Rickets in Breast-Fed and Artificially Fed Infants

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Rickets is by definition a disorder of growing bones. The disease is characterized by disturbances in calcium and/or phosphorus homeostasis. As a consequence, abnormalities in endochondrial bone formation arise, together with a defect in and/or delay of mineralization of preformed osteoid. It is generally accepted that rickets refers to the characteristic radiological and histological features that develop in the epiphyseal and metaphyseal regions of growing long bones. Osteomalacia, on the other hand, refers to the changes following the delay in mineralization of preformed osteoid and the associated abnormalities in bone remodeling. The latter occur at the trabecular bone surface and within cortical bone (1,2).

Although rickets is often regarded as synonymous with vitamin D deficiency, the clinical, radiological, and biochemical syndrome of rickets has numerous different pathogenetic mechanisms and etiologies (Table 1). These may be divided broadly into two large groups, depending on whether the basic defect is a failure to maintain serum ionized calcium or phosphorus concentrations within the normal range for age (3). The present review will not discuss the more complex causes of rickets, but will concentrate on the problems of Vitamin D and substrate (calcium and/or phosphorus) deficiency as seen in breast- and artificially fed infants. It will, moreover, highlight some of the controversies concerning prevention, diagnosis, and management.

RICKETS AS A GLOBAL PROBLEM

Rickets in children has been a well-recognized problem in Europe for many centuries. Over three hundred years ago, Francis Glisson in London described the classic features of the disease, but it was not until the early part of the twentieth century that the role of sunlight in its prevention was in-
TABLE 1. The etiology of rickets

<table>
<thead>
<tr>
<th>Etiology</th>
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<tbody>
<tr>
<td><strong>Calcioopenic</strong></td>
</tr>
<tr>
<td>Inadequate formation of 1,25-(OH)$_2$D</td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
</tr>
<tr>
<td>Dietary lack</td>
</tr>
<tr>
<td>Lack of sunlight exposure</td>
</tr>
<tr>
<td>Malabsorption syndromes</td>
</tr>
<tr>
<td>Failure of hepatic hydroxylation</td>
</tr>
<tr>
<td>Severe liver disease</td>
</tr>
<tr>
<td>Increased vitamin D metabolism</td>
</tr>
<tr>
<td>Anticonvulsant therapy</td>
</tr>
<tr>
<td>Failure of renal hydroxylation</td>
</tr>
<tr>
<td>Renal failure</td>
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<tr>
<td>1-α-Hydroxylase deficiency (vitamin D dependency type I)</td>
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<tr>
<td><strong>Phosphopenic</strong></td>
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<tr>
<td>Inadequate phosphate absorption</td>
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<tr>
<td>Dietary lack</td>
</tr>
<tr>
<td>Binding of dietary phosphate within the gastrointestinal tract</td>
</tr>
<tr>
<td>Increased renal losses</td>
</tr>
<tr>
<td>Renal phosphate leak</td>
</tr>
<tr>
<td>Genetic or sporadic hypophosphatemic vitamin D-resistant rickets</td>
</tr>
<tr>
<td>Hypercalciuric vitamin D-resistant rickets</td>
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<tr>
<td>Adult-onset hypophosphatemic vitamin D resistant rickets</td>
</tr>
<tr>
<td>Tumor associated</td>
</tr>
<tr>
<td>Associated with neurofibromatosis or polyostotic fibrous dysplasia</td>
</tr>
<tr>
<td>Complex renal tubular abnormalities</td>
</tr>
<tr>
<td>Proximal tubular defect: primary (e.g., Fanconi’s syndrome, cystinosis, Lowe’s syndrome); secondary (e.g. galactosemia, Wilson’s disease)</td>
</tr>
<tr>
<td>Distal tubular defect: distal renal tubular acidosis</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
</tr>
<tr>
<td>Hypophosphatasia</td>
</tr>
</tbody>
</table>

In the third decade of this century, vitamin D and a number of its related sterols were isolated, and the realization that rickets was due to a deficiency of these sterols was then established. Over the subsequent 30 years, the introduction of vitamin D supplements, the fortification of infant milk formulas, and the supplementation of other food substances with vitamin D in both Europe and America almost eradicated infantile rickets as a public health problem in the industrialized nations of the world.

Recently, however, attention has once again been focused on the problem of rickets in children of immigrant families in Europe. This problem has been particularly highlighted in Great Britain, with numerous reports of both clinical and subclinical rickets in Asian children (4–16). It has also been reported in such countries as Norway (17) and Australia (18). In America, attention
is being drawn to the greater risk of developing rickets in certain groups, especially in infants of black mothers, in those who are breast-fed for prolonged periods, and in those who are on vegetarian diets (19–22).

The marked reduction in the prevalence of vitamin D deficiency rickets in the developed countries of the world has not been seen in a number of third world countries, where the climate would normally have been thought to militate against the development of rickets. However, social customs, such as dress and keeping children indoors to ward off the "evil eye," urbanization, prolonged breast-feeding, and the lack of vitamin D supplements have led to reports of rickets being prevalent in such countries as Pakistan (23), Israel (24), Greece (25), Iran (26) Ethiopia (27,28), and South Africa (29,30).

With the increased care and facilities provided by neonatal intensive care units, the survival of very low birthweight (VLBW) babies has increased dramatically, but with increased survival has come the recognition of the high prevalence of osteopenia, rickets, and fractures in these very small, rapidly growing infants (31). Over the past two decades, our understanding of the vitamin D, calcium, and phosphorus requirements of these infants has increased sharply, and although much research still needs to be done to unravel the interrelationship among the various factors involved in the pathogenesis of the disease, guidelines have been produced on feeding these infants (32).

VITAMIN D PHYSIOLOGY

The physiology, metabolism, and action of vitamin D have been extensively reviewed (33,34); however, certain aspects need to be highlighted in a discussion of rickets in infants.

Photosynthesis of Vitamin D₃

Vitamin D requirements in infants are met by a combination of dietary intake (usually in the form of vitamin D₂) and the photosynthetic formation of vitamin D₃ from 7-dehydrocholesterol in the skin (35,36). The relevant importance of each of these sources is discussed later. Under the influence of ultraviolet light (290–320 nm), 7-dehydrocholesterol in the stratum Malpighii is converted to previtamin D₃ by the cleavage of the C₉—C₁₀ bond. Previtamin D₃ is then slowly thermally converted to vitamin D₃ (Fig. 1), which is released into the circulation over 2 to 3 days following ultraviolet irradiation.

Once vitamin D₃ has been formed in the skin, it is preferentially removed into the circulation by the circulating vitamin D-binding protein (α₁-globulin), allowing further formation of vitamin D₃ from previtamin D₃. Factors af-
fecting the formation of vitamin D₃ in the skin include the amount of skin exposed to ultraviolet light, the intensity of the irradiation, and the degree of pigmentation of the skin. Holick (36) has demonstrated in in vitro experiments that with increasing intensity of ultraviolet light, previtamin D₃ formation reaches a plateau, and the sterol is further isomerized to two biologically inactive metabolites, lumisterol and tachysterol. This mechanism prevents the formation of excessive vitamin D₃ in situations of prolonged exposure to ultraviolet light. Further, the gradual release from the skin allows for a continuous supply of the prohormone during periods of limited ultraviolet exposure (37).

Whether or not pigmentation of the skin plays a major role in limiting vitamin D₃ formation in countries of high latitudes with limited sunshine is a contentious issue. The pigmentation of Asians in Britain (9) and blacks in America (21) has been put forward as a reason for their predisposition to vitamin D deficiency in those countries. Certainly, circulating 25-hydroxy vitamin D concentrations [25-(OH)D] are lower in Asians than whites living in Britain (7,38); however, Asians also tend to have lower intakes of vitamin D (39), and their diets are high in phytate. Also, mothers and children tend to spend more time indoors, and their clothing covers a greater extent of their bodies (11).
Stamp (40) showed a fivefold rise in circulating 25-(OH)D in an Asian after ultraviolet irradiation, a rise of twofold having been reported by Holick (36) in Caucasians given three minimal erythematous doses of ultraviolet irradiation. It should be noted, however, that the rise in 25-(OH)D in the Caucasians would be expected to have been lower, since their initial 25-(OH)D values indicated a vitamin D replete state, whereas the Asian was vitamin D depleted and would thus have formed 25-(OH)D more readily (41).

The evidence to date indicates that given adequate ultraviolet exposure, melanin pigmentation does little to inhibit vitamin D₃ formation in the skin. Whether relatively unpigmented skins form vitamin D more efficiently when the area of skin exposed and the amount of irradiation are suboptimal has not been clearly established.

Absorption of Dietary Vitamin D

Dietary vitamin D is generally in the form of vitamin D₂. The normal Western diet contains little vitamin D, unless foods have been supplemented. Being a fat-soluble vitamin, its absorption is affected by factors that alter fat absorption (42,43). Thus, steatorrhea is associated with decreased absorption of vitamin D, whether the former be due to intestinal, pancreatic, or hepatic dysfunction.

It appears that most vitamin D is transported from the intestine via the lymphatics bound to chylomicrons (44). Orally administered 25-(OH)D₃ absorption is less affected by steatorrhea and is probably absorbed directly into the portal vein (43,44). Animal studies have raised the question as to whether vitamin D₂ and D₃ are equally absorbed and metabolized and have similar actions (45). The rat appears to discriminate in favor of vitamin D₂, whereas the pig and chick favor vitamin D₃. This may have relevance in infants, since foods are generally supplemented with vitamin D₂.

Tjellesen et al. (46) have demonstrated different effects of vitamin D₂ and vitamin D₃ on the bone disease associated with anticonvulsant therapy. Hillman et al. (47) have suggested that differences in premature infants treated with either D₂ or 25-(OH)D₃ might be due to different actions of vitamin D₂ and D₃. Further studies in this area are obviously needed.

Vitamin D Metabolism in the Newborn Infant

The fetus obtains its vitamin D stores from its mother. This is evidenced by the good correlation between maternal 25-(OH)D levels and those in cord blood (48,49). Further confirmation is given by the occurrence of neonatal rickets in infants born to vitamin D-deficient mothers (50,51). Whether or not the vitamin D-replete neonate has significant stores of vitamin D, and how long these stores will last without an exogenous source of vitamin D,
TABLE 2. Breast-fed infants and mothers 25-(OH)D levels at birth and 6 weeks after varying vitamin D supplementation

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th>No vitamin D supplementation</th>
<th>Infant receiving 400 IU/day D₂</th>
<th>Mother receiving 1,000 IU/day D₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant 25-(OH)D (ng/ml)</td>
<td>8.9 ± 7.1</td>
<td>1.1 ± 1.4</td>
<td>15.2 ± 3.7</td>
<td>9.4 ± 2.1</td>
</tr>
<tr>
<td>Mother 25-(OH)D (ng/ml)</td>
<td>11.9 ± 6.1</td>
<td>10.0 ± 5.5</td>
<td>11.0 ± 3.5</td>
<td>14.7 ± 4.0</td>
</tr>
</tbody>
</table>

* From ref. 53.

is unclear. In studies from Finland (52) and South Africa (53), breast-fed infants born to mothers who neither had vitamin supplementation during pregnancy nor received any during the first few months of life had very low levels of 25-(OH)D at 8 and 6 weeks of age, respectively (Table 2).

Because 25-(OH)D is not the major storage form of the vitamin, it is not surprising that 25-(OH)D levels in the neonate fall rapidly, since turnover time is approximately 3 to 4 weeks (54). Information on the amount of vitamin D stored in the neonate at the time of birth is not available, but considering that it must be obtained transplacentally, and that circulating adult levels are under most circumstances low (47), it would be surprising if the neonate had large stores of the vitamin. This might be particularly true for the premature infant who has little fat and muscle, the major sites of vitamin D storage (55).

Cord levels of the other major vitamin D metabolites, 1,25-dihydroxy vitamin D [1,25-(OH)₂D] and 24,25-dihydroxy vitamin D [24,25-(OH)₂D], correlate poorly or not at all with circulating maternal levels (48,56,57). These results would suggest that at birth, the infant's levels are controlled by the neonatal kidney, and with regard to 1,25-(OH)₂D, possibly by the placenta, which has been shown to synthesize the active metabolite of vitamin D (58).

RICKETS IN THE NORMAL INFANT

As discussed in the first part of this chapter, rickets during the first year of life is an uncommon problem except in certain high-risk groups of infants. Vitamin D deficiency can easily be prevented by the provision of vitamin D supplements (400 IU/day; 1 µg vitamin D = 40 IU). This is especially so in those infants who are at risk because of inadequate skin exposure to ultraviolet light due to social customs, or because they live in countries at high latitudes where sunshine is limited. However, with the recent trend of encouraging breast-feeding and delaying the addition of solids until 5 to 6
months of life, attention is once again focused on the vitamin D content of breast milk.

Prior to the era of vitamin supplementation of infant milk formulas, breast-milk-fed infants were reported to have a lower prevalence of rickets than those fed cow's milk preparations. Breast milk was thus assumed to have protective properties against the development of rickets. Yet, from various biological tests, the vitamin D content of milk was found to be low [ranging from 4 IU/liter (59) to 35 IU/liter (60)]. Over the past decade, the vitamin D content of breast milk has been reassessed using newer techniques that are able to separate out the known metabolites of vitamin D.

Several authors have reported that breast milk contains considerable amounts (9–22 μg/liter) of water-soluble vitamin D sulfate (61,62), but these reports have not been confirmed by other workers (63). Furthermore, vitamin D sulfate has been shown to be inactive biologically (64). Although the actual concentrations of the different metabolites in breast milk vary in the various reports, the general consensus is that results obtained by the old biological tests reasonably reflect vitamin D activity in breast milk (Table 3).

Various methods for calculating the biological activity of the metabolites have found the total vitamin D activity to range from 27 to 68 IU/liter (65–69). Hollis et al. (68) assessed the relationship between maternal serum vitamin D metabolite concentrations and those in breast milk and were able to show significant correlations among maternal vitamin D$_2$, D$_3$, 25-(OH)D$_2$, and 25-(OH)D$_3$ and the breast milk concentrations of these metabolites. Vitamin D appeared to cross into breast milk more readily than 25-(OH)D, and vitamin D$_2$ crossed at twice the rate of vitamin D$_3$. This latter finding once again raises the question as to whether vitamin D$_2$ and D$_3$ are handled equally in humans. Breast milk 25-(OH)D normally accounts for some 75% of the total vitamin D activity of breast milk.

In another study, it was shown that the vitamin D content of bovine milk could be increased markedly by supplementing cows with vitamin D (40,000

### TABLE 3. Mean vitamin D metabolite concentrations in human breast milk

<table>
<thead>
<tr>
<th>Vitamin D (pg/ml)</th>
<th>25-(OH)D (pg/ml)</th>
<th>24,25-(OH)$_2$D (pg/ml)</th>
<th>1,25-(OH)$_2$D (pg/ml)</th>
<th>Activity (IU/liter)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>311</td>
<td>52</td>
<td>5.1</td>
<td>27</td>
<td>65</td>
</tr>
<tr>
<td>379</td>
<td>~170</td>
<td>&lt;21</td>
<td>&lt;0.6</td>
<td>48</td>
<td>66</td>
</tr>
<tr>
<td>NM*</td>
<td>&lt;500</td>
<td>NM</td>
<td>&lt;3</td>
<td>—</td>
<td>67</td>
</tr>
<tr>
<td>460</td>
<td>250</td>
<td>NM</td>
<td>NM</td>
<td>33–68</td>
<td>68</td>
</tr>
<tr>
<td>558</td>
<td>206</td>
<td>NM</td>
<td>NM</td>
<td>69 (white mothers)</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>153</td>
<td>NM</td>
<td>NM</td>
<td>34</td>
<td>69 (black mothers)</td>
</tr>
</tbody>
</table>

* (NM) not measured.
IU/day) (65). A similar relationship between maternal intake and the vitamin D activity of breast milk has been shown in humans. Specker et al. (69) demonstrated a correlation between maternal vitamin D intake and breast milk vitamin D concentrations but not between maternal intake and breast milk 25-(OH)D. These authors also found that black mothers had significantly lower breast milk vitamin D$_2$, vitamin D$_3$, and 25-(OH)D concentrations than white mothers. They postulated that the difference might relate to the amount of sun exposure or to the degree of skin pigmentation since the dietary intakes of vitamin D were similar in the two groups.

Hollis et al. (65) was unable to detect any vitamin D metabolites in the breast milk of a proven vitamin D-deficient mother. These studies thus confirm that breast milk is relatively low in vitamin D and that the concentrations of it and its metabolites are dependent on maternal vitamin D status; but does this indicate that breast-fed infants should be supplemented with vitamin D?

Some of the answers to this question are provided by a study from Cincinnati (70). In a report on sunlight exposure and vitamin D status of 48 exclusively breast-fed infants less than 6 months old, it was found that infant 25-(OH)D levels did not correlate with maternal 25-(OH)D concentrations. Black infants had lower 25-(OH)D concentrations than white infants. This difference could be explained by differences in ultraviolet exposure. The authors estimated that in order to maintain an adequate 25-(OH)D concentration, an infant would need to be out of doors for either 30 min/week wearing a diaper only, or 2 hr/week if fully clothed but not wearing a hat.

The importance of sunlight exposure in maintaining breast-fed infant 25-(OH)D levels probably explains the differences that have been observed among several studies reporting on serum 25-(OH)D levels in breast-fed infants. Rothberg et al. (53) and Ala-Houhala (52) conducted their studies in winter and reported that 25-(OH)D concentrations were not maintained in unsupplemented infants. Yet, a study from New Zealand (71) was unable to show a fall in levels after the initial drop in the first 3 weeks postpartum (the time of year when the study was done is not recorded).

A study from the United States (72) documented a significant fall in 25-(OH)D concentrations at 12 and 26 weeks in unsupplemented breast-fed infants, compared with values in vitamin D-supplemented infants (400 IU/day), which remained relatively constant over that period. Two of the nine unsupplemented infants had 25-(OH)D concentrations at 26 weeks of less than 4 ng/ml (a level that has been taken to signify marked vitamin D depletion and at which clinical rickets might develop). The bone mineral content at 12 weeks of age in the unsupplemented infants was significantly lower than that in the supplemented group.

The findings of this study were not supported by another report from America (73), which was unable to demonstrate any difference in bone min-
eral content in unsupplemented white breast-fed infants at 16 weeks compared with either formula-fed or supplemented breast-fed infants. Although serum 25-(OH)D levels were lower in the unsupplemented breast-fed group than in the other two groups, the values remained within the normal range.

Thus, the conclusions reached from the studies on the vitamin D content of breast milk and on serum 25-(OH)D concentrations in unsupplemented breast-fed infants, are as follows:

1. Breast milk alone does not contain sufficient vitamin D to guarantee adequate vitamin D intake for preventing the development of vitamin D deficiency.
2. Ultraviolet exposure of the infant during the first 6 months of life is probably the most important source of vitamin D in the unsupplemented breast-fed baby. Such exposure is necessary to maintain adequate vitamin D status.

Although some workers in the field believe that vitamin D supplementation of the breast-fed baby is unnecessary, especially when environmental and social conditions are favorable (71,73,74), it would probably be prudent to follow the advice of Finberg (75), who commented that he would "continue to recommend routine daily supplementation of 400 IU of vitamin D for breast-fed infants pending further information." He is supported by similar comments from Britain (76) and Turkey (77). Routine supplementation would probably eradicate vitamin D-deficiency rickets in at-risk communities, especially in black American infants, infants of Asian mothers in Britain, immigrant infants in European countries, and children in countries where rickets is still common, such as Greece and Turkey.

Considerable literature exists concerning the potential toxic effects of excessive vitamin D supplementation in the mother and infant. The subject was extensively reviewed by Seelig in 1969 (78). Utilizing the more recently available assays for determining serum 25-(OH)D concentrations in infants, a daily supplement of vitamin D (400 IU/day) does not appear to increase circulating 25-(OH)D concentrations above those normally found in the population (53,72,73) and at which normal mineral homeostasis is maintained. It is possible, however, that the recommended 400 IU/day is unnecessarily high; a study of elderly patients in Britain suggested that 160 IU/day (4 μg vitamin D₂) would maintain 25-(OH)D concentrations above the deficient range (>5 ng/ml) (79).

RICKETS IN VERY LOW BIRTHWEIGHT INFANTS

As early as 1946, von Sydow (80) reported on the higher incidence of rickets in breast-fed premature infants compared with those fed cow’s milk. He also noted that the rickets was not eliminated by vitamin D supplemen-
RICKETS IN INFANTS

Since that time, and particularly over the past decade, considerable attention has been paid to the problems of osteopenia, rickets, and elevated alkaline phosphatase values in VLBW infants.

Considerable confusion exists as to what comprises the syndrome of rickets in the premature infant, since frank radiological rickets and fractures (Fig. 2) are probably at one end of the spectrum, and elevated serum alkaline phosphatase values, hypophosphatemia, and radiological rarefaction of bones are at the other (31).

Gross (81), in a study of growth rates in preterm infants (<1,600 g) on three different feeds (pasteurized milk from mothers delivering preterm infants, pasteurized milk from mothers in well-established normal lactation, and a whey-based formula), found significantly higher alkaline phosphatase values and lower serum phosphorus values in the infants receiving breast milk than those receiving the whey formula. These differences persisted throughout the duration of the study (until 1,800 g).

Similarly elevated alkaline phosphatase values were noted in VLBW infants (<1,500 g) fed either pooled pasteurized or fresh preterm breast milk (82). It has been suggested that an alkaline phosphatase value of up to five times normal adult values may be considered normal in preterm infants, a
value of six times normal adult values would indicate the need for radiological assessment to exclude rickets, and a value seven and one-half times adult values probably indicates active rickets (83).

It should be noted that in this study, all the infants were fed breast milk, and thus the so-called normal values might actually reflect elevated values if compared to values obtained from similar infants fed a formula supplemented with calcium and phosphorus. At 5 to 10 days after birth, alkaline phosphatase values correlated inversely with gestational age (84). In 51 infants (<1,500 g) followed sequentially during hospitalization, the peak in alkaline phosphatase values in a particular infant correlated inversely with gestational age and birthweight, and directly with the duration of parenteral feeding. The degree of osteoporosis, the presence of metaphyseal changes, and periosteal reaction were all significantly related to maximum alkaline phosphatase activity. It would thus appear that elevated alkaline phosphatase levels in the premature infant are an indicator of metabolic bone disease.

At its severest, bone disease in premature infants takes the form of marked rickets and fractures (Fig. 2). More frequently, there is radiological evidence of osteoporosis, which has been diagnosed in up to 75% of VLBW infants at 3 weeks of age (85). The frequency of osteopenia is greatest in infants of the lowest birthweights (<1,000 g). The high prevalence of osteopenia in premature infants fed standard infant formulas has been confirmed by photon absorptiometry (86). The nature of the osteopenia present in premature infants is unclear, although its association with elevated alkaline phosphatase levels and the subsequent appearance of rickets in a number of infants would suggest that the bone disease is one of impaired mineralization.

In an attempt to standardize reports of the frequency of bone changes in premature infants, several grading systems have been proposed. Perhaps the easiest is that put forward by Koo et al. (87), who graded the changes at the wrists and distal tibiae as follows:

Normal. Normal density of bony cortex along the shaft, with normal dense white line at metaphyses and a normal band of lucency in the submetaphyseal region.

Grade 1. Loss of the dense white line at metaphyses, increased submetaphyseal lucency, and thinning of the cortex.

Grade 2. Changes of grade 1 plus irregularity and fraying of metaphyses, with splaying and cupping (typical rickets).

Grade 3. Changes of grade 2 with evidence of fractures.

Hillman et al. (85) grade the severity of osteopenia as mild, moderate, and severe, but these grades may be difficult to assess unless a single radiologist interprets the radiographs.

Several factors appear to be important in the development of bone disease:

1. It becomes progressively more common with decreasing gestational age (85).
TABLE 4. Possible pathogenetic mechanisms for rickets in VLBW infants

<table>
<thead>
<tr>
<th>Abnormalities of vitamin D absorption or metabolism</th>
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</thead>
<tbody>
<tr>
<td>Impaired vitamin D intestinal absorption</td>
</tr>
<tr>
<td>Decreased hepatic hydroxylation of vitamin D</td>
</tr>
<tr>
<td>Decreased 1α-hydroxylase activity in the kidney</td>
</tr>
<tr>
<td>Inadequate calcium and/or phosphorus content of the diet.</td>
</tr>
</tbody>
</table>

2. It is most frequent in breast-milk-fed premature infants (81), but has been reported in infants on standard infant milk formulas (86) or soy milk preparations (88).

3. It occurs more frequently in infants who have had prolonged periods of parenteral nutrition (84) or who have had prolonged illness, such as severe respiratory distress (89).

Several pathogenetic mechanisms (Table 4) have been put forward to explain the prevalence of the bone disease in premature infants and can be grouped as follows: (a) vitamin D deficiency or abnormality of vitamin D metabolism; and (b) dietary calcium and/or phosphorus deficiency.

Vitamin D Deficiency or Abnormality of Vitamin D Metabolism

Because of the higher prevalence of rickets in preterm than in full-term infants, it has been suggested that preterm infants might have higher vitamin D requirements or that there might be abnormalities in absorption of vitamin D or problems with adequate hydroxylation at the liver or kidney level.

At birth, cord 25-(OH)D concentrations are similar in preterm and term infants, although race and season affect both maternal and cord concentrations (48). Serial determination of serum 25-(OH)D concentrations after birth suggest that despite vitamin D supplementation (400–800 IU/day), levels do not rise until a postconceptual age of 36 to 38 weeks is reached (82,90). However, Glorieux et al. (91) were able to demonstrate a marked rise in serum 25-(OH)D levels in preterm infants in the first week of life after 5 days of oral administration of vitamin D (2,100 IU/day). In a group of four infants (<1,500 g) given vitamin D (400 or 800 IU/day) (47), serum vitamin D levels were elevated but lower than those found in adults given a similar dose on a kilogram weight basis. The authors speculate that these results might indicate impaired absorption of vitamin D in the premature infant.

Evidence for decreased hydroxylation of vitamin D in the liver of the premature infant is suggested from several sources. 25-(OH)D concentrations either continue to fall or fail to rise in VLBW infants despite the administration of vitamin D (400–800 IU/day) (82,90). Low but detectable levels of vitamin D, which in adults would maintain normal 25-(OH)D levels,
are associated with low concentrations of 25-(OH)D in the premature infant (47).

Although Glorieux et al. (91) were able to show a significant rise in 25-(OH)D after vitamin D administration, all the premature babies studied weighed more than 1,600 g at birth, and the dose of vitamin D administered was larger than that given by Hillman et al. (47) (400–800 IU/day, compared with 2,100 IU/day). These results suggest that the degree of prematurity is an important factor in determining the impairment of hydroxylation by the liver. Although prematurity might be associated with decreased hepatic hydroxylation, there is no evidence to exclude increased excretion of 25-(OH)D as a cause of the lack of rise in 25-(OH)D concentrations after vitamin D administration in VLBW infants.

Concrete evidence for impaired renal hydroxylation of 25-(OH)D in premature infants is not available. 1,25-(OH)2D concentrations have been reported to be either normal or elevated in VLBW infants suffering from rickets (92,93). Further, a significant rise in 1,25-(OH)2D levels has been demonstrated after vitamin D administration to premature infants (91). The data also suggest that these infants may require higher circulating levels of 25-(OH)D than adults to achieve maximal 1,25-(OH)2D production in the face of hyperparathyroidism. Following vitamin D supplementation, which increased 25-(OH)D levels from 8 ± 2 ng/ml (mean ± SE) to 29 ± 3 ng/ml, 1,25-(OH)2D concentrations rose from 56 ± 8 pg/ml to 154 ± 17 pg/ml, while parathormone (PTH) levels remained constant at an elevated value of approximately 160 mEq/l.

In adult patients with vitamin D deficiency and secondary hyperparathyroidism, Stanbury et al. (94) were able to show a significant relationship between 25-(OH)D and 1,25-(OH)2D concentrations, when 25-(OH)D concentrations were below 10 ng/ml. Extrapolating from their data, a 25-(OH)D value of 8 ng/ml (as found in the preterm infants) would have been associated with a 1,25-(OH)2D value of between 120 and 176 pg/ml. Direct comparisons between the two studies are difficult to make, however, as it is not possible to assess whether or not the degree of hyperparathyroidism was similar in the two groups. In summary, the results do raise the possibility that premature infants might require higher substrate levels [25-(OH)D] to achieve similar 1,25-(OH)2D concentrations in the face of similar PTH values.

Thus, abnormalities of vitamin D absorption and/or metabolism probably do occur in premature infants, the severity of which are related inversely to the gestational age of the neonate. The recommended vitamin D intake of the preterm infant is still open to debate. Hoff et al. (95) documented rickets in VLBW infants receiving mean vitamin D intake of 300 ± 181 IU/day. Seven of the eight infants in whom 25-(OH)D levels were measured had values below 7 ng/ml. All responded by increasing 25-(OH)D levels and healing of the rickets when given vitamin D2 (4,000 IU/day).

In another report, a VLBW infant on a vitamin D intake of 390 IU/kg/day
developed rickets, which healed on 500 IU/kg/day (96). On a vitamin D intake of 400 IU/day, 55% of infants weighing less than 1,600 g at birth failed to maintain circulating 25-(OH)D concentrations or increase initially low levels (85). These infants had lower serum calcium, higher alkaline phosphatase concentrations, and more severe radiological abnormalities than those who were able to maintain normal 25-(OH)D values (>15 ng/ml).

VLBW infants given vitamin D (800 IU/day) have higher 25-(OH)D concentrations than those given 400 IU/day and have less hypophosphatemia and less radiographic evidence of osteopenia (47). From the same study, the authors concluded that there appeared to be no advantage in giving 25-(OH)D (2 μg/kg/day) over vitamin D (800 IU/day) (47). Based on the available data, vitamin D (800 IU/day) would seem to be a safe and efficacious recommended intake for VLBW infants.

Dietary Calcium and Phosphorus Deficiency

Ziegler et al. (97) have estimated from fetal analysis that the daily increment of total body calcium and phosphorus increases from 129 mg and 83 mg, respectively, at 28 to 29 weeks gestation to 302 mg and 179 mg, respectively, at 39 to 40 weeks (Table 5).

Breast milk contains between 250 and 300 mg/liter calcium, whereas the phosphorus content varies between 140 and 200 mg/liter (98,99). Thus the preterm infant receiving approximately 200 ml/kg/day will ingest between 50 to 60 mg/kg/day calcium and 28 to 40 mg/kg/day phosphorus. From the above calculations, and assuming an intestinal absorption of calcium and phosphorus of approximately 50% and 80% of intake respectively, it is apparent that breast milk cannot meet the incremental increases of these elements that occur in the fetus. Standard modified infant milk formulas, although varying considerably in their composition, usually contain twice or more of the concentrations of calcium and phosphorus found in breast milk. Thus, although these concentrations may still not meet the calculated re-

<table>
<thead>
<tr>
<th>Gestational age (Weeks)</th>
<th>Weight (g)</th>
<th>Calcium (mg)</th>
<th>Phosphorus (mg)</th>
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<tr>
<td>24–25</td>
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<td>39</td>
</tr>
<tr>
<td>28–29</td>
<td>22.6</td>
<td>129</td>
<td>83</td>
</tr>
<tr>
<td>32–33</td>
<td>27.1</td>
<td>193</td>
<td>123</td>
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<tr>
<td>36–37</td>
<td>35.7</td>
<td>341</td>
<td>212</td>
</tr>
<tr>
<td>39–40</td>
<td>17.1</td>
<td>302</td>
<td>179</td>
</tr>
</tbody>
</table>

*From ref. 97.
requirements of the rapidly growing premature infant, they do go some way toward meeting them. Despite this, the bone mineral content of premature infants fed a standard infant formula failed to increase at the expected rate for postconceptional age (86).

Even in the face of an adequate vitamin D intake, biochemical evidence of disturbed mineral and bone metabolism as manifested by hypophosphatemia and elevated alkaline phosphatase levels is a frequent finding in premature infants fed breast milk (80,82,100), and several authors have reported hypophosphatemic rickets in breast-milk-fed preterm infants (101,102). The pathogenesis of these abnormalities has been ascribed to both dietary calcium and phosphate deficiency, but phosphate depletion is probably the major component. Renal phosphate excretion is significantly lower in breast-milk-fed preterm infants than in formula-fed infants (103). This is in keeping with their low serum phosphorus concentrations, whereas renal calcium excretion is elevated (~9 mg/kg/day) (104). Supplementation of the breast-milk fed VLBW infants with oral phosphate (0.8 mmol/kg/24 hr) reduces urinary calcium loss, increases renal phosphate excretion, and elevates serum phosphate values without decreasing serum calcium concentrations. PTH values nevertheless rise significantly but remain within the normal range (104).

Similar features of disturbed mineral and bone metabolism are seen in VLBW infants fed standard infant milk formulas, especially in those weighing less than 1,000 g (85). Those infants receiving less than 80 mg/kg/day calcium orally had more bone demineralization, lower serum calcium and phosphorus values, as well as higher alkaline phosphatase concentrations than those with higher calcium intakes (85). Bone mineral content is also reduced in preterm infants fed standard milk formulas (86).

Hypophosphatemic rickets (88), hypophosphatemia, and elevated alkaline phosphatase levels (105) are more frequent in VLBW infants fed a soy-based formula than in those fed modified cow’s milk-based formulas, despite slightly higher calcium and phosphorus concentrations in the soy formula (calcium 700 mg/liter compared with 510 mg/liter in the milk-based formulas; phosphorus 500 mg/liter, compared with 390 mg/liter in the milk-based formulas). The pathogenesis of the hypophosphatemia in soy-formula-fed infants appears to relate to impaired intestinal phosphate absorption (106). Supplementation of a soy formula with calcium (92 mg/kg/day) and phosphorus (44 mg/kg/day) largely prevented the development of hypophosphatemia and elevated alkaline phosphatase concentrations in a group of VLBW infants (107), but weight gain and serum albumin concentrations were not as good as those achieved in a control group receiving a whey-based formula.

Over the past decade, a number of reports have documented a decrease in the prevalence of radiological and biochemical abnormalities indicative of metabolic bone disease in preterm and VLBW infants by increasing the calcium and phosphorus content of standard modified cow’s milk formulas. Steichen et al. (86) were able to approximate extraterine bone minerali-
zation rates in premature infants to intrauterine rates by feeding the infants a formula containing 1,260 mg/liter calcium and 630 mg/liter phosphorus. In a group of smaller infants, Greer et al. (108) showed an increase in bone mineral content in eight of 12 babies fed a high-calcium and high-phosphorus-containing formula (calcium, 1,400 mg/liter; phosphorus, 750 mg/liter) for a period of 3 to 5 weeks. Serum calcium, phosphorus, and alkaline phosphatase concentrations remained constant for the duration of the study.

By supplementing a low-solute milk formula (calcium, 440 mg/liter; phosphorus, 330 mg/liter) with calcium, Laing et al. (109) were able to reduce radiological bone changes but not alkaline phosphatase values in VLBW infants. When both calcium and phosphorus were added to the formula, the prevalence of abnormalities in both variables was reduced.

**CONCLUSIONS**

Biochemical and radiological features of metabolic bone disease are common in breast-milk-fed premature infants, especially in those weighing less than 1,200 g at birth. Similar, but less severe changes are seen in VLBW infants fed standard modified cow's milk formulas. Soy-based formulas appear to exacerbate the problems.

The pathogenesis of the bone disease is probably multifactorial, since abnormalities of vitamin D absorption and metabolism and substrate (calcium and particularly phosphorus) deficiency both play a role. Problems related to vitamin D metabolism can be safely and efficaciously overcome by providing preterm infants with vitamin D at a dose of 800 IU/day. If breast milk is to be used as a feed for VLBW infants, then phosphorus supplementation of the feed should be routine. However, careful monitoring may be necessary, as hypocalcemia following the addition of phosphate has been reported (110). Where artificial feeds are to be used in VLBW infants, it is probably preferable to use one of the specifically formulated premature infant feeds, since these lead to better rates of growth (111). The higher calcium and phosphorus content, moreover, appears to decrease the frequency of radiological and biochemical evidence of metabolic bone disease. Soy-based formulas should be considered only where a specific indication for their use exists, and mineral homeostasis should be carefully monitored.

On the basis of the following four findings, it could be argued that most premature infants do not require any special attention or treatment with regard to abnormalities in bone and mineral homeostasis: (a) biochemical evidence of impaired mineralization is a common finding in breast-milk-fed premature infants; (b) most do not develop any clinical complications related to these changes; (c) biochemical abnormalities generally return to normal by about 3 to 4 months of age; (d) follow-up of these infants does not demonstrate any long-term sequelae.
These findings are probably true, but with the increased survival rates of VLBW infants (<1,500 g), especially those weighing less than 1,000 g at birth, severe osteopenia, rickets, and fractures are becoming well-recognized problems. It is in this group that special attention to the features of metabolic bone disease should be paid and appropriate preventative therapy given.

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REFERENCES


