Impact of Micronutrient Deficiencies on Bone Growth and Mineralization

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Introduction

The first 2 years of life are a period of significant bone growth and mineralization. Garn [1] was one of the first researchers who eloquently described early bone growth based on over 25,000 radiographs of the metacarpals. He measured the mid-shaft width (T) and medullary cavity width (M) of metacarpals and, assuming a cylindrical shape for tubular bones, estimated periosteal diameter, cortical area and cortical thickness (T–M) [1]. Changes in mid-shaft width, or periosteal bone apposition, with age are shown in figure 1. The pattern of periosteal expansion during childhood parallels that of growth curves, with rapid apposition during the first year of life followed by a phase of slower growth until the sex hormone-mediated growth spurt during adolescence occurs. Resorption at the endosteal surface, as measured by the width of the medullary cavity (M), occurs from birth to the second decade of life. The width of the medullary cavity is larger in males compared to females and changes are more rapid during the first half of the first year of life compared to later in infancy. The increase in medullary cavity during the first year of life exceeds that of periosteal formation leading to a transient decrease in cortical thickness (fig. 2). Although the decrease in cortical thickness was originally thought to be a result of protein-energy malnutrition, this decrease has been observed in other pediatric studies in well-nourished infants and children [1].

Single photon absorptiometry (SPA) measures bone mineral density (BMD) at peripheral bone sites and was used extensively in pediatric nutritional intervention studies during the 1970s and 1980s. Since the early 1990s dual energy X-ray absorptiometry (DXA) methods have been used to measure bone mineral content (BMC) in the total body or at a particular skeletal site such as the lumbar spine, hip or radius. The DXA method has been shown
To be precise and accurate [2], and changes in total body BMC with growth provide data on bone mineral accretion. Areal BMD (aBMD) measured by DXA is BMC divided by the bone area of the scanned region and thus is not a measure of true volumetric density. There are concerns on the use of aBMD measures in children due to the artifact that larger bones may erroneously appear to have greater density. Figure 3 illustrates this phenomenon.

Peripheral quantitative computed tomography (pQCT) has been used in some pediatric studies and provides measures of bone size (periosteal and

Fig. 1. Garn [1] measured mid-shaft width (T) and medullary cavity width (M) of the metacarpals to estimate sub-periosteal diameter and cortical area. Changes in metacarpal width, or periosteal bone apposition, with age among Central American infants and children are shown, as well as data obtained from young Ohio children.

Fig. 2. The increase in medullary cavity width during the first year of life exceeds that of periosteal formation leading to a transient decrease in cortical thickness. Data are from Garn [1].
endosteal circumferences) and volumetric BMD (vBMD) at peripheral sites. However, the current pQCT technology does not allow for accurate measurement of cortical vBMD when the cortical thickness is less than 1.5 mm (up to approximately age 8 years) [3]. However, accurate measures of bone size can be obtained and results confirm many of Garn’s [1] original findings. It has been suggested that differences in bone size due to periosteal bone apposition and endosteal expansion is determined early in life and could explain some of the gender and ethnic differences in fracture risk [1]. Insulin-like growth factor-1 and growth hormone are also thought to influence periosteal expansion prior to puberty, while sex hormones are important regulators of periosteal expansion later in childhood. Androgens are thought to increase periosteal expansion while estrogens are thought to decrease endosteal expansion. Effects of vitamin D, calcium and phosphorus on these measures of bone size, as well as on total body and site-specific BMC are described below. Other factors that may influence bone growth or mineralization that are not discussed here include protein, vitamin C, magnesium, zinc, and other trace minerals.

**Vitamin D**

Vitamin D is either synthesized in the skin upon exposure to sunlight or is obtained from the diet. Vitamin D is converted to 25-OHD in the liver and further hydroxylated in the liver to 1,25-(OH)2D, the biologically active form of vitamin D. 1,25-(OH)2D increases intestinal absorption of calcium and phosphorus, and along with parathyroid hormone (PTH) increases bone turnover. PTH also acts in the kidney to increase calcium reabsorption and increase phosphorus excretion. Thus, adequate vitamin D status is necessary to maintain blood calcium and phosphorus at sufficient concentrations that enable their deposition in bone as hydroxyapatite. Serum 25-OHD concentrations are used as the biochemical indicator of an individual’s vitamin D status.
Breast-fed infants are at increased risk of vitamin D deficiency rickets due to the low amounts of vitamin D in human milk [4]. Conservative estimates of 2 h/week of sunlight exposure with only the face exposed, or 30 min/week with just a diaper on, are sufficient to maintain serum 25-OHD concentrations within the lower limit of the normal range [5]. Although sunlight exposure is a natural method for obtaining vitamin D, there are concerns on the long-term cancer risk associated with sunlight exposure early in life. Due to these concerns, and the recent resurgence of the numbers of cases of vitamin D deficiency rickets, the Centers for Disease Control in the United States has recommended that all infants receive vitamin D through the first year of age [6].

Serum PTH concentrations are high with vitamin D deficiency and the 25-OHD concentrations at which PTH concentrations begin to increase is considered the lower limit of normal in adults [7]. An observational study of 8-year-old children found that serum PTH increased when serum 25-OHD concentrations were below 8 ng/ml (20 nmol/l) [8]. Although there are numerous case reports and case studies on vitamin D deficiency and growth in the literature, few studies have systematically determined the relationship between vitamin D status and either linear growth or bone mineralization in infants or young children.

### Linear Growth

There are few studies on the relationship between linear growth and vitamin D status during the weaning period and the first years of life. Vitamin D status theoretically may affect fetal growth, as well as linear growth during infancy. Several observational studies and vitamin D supplementation trials have reported associations between maternal vitamin D status during pregnancy and neonatal calcium homeostasis and fetal growth [9–15]. In general these studies have reported lower serum calcium concentrations in neonates of mothers who are vitamin D-deficient, along with decreased birth weight and birth size.

In 1936 Stearns et al. [16] summarized a series of longitudinal studies investigating the relationship between linear growth and vitamin D supplementation. They found that infants who were given 340 IU vitamin D/day (as either cod liver oil or via irradiated milk) in their whole milk feedings grew at a faster rate than infants who were given 60–135 IU/day. Exposure to sunlight increased the linear growth rate among those infants receiving 60–135 IU/day. Slyker et al. [17] analyzed linear growth rates among 414 infants and found that those infants who received between 250 and 810 IU/day appeared to have greater gains in length over the first year of life than those infants receiving 95–162 IU/day, which was greater than those infants not receiving vitamin D. The adverse effect of vitamin D deficiency on linear growth appeared to occur from 6 to 12 months of age. However, no results of statistical analyses were presented for either of these two studies. More recent studies on vitamin D supplementation in human milk-fed infants have found no differences in bone growth and mineralization.
linear growth rates, but the infants were only studied until 6 months of age [18, 19].

Large amounts of vitamin D supplementation, in excess of 1,800 IU/day, have been shown to lead to decreased linear growth rates, similar to that of infants receiving no vitamin D [20]. In 1966 Fomon et al. [21] reported the results of a quasi-randomized trial among 60 newborn infants: 26 were breast-fed and received 300 IU/day in a vitamin preparation; 25 were alternatively assigned to receive either 400 (350–550 IU/day) or 1,600 (1,380–2,170 IU/day) IU vitamin D/can of evaporated milk. All infants were followed to 5.5 months of age and no differences in rates of gain in length were observed among the 3 groups. Whether decreased linear growth would be observed after longer periods of vitamin D supplementation is not known.

In summary, infants with vitamin D intakes between 250 and 810 IU/day have linear growth rates greater than infants with vitamin D intakes of <162 IU/day. Vitamin D intakes of >1,800 IU/day may lead to linear growth rates comparable to those observed in infants not receiving vitamin D. The effects of vitamin D on linear growth appear to occur after 6 months of age.

**Bone Mineralization**

Decreased bone mineral accretion in utero may be manifested as rickets or osteopenia in the newborn infant [22]. Although congenital rickets of the newborn are rare, case reports in newborn infants of mothers with severe nutritional osteomalacia associated with vitamin D or calcium deficiency have been reported [15, 23]. Results from several [12, 24, 25], but not all [10], observational studies have suggested an association between bone ossification and maternal vitamin D deficiency.

Vitamin D deficiency leads to increased serum PTH concentrations and theoretically should increase bone resorption and decrease bone density or bone mass accretion. Very few pediatric studies have correlated BMD with serum 25-OHD concentrations and the results are not consistent among those studies that have. The only studies that could be located among healthy infants or young children were conducted within the first 6 months of life. In 1981 Greer et al. [26] conducted a vitamin D supplementation trial and found that 25-OHD concentrations of human milk-fed infants not receiving supplemental vitamin D (n = 9) decreased during the winter months, whereas the 25-OHD concentrations did not change among the infants randomized to receive 400 IU/day (n = 9). By 12 weeks of age, BMC at the 1/3 distal radius (measured by SPA), was lower in infants randomized to placebo compared to infants randomized to 400 IU vitamin D/day. By 26 weeks of age the differences in radius BMC between those infants receiving vitamin D and those receiving placebo was no longer apparent [27]. These same investigators conducted an additional randomized vitamin D supplementation trial among 46 human milk-fed infants from birth to 6 months of age and found no difference in BMC at the 1/3 distal radius between supplemented and non-supplemented infants, despite
significant differences in serum 25-OHD concentrations [28]. Park et al. [29] measured BMC of the lumbar spine (measured by DXA) in 2- to 5-month-old Korean infants who were either breast-fed without vitamin D supplementation or received infant formula containing 400 IU vitamin D/l. They found no significant difference in lumbar BMC between the 2 groups despite a greater serum 25-OHD concentration among the formula- vs. breast-fed infants. Lumbar BMC was not correlated with serum 25-OHD concentrations.

It is possible that these conflicting results are due to differences in the bone sites measured or the methodologies used. Greer et al. [26–28] measured BMD at a cortical bone site, whereas Park et al. [29] measured BMC at a predominantly trabecular bone site. Both SPA and DXA obtain areal measures of BMD and BMC and it is not possible to obtain separate measurements for both cortical and trabecular bone. Serum PTH concentrations are increased in vitamin D deficiency and the effect of PTH differs depending upon whether it is cortical or trabecular bone. Hyperparathyroidism (HPT) is one of the more prevalent diseases related to altered bone metabolism. Primary HPT is a disease condition caused by the parathyroid glands overproducing PTH, whereas vitamin D deficiency and the need to regulate blood calcium concentrations cause secondary HPT. Cortical and trabecular bone appear to be affected differently in patients with primary HPT. A selective reduction in cortical BMD and preservation of trabecular BMD in patients with primary HPT has been reported [30, 31]. Bilzkejian et al. [32] reported significant cortical thinning, despite maintenance of trabecular architecture, based on the histomorphometric studies conducted in a subset of these patients. Not only was trabecular architecture maintained, there was a significant increase in trabecular volume that appeared to be due to increased trabecular number rather than thickness. Duan et al. [33] reported similar findings using CT measurements of the spine and suggested that assessment of BMD in relation to PTH requires separation of cortical and trabecular bone. Whether these disparate effects of elevated PTH concentrations on trabecular and cortical bone occur in infants and children with secondary hyperparathyroidism resulting from vitamin D deficiency is not known, but these differing effects may be why bone studies using SPA or DXA technology do not find a relationship between bone density and vitamin D status.

Although primary hyperparathyroidism in adults is associated with an increase in trabecular volume due to increased trabecular number, this is not what is observed in vitamin D deficiency rickets. In rickets histologically there is a thinning of the cortical width in the diaphyseal region, a widening of the epiphyseal plate, and a thinning out and loss of trabecular bone in the metaphyseal region. This thinning and loss of trabecular bone in rickets is not consistent with the histological findings in adult patients with secondary hyperparathyroidism described above.

Historically there have been hypotheses that vitamin D deficiency is not the sole cause of rickets. During the early 1920s it was thought that rickets
was a result of overeating, was caused by an acidosis, was a manifestation of congenital syphilis, or was a result of infection. In 1923, Parks [34] presented a summary of the epidemiological findings and animal studies regarding the etiology of rickets and although vitamin D had not yet been identified he was able to deduce that the absence of ‘Factor X’ was necessary for the development of rickets. Parks further proposed, based on studies available during this period, that: (1) ‘When the organism is deprived of the influence of X in the food and of radiant energy, defects in the diet become reflected in the blood’; (2) ‘In the absence of X and of radiant energy, rickets can be made to develop by altering the composition of the diet in several ways’. He further described several conditions that could produce rickets, including decreasing the phosphorus content of the diet and administering calcium or decreasing the calcium content of the diet while maintaining the phosphorus content. (3) ‘In the absence of X and radiant energy, rickets can be prevented by means of the diet’. Additionally, he described that the organic portion of the diet, if given correctly, could prevent the development of rickets. In 1976 Bronner [35] proposed that rickets was not the result of a simple vitamin D deficiency, but rather a combination of vitamin D and phosphate deficiency. He provided evidence from animal studies showing that simple vitamin D deficiency leads to biochemical alterations without the classical bone lesions that are observed in rickets. He also provided evidence that the bone lesions associated with phosphate deficiency are similar to those observed with classical rickets. These previous hypotheses of Parks [34] and Bronner [35] are supported by findings that vitamin D deficiency alone, as reflected by low serum 25-OHD concentrations, are not associated with BMD.

**Calcium and Phosphorus**

During the first 6 months of life the average calcium intake of breast-fed infants is used to define an adequate intake (AI) level (210 mg Ca/day and 100 mg P/day), while the average calcium intake from both human milk and foods define the AI at 7–12 months of age (270 mg Ca/day and 275 mg P/day) [36]. Breast milk calcium concentrations are fairly consistent with increasing length of lactation, while phosphorus concentrations decline. Beyond the first year, calcium and phosphorus intakes that maximize bone mass accretion are considered optimal. Total body calcium is approximately 30 g at birth [37] and mean net calcium retention during the first year of life is estimated at approximately 70 mg Ca/day [38]. Calcium retention at 1 and 2 years of age is approximately 85 mg/day and increases to approximately 110 mg/day in 3- and 4-year-olds; male children have greater calcium retention than female children [1]. Calcium and phosphorus are the most abundant minerals in bone and adequate intake of these minerals is essential for appropriate bone mineral accretion. DXA measurements provide reasonable estimates of total body
Bone mass in infants and young children and several studies have used this measure to assess the impact of micronutrients on bone mass accretion early in life.

**Linear Growth**

No studies specifically looking at the effect of calcium and phosphorus intake on linear growth were found. In a recent study by Kramer et al. [39], the growth of infants exclusively breast-fed for either 3 or 6 months differed: infants breast-fed for only 3 months had greater gains in length (20.3 ± 7.0 mm/month) between 3 and 6 months of age compared to infants exclusively breast-fed through 6 months (19.2 ± 6.4 mm/months). Once complimentary foods were introduced there was equalization in length so that by 12 months of age there was no difference. There are numerous differences in nutrient composition between human milk and infant formula other than mineral content, making it impossible to ascribe these differences to an influence of mineral intake on length. However, one randomized trial described in detail below did not find significant differences in length gain among infants receiving different formulas with varying mineral intake [40].

**Bone Mineralization**

In 1993, Lee et al. [41] reported the results of a longitudinal observational study of 128 children from Hong Kong for whom they had collected extensive dietary intake data during the first 5 years of life. They found that BMC at the 1/3 distal radius was associated with the cumulative calcium intake during the first 5 years of life. Calcium intake during the second year of life had the strongest correlation with BMC at 5 years.

In 1997 the results of a randomized trial of varying mineral intake in 101 infants during the first year of life were reported [40]. This trial was conducted in two phases: phase I was conducted during the first 6 months of life when infants were either breast-fed or randomized to one of two infant formulas containing different amounts of calcium and phosphorus (table 1). Phase II involved the same infants who were re-randomized at 6 months of age to 1 of 3 feeding groups that differed in mineral content (table 1). During the first 6 months the infants who received the moderate mineral containing formula had a greater bone mass accretion than the other 2 feeding groups (human milk and low mineral containing formula). Although there was no effect of the type of feeding on BMC gain during the second 6 months, there was a difference during the second 6 months by the first 6 month feeding groups: infants who received breast milk had a greater gain in BMC than either the low- or moderate-mineral formula groups. By 12 months of age there were no differences in BMC among either the first or second 6-month feeding groups. These results indicate that early mineral intake is associated with early bone mass accretion, but when mineral intake is increased later in infancy these differences disappear.
Nutrients may interact with other environmental factors in their effect on bone growth and mineralization. Increased bone loading through physical activity is one of the major factors influencing bone mass accretion during growth. Early studies found that physically active children had greater BMD or BMC than less active children [42]. A randomized trial on the effect of gross motor activities on bone mass accretion in infants found evidence that calcium intake during infancy may modify the bone response to activity [43]. Infants were randomly assigned to an intervention of either gross motor or fine motor activities for 5 days/week for 1 year. Gross motor activity had no effect on bone mass accretion among infants receiving moderately high calcium intakes, whereas among infants with moderate to low calcium intakes, gross motor activity actually resulted in less gain in BMC than the fine motor activity group (fig. 4). The results of this study lead to the hypothesis that calcium intake modified the bone response to physical activity in young children. This hypothesis was formally tested in a randomized trial using a factorial design.

The randomized trial to formally test for an interaction between calcium intake and physical activity involved 239 children aged 3–5 years [44]. Children

### Table 1. Randomized trial of varying mineral intake during the first year of life (data from Specker et al. [40])

<table>
<thead>
<tr>
<th>0–6 months of age</th>
<th>Human milk</th>
<th>Low mineral</th>
<th>Moderate mineral</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca, mg/l</td>
<td>300</td>
<td>430</td>
<td>510</td>
<td></td>
</tr>
<tr>
<td>P, mg/l</td>
<td>150</td>
<td>240</td>
<td>390</td>
<td></td>
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<tr>
<td>Changes in</td>
<td></td>
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<tr>
<td>Length, cm</td>
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<tr>
<td>BMC, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gain in BMC at 6–12 months¹</td>
<td>11.1 ± 1.7ᵃᵇ</td>
<td>12.5 ± 1.9ᵃ</td>
<td>11.8 ± 2.9</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>59 ± 17ᵃᵇ</td>
<td>66 ± 18ᵃ</td>
<td>82 ± 25ᵇ</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6–12 months of age</th>
<th>Moderate mineral</th>
<th>High mineral</th>
<th>Cow’s milk</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca, mg/l</td>
<td>510</td>
<td>1,350</td>
<td>1,230</td>
<td></td>
</tr>
<tr>
<td>P, mg/l</td>
<td>390</td>
<td>900</td>
<td>960</td>
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<td>Changes in</td>
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<td>Length, cm</td>
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<tr>
<td>BMC, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gain in BMC at 6–12 months¹</td>
<td>8.4 ± 1.8</td>
<td>8.6 ± 1.6</td>
<td>8.6 ± 1.3</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td>72.9 ± 21.1</td>
<td>75.9 ± 24.9</td>
<td>71.7 ± 38.1</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

Significance values are for group differences. 
ᵃᵇMeans with the same symbols on each row are different at p < 0.05. ¹Gain in BMC after re-randomization to one of the three feeding groups at 6 months of age.
were randomized to participate in gross motor or fine motor activities for 30 min/day, 5 days/week for 1 year. Within each group, children were blindly assigned to receive either a placebo or 1,000 mg/day of calcium carbonate. Total body and regional BMC measurements using DXA and tibia cross-sectional measurements were obtained at baseline and study completion. Three-day diet records and accelerometer readings were obtained at 0, 6 and 12 months and showed a high baseline calcium intake among this population and a higher percent time in moderate plus vigorous activity among those children in the gross motor vs. fine motor group. Overall, calcium intake did not influence bone mass accretion. However, the difference in leg BMC gain between gross motor and fine motor was more pronounced in children receiving calcium vs. placebo (fig. 5; interaction, \( p < 0.05 \)). At study completion, children in the gross motor group had greater periosteal and endosteal circumferences at the tibia compared to children in the fine motor group. There was also a significant interaction between the calcium intake and activity groups in both cortical thickness and cortical area: among children receiving placebo, thickness and area were smaller with gross motor vs. fine motor activity, but among children receiving calcium, thickness and area were larger with gross motor activity (fig. 5). These results indicate that the relationship of bone growth or mineralization and calcium intake is not simple and may depend upon other environmental factors that influence bone development, such as physical activity.

![Fig. 4. Illustration of the effect of calcium intake on changes in total body BMC over the 12-month study period of infants in the gross motor (GM) and fine motor (FM) activity programs. The interaction term of age, activity group, and calcium intake was significant at \( p = 0.07 \). Reprinted from Specker et al. [43].](image-url)
Both low and high phosphorus intakes may theoretically lead to reduced bone mass accretion. A low phosphate intake will limit the substrate availability for bone formation, while a high phosphate may decrease serum calcium leading to an increase in serum PTH. A randomized trial tested whether the introduction of cereals between the ages of 16 and 26 weeks would lead to higher PTH and lower BMC [45]. Forty-one infants were randomized to either formula alone or formula plus infant cereal beginning at 16 weeks of age. Although serum PTH concentrations increased significantly in the cereal-fed group, there was no difference between groups in BMC changes (1/3 distal radius using SPA).

**Early Diet and Bone Later in Life**

While it is possible to increase bone mass accretion early in life, the long-term effect on bone health is not known. There are no studies showing that increasing bone mass in the first years of life is associated with greater bone mineralization later in childhood or adolescence. Bishop et al. [46] reported that preterm infants fed human milk with a high mineral fortifier in the neonatal period had lower bone mass at 5 years of age compared to preterm infants fed only human milk with its relatively low mineral content. These authors suggested that ‘programming’ may occur in the early neonatal period with infants who have low mineral intakes developing improved calcium retention later in life. However, this hypothesis has not been confirmed in other studies [47, 48], including one by the same authors involving a larger number of children who were aged 8–12 years [47].
There are single studies that report both a positive relationship between childhood BMD and length of breast-feeding or infant vitamin D supplementation. Jones et al. [49] studied 330 eight-year-old children in Australia and found that total body, femoral neck and spine BMD was higher among prepubertal 8-year-old children who were breast-fed for more than 3 months compared to those children who were breast-fed for less than 3 months. These findings were only observed among the 245 children born at term and not among those born preterm (n = 85). Zamora et al. [50] reported the results of a retrospective study and found that the 91 prepubertal girls (median age 8 years) who received vitamin D supplements during the first year of life had greater aBMD at the radius, femoral neck, and greater trochanter than the 15 girls who were not supplemented with vitamin D. Both of these studies used retrospective designs and the issue of bias needs to be considered, in particular recall bias and selection bias.

Conclusions

Vitamin D, calcium and phosphorus are the major micronutrients involved in bone growth and mineralization. There are few studies that have systematically determined the effect of vitamin D deficiency on either linear growth or bone mineralization. Maternal vitamin D deficiency during pregnancy is associated with fetal bone growth and ossification. Vitamin D supplementation of infants at high risk of vitamin D deficiency may improve linear growth, but this effect does not become apparent until the second half of the first year of life. There are no consistent findings of a relationship between BMC or BMD measured by DXA and vitamin D status of infants, possibly due to differences in the bone sites that have been measured. Calcium and phosphorus intakes early in life are associated with early bone mass accretion, but the long-term implications of early infant feeding on bone size and mass are not clear.

References

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Discussion

Dr. Zlotkin: One of the characteristics of children in the developing world, especially rural children, which differentiate them perhaps from a typical American population, is the fact that the majority are breast-fed and don’t receive any formula, and as they get older they exercise a lot. One of the groups you didn’t talk about would be in terms of periosteal impact, decreased calcium and increased exercise. Could you postulate on what you think you would find for that group?

Dr. Specker: I think you will still find an increase in bone circumference with periosteal expansion from exercise, but it may not be as great if calcium is low. The body has to adapt to the low calcium intake, perhaps by removing calcium from bone. Exercise will increase both periosteal and endosteal expansion, low calcium intake is likely to increase endosteal expansion. If the cortical thickness is too small then periosteal expansion is going to slow down, so calcium must be going into the bone in order for it to expand. Therefore I would speculate that periosteal expansion will not be as large on a calcium-deficient diet, but there would still be some expansion.

Dr. Guesry: You selected to speak only on vitamin D, phosphate and calcium. We did animal studies 20 years ago on zinc deficiency, the elasticity module of the bone, and resistance to fracture. Do you know if there are new data on that, or human data related zinc deficiency and bone fragility?
Dr. Specker: I am not aware of any, but Dr. Abrams might.

Dr. Abrams: There are several animal studies suggesting that zinc supplementation improves bone strength. There was one control trial on zinc supplementation in adults suggesting an increasing bone mass, but that is an area that needs considerable more work.

Dr. Pettifor: A couple of issues, first of all just to pick up on what Dr. Zlotkin asked which is that one generally perceives children as being more active in less developed countries or in developing countries than in developed countries. We are busy doing a longitudinal study of children through puberty. At age 9 years our black children from a poor socioeconomic environment in fact exercised less than our white children, basically because they had no formal physical activity at school, which was a major component of the exercise that white children had. So the high socioeconomic group was going to schools that had organized physical activity with high stress and strain which is important for bone development. If we look at actual bone mineral density in those children, the black children showed no change in bone mass with quartiles of physical activity, while the white children did. So the children with the highest physical activity, the highest peak strain on the bone, had higher bone mineral density. It appears that the black children didn't get up to those physical activity levels that were seen in the last quartile of the white children, suggesting that perhaps it is the extent of exercise that is important, and again the issue of calcium intake. Our black children were on a significantly lower calcium intake than the white children, but the bone mass was similar in both groups, except at the hip where black children have a higher bone mass. We have seen this as well in adults, both pre- and postmenopausal adults, suggesting that a genetic difference rather than an environmental factor is influencing the hip change. A question I want to ask is about calcium intake and changes with exercise. You said the subjects were already on a very high calcium intake. Was there any evidence that if you looked at those on a low calcium intake that exercise had less effect on bone, or was there any effect of dietary calcium intake on bone at all?

Dr. Specker: No, there was no main effect of dietary calcium on bone. If calcium is interacting with physical activity that means that there is an effect of calcium, but it is through modifying the relationship between bone changes and physical activity. If we grouped all the children and looked at changes in leg bone mass by quartiles of calcium intake, based on both the supplement and the diet, there was a very nice dose-response relationship. The children in the exercise group had a greater increase in bone with increasing quartile of calcium intake. However, at the lowest quartile of calcium intake there was no benefit, it was actually detrimental, and the children in the gross motor group had less bone accretion than the fine motor group. This may explain part of your findings. The other thing I just want to mention is when looking at bone mass using DXA, as in our study, we didn't see any difference in the change in leg bone mass between the calcium and the no calcium groups among the children in the fine motor group who had similar changes as the gross motor, no calcium group. It wasn't until bone geometry was looked at that differences in bone size were actually found, which explains those findings, or lack of findings, a little bit better. If there is this expansion of bone and a smaller cortical thickness then mass can be the same. I am trying to make the point that bone size itself is a very important outcome parameter that needs to be looked at in pediatric studies.

Dr. Mogrovejo: What is the relation among other nutrients like protein, energy, vitamin C or copper in bone accretion?

Dr. Specker: Protein deficiency should have an effect on bone through the IGF system. IGF is important for this periosteal expansion that occurs during growth, it is one of the driving factors. I know the people at the USDA Human Nutrition Research Center in Grand Forks, North Dakota, are interested in the effects of trace minerals.
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on bone. Magnesium deficiency appears to affect trabecular bone at least in adults, but it doesn’t seem to affect cortical bone. I am not sure on the mechanism, but perhaps it relates to the role of magnesium in ensuring adequate functioning of the parathyroid gland. Dr. Abrams, do you have any information on copper?

Dr. Abrams: There are considerable animal data related to copper deficiency, but there aren’t any good human studies.

Dr. Specker: Very few pediatric studies have been done looking at calcium and vitamin D on bone, and those are the big nutrients in terms of pediatric bone research. There just aren’t that many infant bone studies for the other micronutrients.

Dr. Castillo-Durán: Some countries even now use a high dose of vitamin D for the prevention of rickets. The effects for the prevention of rickets have been demonstrated, but I don’t know about the safety of the high doses for the bone metabolism or the long-term effects on the bone metabolism. Do you have some information?

Dr. Specker: I don’t think there is anything out there looking at the effects of Stoss therapy on bone. At least in later in childhood and adulthood it has been shown to be effective for the prevention of rickets and osteomalacia, especially in high-risk populations where you might have a problem with compliance.

Dr. Castillo-Durán: A study in our country some years ago compared those infants who were supplemented with daily doses and high doses, 2 or 3 times a day, and they demonstrated some effect in length, about 2 cm during the first years of life. Maybe it was related to the hypercalcemia and the effect on bone.

Dr. Specker: So the high-dose ones grew better?

Dr. Castillo-Durán: With the daily supplementation they grew better than the high dose of vitamin D supplementation.

Dr. Specker: So it is not a compliance issue.

Dr. Gebre-Medhin: If I were to start from the disease perspective and use rickets as an example, is it always possible to completely heal vitamin D deficiency rickets using only vitamin D? Is it also possible to treat vitamin D deficiency rickets at least in infants only using sunlight? What are the metabolic and bone consequences of these two statements?

Dr. Specker: You can effectively treat vitamin D deficiency rickets with vitamin D and without the minerals. By treating the vitamin D deficiency, you are correcting the mineral deficiencies because you increase 1,25-dihydroxy-vitamin D and absorb more calcium. In effect you are giving the infant more calcium. As far as sunshine is concerned, it is very effective treatment. In Scandinavia they use UV lights to prevent and treat rickets.

Dr. Batubara: I would like to ask you about attaining big bone mass in early adulthood for the prevention of osteoporosis. What is the optimal upper level calcium intake suggested, because as you know a higher calcium intake will interfere with the zinc absorption?

Dr. Specker: The new dietary reference intakes for North America are based on maximizing calcium retention, or at what level of calcium you no longer see additional retention by giving more, and that value around the time of peak bone mass is between 1,000 and 1,200 mg/day for both women and men.

Dr. Gibson: I just have a question in relation to a study that was carried out in New Zealand that you may know about: a multidisciplinary study on 1,000 infants who have been followed every 5 years up to 26 years of age [1]. Are you familiar with this study?

Dr. Specker: This last study I mentioned with the breast-fed and bottle-fed infants is from New Zealand? I am not sure if that is the study you are referring to.

Dr. Zlotkin: You showed data suggesting that there was some tracking at early infant nutrition on bone growth at age 8. There was a controversy about whether early breast-feeding had an impact on the bone mineral content at age 8. Is there any further
tracking to an older age? Does it make any difference if we feed the infants in the first 2 years of life with regard to the two important outcomes which will be number of fractures and risk of osteoporosis or height growth for example? Does it matter if we feed the infants during the first 2 years?

Dr. Specker: When you say tracking, the data I showed at age 8 actually were opposite to what you would have expected if there was tracking. What I was showing was that those infants who had low mineral intake early in life should have had lower bone mass than those infants fed higher mineral, but at age 8 it had switched, so that the ones that had the low mineral early in life had high bone mass and vice versa. However, we have data showing that over 3 years during the toddler period there appears to be tracking. There are several studies done at different ages in childhood, so if you combine them all together you could speculate that in fact there is a point at which bone mass begins to track. What age that is, is not clear, but it is probably before puberty. Significant changes in bone occur during puberty. Those children with high values before puberty have high values after puberty. But no one has done a study looking over the entire pediatric age range. There are more mixed longitudinal studies and those tend to show that there is some tracking. In the adult literature, it has been found that the bone density made 11 years earlier predicts fracture risk just as well as the most recent bone density measurement. So we know that fracture risk is associated with bone density measurements up to 11 years earlier in adults. This has not been done in children. There also are studies, including some from New Zealand, that are case-control studies in which the bone density of children who fractured versus those who did not were compared [2, 3]. These studies find a lower bone density in the children with fractures versus those without fractures. In addition, those who fracture are then at a higher risk of fracturing later in childhood [4]. So early bone density is also thought to be a predictor of fracture risk in childhood, and once fractures occur then there is a higher risk of subsequent fractures. Therefore, there is immediate benefit of having a high bone density during childhood on a child's fracture risk, not necessarily just the prevention of osteoporosis later in life.

Dr. Zlotkin: Are you suggesting that early breast-feeding would decrease the risk of fracture based on that argument?

Dr. Specker: I am not willing to say that yet because I don't think there are enough studies showing the long-term impact of feeding during the neonatal period on bone mass later in childhood.

Dr. Pettifor: To follow up on Dr. Zlotkin's comments. I am left with the impression that you are suggesting that in fact a high calcium intake may not be beneficial in early life. Certainly from an exercise point of view you worry that calcium supplementation may not be appropriate. And yet in the food industry there is a push to increase calcium intake in this age group. Would you comment on that and speculate on whether one should be promoting high calcium?

Dr. Specker: It depends on what you mean by high calcium, but I think that it is very early to promote the feeding of high amounts of calcium early in life. I don't think the long-term effects on bone growth and size have really been determined. I think it would be premature.

Dr. Endres: I am delighted that you said for the treatment of rickets you would recommend the use of minerals; you didn't say calcium but you did mean calcium and phosphorus?

Dr. Specker: I was talking more biochemically. If you were to just treat with vitamin D then you would be correcting mineral deficiencies. You don't have to actually treat with calcium and phosphorus as well.

Dr. Endres: I think that calcium together with vitamin D would be enough, let's say 5,000 units/day over 3 or 4 weeks, but we never tried sunlight only because it is
so easy over 3 or 4 weeks. One of the initial problems with vitamin D deficiency rickets is that nobody knows that. So we assume that in a first step, it is hypocalcemia, and then due to secondary hypothyroidism calcium is normalized and phosphorus decreases, and that is what we always find initially, i.e. hypophosphatemia. I think to prevent this tetany it is necessary, at least it is not harmful, to give calcium in addition to vitamin D. When we had more cases of rickets in Germany, we saw some children with tetany.

*Dr. Specker:* Venkataraman et al. [5] in Cincinnati found that treatment of vitamin D deficiency rickets with 400 IU vitamin D/day was sufficient to heal rickets. What I was trying to say about calcium is that if vitamin D is given to correct the vitamin D deficiency then 1,25-dihydroxyvitamin D increases and this will lead to an increase in intestinal calcium absorption, normalizing serum calcium concentrations, decreasing parathyroid hormone, and normalizing serum phosphorus. Initially, during early stages of vitamin D deficiency, 1,25-dihydroxyvitamin D concentrations are high, but then they drop. When you give vitamin D, 1,25-dihydroxyvitamin D will increase, intestinal absorption of calcium will increase and you have corrected the calcium deficiency.

*Dr. Tolboom:* I have a question on the fetal origin hypothesis of adult disease. What do you know of the fetal origin hypothesis in terms of bone disease or infections?

*Dr. Specker:* There was an abstract presented at the American Society for Bone and Mineral Research by Javaid et al. [6] from Southampton. They reported that maternal vitamin D status in late pregnancy was correlated with the child’s whole body bone mineral content at the age of 9 years. It has not been published or peer-reviewed, only presented as an abstract.

*Dr. Pettifor:* I have a comment on the general data on the fetal origin of bone disease. The problem with the majority of studies is that they are influenced by the failure to adjust for bone size. So that actually if you adjust for bone size in children, later age groups in childhood and in adulthood, you adjust for differences in body stature which may be influenced by fetal environment. Then there is very little difference in bone mass if you are a low birth weight baby as opposed to a full size baby. I think most of the effects are seen on body size and therefore on bone mass, but once you adjusted for these there is very little evidence that there are major effects on bone density per se.

*Dr. Abrams:* I agree entirely. There have been several studies in preterm infants on bone mass up until early adolescence and they all show a decreased bone mass proportional to the decreased body size.

*Dr. Specker:* The abstract presented at the bone meeting had to do with maternal exposure to either vitamin D or calcium during pregnancy and long-term effects on the bone of the child, but I am not sure exactly what that was.

*Dr. Gebre-Medhin:* I am happy the issue of rickets and calcium and phosphorus came up again, that was my intention. The impression I have now is that you can effectively treat rickets on a pre-rickets diet by only providing vitamin D or sunshine, that is what we have had in Sweden, that is also the standard we have right now. Having said that, regarding bone and fractures and osteoporosis there is a heated debate concerning vitamin A along with increased calcium intake. You know the work by Melhus et al. [7] and the others. What is your comment on that?

*Dr. Specker:* There are quite a few studies. Melhus et al. [7] from Sweden originally reported this potentially adverse effect of vitamin A. Another study that supports these findings recently came from the Rancho Bernardo Study [8]. However, there are contradictory studies from Iceland [9]. There may be a possible vitamin A toxicity on bone at very high levels, at least in animal studies, but I am not sure about the human evidence because of these conflicting reports.
References
