Copper, iron, and selenium dietary deficiencies negatively impact skeletal integrity: A review

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Abstract

Nutrients have been known to have a significant role in maintaining the health of the skeleton, both bone and cartilage. The nutrients that have received the majority of the attention are Vitamin D and calcium. However, limited attention has been directed toward three trace elements that may have mechanistic impact upon the skeletal tissues and could compromise skeletal health resulting from inadequate intakes of copper, iron, and selenium. The role of copper and selenium has been known, but the role of iron has only received recent attention. Copper deficiency is thought to impact bone health by a decrease in lysyl oxidase, a copper-containing enzyme, which facilitates collagen fibril crosslinking. Iron deficiency impact upon bone has only recently been discovered but the exact mechanism on how the deficient states enhance bone pathology is speculative. Selenium deficiency has an impact on cartilage thereby having an indirect impact on bone. However, several studies suggest that a mycotoxin when consumed by humans is the culprit in some cartilage disorders and the presence of selenium could attenuate the pathology. This review summarizes the current knowledge base with respect to skeletal integrity when each of these three trace elements are inadequate in diets of both animals and humans.

Keywords: Copper, iron, selenium, skeleton, bone, chondrocytes

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Introduction

Osteoporosis is a disease that affects millions of Americans and it develops over a life time. More than half of the US population is thought to have this disease.¹ In the nutrition literature, most research has focused upon the nutrients vitamin D and calcium. Other nutrients have a significant role in bone development and maintenance. Vitamins and minerals such as magnesium, vitamin C, vitamin A, boron, zinc, and vitamin K play a role in bone health.^{2–4}

There are two trace elements that have been studied as it relates to bone health and one related to cartilage that impacts the skeleton indirectly that have not received much attention. These trace elements are copper, iron, and selenium. Copper and iron deficiency lead to compromised bone health. A lack of selenium is thought to lead to cartilage pathology. Here, we review the impact that these trace elements have upon the skeleton in terms of either cartilage or bone in both animal and human studies and potential mechanisms that may explain any pathologies.

In this review, articles were searched via Web of Science and PubMed using key words such as copper, iron, bone selenium, and cartilage. References to some articles in later

published research were obtained by checking citations through Web of Science.

Copper and bone

Much of our understanding of copper as it relates to bone comes from some early ground breaking research. Studies conducted and published by Rucker,⁵ Rucker et al.,^{6–8} Opsahl et al.,⁹ and Jonas et al.¹⁰ developed the mechanistic foundation on how copper impacts bone integrity. These researchers demonstrated that in elastin- and collagen-containing tissues, such as blood vessels, tendon, and bone, there was decreased mechanical strength of these tissues with copper deficiency. This decreased strength was associated with deficient collagen and elastin crosslinking. Torsional strength of bone in copper deficiency was noted to be decreased in chick tibia⁹; and this was correlated with decreased lysyl oxidase activity, which is a copper-containing enzyme. Lysyl oxidase oxidizes the epsilon amino group of lysine to produce an aldehyde group, or allysine. Subsequently, the aldehyde groups of two molecules of allysine react to produce an aldol crosslink which impacts greater strength in the collagen. Later, Jonas et al.¹⁰ reported

decreased torsional loading in femurs from copper-deficient rats and suggested that since the calcium content of the femurs did not differ by copper treatment, this difference was likely due to a decrease in collagen crosslinking; via a decrease in lysyl oxidase activity. However, decreased bone mineralization in copper deficiency was reported earlier.¹¹ That study reported lower bone mineral density (BMD), as measured by dual-energy X-ray absorptiometry in the fifth lumbar vertebra and the proximal femur in copper-deficient rats. Changes in markers of bone formation such as blood alkaline phosphatase activity were lacking which suggested an accelerated bone resorption as a potential mechanism for decreased BMD in the bones from copper-deficient rats.

Recent studies on copper and bone are limited in both humans and animals. One of the major reasons for this could be the fact that copper deficiency in humans is not a major problem in most US groups. The Recommended Dietary Allowance for copper is 0.9 mg/day. Whether the majority of Americans consume this level of copper has been the subject of much debate.¹² A more recent study indirectly implicates the role of copper in bone integrity.¹³ Increased cytoplasmic superoxide in Cu, Zn-SOD^{-/-} mice appears to compromise bone integrity. Mice with a decrease in Cu, Zn-SOD had greater weakness in bone stiffness and decreased BMD. Osteoblasts and osteoclasts were decreased on the surface area in the lumbar vertebrae of the SOD knockout mice. Enhanced cell death and decreased proliferation in primary osteoblasts, but not in osteoclasts, resulted perhaps due to decreased antioxidant activity. The same study demonstrated that treatment with vitamin C as an antioxidant nutrient reversed the compromised bone fragility and osteoblastic survival. The study did not discuss the role of copper, but a copper deficiency could likely result in similar findings. These findings are novel in that bone integrity due to copper deficiency may not in itself be due to less collagen crosslinking but increased free radical production. This could explain the decreased BMD as affected by decreased osteoblasts and osteoclasts.

There are a number of early studies that suggested in infancy that a copper-deficient diet could lead to brittle bones resulting in fractures, forms of osteogenesis imperfecta, and osteoporosis. Many of the publications on this topic focused on whether the bone anomalies were due to child abuse or low-copper diet and/or absorption of copper.¹⁴⁻¹⁷ In some cases, the lack of copper was traced back to prolonged use of enteral nutrition. Marquardt et al.¹⁸ reported that infants with short gut syndrome and placed on prolonged enteral nutrition developed osteoporosis and metaphyseal changes. Low levels on copper in the parenteral nutrition feeding and diarrhea were most likely the cause. Supplementation with copper improved or reversed the bone disorders. Another study reported skeletal abnormalities in premature infants presenting with depressed serum copper and ceruloplasmin. Copper supplementation reversed these abnormalities.¹⁹

It is not uncommon for copper deficiency in humans to initially be reported in those infants or adults receiving either total parenteral nutrition or some form of enteral nutrition. Reports of adults receiving feeding via a

nasopharyngeal tube or long-term enteral nutrition with a jejunostomy were diagnosed with copper deficiency as determined by low blood copper and ceruloplasmin levels.^{20,21} There were no reports of bone abnormalities in these adults. Children receiving either total parenteral nutrition or some type of enteral nutrition are especially vulnerable to copper deficiency.^{22,23} Wiss and Ledesma-Medina²³ reported histological changes in bone from infants receiving total parental nutrition.

Studies with adults have suggested that copper may impact bone health. A study on elderly patients with low blood copper levels revealed a significant increased incidence of femoral-neck fractures compared to age-matched controls.²⁴ Another study on elderly patients who were bed ridden for 12 months or more and exhibited signs of copper deficiency revealed that copper supplementation improved copper status and bone markers of bone resorption and formation.²⁵

With obesity being a growing national concern, various weight loss approaches have been introduced. One invasive method has been collectively known as gastric bypass. Patients undergoing these procedures develop compromised copper status, but no impact on bone has been reported.^{26,27}

A more recent study revealed a possible role of copper in dental health.²⁸ Fifty patients with significant tooth wear were age, sex, and body weight matched to 20 control subjects with normal dental wear. Vertebral BMD was measured and copper content in tooth enamel, saliva, and serum. Those subjects with significant tooth wear had reduced vertebral BMD and significantly lower enamel copper concentrations than controls. The authors suggested that decreased activity of lysyl oxidase could lead to decreased collagen crosslinking in the teeth. These observations were independent of serum Ca levels, osteocalcin, and vitamin D.

Iron and bone

Animal studies

Medeiros et al.²⁹ initially reported that iron could have a role in bone integrity. Iron is a component of prolyl hydroxylase that converts proline to hydroxyproline that is subsequently used in collagen crosslinking. Hydroxylases are required for collagen crosslinking and this could be compromised and lead to increased bone fragility in iron deficiency.

Weanling rats fed control, iron-deficient, or copper-deficient diets for five weeks thereafter revealed decreased strength in femurs from iron- and copper-deficient rats. Additionally, there were smaller cortical, but larger medullary areas in portions of the femurs.²⁹ It was unclear as to how this increased fragility in the iron-deficient state compared to rats fed a calcium restricted diet either singly or in combination with an iron-deficient diet. To address this issue, Medeiros et al.³⁰ used a very low-iron diet and a low-calcium diet containing 5–8 mg Fe/kg diet or 1 g Ca/kg diet, respectively. Experimental groups had reduced cortical, femur, and tibia, but greater medullary widths with the combined calcium restricted and iron-deficient groups

Figure 1 Radiographs of femurs from rats fed control, calcium-restricted (Ca-), iron-deficient (Fe-), and both calcium-restricted and iron-deficient diets (Ca-Fe-).³⁰

having the greatest reduction. Reduced BMD and cortical bone area was reported in calcium restriction and iron deficiency combined or singly (Figure 1). Since the level of iron in the study was very low, there was a possibility that the results could be due to reduced body weight of the iron-deficient group which could coincide with reduced food intake. Using pair-feeding to correct for potential differences in food intake, similar findings were reported.³¹

Micro-CT imaging revealed decreased bone, total bone volume, trabecular number and thickness, and lower structural model index, but increased trabecular separation in each of the nutrient-deficient groups compared to the control and pair-fed groups.³¹ This suggested that not only was there less bone but also a greater porosity of bone due to calcium restriction or iron deficiency.

Finite element analysis is a computer simulation technique used to predict how materials and structures will respond when loads are placed upon them. Finite element analysis revealed the vertebrae had lower stiffness (or a lower force was needed to compress the vertebrae), but a greater von Mises stress, in the calcium-restricted and iron-deficient groups compared to the control and pair-fed groups. This suggested that in the calcium-restricted and iron-deficient groups, there were greater internal stresses which compromised bone structural integrity. An example of vertebral trabecular bone as affected by calcium or iron deficiency compared to controls is shown in Figure 2.

It had been reported that 1α -hydroxylase depends upon an iron-sulfur containing flavoprotein and cytochrome P-450,³² which suggests that decreased hydroxylation of vitamin D could explain results observed with iron restriction. Calcium restriction lowered vitamin D-3 levels, but iron restriction did not.³¹

A relevant question is whether feeding a marginal iron diet, not deficient levels, over a greater period of time produce similar results? Feeding marginal levels of calcium and/or iron diets for 10 weeks resulted in detrimental effects of marginal dietary iron upon bone.³³ Marginal-

calcium or marginal-iron intake resulted in decreased BMD of the femur. Micro-CT analysis suggested both marginal iron L₄ and marginal calcium L₄ had reduced connectivity of the trabeculae. Trabecular number was decreased and trabecular separation was increased, which results in enhanced porosity in marginal iron fed rats. As was the case in outright iron deficiency, the marginal iron group was less likely to withstand compression force and could break at lower external stress than the control group, as determined using finite element analysis.³³

Díaz-Castro et al.³⁴ reported that in severe iron-deficiency anemia, bone abnormalities from the sternum and femur were readily apparent. Iron-deficient rats had lower blood levels of type I procollagen N-terminal propeptide which suggests less bone matrix was formed. Other markers of bone resorption such as increased blood parathyroid hormone, tartrate-resistant phosphatase (a measure of bone-resorbing osteoclasts), and breakdown of C-terminal peptides of type I collagen and release into the blood were reported. The femurs, but not sternum, revealed significant decreases in calcium and phosphorus in iron-deficient rats. However, there were no differences reported in blood 25-hydroxycholecalciferol. Another group³⁵ was able to determine that osteoblasts in culture treated with the iron chelator deferoxamine during differentiation developed a phenotype that resulted in downregulation of genes that favored the osteoblast phenotype (e.g. genes encoding osteocalcin and alkaline phosphatase) along with decreased mineralization compared to untreated cells.

One investigator did not have similar results with respect to bone breaking strength of rats fed marginal iron diets.³⁶ This could be due to initiation of iron-deficient diets at 10 weeks of age instead of at weaning. Also, anemia was not present in rats from their study compared to others^{30,31,33,34} suggesting the degree of iron status was lower in these other studies. The age of the animals may be a factor in that younger rapidly growing animals may be more susceptible to nutrient restriction compared to older rats as used in McClung et al.³⁶ study. Other laboratories

Figure 2 Represented three-dimensional vertebral trabecular bone from controls, calcium-restricted and iron-deficient diets. Images obtained from micro-CT scans. Red indicates predicted breaking or fracture points if a load is applied to the bones.³¹

have reported the negative impact of a postweaning iron-deficient diet upon bone development.^{37,38} The Katsumata group reported that BMC and BMD were significantly lower in the femurs of the iron-deficient groups compared to control and pair-fed groups.³⁷ They reported lower bone volume to total bone volume ratio in the iron-deficient group, but trabecular thickness did not differ among the three groups. Trabecular number was significantly decreased but trabecular separation was increased in the iron-deficient group. The increased porosity in iron-deficient rats is in agreement with previous studies.^{31,33} The Katsumata group also reported that osteoid volume, surface, and thickness were all reduced in the iron-deficient group compared to those of the control.^{37,38} The iron-deficient groups had decreased mineralizing surface, mineral apposition rate, bone formation rate, adjusted apposition rate percent of bone surface occupied by osteoclasts and osteoclast number. Blood osteocalcin concentrations and urinary deoxypyridinoline levels were decreased in the iron-deficient group. The C-terminal telopeptide of type I collagen was higher in the iron-deficient group. Lower osteocalcin levels could suggest lower bone formation. Additionally, decreased deoxypyridinoline and C-terminal telopeptide of type I collagen could suggest that bone resorption is decreased in iron deficiency.

Human studies

Several studies suggest that there could be a link between adequate diet iron intake and bone health in humans. Harris et al.³⁹ assessed 242 women 40 to 66 years of age for a variety of bone-related variables as part of the randomized clinical trial known as the Bone, Estrogen, and Strength Training (BEST) Study. Diet records and various bone measures were assessed in this study. After controlling for possible confounding variables, subjects who consumed more than 20 mg of dietary iron per day and had a calcium intake between 800 and 1200 mg per day had the most significant increases in BMD.

Another study using subjects from the BEST trial⁴⁰ supported the contention that iron may be related to bone mass and density. Hormone replacement therapy was studied for a one-year longitudinal study of women ($n = 116$) who received hormone replacement and women ($n = 112$) who did not receive replacement therapy. This study had access to both eight-day dietary records and the same BMD measurements as in the previous report.³⁹ Iron intake was positively correlated with BMD in the greater trochanter and Ward's triangle in women receiving hormone-replacement therapy, but not in those who did not receive hormone replacement therapy. Femur neck BMD increased as iron intake increased in women in the lowest calcium intake group.

A British study⁴¹ using 32 women ages 46 to 55 years not on hormone-replacement therapy reported a positive association between dietary iron and BMD after adjustment for potential confounding variables (e.g. calcium and protein intakes). This study was longitudinal in that they were followed for a period of 11 to 14 years. Subjects provided weighed-food intakes and BMD measurements at L₂ to L₄ of the vertebral column. Results revealed that dietary iron intake was positively correlated with BMD. A second but larger cross-sectional study of the same subjects revealed that, among 244 females within the same age range, a positive correlation between dietary iron with BMD was found at all bone sites.⁴²

While randomized clinical trials for studying if iron supplements improve bone integrity and strength are lacking, there are two studies that offer strong indirect evidence that iron may enhance bone strength. Moran et al.⁴³ evaluated risk factors that might predict stress fractures among Israeli soldiers during the four-month basic training period in both males and females. Males did not develop stress fractures during basic training, but 27 females developed stress fractures. Furthermore, those that had the greatest risk of developing stress fractures were females with iron deficiency. A second study by the same group revealed that both iron deficiency and anemia lead to increased incidence

of stress fractures in female Israeli soldiers.⁴⁴ These results are intriguing and should be further investigated because of relevance to other military units as well as to athletes who may be consuming an iron-poor diet.

Selenium and cartilage

Selenium does play a role in bone health indirectly, as it effects cartilage integrity. The bone disorder, Kashin-Beck disease, was linked to low selenium and osteoarthritis in low-selenium areas of northern China. Here, the impact of the disease does not appear to be on the bone itself but upon the chondrocytes of the cartilage. The impact upon bone is indirect via necrosis of the chondrocyte that composes the growth plate, thereby impacting the skeleton. Depending on severity, this pathology can lead to impaired growth, osteoarthritis, and disability in terms of mobility.^{45,46} Joints are deformed and it is not uncommon for dwarfism to be presented by growing children in low-selenium areas. However, there is some dispute that this disease may not be due to selenium deficiency per se, but may be due to a combination of factors. High mycotoxin content in cereals has been implicated.⁴⁷ Additionally, iodine deficiency combined with selenium deficiency have also been suggested.⁴⁷ For instance, rats fed a diet with the mycotoxin T2 developed minor signs of cartilage pathology. When a low protein, iodine, and selenium diet were fed to another group of rats with or without T2 toxin, chondrocyte necrosis was evident in both cases and the tibia length was decreased. However, rats fed the T2 plus low-nutrition diet showed signs of human Kashin-Beck disease, including a decrease in tibia length. Both T2 addition to the diet and a low protein, iodine, and selenium diet resulted in a blurring, thinning, and irregularity of the epiphyseal plate in this group compared to other groups. The metaphyseal trabecular bone was sparse, disordered, and disrupted.⁴⁸ This suggested that nutrition and T2 toxin interact in some manner to impact cartilage.

Another animal study used Dark Agouti rats which is a model often used to study rheumatoid arthritis and other joint disorders.⁴⁹ Here, two generations of rats were fed selenium-deficient diets. The F0 generation revealed decreased thickness of both the femur and tibia epiphyseal plate in the selenium-deficient rats. The F1 generation had decreased proliferative areas in the epiphyseal plate of selenium-deficient rats. Both generations of selenium-deficient rats had decreased expression of collagen II mRNA as did the antioxidant enzymes glutathione peroxidase 1. The enzyme known as ADAMT-4 was upregulated in both generations of selenium-deficient diets. This is significant in that this latter enzyme degrades proteoglycans in the articular cartilage in osteoarthritis disorders.⁴⁹

Children are most vulnerable to this disease since they are in the growing phase. However, there are mixed results as to whether selenium supplementation results in benefits. This leads to questions as to whether lack of dietary selenium is the primary etiology of the disease even though animal studies suggest selenium deficiency results in cartilage defects. Zou et al.⁵⁰ published a meta-analysis suggesting that selenium supplementation does prevent the

disease but cautioned there are probably other confounding events. Another study compared Chinese children with Kashin-Beck disease to three other groups of children: (1) children without the disease, (2) children without the disease but from Kashin-Beck areas of China, and (3) healthy children in non-endemic areas of Kashin-Beck disease. Results revealed that blood levels of 4-hydroxyl-2-nonenal and 8-hydroxydeoxyguanosine were elevated in the cartilage of children with Kashin-Beck disease and healthy children from high Kashin-Beck disease areas. These chemicals are produced in response to oxidative stress, which suggest the disease is oxidative stress related.⁵¹ Another human study on subjects with either Kashin-Beck or osteoarthritis revealed downregulation of the expression and synthesis of two important mRNAs and corresponding enzymes, respectively. The first enzyme was chondroitin sulfate N-acetylgalactosaminyl-transferase I. This enzyme facilitates chondroitin sulfate chain formation in cartilage. The second enzyme was hyaluronan and proteoglycan link protein 1. This enzyme is critical in stabilizing the collagen matrix as it stabilizes the aggregation of aggrecan and hyaluronic acid.⁵² Collectively, there appears to be decreased antioxidant activities and changes in expression of proteins involved with chondrocyte integrity in Kashin-Beck disease. Another study gives further insight as to the linkage between Kashin-Beck disease caused either by selenium deficiency or T2 toxin. Human chondrocytes cultured with T2 toxin resulted in decreased mitochondrial activities of complex III, IV, and V; decreased ATP levels and mitochondrial membrane potential; but increased reactive oxygen species. Apoptosis was clearly evident in the T2 toxin-treated group. However, when selenium was added to these cultures, this partly blocked the pathologies including mitochondrial dysfunction observed in T2-treated cultures and reduced oxidative damage and chondrocyte apoptosis.⁵³ This study in addition to the previous study above⁴⁸ could suggest that the toxin is the primary cause of Kashin-Beck disease, but in the presence of selenium, the pathology is attenuated and possibly absent.

Summary

All three trace minerals reviewed have an apparent impact upon skeletal integrity; with iron and copper affecting the bone directly, and selenium affecting cartilage. Copper deficiency is linked to bone abnormalities due to its role as a co-factor in the rate limiting enzyme lysyl oxidase, which strengthens collagen fibrils and thereby bone by promoting crosslinks with lysine and other amino acids. The studies with animals have been convincing, but whether copper deficiency to the level needed affects humans has been a subject of debate. However, there does appear to be evidence that copper does have a practical role in infants afflicted with malabsorption syndrome and in some elderly humans. While recent data on gastric bypass surgery negatively impact copper status, the degree of the impact is not likely to clinically impact bone. The influence of iron deficiency has clearly been demonstrated in animal models. However, the mechanism is unclear as it is not likely due to an impairment of collagen crosslinking. While the

hydroxylases do contain iron, it apparently does not influence hydroxylated forms of vitamin D, which would be the case if such a catalytic or rate limiting role was apparent. Rather, decreased bone formation or increased bone resorption is more likely the cause; but the nature of how iron plays a role with osteoblasts and/or osteoclasts, if any, remains elusive. The relevance to human bone issues is indirect with descriptive studies providing the majority of the evidence. A lack of selenium has been linked to chondrocyte pathology in the form of necrosis. Studies have clearly indicated some mechanistic features that occur both in the epiphyseal plates and the chondrocytes. The issue is complicated as to whether the presence of the mycotoxin T2 that could be present in diets may lead to Kashin-Beck like symptoms and not selenium deficiency. The majority of the evidence does suggest that a lack of selenium affects human populations in selenium-deficient areas of China. However, one study suggests that the mycotoxin and selenium deficiency must be present together for signs of Kashin-Beck disease to become apparent. The presence of selenium appears to attenuate T2-induced cartilage pathology.

Regardless, it is important to note that these trace elements, and not simply the macroelements calcium and vitamin D, may have roles in maintaining skeletal integrity. While this review presents novel findings, they also pose challenging questions for future research.

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