenia of prematurity are more problematic. Although AP is an enzyme found in many tissues, most circulating AP is derived from bone or liver. Furthermore, unless overt liver disease is present, a possibility that can be ruled out by screening for other liver enzymes, the majority of circulating AP is of bone origin, and measurement of the specific bone isoenzyme is unnecessary. Bone-forming osteoblasts have high amounts of AP, and plasma AP activity correlates with bone formation.

The major drawback to using AP to screen for osteopenia is poor sensitivity and specificity of AP activity in relation to the radiographic bone changes of osteopenia. Furthermore, the relationship between postconceptional age and AP serum concentration remains equivocal (35, 62). From a study of 22 premature infants with rickets, Kulkarni et al (36) concluded that AP levels were a good indicator of rickets. Glass (22) and Callenbach (8) found that high AP levels above 750 and 1000 IU/L, respectively, are indicative of severe osteopenia and may precede the radiologic signs of rickets by two to four weeks. In contrast, Walters (62) followed 84 infants, including 3 with radiographic changes of rickets. Five infants without radiologic evidence of rickets had peak plasma AP levels more than two times the upper limit of the adult normal range. Three of these five infants had peak AP levels above those of

Table 1 Diagnosis of osteopenia prematurity

Indications	Results
Radiographic	
Standard X ray	Rickets (knees, wrist) Fractures (ribs, long bones) Hypomineralization
Single photon absorptiometry	↓ BMC compared to intrauterine rate
Dual energy X-ray absorptiometry (DEXA)	↓ total body mineral compared to intrauterine value
Serum	
Ca	Normal (may be increased with hypophosphatemia)
P	Low (<4.0 mg%) or normal
AP	Normal or increased
PTH	Normal or increased
25-OH vitamin D	Normal (intakes 400–800 IU/day)
$1,25(OH)_2$ vitamin D	Increased
OC	Normal (compared to full-term infants)
Renal	
Fractional excretion of Ca (%)	Increased (>2%)
Renal tubular reabsorption of P (%)	Increased (99–100%)

the three rachitic infants. In the largest study of its type, Lucas et al (38) studied 857 preterm infants assigned to various feeding regimens. These authors reported that AP concentrations over 1200 IU/L correlated epidemiologically with radiographic evidence of bone disease. However, in this same study, 66% of the human milk–fed infants under 1220 g at birth had AP levels over 1000 IU/L, but only 2% exhibited overt radiologic manifestations of bone disease. Others authors, including Lindroth et al (37), Lyon et al (56, 40), Evans et al (21), and Pittard et al (48), have found poor correlations between AP levels and the degree of hypomineralization.

PTH concentrations have been infrequently reported in osteopenia of prematurity. Reported values have been normal to increased compared with control values (11, 60). However, serum values of 25-OHD and $1,25(OH)_2D$ have

been extensively reported in this population. $1,25(OH)_2D$ values have been uniformly elevated in infants with osteopenia of prematurity compared with controls, with intakes of vitamin D ranging from 400–800 IU/day. These high $1,25(OH)_2D$ values are often associated with a relatively low serum phosphate concentration (32), thereby confirming phosphate insufficiency as a primary factor in the pathogenesis. 25-OHD concentrations have generally been reported to be normal in studies of osteopenia of prematurity in which infants received up to 400 IU/day of vitamin D (33), although elevated values have been noted in infants receiving as much as 2000 IU/day (21). 25-OHD is the most abundant circulating metabolite of vitamin D and is generally thought to reflect overall vitamin D status. In the growing, hospitalized premature infant, the source of vitamin D is almost exclusively exogenous (dietary supplements).

Serum OC has also been proposed as a biochemical marker for osteopenia of prematurity. This vitamin K–dependent, noncollagenous bone protein synthesized by osteoblasts may correlate with bone mineralization and turnover. However, studies relating OC concentration to radiologic bone status have been disappointing (47, 48). A recent study of 40 very LBW infants found no differences in serum OC concentrations between preterm infants at six weeks of age and those of term infants. Likewise, no significant correlation was observed between OC and the presence or absence of bone disease (21).

Several urinary values have been examined in relation to osteopenia of prematurity. These include fractional excretion of calcium and tubular reabsorption of phosphate as well as calcium/creatinine ratios in studies in which 24-h urines have not been collected. In general, these values depend on the patient's calcium and phosphate status and most particularly on the presence or absence of hypophosphatemia/phosphate depletion syndrome. In the presence of hypophosphatemia, tubular reabsorption of phosphate reaches nearly 100%, and fractional excretion of calcium is paradoxically increased. The presence of a normal fractional excretion of calcium ($\leq$2%) argues against significant phosphate depletion. A highly significant negative correlation has been established between phosphate intake and urinary calcium excretion in very LBW infants at risk for osteopenia (21).

TREATMENT/OUTCOME

Although the treatment of osteopenia of prematurity is somewhat controversial, in the US and Canada, where the maternal intake of vitamin D is higher than many other parts of the world, very LBW infants on oral feedings clearly do not need more than 400–500 IU/day of vitamin D as recommended by the American Academy of Pediatrics Committee on Nutrition (13). The high $1,25(OH)_2D$ concentrations and adequate levels of 25-OHD in almost all

infants diagnosed with osteopenia of prematurity support this recommendation, which assumes that very LBW infants will not be maintained on diets with grossly inadequate concentrations of calcium and phosphorus such as unfortified human milk. Increasing the intakes of vitamin D to 800 IU/day or greater has not proved beneficial (21), and based on the present information, improved absorption of calcium and phosphorus is not likely to occur with these increased intakes. Parenteral requirements of vitamin D are less clear, though with the present multivitamin preparations available in the US for i.v. use in LBW infants, it is difficult to exceed 400 IU/day without concerns for increased amounts of other vitamins. Nevertheless, premature infants on long-term TPN have exhibited adequate vitamin D status on solutions supplying as little as 30–35 IU/kg per day (34). Such low amounts of vitamin D in the infusates would tend to minimize any potential toxic effects of vitamin D that have been reported in adults (58).

If the primary etiology of osteopenia of prematurity is a deficiency of minerals, then the most important component of prevention and treatment of this disease is the supply of adequate amounts of calcium and phosphorus. The logical goal of such therapy would be to attain the intrauterine rate of bone mineralization. To do so would require enteral intakes of ~ 200 mg/kg per day of calcium and 90 mg/kg per day of phosphorus, assuming 65% absorption of calcium (at best) and 80% absorption of phosphorus. A number of specially designed formulas for LBW infants have sufficient amounts of calcium and phosphorus to potentially achieve the intrauterine rate. However, even with these formulas, achievement of this rate of bone mineralization as determined by using techniques such as SPA has been less than satisfactory, particularly in the very LBW infant during the first six weeks of life (24–26). As stated above, human milk requires supplementation with calcium and phosphorus to achieve intakes that would allow the achievement of the intrauterine rate of bone mineralization. Several commercial fortifiers are available for this purpose in both liquid and powdered forms.

One may argue that achievement of the intrauterine accretion rate of bone mineral in very LBW infants during the first six to eight weeks of life is an unreasonable goal because of the difficulty involved. Preventing severe osteopenia with fractures and rickets may prove adequate. Subsequent studies (see below) at 40 weeks postconception have shown catch-up bone mineralization in these infants, particularly if they are not maintained on unfortified human milk.

Although the majority of research effort has been devoted to increasing the calcium and phosphorus intakes of very LBW infants early in life, such high mineral intakes may prove harmful. Recent anecdotal reports have described hypercalcemia in extremely LBW infants on human milk fortified with calcium

and phosphorus (4). Moreover, osteochondrosis accompanied by fetal hypercalcitonemia and supraphysiologic levels of 24,25-dihydroxyvitamin D has been reported in fetuses of ewes overfed with calcium (15).

The prevention and/or treatment of osteopenia in the very LBW infant on TPN is subject to the limitations in the preparations of TPN solutions as discussed above. In these infants, the concentrations of calcium and phosphorus obtainable in these solutions will not likely achieve the in utero rate of accretion of bone mineral. However, enough calcium and phosphorus can be infused with TPN solutions to prevent overt fractures and rickets in these patients. Solutions containing 60 mg/dl (15 mmol) of calcium and 46.5 mg/dl (15 mmol) of phosphorus will maintain desired biochemical and calciotropic hormone indices of mineral homeostasis. These include normal serum concentrations of calcium, phosphorus, PTH, 25-OHD, and $1,25(OH)_2D$ as well as normal renal tubular reabsorption of phosphate (34). Studies using standard radiographs have also revealed less severe metabolic bone disease in premature infants on these TPN solutions (41). Balance studies with these quantities of calcium and phosphorus in TPN solutions have documented good retentions for calcium (88–94%) and phosphorus (83–97%) (12, 45, 46). Thus, 50–60% of the in utero accretion of calcium and phosphorus can be obtained by using these TPN solutions. The ratio of calcium and phosphorus in TPN solutions may also be important. Maximal retention appears to be accomplished with a ratio ranging from 1.3:1 to 1.7:1 by weight (45, 31).

Relatively few studies have documented long-term outcome in very LBW infants with osteopenia, despite the fact that optimal mineral status is difficult to achieve. James et al (30) found that very LBW infants (birth weight <1000 g) have a BMC (as measured by SPA) 35–40% lower than that of term infants at 40 weeks postconception, despite mineral supplementation. In a subsequent study in slightly larger premature infants, BMC (radius) as measured by SPA did not differ from that a control group of full-term infants at 65 weeks postconception (29). This finding implies a period of catch-up mineralization in the premature group at 40 weeks postconception. In a study by Chan and Mileur (9), very LBW infants fed human milk as opposed to standard formula after hospital discharge had significantly lower BMC at 42–56 weeks postconception. Similarly, in a study by Abrams et al (1), a group of very LBW infants fed unfortified human milk after discharge were compared with a group fed standard infant formula. At 52–53 weeks postconception, the infants fed human milk had significantly lower BMC as measured by SPA than those fed cow-milk formula. The group fed formula at this age resembled a control group of full-term infants at the same postconceptional age. However, by two years of age, the BMC of the unfortified human milk group had finally achieved a value equal to that of a formula-fed group of LBW infants. (53) The long-term effects of osteopenia of prematurity, including the effects on bone mineraliza-

tion and stature during adolescence as well as the effects on the risks of osteoporosis in adult life, remain unknown.

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