Nutritional zinc plays a pivotal role in bone health and osteoporosis prevention

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Bone homeostasis is maintained through a delicate balance between osteoblastic bone formation and osteoclastic bone resorption [1, 2]. Bone mass is reduced by decrease in osteoblastic bone formation and increase in osteoclastic bone resorption. Numerous pathological processes have the capacity to disrupt this equilibrium leading to conditions where the rate of bone resorption outpaces the rate of bone formation. Osteoporosis is induced with decrease in bone mass. The most dramatic expression of osteoporosis is represented by fractures of the proximal femur for which the number increases as the population ages [3, 4]. Osteoporosis is characterized by reduced bone strength and an increased risk for low-trauma fractures. Bone mass is dramatically reduced after menopause, which depresses the secretion of ovarian hormone (estrogen) in women [5]. Deficiency of estrogen advances osteoclastic bone resorption. This is very important as a primary osteoporosis. Postmenopausal osteoporosis, a consequence of ovarian hormone deficiency, is the archetypal osteoporotic condition in women after menopause and leads to bone destruction through complex and diverse metabolic and biochemical changes. About 40% of women in developed countries will experience an osteoporosis-related fracture in the course of their lifetime, with men experiencing approximately one-third to one-half the risk of women. According to a recent World Health Organization report, osteoporosis has become a global health problem with a disease incidence and mortality rate similar to that of cardiovascular diseases, cancer and diabetes affecting the aging population [6–8].

Osteoporosis has also been shown to induce after diabetes (type I and II), obesity, inflammatory disease, and various pathophysiological states. Diabetic osteoporosis is noticed in recent years [7, 8]. Diabetes is frequent in the elderly, and therefore frequently coexists with osteoporosis. Furthermore, there has also been a global increase in the prevalence of obesity, with obesity-related diabetes currently affecting over 366 million adults worldwide and projections that this will reach 552 million by 2030 [9]. Type 1 diabetes, and more recently type 2 diabetes, has been associated with increased fracture risk. In Western societies, mean body weight has dramatically increased in older people, and a similar trend exists in Asia. Yet insufficient attention has been directed to the problem of osteoporotic fractures in the over weight and obese. Osteoporotic fractures occur in overweight or obese people, and obese men may be particularly susceptible [10, 11]. The National Health and Nutrition Examination Survey have reported that 63% of osteoporotic patients have hyperlipidemia. Epidemiological studies reveal an inverse relationship between serum cholesterol levels and bone mineral content and density, independent of age and body mass index. Diet-induced hyperlipidemia is also associated with a reduction in bone mineral content and density in animals [9, 10]. Hyperlipidemia induces secondary hyperparathyroidism and impairs bone regeneration and mechanical strength.

Bone mass is reduced due to decreased osteoblastic bone formation and increased osteoclastic bone resorption. Malnutrition or undernutrition is often observed in the elderly, and it appears to be more intense in patients with bone fracture than in the general aging population [12]. Deficiency in both micronutrients and macronutrients appears to be strongly implicated in the pathogenesis and the consequences of bone fracture in the osteoporotic elderly. Nutritional and functional food factors may have potential effects to delay degenerative bone disorders such as osteoporosis. There is growing evidence that nutritional and functional food factors regulate bone homeostasis and have restorative effects on bone loss with various pathophysiologic conditions.
Zinc, genistein and vitamin K2 (menaquinone-7) have been shown to have osteogenic effects and these factors play a role in the prevention of bone loss in animal model for osteoporosis and human subjects [13, 14]. Interestingly, their combination with zinc has been found to reveal potential synergistic effects on osteogenesis [13-15]. Supplemental intake of these ingredients may be a useful tool in bone health and osteoporosis prevention.

Zinc plays a pivotal role in the regulation of bone homeostasis. Many zinc-related proteins are found to involve in the regulation of cellular function in osteoblasts and osteoclasts. These factors play an essential role in bone homeostasis, and those are known as zinc finger transcription factors (Osterix, Runx2/Cbfa1 and Cas-interacting zinc finger protein), zinc transporter, Schnurri-3, an essential regulator of adult bone formation, and TRAF6-inhibitory zinc finger protein, a tumor necrosis factor receptor-associated factor 6 [16–22]. Nutritional conditions of zinc may influence function of osteoblasts and osteoclasts that are related to zinc finger proteins. Zinc is required for the growth, development and maintenance of healthy bones. Retardation of bone growth is a common finding in various conditions associated with zinc deficiency [23]. Skeleton contains a large proportion of the total body burden of zinc. Bone zinc has been shown to concentrate in the layer of osteoid prior to calcification [24]. Zinc deficiency is associated with many kinds of skeletal abnormalities in fetal and postnatal development. Nutritional zinc plays a physiologically important role in bone growth. Osteoporotic patients have been shown to have lower levels of skeletal zinc than control. In postmenopausal women, urinary zinc has been suggested as a marker of bone resorption, since women with osteoporosis excrete over than 800 μg zinc per g creatinine in urine [25].

Zinc stimulates osteoblastic bone formation and osteoclastic bone resorption in vitro and in vivo [26, 27]. Bone calcium content, alkaline phosphatase activity and collagen content have were increased after culture with zinc, and these increases were depressed in the presence of an inhibitor of protein synthesis. Endogenous zinc in the bone tissues was shown to reveal direct stimulatory effects on bone formation and mineralization due to stimulating protein synthesis [28-30]. Zinc was shown to stimulate differentiation and proliferation in osteoblastic MC3T3-E1 cells [31, 32]. Zinc activated aminoacyl-tRNA synthetase, which is a rate-limiting enzyme at translational process of protein synthesis, in osteoblastic cells [33]. Zinc increased various protein components including osteocalcin, insulin growth factor-I (IGF-I) and transforming growth factor-β1 in osteoblastic MC3T3-E1 cells [34]. Zinc stimulated DNA synthesis in osteoblastic cells in vitro [35]. Moreover, zinc was found to stimulate the mRNA expression of Runx2, a transcription factor, which is related to the differentiation from mesenchymal stem cells to preosteoblast cells [36]. Thus, zinc stimulates cell differentiation, cell proliferation, and mineralization in osteoblasts, thereby promoting bone formation.

Zinc has been shown to reveal a suppressive effect on osteoclastic bone resorption in vitro [37]. Calvaria, which were removed from weanling rats, were cultured for periods of up to 48 hours in a medium containing various bone-resorbing factors [PTH, prostaglandin E2 (PGE2), interleukin-1α (IL-1α), and lipopolysaccharide (LPS)]. Decrease in bone calcium content caused by these factors was suppressed in the presence of zinc. Osteoclasts, bone-resorbing cells, are formed by differentiation of bone marrow cells. Zinc revealed suppressive effects on osteoclast-like cell formation enhanced by various bone-resorbing factors in mouse marrow culture in vitro [37, 38]. Suppressive effects of zinc on osteoclast-like cell formation in mouse bone marrow culture were equal in comparison with the effect of other anti-bone resorbing agents (calcitonin, 17β-estradiol, or acetazolamide) [35]. In addition, zinc caused apoptotic cell death of mature osteoclast-like cells isolated from rat femoral tissues [39]. Thus, zinc was found to reveal suppressive effects on osteoclastogenesis and osteoclastic cell death. The receptor activator of nuclear factor-κappaB ligand (RANKL) plays a pivotal role in the differentiation from preosteoclasts to mature osteoclasts [2]. RANKL is expressed in osteoblastic cells and bone marrow stromal cells in response to osteotropic factors. RANKL/RANK pathway is essential for osteoclast differentiation [2]. The effect of RANKL is abrogated by osteoprotegerin (OPG), a natural antagonist of RANKL that is produced in osteoblastic cells [2]. TNF receptor-associated factor (TRAF) family proteins are adaptor molecules. TRAFs bind to the membrane-proximal region of RANK and IL-1R-associated kinase and are critically involved in the intracellular signal transduction including NF-κB and mitogen-activated protein kinase (MAPK) activation [2]. Zinc was found to reveal suppressive effects on RANKL-induced osteoclast-like cell formation in mouse marrow culture [40]. Also, zinc inhibited TNF-α-induced osteoclastogenesis [40]. Suppressive effects of zinc on osteoclastogenesis may be involved in inhibitory effect on RANKL stimulation. Culture with zinc has been shown to have stimulatory effects on the expression of OPG mRNA in osteoblastic cells. The mechanism by which zinc suppresses osteoclastogenesis may also be related to production of OPG in osteoblastic cells.

Zinc supplementation has been shown to prevent bone loss in various pathophysiological states. Fracture healing can be envisioned as involving five distinguishable processes, including the immediate response to injury, intramembranous bone formation, chondrogenesis, endochondral bone formation leading to the reestablishment of load bearing function, and bone remodeling [41]. These processes may occur simultaneously during fracture repair. The role of zinc in fracture healing was examined using the diaphyseal tissues obtained at 7 or 14 days after the fracture of...
femoral diaphysis of rats [42, 43]. Oral administration of zinc acetamate (100 mg Zn/kg) for 28 days enhanced fracture healing [42, 43]. Supplementation of zinc may have a role in the promotion of the healing of femoral fracture. Zinc plays a role in the deterioration of bone metabolism with increasing age. Bone cellular zinc was reduced in the femoral diaphysis of elderly rats (age of 30 weeks) as compared with that of weanling rats (age of 3 weeks) [26]. Bone protein synthesis was deteriorated with increasing age, and this reduction was restored by oral administration of zinc sulfate. Supplementation of zinc may be important in the prevention of bone loss with aging. Skeletal unloading induces osteopenia after immobilization, spaceflight, bedrest, or hindlimb suspension. Skeletal unloading results in an inhibition of bone formation and induces an increase in bone resorption, thereby a loss of bone mass. Bone zinc content was decreased in the femoral-metaphyseal tissues of rats with skeletal unloading [26]. Oral administration of zinc prevented bone loss induced by skeletal unloading. Zinc revealed preventive effects on bone loss in type 1 diabetic conditions. Oral administration of zinc (25 mg Zn/kg body weight) for 14 or 21 days with once daily was found to reveal preventive effects on the increase in serum glucose and triglyceride levels and the reduction of bone components induced in type 1 diabetic model animals [44]. Thus, supplemental intake with zinc may be a useful tool in the prevention and treatment of bone loss with various bone diseases.

Interestingly, anabolic effects of zinc on bone have been found to be enhanced by soybean genistein. Isoflavones (including daidzin, daidzein, genistein and genistein) are present in soybeans at relatively high concentrations. Daidzin or genistin are hydrolyzed to daidzein or genistein by β-glucosidase in the gastrointestinal system, respectively. Genistein has been shown to stimulate osteoblastic bone formation and suppress osteoclastic bone resorption in vitro, thereby increasing bone mass [45–48]. Prolonged intake of dietary genistein revealed preventive effects on ovariectomy (OVX)-induced bone loss, an animal model for postmenopausal osteoporosis [49]. Moreover, the effects of dietary genistein on bone metabolism in human subjects were estimated with change in circulating biochemical markers of bone metabolism in aged individuals [50]. Sixty-three volunteers (31 men and 32 women) were divided into four groups of 15 or 16 male volunteers and 16 or 16 female volunteers, and each group was sequentially given natto (40 g pack) containing two different levels of zinc once a day for 4 or 8 weeks as follows: either regular natto with naturally occurring isoflavone 35.0 mg, zinc 0.8 mg and calcium 51.4 mg or supplemented natto containing isoflavone 35.0 mg, zinc 3.6 mg, and calcium 60.0 mg. Osteoblastic bone formation markers (alkaline phosphatase and γ-carboxylated osteocalcin) and osteoclastic bone resorption markers [tartrate-resistant acid phosphatase (TRACP) and N-telopeptide of type I collagen] were assayed. Intake of zinc-supplemented natto for 8 weeks in men or women caused a significant increase in serum bone-specific alkaline phosphatase activity and γ-carboxylated osteocalcin concentration and a significant decrease in serum bone TRACP activity and N-telopeptide of type I collagen, as compared with the values with the intake of regular natto [50]. This study demonstrated that the intake of regular natto with genistein-rich soybean reveals stimulatory effects on bone formation and suppressive effects on bone resorption in aged individuals, and that such effect is synergistically enhanced with supplementation of zinc. As described above, combination of zinc and genistein was found to reveal synergistic effects on prevention of osteoporosis. Supplementation with zinc compound and genistein may reveal potential effects in the prevention and therapy of osteoporosis with various pathophysiological conditions.

Moreover, anabolic effects of zinc on bone metabolism have been synergistically enhanced by vitamin K2 (menaquinone-7; MK-7). Vitamin K is a fat-soluble vitamin that was originally identified as an essential factor for blood coagulation. Vitamin K is an essential cofactor for the post-translational carboxylation of certain protein-bound glutamate residues of osteocalcin, a synthesized by osteoblasts, which are converted into γ-carboxy glutamate (Gla) by γ-carboxylase [51]. These Gla residues form calcium-binding sites that are essential for the activity of the proteins. There are three types
of vitamin K: vitamin \( \text{K}_1 \) (phyllolquinone), vitamin \( \text{K}_2 \) (menaquinone), and vitamin \( \text{K}_3 \) (menadione). Vitamin \( \text{K}_1 \) is a sole compound, but vitamin \( \text{K}_2 \) is a series of vitamins with multi isoprene units (one to four) at the 3-position of the naphthoquinone. Vitamin \( \text{K}_2 \) (menaquinone-4; MK-4) has four isoprene units. MK-4 is essential for the \( \gamma \)-carboxylation of osteocalcin. MK-4 has been shown to inhibit bone loss, which may be related to its side chain, in ovariectomized rats. Natural menaquinone-7 (MK-7; vitamin K2) with seven isoprene units is very abundant in the fermented soybean (natto). There is growing evidence for the roles of vitamin \( \text{K}_2 \) in bone health in human subjects. Clinically, vitamin \( \text{K}_2 \) maintains lumbar bone mineral density (BMD) and prevents osteoporotic fractures in patients with osteoporosis. Osteocalcin, which is newly synthesized by osteoblasts, is considered sensitive markers of bone formation [52]. A poor vitamin K status will lead to production of under-carboxylated (inactive) osteocalcin (unOC) [53]. In postmenopausal women, a clear association between elevated unOC and increased fracture risk is found [54]. A daily vitamin \( \text{K}_1 \) supplement of 80 \( \mu \)g seems to be necessary to reach a premenopausal carboxylated osteocalcin/total osteocalcin ratio [55]. An adult daily intake of about 100 \( \mu \)g of vitamin \( \text{K}_1 \) is recommended for the maintenance of hemostasis [56].

MK-7, which was isolated from fermented soybean (natto), has been found to have a stimulatory effect on calcification in the femoral tissues obtained from normal young rats in vitro [57, 58]. The action of MK-7 on bone calcification has been shown to have the same effect as MK-4. MK-7 has partially been converted to MK-4 in the body. Culture with MK-7 (10^{-6} \text{ or } 10^{-5} \text{ M}) caused a significant increase in biochemical components (alkaline phosphatase activity, DNA and calcium contents) in the femoral tissues obtained from aged rats in vitro [59]. Anabolic effect of MK-7 on bone was enhanced in the presence of genistein (10^{-6} \text{ or } 10^{-5} \text{ M}) [60]. MK-7 was shown to reveal a stimulatory effect on osteoblastic bone formation due to increasing protein synthesis including osteocalcin [60]. Moreover, MK-7 was found to reveal suppressive effects on osteoclastic bone resorption in vitro [61]. Osteoclast-like cells are formed from bone marrow cells in the presence of bone-resorbing factors [61]. This osteoclast-like cell formation was significantly suppressed after culture with MK-7 [61]. Thus, MK-7 was shown to stimulate osteoblastic bone formation and osteoclastic bone resorption. MK-7 may activate \( \gamma \)-carboxylase that glutamate residues of osteocalcin are converted into \( \gamma \)-carboxyglutamate in osteoblastic cells. MK-7 stimulates protein synthesis including osteocalcin in osteoblastic cells [60]. This action may be important as a mechanism by which MK-7 regulates bone homeostasis. Activation of NF-\( \kappa \)B signal transduction pathway is essential for osteoclast formation and resorption [2]. The action of MK-7 on osteoblast and osteoclast formation and activity was accomplished by downregulating basal and cytokine-induced NF-\( \kappa \)B activation, by increasing \( \kappa \)B mRNA, in a \( \gamma \)-carboxylation-independent manner [62]. Moreover, suppressive effect of MK-7 on mature osteoclasts may be partly mediated through the pathway of \( \text{Ca}^{2+} \)- and cyclic AMP-dependent signalings [62]. Vitamin \( \text{K}_2 \) has also been shown to be a transcriptional regulator of bone-specific genes that act through steroid and xenobiotic receptors (SXRs) to promote expression of osteoblastic markers [63].

Dietary MK-7 has been shown to have preventive effects on osteoporosis [64, 65]. OVX rats were given experimental diets containing natto (including MK-7, 9.4 \( \mu \)g/100 g diet) with or without supplemental MK-7 (containing 14.1 or 18.8 \( \mu \)g/100 g diet) for 150 days [65]. Feeding produced a significant elevation of the serum MK-7 concentration of OVX rats [65]. Serum \( \gamma \)-carboxylated osteocalcin concentration was significantly decreased after OVX. This decrease was significantly prevented after supplementation of MK-7 (18.8 \( \mu \)g/100 g diet) [65]. OVX caused a significant decrease in femoral dry weight, femoral calcium content, and mineral density. These decreases were prevented after supplementation of MK-7 (total, 18.8 \( \mu \)g/100 g diet) [65]. Thus, prolonged intake of MK-7 has been shown to have a preventive effect on bone loss induced by OVX. MK-7 may be useful in the prevention and treatment of osteoporosis. Change in circulating MK-7 and \( \gamma \)-carboxylated osteocalcin (Gla osteocalcin) concentrations in normal individuals with the intake of fermented soybean was examined [66, 67]. Forty-eight volunteers (45 men and 3 women) were divided into three groups of 16 volunteers each (15 men and 1 woman), and each group was given sequentially natto (50 g) containing three different amounts of MK-7 once a day for 14 days as follows: either regular natto with MK-7 865 \( \mu \)g/100 g diet of natto, reinforced natto containing MK-7 1295 \( \mu \)g/100 g, or MK-7 1730 \( \mu \)g/100 g [67]. Serum MK-7 was not found in normal individuals who had not eaten natto. Serum MK-7 and \( \gamma \)-carboxylated osteocalcin concentrations were significantly raised 7, 10, and 14 days after the start of the intake of reinforced natto containing MK-7 1295 or 1730 \( \mu \)g/100 g [68]. Serum \( \gamma \)-carboxylated osteocalcin concentration was elevated at 14 days after the intake of natto containing either 1295 or 1730 \( \mu \)g/100 g diets as compared with that after regular natto intake [68]. Intake of reinforced natto that contains more MK-7 than regular natto may play a role in the prevention of age-related bone loss.

Zinc has been shown to synergistically enhance the effect of MK-7 in increasing bone calcium content in vitro and in vivo [68]. Rats were orally administered with vehicle (distilled water), zinc sulfate (10 mg Zn/kg body weight), MK-7 (5 mg/kg), or zinc (10 mg/kg) plus MK-7 (5 mg/kg) once a day for 7 days [68]. Femoral dry weight was increased after the administration of both zinc and MK-7, although a significant change was not seen after the administration of zinc or MK-7 alone [68]. Calcium content in the femoral-diaphyseal and metaphyseal
tissues was increased after zinc administration [68]. Such an increase was not found after MK-7 alone. Bone calcium content was synergistically enhanced after the administration of both zinc and MK-7 [68]. Moreover, supplemental intake containing both zinc (16.75 mg/kg) and MK-7 (16.88 μg/kg) once a day for 15 days caused synergistic increase in femoral dry weight, alkaline phosphatase activity, DNA, calcium and zinc contents in the diaphyseal and metaphyseal tissues of female elderly rats [68]. Thus, supplemental intake with the combination of MK-7 and zinc may be useful in the prevention and treatment of osteoporosis.

As described above, zinc, an essential trace element, plays a pivotal role in the regulation of bone metabolism. Deficiency of nutritional zinc induces retardation of bone growth, and bone zinc is reduced with increasing age. Many proteins, which are related to regulation of osteoblasts and osteoclasts, require zinc in zinc-finger proteins and zinc-activating enzymes. Function of such proteins may be attenuated by conditions of nutritional zinc. Zinc may play a pivotal role in maintaining of bone health and bone mass with aging in complication with prevention of osteoporosis. Interestingly, anabolic effect of zinc on bone is synergistically enhanced by combination with genistein or MK-7, which is functional food factor. Supplemental intake of these combined factors may play preventive and therapeutic roles for bone loss that are induced by aging, postmenopausal, obesity, diabetes, inflammation, cancer bone metastasis and other diseases.

REFERENCES


46. Gao YH, Yamaguchi M. Inhibitory effect of genistein on osteoclast-like cell formation in mouse marrow