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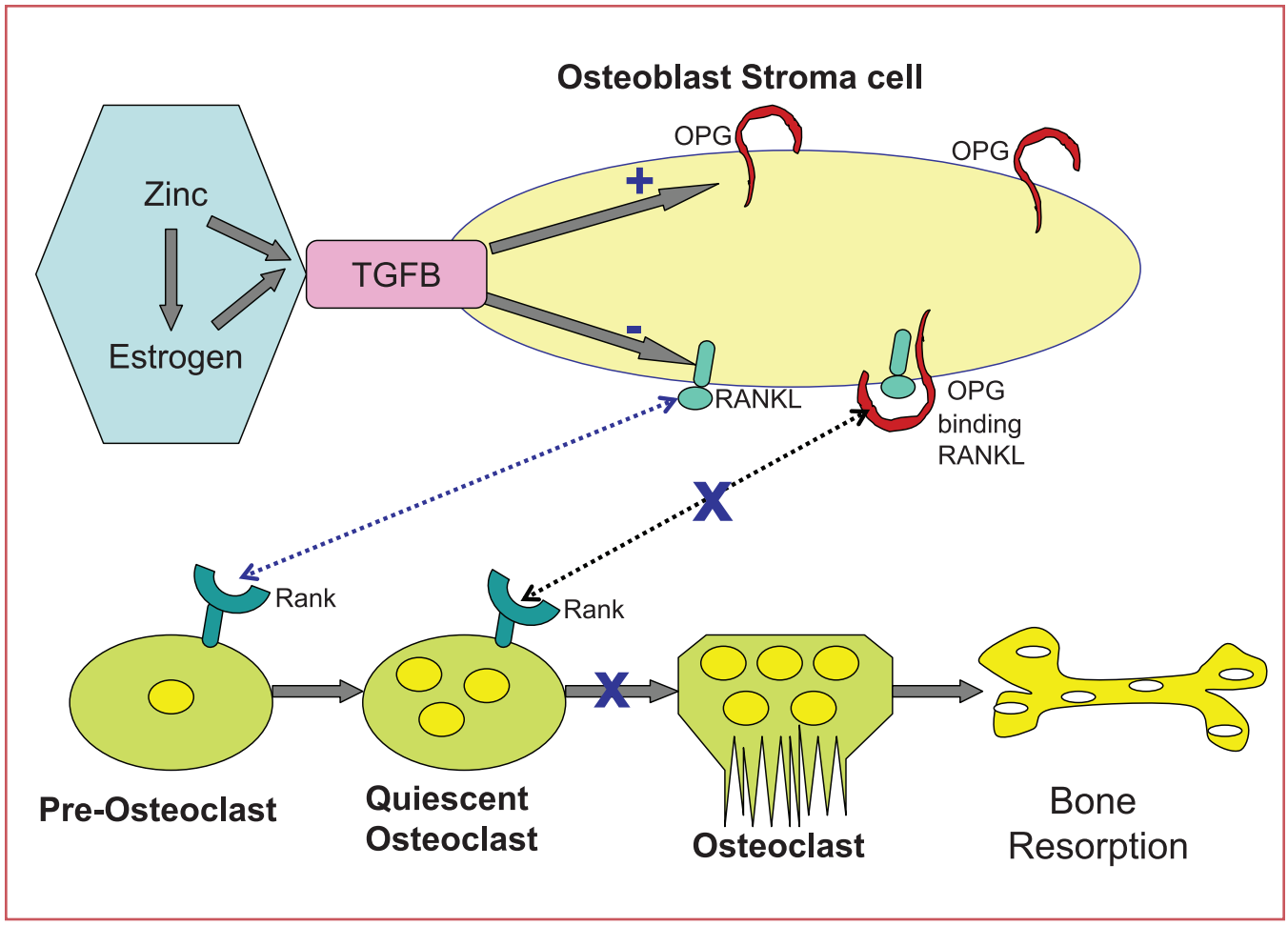

Zinc Homeostasis and Bone Mineral Density

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Abstract Zinc is an essential transition metal in humans, playing a catalytic, structural and regulatory role in the biological system. Zinc is abundant in bone tissue and is needed to maintain bone mineral density and bone metabolism. Every step of bone metabolism utilizes zinc, and its deficiency is implicated in osteoporosis. The organic matrix of bone is comprised of proteins that require adequate amounts of zinc for optimal function. Zinc acts as a cofactor for osteoblast activity during bone formation and is required for maintaining peak bone density and reducing the risk of age-induced osteopenia or fracture. Effective reversal of osteopenia in osteoporotic-prone women requires estrogen therapy with concomitant doses of divalent cations such as calcium and zinc. Although zinc is less known for its effects on maintaining bone integrity than is calcium, it is emerging as a vital nutrient for the prevention and reversal of the osteoporotic process. Recent evidence demonstrates that zinc may act as a local regulator of bone cell formation by stimulating the proliferation and differentiation of osteoblasts while at the same time inhibiting osteoclast differentiation. Thus, the effect of zinc on bone mineral density could be a missing link for a wide array of clinical anomalies encountered in the practice of osteopathic medicine. This review highlights the recent growth in understanding the significant role that zinc plays in bone metabolism and improvement of bone quality.

Key Words zinc, osteopenia, calcium, osteoporosis, bone, mineral

A great deal of emphasis has been placed on the need for adequate dietary calcium as an important means of preventing osteoporosis. However, in addition to calcium and vitamin D, which is required for its absorption, other minerals appear to be important for optimal bone formation and strength. Although zinc's effect on the maintenance of bone integrity is less well understood than is calcium's, recent studies are underscoring zinc's potential significance in both the prevention and reversal of the osteoporotic process. Zinc is abundant in every part of the body, second only to iron in terms of concentration of transition metals, and is essential for human growth and development.[1-4] Numerous enzymes and proteins utilize transition elements to carry out their biological function. Zinc is the most widely used of these elements. Therefore, most of the zinc in the body is bound up in metal-protein complexes and is comprised largely of metalloenzyme- and metalloprotein-bound zinc, such as zinc-finger proteins, in which zinc is used for enzymatic catalysis or for structural stability.[5-7] For example, zinc is a cofactor in at least 300 enzymes from all 6 enzyme classes. It is essential for DNA and protein synthesis in addition to its use in the maintenance of DNA and RNA. As a component of many enzymes, zinc is involved in the metabolism of proteins, carbohydrates, and lipids.[8-13] Hence, maintaining a constant state of cellular zinc homeostasis becomes essential for normal function.

Zinc biology is a rapidly developing field, and recent research reveals zinc's strategic role in most organ systems.[7,11] Physiologically, zinc is vital for growth and development and for healthy functioning of many body systems,[14-16] encompassing insulin storage and release, cognition, cell membrane integrity, sexual maturation and reproduction, dark vision adaptation, olfactory and gustatory activity, thyroid function, blood clotting, taste acuity and for a variety of host immune defenses, among others.[16] Clinical manifestations of zinc deficiency in humans include growth retardation, male hypogonadism, mental lethargy, abnormal neurosensory changes, delayed wound healing, skin changes, poor appetite, oligospermia, anorexia, weight loss, and susceptibility to infection.[15,17] The average adult body contains about 2-3 g of zinc.[18] Zinc is found in all parts of the body but is more concentrated in muscle (60% of total body zinc), bone tissue (30%), and skin (5%). As there are no specific cellular storage sites yet known for zinc, a regular supply in the

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diet is required. Approximately 20% of dietary zinc is actually absorbed by the body.[19,20]

Zinc deficiency is associated with many diseases affecting both young and old people, including osteoporosis.[21-23] As zinc acts as a local regulator of bone cells, bone growth retardation is a common finding in various conditions associated with zinc deficiency.[24] Of the many essential transition metals, zinc effectively stimulates bone growth and mineralization, implying both nutritional and physiological roles.[25,26] The oral administration of a zinc compound in an animal model of osteoporosis has been shown to prevent bone loss in ovariectomized rats.[27] As such, the effect of zinc on bone mineral density could be a missing link for a wide array of clinical anomalies encountered in the practice of osteopathic medicine. The number of biological functions, health implications and pharmacological targets pertaining to zinc indicate a need for further studies to solve the mystery of this important metal in biological systems, particularly those related to the maintenance of bone mineral density.

Zinc and Bone Mineral Density

Essential trace minerals such as copper and manganese are required along with zinc for the maintenance of healthy bone tissue (Figure 1). These minerals are involved in the formation of the bone framework structure contributing to the organic component of the osseous matrix. The hard mass characteristic of healthy bone is formed by inorganic minerals such as calcium and phosphorous, a component typically referred to as the "mineral mass." The structural framework around which the "mineral mass" deposits is termed the "organic bone matrix." The organic matrix is comprised of proteins that require zinc, manganese and copper as essential co-factors for enzymes involved in their synthesis.[28,29] Deficiency in zinc will lead to bone growth retardation and osteopenia due to insufficient bone mineral mass.[2,30-33]

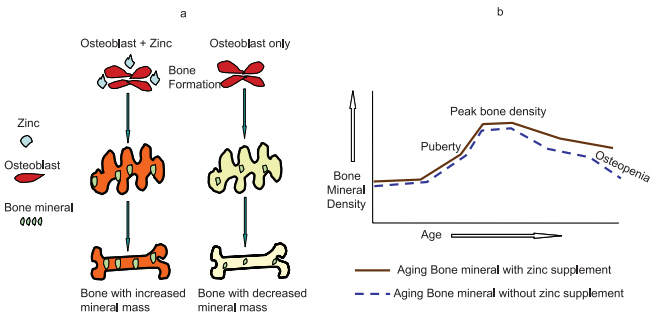

Figure 1: A schematic model for the association of zinc with bone mineral density. A. Shows a representation of zinc acting as a local regulator of an osteoblast at the same time acting as a template with other transition metal to form the bony framework for organic matrix formation. Deficiency in zinc will lead to bone growth retardation and osteopenia due to insufficient mineral mass in bone. B. Shows how bone mineral density decreases with aging and the effect of zinc on aging bone. The mineral mass in bone peaks after puberty but decreases with old age. Zinc supplementation prevents/improves osteoporosis.

Zinc status – a combination of serum and urine zinc concentrations – may play a substantial role in several indicators of bone mineralization including bone mineral content, bone mineral density, height, weight and insulin-like growth factor. In a 2-year French study of 139 healthy premenarcheal girls, zinc status was significantly related with bone mineral density increase, suggesting that during puberty onset, zinc is important to normal growth and bone mineralization.[14] In another study, a group of 396 men, age 45-92, completed bone mineral density measurements at baseline and again 4 years later.[23] Dietary zinc intake was evaluated and plasma zinc concentrations were measured with plasma spectroscopy. Men in the lowest plasma zinc quartile had significantly lower bone mineral density measured at the hip, spine and distal wrist. These results were independent of age or body mass index and suggest that dietary zinc intake and plasma zinc are positively associated with bone mineral density in men.[23]

Osteogenesis, the process of bone formation, involves 3 main steps: 1) the production of the extracellular organic matrix – osteoid; 2) the mineralization of the matrix to form bone; and 3) bone remodeling by resorption and deposition. The cellular activities of osteoblasts, osteocytes, and osteoclasts are necessary for this process. Current research indicates a role for zinc in every step of bone metabolism. Zinc is known to play a key role in regulating cellular activity by acting as a cofactor, stimulating protein synthesis needed for organic matrix formation (eg, the production of collagen).[17,18,34,35] Collagen comprises 90% to 95% of the organic matrix of bone and forms the structural framework around which mineralization occurs. The main mineral component of bone is a crystalline salt made up of calcium hydroxyapatite [Ca_{10} $(PO4)_6(OH)_2$]. Hydroxyapatite crystals also contain zinc along with other transition metals.[36] Zinc also functions as a metal component of alkaline phosphatase, a metalloenzyme that plays a key role in the formation of new bone. Alkaline phosphatase structure incorporates 4 zinc atoms per molecule, 2 of which are essential for enzyme activity.[37,38] Zinc may induce the increase in alkaline phosphatase-related DNA synthesis and, as a result, stimulate bone growth.[39] Recent studies indicate that zinc supplementation can have direct stimulatory effects on alkaline phosphatase and osteocalcin.[40] It has also been shown to promote bone mineralization through its role as a cofactor for alkaline phosphatase.[39,41]

Zinc also acts in regulating the activity of cells involved in the formation of the bony framework. Generally, it increases the synthesis of growth factors such as insulinlike growth factor 1 (IGF-1) and the effect on target tissues.[35] It also stimulates the proliferation and differentiation of osteoblasts via mechanisms influencing the activity of the calcium-regulating hormones 1, 25-vitamin D and parathyroid hormone (PTH).[18,39,42,43] Zinc also prevents PTH-induced bone resorption.[14,35,42,44] Zinc is required for osteoblastic activity. Aminoacyl-tRNA synthetase, which is the first step in protein biosynthesis, is directly activated by zinc in osteoblasts.[45-48] A more detailed description of this process is discussed under the section of "zinc and osteoblasts." Thus, zinc appears to have multiple important functions in bone development, formation, and metabolism.

IGF-1 is a hormone-like agent that is primarily produced in the liver and carried to body tissues via the bloodstream. IGF-1 is a critical factor in the regulation of bone formation, resorption, and calcium homeostasis. Recent studies have suggested that low intakes of zinc are associated with decreased production and secretion of IGF-1.[49-51] Lower amounts of circulating IGF-1 lead to more rapid loss of calcium from bone with aging and the subsequent development of osteoporosis. To shed light on the relationship between zinc, IGF-1, and osteoporosis, Devine and colleagues examined 119 postmenopausal women for dietary intake and IGF-1 blood levels. The results indicated that zinc intake strongly correlated with IGF-1 blood levels.[49] Other research involving malnutrition in animal models and children supports the association between zinc intake and IGF-1 blood levels.[52,53] Notably, zinc deficiencies were correlated with reduced IGF-1 concentrations and the concomitant decrease in total bone growth. With zinc supplementation, however, concentrations of IGF-1 and growth were significantly improved.

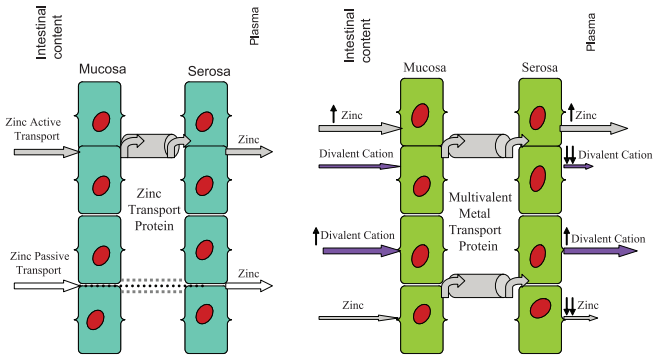

Figure 2: Diagrammatic representation of zinc absorption in the intestinal lumen and zinc competing with other divalent cations for the multivalent metal transporter in intestinal lumen. A. The drawing depicts the passive absorption of zinc and the active transport of zinc via a zinc transporter in the jejunum of the gastrointestinal tract where the majority of zinc absorption occurs. B. The increasing zinc concentration inhibits luminal absorption of divalent cations (Ca, Fe, Cu, Mg) by competing for the multivalent metal channel transporter and vice-versa, with an increase in divalent cations inhibiting zinc absorption.

Zinc Absorption and Interaction with Other Divalent Cations

Zinc absorption

Zinc homeostasis, like that of other divalent cations, is primarily maintained via the gastrointestinal system by the processes of absorption of exogenous zinc and secretion and excretion of endogenous zinc.[54] Although these processes modulate net absorption and the size of readily exchangeable zinc pools, there are limits to the effectiveness of such homeostatic mechanisms. The absorption of zinc at the intestinal level has been shown to take place via a saturable active transport as well as a non-saturable passive diffusion process. The passive diffusion process depends on the concentration gradient of the zinc cation available for absorption.[3,55,56] Hence, there are multiple factors that are now thought to influence zinc absorption, including (1) increased zinc concentration saturating the active transport system; (2) reduced absorptive rate of intestinal mucosa due to disease processes; and (3) increased concentration of other divalent cations (e.g., calcium) that compete with zinc for the nonspecific multivalent cation channel.

Effect of divalent cation on zinc absorption

Minerals may compete for absorption sites in the intestine via the multivalent metal channel. The absorption sites for zinc are the same ones used by calcium, iron and copper. Therefore, excess intake of iron or copper can adversely interfere with zinc absorption. Likewise, excess intake of zinc can impair calcium, iron and copper absorption. Various zinc-mineral interactions have been investigated, and it has been shown that there is a divalent cation transporter in the brush border membrane possessing a broad substrate range including Fe^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+}. This transporter is a multidivalent metal channel and accounts for the observed interactions between zinc and other divalent cations in competition for this common transporter.[57-61] Zinc is an essential trace element in human nutrition, and zinc deficiency is a worldwide nutritional problem. However, the zinc content in food is low, and zinc's availability is affected by several physiologic and dietary factors. Further study is needed to investigate the influence of these factors to try to improve zinc availability. Figure 2 explains how zinc, like any other divalent cation, competes for absorption into the intestinal lumen.

The metal cations calcium, magnesium, copper, and manganese have all been shown to be capable of inhibiting zinc absorption by competing with it for transport across the brush border membrane.[54,62,63] A high concentration of calcium in the lumen may depress zinc absorption by reducing the passive component of intestinal zinc absorption. Several studies indicate that taking a calcium supplement may actually lead to a zinc deficiency, increasing the requirement for zinc. A study from one group found that high calcium intake reduced zinc absorption in the elderly. [64,65] In this study, the addition of a calcium supplement to the diet significantly reduced zinc absorption and balance by more than 50%. Others suggest the use of a zinc supplement along with calcium for people suffering from zinc deficiency or osteopenia.[45,46,48,66,67] Furthermore, because of the crucial role zinc plays in bone mineralization, it is now suggested that postmenopausal woman on estrogen therapy take both calcium and zinc supplements due to the inhibitory effect that increased luminal calcium has on zinc absorption.[65,66,68]

While excess cupric ions have no effect on zinc absorption, excess zinc has an inhibitory effect on both calcium and copper.[69,70] A zinc-rich diet and zinc supplementation have recently been recommended for patients suffering from Wilson's disease to help inhibit cupric ion absorption, a classic presentation of that disease. Also called hepatolenticular degeneration, Wilson's Disease is a rare autosomal recessive inherited disease, primarily caused by an accumulation of copper in tissues throughout the body. Several studies support the hypothesis that zinc supple-

mentation has a deleterious effect on copper status or absorption.[71] It is possible that zinc acts both to compete with copper for absorption on the luminal side of the intestinal epithelium and to induce the synthesis of metallothionein, which chelates excessive copper.[70]

Zinc interacts not only with other divalent cations, but with other organic compounds involved in the zinc absorption pathway.[72] Several key compounds that inhibit the absorption of zinc include selenium, folic acid, phytic acid (from cereal), tannins, and fiber. Phytates and fiber found in unprocessed grains inhibit the bioavailability of zinc and other minerals. Whole grain yeast breads enhance the absorption of zinc by producing enzymes that destroy phytates.[73] Researchers have proposed that some saccharides such as lactose and glucose polymers could improve zinc absorption at the brush border membrane by forming water-soluble compounds with zinc and preventing the formation of the insoluble (non-absorbable) zinc phytate compounds.[55,73,74] Exposure to toxic metals such as lead or cadmium can interfere with the absorption of zinc and displace zinc in its metabolic functions.[56,75]

Role of Zinc and Estrogen Therapy on Bone Density

Bone loss is a major health issue and cause of death in the elderly. The rate of bone loss increases in both men and women around 40-45 years of age, but is more pronounced in women because of hormonal changes occurring with menopause. Bone loss is accelerated to 2% to 5% each year immediately before and for up to 10 years postmenopause.[76] Osteoporosis that occurs among postmenopausal women is the most prevalent type of disturbance to the bone remodeling process. Estrogen deficiency hastens the loss of bone in postmenopausal women (Figure 3). Estrogen, given in the form of hormone therapy (HT), reduces the risk of osteoporosis in postmenopausal woman. Thus, estrogen plays a major role in bone remodeling. This action is thought to reflect the ability of estrogen to prevent, and even partially reverse, postmenopausal bone loss.[77-82]

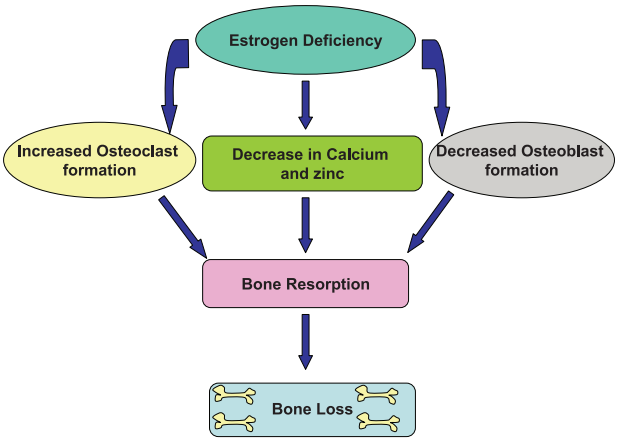

Figure 3: The effect of estrogen deficiency on postmenopausal women. Due to loss of the normal physiological role of the steroid hormone, estrogen deficiency leads to bone loss by increasing osteoclast action in bone and decreasing osteoblast activity.

Effective reversal of osteopenia in osteoporotic-prone women requires the use of estrogen therapy concomitantly with divalent cations like calcium and zinc. Initial clinical studies demonstrated a therapeutic role of zinc in experimental animals with osteoporosis by stimulating proliferation and differentiation of osteoblasts, inhibiting bone resorption and stimulating calcification.[13,42,45,47,66,83-85] Several studies have been conducted on the role of estrogen and zinc on bone mineral density, but not much research has been conducted on the interaction of estrogen and zinc in postmenopausal woman using HT.

Estrogen and Bone Density

Estrogen therapy can help prevent and treat osteoporosis. After menopause, the ovaries stop producing estrogen. HT started within the first few years after menopause has been shown to significantly reduce the risk for developing osteoporosis.[78-82] It both prevents and treats osteoporosis and increases bone mineral density. The way estrogen stimulates osteoblast activity in postmenopausal women is consistent with other evidence which suggests that this hormone is an important physiological regulator of osteoblast activity. In particular, recent findings suggest that following puberty in females, estrogen is required for the maintenance of bone density during skeletal growth,[86] an action that presumably involves increased osteoblast activity. There are some downsides to estrogen replacement therapy; unopposed estrogen therapy has been associated with an increased risk of breast and ovarian cancer.[78-82]

Estrogen plays a large role in regulating the population of osteoclasts by acting on estrogen receptors in osteoclast progenitors and via the reciprocal relationship between osteoclasts and osteoblasts.[87] Estrogen receptors are now known to be present on osteoblast-like cells.[88,89] Recent studies suggest that postmenopausal women on HT have relatively high estrogen levels, which stimulate osteoblast function, presumably as a consequence of a rise in the number or increase in activity of osteoblasts as individual remodeling units.[90] An increase in the number of osteoblasts prevents the accumulation or increase in number of osteoclasts involved in bone resorption. Estrogen also directly interacts with osteoclast progenitors through their estrogen receptors. These receptors operate in the nucleus and on the surface of the cell. They activate target genes in the nucleus and send signals to specific sites from the cell surface. Signals from these nuclear and surface estrogen receptors limit the size of the preosteoclast population by stimulating apoptosis driven by transforming growth factor-β (TGF-β).[87] Osteoblast precursors also secrete receptor activator of nuclear factor-κβ ligand (RANKL). Preosteoclasts have membrane receptors called receptor activator of nuclear factor-κβ (RANK). When RANKL activates these receptors, the cells fuse and differentiate into mature, multinucleated osteoclasts that develop a ruffled border and absorb bone. Osteoprotegrin (OPG) is a free-floating decoy receptor, related to the tumor necrosis factor (TNF) family, which can bind the RANKL and prevent it from activating the RANK. Estrogen also stimulates the production of TGF-β, which causes the osteoblastic stromal

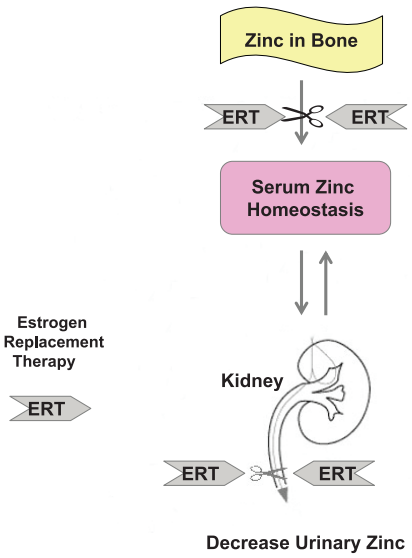

Figure 4: Diagrammatic representation showing how zinc is lost from bone in postmenopausal women deficient in estrogen, and how estrogen from HT is able to reduce bone mineral loss via decreasing urinary zinc excretion.

cells to make OPG instead of RANKL.[91] Thus, with estrogen loss, the levels of OPG decline, leaving RANKL to bind to RANK and allowing differentiation of osteoclasts to proceed.[88,92-96]

Estrogen Interaction with Zinc

As a dietary component or supplement, zinc is important for bone health throughout life (Figure 1). Many nutrients are interdependent; no one mineral, by itself, is sufficient to maintain healthy bones. Clinical studies have shown that supplementing calcium along with zinc, copper, and manganese slows the loss of spinal bone mineral density in postmenopausal women more effectively than calcium alone.[20,54,65,76] A clinical study by Rico and colleagues has showed that women with osteoporosis excrete more zinc in their urine than non-osteoporotic women.[22] They examined plasma zinc levels and urinary excretion of zinc in 30 menopausal women with osteoporosis compared to 30 healthy postmenopausal women. They reported that plasma levels were not significantly different between the 2 age groups, but urinary excretion of zinc was significantly higher in the osteoporotic menopausal group when compared to the controls. Urinary zinc concentration correlated significantly with serum tartrate-resistant acid phosphatase levels in women with postmenopausal osteoporosis but not in controls. Total body bone mineral content (TBBMC) correlated with urinary zinc concentration significantly in women with postmenopausal osteoporosis, but the correlation was not significant in healthy postmenopausal controls. These findings indicate that the elevation of urinary zinc elimination in osteoporosis is dependent on bone resorption (Figure 4).

Another study by Herzberg and colleagues examined the influence of HT on blood and urinary zinc in postmenopausal women.[68] They found a significant decrease in zinc excretion after 3 months of HT. This change was more pronounced in the osteoporotic women who had elevated urinary zinc excretion.[68] Figure 4 summarizes the effect of estrogen on urinary zinc loss from bone.

Estrogen has a direct stimulatory effect on bone DNA, promoting the proliferative ability of bone protein content. Zinc can also stimulate bone protein synthesis directly or by enhancing the effect of estrogen to increase DNA content.[13,22,28,47] Recent studies have shown that zinc can compete with estrogen via the steroid transport protein binding site,[97,98] while other studies have shown that estrogen decreases the amount of zinc loss from bone via urinary zinc excretion.[22,68] Zinc competes with estrogen transport via sex hormone-binding globulin (SHBG), a protein that transports estrogen in the blood and regulates its access to the target tissues.[97,98] SHBG reduces bioavailability of estrogen, especially in menopausal woman who have a high level of SHBG.[99] SHBG has specific binding sites for divalent cations. Zinc binding to a site at the entrance of the steroid-binding pocket in human SHBG has been shown to reduce its affinity for estrogens.[97,98,100,101] Taken together, zinc may increase the bioavailability of estrogen by interacting on SHBG.

HT contains estrogen with or without progesterone and is available in many brands and forms. It now appears that estrogen must be taken indefinitely for maximum protection against osteoporosis, which may increase the risk for breast cancer. Experiments conducted on postmenopausal woman have shown estrogen to significantly reduce the amount of mineral loss, which helps to maintain zinc concentration in bone tissue.[68] This ultimately might affect zinc homeostasis and eventually increase serum zinc concentration over time. It would be interesting to determine if the increase in zinc concentration via zinc supplementation in postmenopausal women acts as a positive feedback mechanism to reduce the dosage of estrogen and to counteract the negative effect of estrogen accumulation.

Zinc and Osteoblasts/Osteoclasts

Zinc increases the number of osteoblasts by inhibiting osteoclast-like cell formation in mouse marrow culture in vitro.[13,46] Osteoclast cell formation was estimated with staining for tartrate-resistant acid phosphatase, a marker enzyme for osteoclasts. The decrease in the marker enzyme indicates the inhibitory effect zinc has on osteoclasts, which was more pronounced at the stage of differentiation of preosteoclasts in the bone marrow cell culture system. This effect was reversed using a potent zinc chelating compound, CaEDTA, during the preosteoclast cell differentiation stage of osteoclast cells in vitro. The cellular mechanism of zinc's action stimulates bone protein synthesis. Aminoacyl-tRNA synthetase, which is the first step in protein biosynthesis, is directly activated by zinc in osteoblasts.[45-48, 102, 103]

Osteoblasts and Osteoclasts

Osteoblasts form from a precursor cell which then differentiates into a mature osteoblast cell. The precursor is a stem cell from bone marrow called a mesenchymal stem cell. Some osteoblasts differentiate further until they become osteocytes. Osteocytes make up over 90% of the bone cells in adults. Osteocytes are now believed to be involved in bone's response to mechanical

loading because of their distribution throughout the bone matrix and their ability to respond to strain with biochemical signals like nitric oxide and prostaglandin E_2 (PGE_2). Osteoblasts and osteocytes in particular, respond to fluid flow in vitro with enhanced nitric oxide and PGE_2 release.[104-106] Both signaling molecules have been shown to be essential for the anabolic response of bone to mechanical loading.[107,108]

Osteoclasts are differentiated from hemopoietic cells of the monocyte/macrophage lineage. They are multinucleated cells and are formed by the fusion of mononuclear pre-osteoclasts. The detailed molecular mechanism underlying the differentiation of osteoclasts is not yet well understood. Their differentiation is thought to be under the control of the bone microenvironment, which is supplied by osteoblasts or stromal cells and local factors such as TNFα, interleukin-1 (IL-1), TGF-β, and zinc.[45,83,109-111] The changes in the bone microenvironment induce intracellular signals (nitric oxide, PGE_2) in the precursor cells for osteoclasts,[104-106] which lead to the expression of target genes and differentiation toward osteoclasts.

The intercellular communication between osteoblasts and osteoclasts is crucial to bone homeostasis. The target cells of osteotropic factors, including 1, 25-dihydroxyvitamin D3 [1,25(OH)2D3], PTH, prostaglandin E_2 (PGE_2) and IL-1 for inducing osteoclast formation are osteoblasts/stromal cells. Cell-to-cell contact between osteoblasts and osteoclast progenitors is then required to induce osteoclastogenesis. Although many factors have been isolated from osteoblasts and shown to regulate the differentiation and function of osteoclasts, the mechanism by which osteoblasts regulate osteoclasts during bone remodeling is still unclear.[112,113] Recent studies show osteoclast differentiation requires the binding of the soluble differentiation factor receptor activator of RANKL to its RANK on osteoclast precursor cells to initiate differentiation.[114-119] The RANKL–RANK interaction confirms that osteoblasts play an essential role in osteoclast differentiation. Osteoblasts express RANKL as a membrane-associated factor. Osteoclast precursors that express RANK, a receptor for RANKL, recognize RANKL through the cell–cell interaction and differentiate into osteoclasts. The binding of the RANKL regulates osteoclast differentiation.

Effect of Zinc on Osteoblasts/Osteoclasts

Many factors are involved in regulating the activity of osteoblasts and osteoclasts. Of particular interest here is zinc's role as a local regulator of osteoblastic/osteoclastic function.[39,42,45,47,106] Zinc has a stimulatory effect on the production of TGF-β in osteoblasts in vitro.[120,121] TGF-β plays a role as a coupling factor in bone formation and bone resorption,[122] and is now believed to have a regulatory effect on osteoclast formation. Conversely, zinc has an inhibitory effect on bone resorption, which is also mediated through TGF-β. Recent literature now shows that TGF-β has a stimulatory effect and an inhibitory effect on osteoclast-like cell formation,[123-127] and that zinc compounds can inhibit the stimulatory effects of TGF-β.[42,45,106,128] Enzyme activity induced by another growth factor, IGF-1, is significantly enhanced by zinc. For example, the effect of IGF-1 in

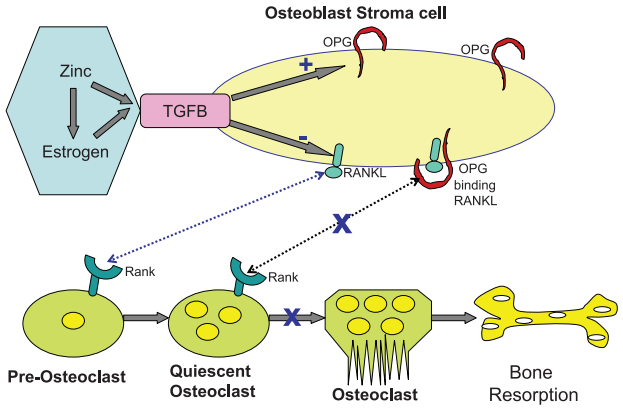

Figure 5: Schematic representation of the effect of zinc on ligand–receptor systems in osteoclast differentiation and the interaction between osteoblast and osteoclast, showing the effect of zinc coupled with estrogen on osteoclast and osteoblast signaling. The RANKL/RANK interaction is responsible for development and differentiation of osteoclast cells. The osteoblast cell in the presence of zinc and estrogen stimulates production of TGF-β, which favors increased production of OPG, a protein that can be secreted outside the cell and then bind RANKL and prevent it from interacting with RANK, thus blocking the formation and activation of osteoclasts. The balance between RANKL and OPG determines how fast bone breaks down.

increasing proliferation of osteoblasts was significantly enhanced by culture with zinc.[129] This study demonstrates that zinc modulates the effect of IGF-1 on protein tyrosine phosphatase activity and cell proliferation. Zinc can stimulate protein synthesis in osteoblasts and bone tissue culture systems in vitro by means of activating aminoacyl–tRNA synthetase which is the first steps in biosynthesis of protein.[45-48,102,103] These results clearly indicate that zinc induces the stimulation of protein synthesis at the translational level in bone cells.

Zinc has an inhibitory effect on bone resorption by preventing the action of osteoclasts; however, the mechanism by which this occurs has not been clarified fully. Zinc can inhibit bone-resorbing factors such as parathyroid hormone and prostaglandin E_2 (PGE_2) induced osteoclast-like cell formation from mouse marrow cells.[39,42,45,47] Zinc can prevent the decrease in bone calcium content induced by various bone-resorbing factors when zinc-containing beta-tricalcium phosphate is used as bone graft substitute.[36,130] Several studies suggest that zinc may have an inhibitory effect on RANKL-stimulated osteoclastogenesis. In an investigation of the role of zinc on RANKL-stimulated osteoclastogenesis in mouse marrow culture in vitro,[131] zinc was added to a cultured media containing RANKL. The presence of RANKL alone caused a significant increase in osteoclast-like cell formation. The stimulatory effect of RANKL was inhibited by the presence of zinc. This suggests that the inhibitory action of zinc on osteoclastogenesis is partly due to suppressing the signaling pathway which is related to RANKL stimulation in osteoclast development. Thus, the inhibitory effect of zinc on RANKL-stimulated osteoclastogenesis is characterized as an indirect effect of zinc on the RANKL/RANK interaction,[131] which is responsible for development and differentiation of osteoclast cells.

Figure 5 summarizes the effect of zinc on ligand–receptor systems in osteoclast differentiation and the interaction between osteoblast and osteoclast. OPG was one of the first factors discovered that regulates osteoclast differentiation. Pre-osteoblasts express a high level of RANKL relative to OPG, which stimulates osteoclast differentiation and function. More mature osteoblasts, by contrast, express high levels of OPG relative to RANKL, which inhibits osteoclast differentiation and function. In healthy bone, OPG helps to maintain the equilibrium of bone resorption and formation.[117,132] OPG acts as a decoy receptor that binds to RANKL, preventing it from interacting with its receptor, or the activity of RANKL is neutralized by binding to the soluble OPG. RANKL and OPG, which are both produced by osteoblast cell differentiation, account for the signaling activity in osteoblast–osteoclast cell communication (Figure 5).[132,133] During osteoblast differentiation, according to a report by Gori and colleagues, RANKL messenger RNA levels decreased 5-fold, whereas OPG messenger RNA levels increased 7-fold, resulting in a 35-fold change in the RANKL/OPG ratio. OPG protein also increased 6-fold.[132] Therefore, the developmental regulation of OPG and RANKL production by stromal/osteoblast cells may contribute to the coordinated sequence of osteoclast and osteoblast differentiation during the bone remodeling cycle. The osteoblast cell in the presence of zinc, which couples with estrogen, stimulates production of TGF-β, which favors increased production of OPG; the OPG binds RANKL and prevents it from interacting with RANK, blocking the formation and activation of osteoclasts.[134,135]

Conclusion

Zinc biology is a rapidly developing field of study with recent research revealing its critical role in most organ systems. Increasing evidence during the last decade suggests that zinc plays an important role in intracellular signaling as an essential and ubiquitous ionic signal, an emerging concept that is gaining acceptance. Zinc is essential for maintaining proper bone health throughout life. Zinc has been shown to stimulate bone formation and inhibit bone loss in animal studies and may prove useful in preventing and/or treating osteoporosis in postmenopausal women. Thus, zinc may represent an underappreciated 'missing link' for the prevention of osteoporosis and the reduction of fracture risk. Therapies based on the manipulation of zinc signals by preventing loss, altering transport and buffering zinc in serum and target tissues are likely to have increasingly important roles in the improvement of bone quality in 21st century medicine.

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