Chapter 25
Assessing Nutritional Requirements for Preterm Infants

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Key Points

- The role of the trace minerals in bone health is much less well developed than for the macrominerals.
- In many cases, studies have used animal models, which are difficult to extrapolate to humans.
- In others, the relationship between serum levels of minerals and markers of bone health or assessment of bone mineral density are described along with their limitations.
- Any relationships are confounded by the other lifestyle and socioeconomic factors that may cause such differences in dietary intakes.
- In addition, low-quality diets may be deficient in more than one nutrient, making it extremely difficult to ascribe the change to any single nutrient.
- There are very few well-designed intervention studies in humans that address the importance of trace and ultratrace minerals in human bone metabolism.
- Strontium, has some good-quality data (i.e. randomized controlled studies) suggesting that high-risk adults may benefit from strontium supplementation.

Keywords Nutritional requirements for preterm infants • Macrominerals • Copper deficiency • Zinc • Boron • Strontium • Silicon • Trace minerals • Skeletal health • Animal studies

25.1 Introduction

Although the macrominerals calcium, phosphorus, and magnesium are of primary concern in bone health, other minerals, including trace minerals can also play an important role. In this chapter, the role of some of these will be considered. In general, the data supporting and defining the role of the trace minerals in bone health is much less well developed than for the macrominerals. In many cases, studies have used animal models, which are difficult to extrapolate to humans. In others, the relationship between serum levels of minerals and markers of bone health or assessment of bone mineral density are described. These are difficult to interpret, and even if a correlation between low serum copper and low bone mineral density (for example) is demonstrated this does not mean that additional

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M.F. Holick and J.W. Nieves (eds.), *Nutrition and Bone Health*, Nutrition and Health,
DOI 10.1007/978-1-4939-2001-3_25,

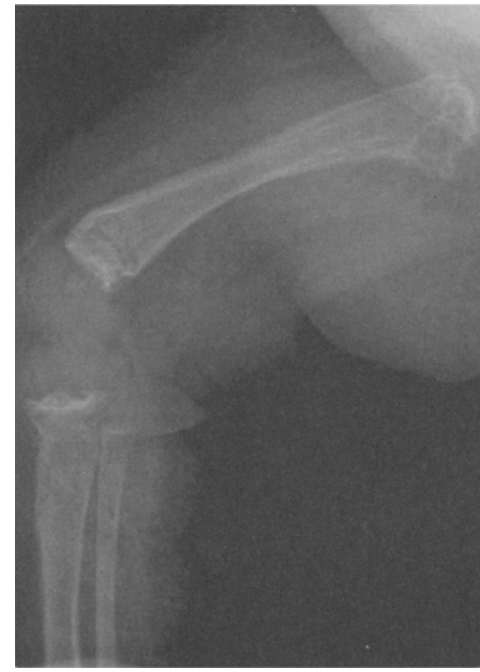

Fig. 25.1 Radiograph of the lower limb of a former preterm infant demonstrating changes of severe copper deficiency

dietary copper would improve bone mineral density. Such relationships are confounded by the other lifestyle and socioeconomic factors that may cause such differences in dietary intakes. In addition, low-quality diets may be deficient in more than one nutrient, making it extremely difficult to ascribe the change to any single nutrient.

There are very few well-designed intervention studies in humans that address the importance of trace and ultratrace minerals in human bone metabolism. The one exception appears to be strontium, where there is increasing good-quality data (i.e. randomized controlled studies) suggesting that high-risk adults may benefit from strontium supplementation.

25.2 Copper

Copper is an essential element in human nutrition and is required by many enzymes, including lysyl oxidase, which is responsible for cross-linking of collagen and elastin [1]. The prototypical disease of copper deficiency is Menkes' kinky hair syndrome.

25.2.1 Copper Deficiency

Menkes' kinky hair syndrome is a congenital cause of copper deficiency resulting from impaired copper absorption, which can present with skeletal changes resembling scurvy, fractures, or delayed bone age. Acquired copper deficiency has also been reported in humans, most commonly in premature or low-birth-weight (LBW) infants who had low enteral or parenterally copper intake [2, 3] or children on prolonged copper-free parenteral nutrition [4]. In premature infants the symptoms of copper deficiency may include osteopenia, fractures, or other bony changes [3, 5].

Figure 25.1 shows an extreme example of copper deficiency-induced bone disease in a former preterm infant who had been on prolonged copper-free parenteral nutrition (PN). This infant had been born extremely prematurely and developed complications including necrotizing enterocolitis—a severe, potentially fatal gastrointestinal infection. This led to the development of widespread gut necrosis requiring multiple surgeries, and ultimately to severe short-gut syndrome. Because of this, establishment of enteral feeds was extremely difficult, and he required prolonged PN, and developed cholestatic liver disease (PNAC, PN-associated cholestasis). Copper is excreted in the bile, and therefore may not always be used in infants with cholestasis due to the possibility that it may worsen liver failure. In this infant, copper had been completely removed from his TPN for a prolonged period of time. After several months on copper-free TPN and minimal enteral copper intake, a routine chest X-ray revealed radiological changes consistent with copper deficiency. A low serum copper level and low ceruloplasmin concentration and characteristic changes in long bone films confirmed the diagnosis of acquired copper deficiency. Radiological features suggestive of copper deficiency include osteopenia, metaphyseal cupping and flaring, spurs and fractures, and retarded bone age [6].

25.2.2 Animal Studies

Several studies have shown that although calcium content may not change, copper-deficient experimental animals have decreased bone strength [7, 8], The cause of this is believed to be the reduced activity of lysyl oxidase, the copper metalloenzyme that is responsible for formation of collagen cross-links. Copper deficiency has been shown to cause decreased collagen cross-linking and this is accompanied by decreased bone strength in chicks [9]. Furthermore, the reduction in bone strength was reversed by chemical induction of cross-links in vitro, suggesting that the decrease in bone strength results from decreased cross-linking [9]. Long-term copper deficiency may also reduce ostrogenesis and reduce osteoclast activity [10].

In ovarectomized rats, copper deficiency increases bone loss [11], whereas copper supplementation may reduce it [12]. However, a similar effect is seen with manganese, with no additional benefit coming from copper supplementation [13].

25.2.3 Human Studies

Although frank copper deficiency clearly has adverse effects of bone health, the importance of mild deficiency or poor copper intakes is much less clear. It has been hypothesized that suboptimal copper nutrition may be a major cause of osteoporosis in Western societies [14], although good evidence for this is lacking. One epidemiological study has described a relationship between copper intake (and indeed iron and zinc intake) and forearm bone mineral content in premenopausal women [15]. Another, has shown that in frail elderly men, higher serum zinc concentrations, relative to zinc concentrations, were associated with *reduced* femoral neck BMD [16]

Two small, randomized studies have examined the effect of copper intake on markers on bone health. In one study, 11 males aged 20–59 years were studied sequentially on diets containing low (0.7 mg/day), medium (1.6 mg/day), and high (6.0 mg/day) copper intakes for 8 weeks each. When these subjects were switched from the medium to low copper intake there was a significant increase in urinary markers of bone resorption, and a significant decrease when they were switched from the low to high copper intake [17]. A further study by the same investigators [18] considered 24 adults, 22–46 years, who were studied three times—following 6 weeks of treatment with 3 mg/day copper sulfate, 3 mg/day copper-glycine chelate, and 6 mg/day copper-glycine chelator.

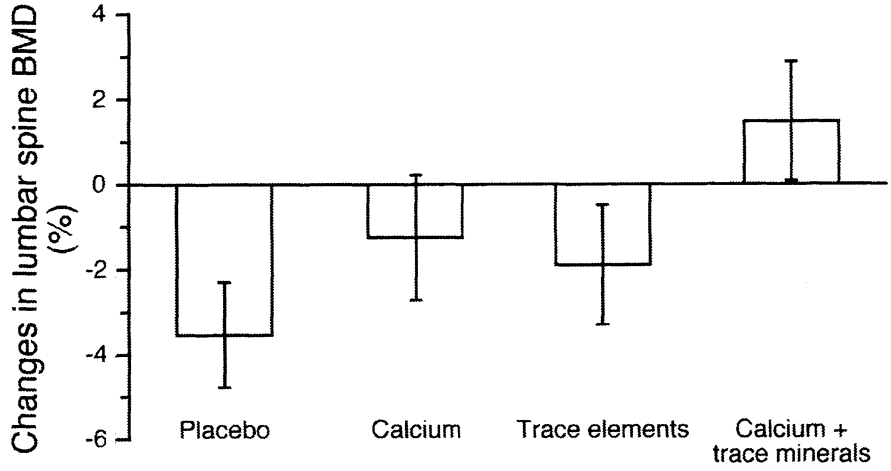

Fig. 25.2 Changes in percent bone mineral density (BMD) from the study of Strause et al. (data extracted from ref. [20]). Mean changes over the 2-year study period are shown for the four treatment groups. Error bars represent ±1 standard error of the mean

There were no differences in serum osteocalcin (a marker of bone formation), or the urinary pyridinoline: creatinine ratio or the urinary deoxypyridinoline:creatine ratio (markers of bone resorption). It is worth noting, however, that markers of copper status did not change among the three treatments [18]. A similar study by Cashman et al. [19] compared changes in markers of bone turnover and resorption after 4 weeks treatment with 3 mg/day copper, 6 mg/day copper, and placebo in 16 healthy females, 20–28 years old. Copper treatment significantly increased the markers of copper status, serum copper, and erythrocyte superoxide dismutase. However, no differences were noted in markers of bone formation (serum osteocalcin) or bone resorption (urinary pyridinoline: creatinine ratio or urinary deoxypyridinoline: creatinine ratio).

Strause and coworkers reported the only long-term interventional study. They studied 59 postmenopausal women randomized to one of four treatments for 2 years [20]. They were given either a placebo, calcium (1,000 mg/day), trace elements alone (15 mg/day zinc, 5 mg/day manganese, and 2.5 mg/day copper), or both calcium and trace elements. After 2 years of treatment lumbar spine bone mineral density fell in all the groups except those receiving both calcium and trace elements (Fig. 25.2). The only significant difference was between the placebo group and the group receiving both calcium and trace elements. The calcium-only and the trace element only groups were intermediate between the placebo and calcium-plus-trace element group (Fig. 25.2).

25.2.4 Conclusions

Although overt copper deficiency has serious effects of the skeleton, the role of milder forms of copper deficiency remains unclear. Although it has been hypothesized that suboptimal copper intake may be an etiological factor in human osteoporosis, direct evidence for this is lacking. Indeed, the few interventional trials that have looked at the effect of copper supplementation on bone health have been small, of generally short duration and they have assessed proxy markers of bone formation and resorption [17, 18] rather than bone density. One study, however, does suggest a benefit to addition of magnesium, copper, and zinc to calcium to reduce postmenopausal bone loss. Whether this benefit is attributable to copper, zinc, or manganese is unclear. Clearly, there is much to learn about the role of

copper in bone health, especially in populations without clinically apparent copper deficiency. What is urgently needed are large-scale, long-term, randomized studies of copper supplementation on bone density or fracture risk. In the absence of such studies the role of copper as an etiological factor in osteoporosis will remain unclear.

25.3 Zinc

Zinc is a component of more than 200 enzymes, and overt zinc deficiency is well characterized. The principal clinical features are diarrhea, dermatitis, alopecia, delayed sexual maturation, and decreased taste acuity [21]. Bony changes do not typically feature as symptoms of zinc deficiency; however, even the earliest reports of human zinc deficiency recognized that short stature was a relatively consistent feature [22].

25.3.1 Observational Human Studies

The possible role of zinc as a cause of osteoporosis has increased as several studies have shown that humans with osteoporosis have reduced plasma zinc concentrations [23, 24] and increased urinary zinc excretion [25, 26]. The latter, however, may be a result of increased bone loss, as approximately one-third of total bone zinc is found in bone [21]. However, a number of studies have suggested that lower zinc intakes are associated with lower bone mineral content [15], lower bone mineral density [27], and may be a risk factor for subsequent fractures [28].

25.3.2 Animal Studies

Data from cell culture, tissue culture, and animal studies have identified a large number of potential beneficial effects of zinc on bone formation and mineralization [29]. Zinc may stimulate osteoblasts proliferation, stimulate bone protein formation [30, 31], increase transcription factors involved in pre-osteoblast differentiation, decrease bone resorption, and reduce osteoclast differentiation [29]. Zinc may increase bone protein content [32], DNA content [32], and insulin-like growth factor-1, and transforming growth factor-β production [33, 34], which may be important for fracture healing.

In rats, experimental zinc deficiency can lead to low-turnover osteopenia [35] and worsen experimental diabetic osteoporosis [36]. Conversely, zinc supplementation may ameliorate the bone loss that accompanies skeletal unloading in rats [37], and increase serum and bone alkaline phosphatase content in mice [38] and rats [39]. Increasing zinc intake can lead to dose-dependent increases in bone strength [40]. However, on a low-calcium diet it had the opposite effect, worsening bone strength and elasticity [41]. One confounding factor may be the anorexia that accompanies zinc deficiency. Zinc-deficient rats have lower femur weights than pair-fed or ad lib-fed controls, but bone volume was similarly reduced in zinc-deficient and pair-fed controls, compared to ad lib-fed controls [42]. In ovarectomized rats, zinc supplementation restored normal bone morphology (improved bone area, perimeter, and max diameter in the tibia and femur), and restored bone zinc and copper levels [43].

In higher animals, zinc deficiency leads to poor bone growth in pigs [44]. In rhesus monkeys made marginally zinc deficient from conception to 3 years of age, bone maturation was delayed. Although bone mineralization was reduced at 6 months of age, by 3 years of age it had largely returned to normal [45].

25.3.3 Human Studies

Given the confusion of the animal and basic sciences literature, what is needed is well-designed, large-scale intervention studies in humans. However, there is a paucity of these. In a study of calcium supplementation, Freudenheim et al. [46] showed that subjects with higher dietary zinc intake have reduced losses in radial bone mineral density. However, the benefit was seen only in those subjects in the placebo limb of the trial, not in those who received calcium supplementation.

The study of Stause et al. [20] is discussed above, although the design of that study does not allow an assessment of whether copper, zinc, or manganese was responsible for the benefits observed. Peretz et al. [47] examined the effect of 12 weeks of zinc supplementation in healthy men. They demonstrated a significant increase in serum alkaline phosphatase in the zinc-treated group, but not in controls. There was no effect of urinary and C-terminal collagen peptide (a measure of bone resorption).

A recent randomized controlled trial in women (51–70 years old) compared the effect of combined zinc (12 mg/day) and copper (2 mg/day) supplementation in women already receiving calcium and vitamin D supplementation [48]. Both groups demonstrated a fall in BMD during the study, but the rate of decline was higher in the group supplemented with zinc and copper [48]. In a secondary analysis, the main determinant of the between-group differences appeared to be zinc intake [48]. In women whose dietary zinc intake was less than 8 mg/day, zinc supplementation was beneficial [48]. But in those whose dietary zinc intake was greater than 8 mg/day, addition zinc and copper supplementation worsened BMD [48].

25.3.4 Conclusions

Profound zinc deficiency appears to lead to reduced bone growth and maturation, probably through an effect on protein synthesis. There is very limited evidence (based on a trial of combined zinc and copper supplementation), that zinc may be beneficial is *some* women [48]. Those most likely to benefit appear to be those with lower dietary zinc intakes [48]. However, the detrimental effect in women with higher zinc intakes is worrisome, and further studies using zinc supplements alone are needed before clear guidance can be given.

25.4 Boron

The role of boron in human nutrition remains uncertain. Boron has been hypothesized to enhance bone mineral balance, although its mechanism of action is uncertain.

25.4.1 Animal Data

A study in ovariectomized rats showed that a combination of boron and estrogen (17-β-estradiol) increased apparent absorption of calcium, phosphorus, and magnesium. This effect was not seen for boron alone or for estrogen alone. No benefit was seen for boron in combination with parathyroid hormone [49]. This study is consistent with previous data in several animal species indicating an increase in mineral balance with supplemental boron [50]. Rats gain more bone mass in response to exercise when provided with boron compared to those without boron [51].

25.4.2 Human Studies

Few human studies have evaluated the role of boron in bone mineral metabolism. In one, providing boron to 12 postmenopausal women, who had been maintained on a low-boron diet for about 4 months, decreased the urinary excretion of calcium and magnesium. A lowered urinary phosphorous excretion was seen in those with a low-magnesium diet. In that study and in one in adults, it has been suggested that boron may act by increasing serum 17-β-estradiol [50].

Two recent studies have reported nutritional interventions that include boron. In the first, women age 40y or aged were recruited to three different nutritional supplements sequentially. The plans all contained about 750 mg calcium, but variable amounts of vitamin D (plan 1 = 1,000 IU, plan 2 = 800 IU, plan 3 = 1,600 IU) [52]. Two of the plans (#2 and #3) contained 780 mg/day strontium, and one (plan #3) contained 3 mg/day boron [52]. After 6 m, the women with the best compliance had higher increases in BMD if they were on plan 3 [52]. It is impossible to say what, if anything, drove this between group difference as the plans varied in several nutrients, particularly in vitamin D [52]. A similar study by the same authors [53] also showed benefits to the nutritional plan containing 3 mg/day boron. However, once again, this was confounded by the higher vitamin D level of the boron containing plan (1,600 IU vs. 800 IU) [53].

Finally, in a randomized trial of supplementation with dried plums (prunes) or dried apples, in women receiving calcium and vitamin D supplementation, the group consuming dried prunes had significantly higher ulnar and spine BMD than those receiving dried apples [54]. Prunes are one of the best dietary sources of boron, but it is not possible to say whether these differences were due to the difference in boron intake.

25.4.3 Conclusion

Although some data support a role for boron on bone health, especially in postmenopausal women, substantial further research, including well designed controlled trials, is needed to clarify the role for this nutrient as well as its physiological mechanisms of action. The quality of the currently reported trials of boron supplementation (or supplementation with boron-rich foods) is inadequate to guide any recommendation.

25.5 Strontium

Strontium has been proposed as effective in enhancing bone health. Stable strontium has been widely used as a marker for assessing calcium absorption, as it appears to be absorbed via similar pathways and share physical properties, including having its absorption stimulated by vitamin D [55].

25.5.1 Animal Studies

Several studies have evaluated the effects of strontium (as strontium ranelate) on bone formation and resorption [56]. These studies have demonstrated a positive effect on bone formation in growing rats as well as prevention of bone resorption in ovariectomized rats [56, 57] The mechanism of action is unknown, but the similarities of calcium and strontium suggest that it may be directly implicated in

physically strengthening bone as well as having hormonal effects. Of significant interest is that bone strontium levels are closely correlated with plasma strontium, a relationship not seen with calcium [58]. A recent study in mice confirmed a significant increase in trabecular bone mass strontium with long-term strontium ranelate treatment [59].

25.5.2 Human Studies

Results of two relatively large randomized controlled trials of strontium supplementation are now available: the SOTI trial [60] and the TROPOS trial [61, 62]. Both trials examined the effect of strontium ranelate (2 g/day) given for 3 years, in subjects who were already receiving calcium and vitamin D supplementation.

In the SOTI trial, 1,649 postmenopausal women with osteoporosis and history of vertebral fracture where randomized to 2 g/day strontium ranelate or placebo. Over the 3 years treatment period, BMD increased in the strontium group, but fell in placebo group [60]. After 3 years, the difference in BMD was 14.4 % at the lumbar spine, 8.4 % at the hip, both favoring strontium treatment [60]. More importantly, not only was BMD improved, the risk of fractures was reduced by 41 % in the strontium group compared to the placebo group [60].

The TROPOS trial was of similar design [61]. A total of 5,091 postmenopausal women were randomized to 2 g/day strontium ranelate or placebo [61]. As in the SOTI trial, BMD increased in the strontium-treated subjects but fell in the placebo-treated subjects [61]. At 3 years, femoral neck BMD was 8.2 % higher in the strontium group than the placebo group [61], very similar to the 8.4 % difference seen in SOTI trial [60]. After 3 years of treatment, strontium lead to significant reductions in the risk of nonvertebral and major fragility fractures. In subgroup with highest risk (most similar to the SOTI population [60]) it reduced risk of hip and vertebral fractures [61]. Slightly over half of the TROPOS population were followed-up at 5 years [62], when strontium reduced nonvertebral fractures by 15 %, reduced hip fracture by 43 %, and reduced vertebral fractures by 24 % [62]. Both the SOTI and TROPOS studies recruited only women. However, one small multicenter study suggests that strontium (2 g/day, as strontium ranelate) has similar effects on BMD in men as it did previously in women [63]. Lumbar spine BMD, femoral neck BMD, and hip BMD were all higher in men receiving strontium than those receiving placebo [63].

Data from the SOTI and TROPOS trials suggests that strontium is well tolerated [60–62], and this also appears to be true after 10 years of follow-up [64]. In one observation study of 1,200 subjects with a mean follow-up of 32 m, strontium seemed well tolerated and compliance with therapy was good [65].

At present, strontium ranelate is supported by expert panels [64], and licensed in the EU, but not in the US [66]

25.5.3 Conclusions

These animal and human studies suggest that relatively large doses of strontium are beneficial in decreasing bone resorption, enhancing bone mineralization, and reducing fractures. They suggest that benefits are maintained for at least 10 years. No toxicity or significant adverse effects have been reported with this therapy, although as this therapy becomes more widespread, ongoing surveillance and follow-up are needed.

25.6 Silicon

Silicon has been suggested as an important trace mineral necessary for bone development, but few specific data are available. Rico and coworkers found that ovariectomized rats that were provided silicon had a lower rate of bone loss [55]. The beneficial effect of silicon on bone health in ovariectomized rats may be limited to those with inadequate calcium intake [67], although data is contradictory [68]. A very small retrospective study suggested a benefit to silicon in bone density in osteoporotic adults [69].

Data from the Framlington offspring cohort has demonstrated a significant positive association between silicon intake and hip BMD in men, and premenopausal women [70]. But no such relationship was seen for postmenopausal women [70]. In men, beer is a significant source of silicon intake and the authors suggest that this may explain the previous reports of a positive association between alcohol intake and bone health [70].

This is supported by a more recent study showing that silicon intake is associated with improved bone mineralization, but only in estrogen-replete women (i.e. premenopausal or postmenopausal women on hormone replacement therapy) [71].

25.7 Other Trace and Ultratrace Minerals

Fluoride may have a role in bone mineralization in rodents [72]. Data in humans in contradictory, and the exact dose may be critical [73].

Early studies examining relatively high fluoride intakes (≥50 mg/day) showed that fluoride supplements significantly increased BMD [74, 75] particularly in cancellous bone [74]. Despite this change in BMD, the rate of fractures was not reduced by fluoride supplementation [74, 75] and side-effects were more common in the fluoride-treated individuals [74, 75].

Three studies have examined low dose fluoride supplementation (≤20 mg/day) using a variety of continuous [76–78] or intermittent (3 m on, 1 m off) [77] dosing schedules, with inconsistent results. One study has shown that 20 mg/day fluoride increased BMD and reduced vertebral fractures over 4 years from 10 to 2.4 % [76], while another suggests that doses of 2.5–10 mg/day have no effect on either BMD or markers of bone turnover [78]. Finally, Ringe et al. compared daily dosing of 20 mg/day fluoride as monofluorophosphate, intermittent dosing of monofluorophosphate (3 m on, 1 m off) or placebo [77]. Both fluoride dosing schedules lead to improved BMD, and the intermittent schedule was better tolerated [77].

Numerous other minerals have been proposed to have either an enhancing or harmful effect on bone (e.g., aluminum). Among these is manganese, although evidence for an effect is very minimal [79]. Because these are uncommonly deficient in diets and are difficult to assess in isolation from other minerals, it has been difficult to obtain solid information regarding their role, and therapeutic use should be considered only in the context of controlled trials.

Conclusion: There are only limited data on the role of trace minerals in bone health. Although overt copper deficiency has serious effects of the skeleton, the role of milder forms of copper deficiency remains unclear. Profound zinc deficiency appears to lead to reduced bone growth and maturation, probably through an effect on protein synthesis. There is very limited evidence that zinc may be beneficial is *some* women probably those with lower dietary zinc intakes. However, the detrimental effect in women with higher zinc intakes is of concern. Although some data support a role for boron on bone health, especially in postmenopausal women, substantial further research, including well

designed controlled trials, is needed to clarify the role for this nutrient as well as its physiological mechanisms of action. These animal and human studies suggest that relatively large doses of strontium are beneficial in decreasing bone resorption, enhancing bone mineralization, and reducing fractures. Silicon may play a role in bone health but data are limited.

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