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EDITORIAL OPEN ACCESS

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 diabetesaffecting 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

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[13, 14]. 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 osteoblastes 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) [33]. 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-kappaB 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 acexamate (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 human aged individuals with sixty-three volunteers (31 men and 32 women) [50]. This study demonstrated that the intake of dietary genistein-rich soybean has stimulatory effects on bone formation and suppressive effects on bone resorption in aged individuals [50]. Supplemental intake of genistein may be a useful tool in the prevention and therapy of osteoporosis with pathophysiological conditions. Interestingly, combination of zinc and genistein was found potential effects in the prevention and treatment of bone loss with various pathophysiologic states. Zinc and genistein largely contain in fermented soybeans (natto). Experimental diets with fermented soybeans containing zinc and genistein prevented OVX-induced bone loss [49]. Experimental diets containing 2.1 to 9.7 mg of zinc per 100 g of diet and 44.6 to 92.4 mg of isoflavones (including genistein, genistein, daidzein, and daidzein) per 100 g of diet was fed to OVX rats for 3 months. OVX caused a significant reduction in the dry weight, mineral density, calcium content, zinc content, and alkaline phosphatase activity in the femoral tissues [49]. These reductions were prevented with feeding a natto diet. Moreover, such effects were significantly enhanced in OVX rats fed a natto diet supplemented with zinc and isoflavone of more amounts.

The effects of intake of fermented soybean (natto), which was made from isoflavone-rich soybean supplemented with zinc were examined 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 K_1 (phylloquinone), vitamin K_2 (menaquinone), and vitamin K_3 (menadione). Vitamin K1 is a sole compound, but vitamin K2 is a series of vitamers with multi isoprene units (one to four) at the 3-position of the naphthoquinone. Vitamin K2 (menaquinone-4; MK-4) has four isoprene units. MK-4 is essential for the γ-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 K2 in bone health in human subjects. Clinically, vitamin K2 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 K1 supplement of 80 μg seems to be necessary to reach a premenopausal carboxylated osteocalcin/total osteocalcin ratio [55]. An adult daily intake of about 100 μg of vitamin K1 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 or 10-5 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 or 10-5 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 γ-carboxylase that glutamate residues of osteocalcin are converted into γ-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-κB 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-–κB activation, by increasing IκB mRNA, in a γ-carboxylation-independent manner [62]. Moreover, suppressive effect of MK-7 on mature osteoclasts may be partly mediated through the pathway of Ca^{2+}- and cyclic AMP-dependent signalings [62]. Vitamin K2 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 μg/100 g diet) with or without supplemental MK-7 (containing 14.1 or 18.8 μg/100 g diet) for 150 days [65]. Feeding produced a significant elevation of the serum MK-7 concentration of OVX rats [65]. Serum γ-carboxylated osteocalcin concentration was significantly decreased after OVX. This decrease was significantly prevented after supplementation of MK-7 (18.8 μg/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 μg/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 γ-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 women), 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 μg/100 g diet of natto, reinforced natto containing MK-7 1295 μg/100 g, or MK-7 1730 μg/100 g [67]. Serum MK-7 was not found in normal individuals who had not eaten natto. Serum MK-7 and γ-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 μg/100 g [68]. Serum γ-carboxylated osteocalcin concentration was elevated at 14 days after the intake of natto containing either 1295 or 1730 μg of MK-7/100 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.

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Conflict of Interest

Authors declare no conflict of interest.

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REFERENCES

1. Raggatt LJ, Partridge NC. Cellular and molecular mechanisms of bone remodeling. J Biol Chem 2010 Aug 13;285(33):25103–8.
2. Zaidi M, Blair HC, Moonga BS, Abe E, Huang CL. Osteoclastogenesis, bone resorption, and osteoblast-based therapeutics. J Bone Miner Res 2003 Apr;18(4):599–609.
3. Riggs BL, Jowsey J, Kelly PJ, Jones JD, Maher FT. Effect of sex hormones on bone in primary osteoporosis. J Clin Invest 1969 Jun;48(6):1065–72.
4. Cooper C, Melton LJ 3rd. Epidemiology of osteoporosis. Trends Endocrinol Metab 1992 Aug;3(6):224–9.
5. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: An inflammatory tale. J Clin Invest 2006 May;116(5):1186–94.
6. Leslie WD, Rubin MR, Schwartz AV, Kanis JA. Type 2 diabetes and bone. J Bone Miner Res 2012 Nov;27(11):2231–7.
7. Nielson CM, Srikanth P, Orwoll ES. Obesity and fracture in men and women: An epidemiologic perspective. J Bone Miner Res 2012 Jan;27(1):1–10.
8. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004 May;27(5):1047–53.
9. Parhami F, Tintut Y, Beamer WG, Gharavi N, Goodman W, Demer LL. Atherogenic high-fat diet reduces bone mineralization in mice. J Bone Miner Res 2001 Jan;16(1):182–8.
10. Pirih F, Lu J, Ye F, et al. Adverse effects of hyperlipidemia on bone regeneration and strength. J Bone Miner Res 2012 Feb;27(2):309–18.
11. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004 May;27(5):1047–53.
12. Bonjour JP, Schurch MA, Rizzori R. Nutritional aspects of hip fracture. Bone 1996 Mar;18(3 Suppl):139S–44S.

13. Yamaguchi M. Nutritional Factors and Osteoporosis Prevention. Nova Science Publishers, Inc., New York, USA 2010.
14. Yamaguchi M. Biomedical Osteoporosis Treatment. Nova Science Publishers, Inc., New York, USA 2013.
15. Yamaguchi M. Osteoporosis treatment with new osteogenic factors. J Mol Genet Med 2013;7:66.
16. Inoue K, Matsuda K, Itoh M, et al. Osteopenia and male-specific sudden cardiac death in mice lacking a zinc transporter gene, Znt5. Hum Mol Genet 2002 Jul 15;11(15):1775–84.
17. Khadeer MA, Sahu SN, Bai G, Abdulla S, Gupta A. Expression of the zinc transporter ZIP1 in osteoclasts. Bone 2005 Sep;37(3):296–304.
18. Nakashima K, Zhou X, Kunkel G, et al. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 2002 Jan 11;108(1):17–29.
19. Komori T, Yagi H, Nomura S, et al. Targeted disruption of Cbfa1 results in a comple lack of bone formation owing to maturational arrest of osteoblasts. Cell 1997 May 30;89(5):755–64.
20. Shen ZJ, Nakamoto T, Tsuji K, et al. Negative regulation of bone morphogenetic protein/Smad signaling by Cas-interacting zinc finger protein in osteoblasts. J Biol Chem 2002 Aug 16;277(33):29840–6.
21. Jones DC, Wein MN, Oukka M, Hofstaetter JG, Glimcher MJ, Glimcher LH. Regulation of adult bone mass by the zinc finger adapter protein Schnurri-3. Science 2006 May 26;312(5777):1223–7.
22. Shin JN, Kim I, Lee JS, Koh GY, Lee ZH, Kim HH. A novel zinc finger protein that inhibits osteoclastogenesis and the function of tumor necrosis factor receptor-associated factor 6. J Biol Chem 2002 Mar 8;277(10):8346–53.
23. Oner G, Bhaumick B, Bala RM. Effect of zinc deficiency on serum somatomedin levels and skeletal growth in young rats. Endocrinology 1984 May;114(5):1860–3.
24. Masters DG, Keen CL, Lonnerdal B, Hurley LS. Release of zinc from maternal tissues during zinc deficiency or simultaneous zinc and calcium deficiency in the pregnant rat. J Nutr 1986 Nov;116(11):2148–54.
25. Herzberg M, Foldes J, Steinberg R, Menczel J. Zinc excretion in osteoporotic women. J Bone Miner Res 1990 Mar;5(3):251–7.
26. Yamaguchi M. Role of nutritional zinc in the prevention of osteoporosis. Mol Cell Biochem 2010 May;338(1-2):241–54.
27. Yamaguchi M. Osteoporosis treatment with new osteogenic factors. J Mol Genet Med 2013;7:66.
28. Yamaguchi M, Osishi H, Suketa Y. Stimulatory effect of zinc on bone formation in tissue culture. Biochem Pharmacol 1987 Nov 15;36(22):4007–12.
29. Yamaguchi M, Oishi H, Suketa Y. Zinc stimulation of bone protein synthesis in tissue culture. Activation of aminoacyl-tRNA synthetase. Biochem Pharmacol 1988 Nov 1;37(21):4075–80.
30. Yamaguchi M, Matsui R. Effect of dipicolinate, a chelator of zinc, on bone protein synthesis in tissue culture. The essential role of zinc. Biochem Pharmacol 1989 Dec 15;38(24):4485–9.
31. Hashizume M, Yamaguchi M. Stimulatory effect of ß-alanyl-L-histidinato zinc on cell proliferation is dependent on protein synthesis in osteoblastic MC3T3-E1 cells. Mol Cell Biochem 1993 May 12;122(1):59–64.
32. Hashizume M, Yamaguchi M. Effect of ß-alanyl-L-histidinato zinc on differentiation of osteoblastic MC3T3-E1 cells: Increases in alkaline phosphatase activity and protein concentration. Mol Cell Biochem 1994 Feb 9;131(1):19–24.
33. Yamaguchi M, Kishi S, Hashizume M. Effect of zinc-chelating dipeptides on osteoblastic MC3T3-E1 cells: Activation of aminoacyl-tRNA synthetase. Peptides 1994;15(8):1367–1.
34. Yamaguchi M, Hashizume M. Effect of ß-alanyl-L-histidinato zinc on proein components in osteoblastic MC3T3-E1 cells: Increases in osteocalcin, insulin-like growth factor-I and transforming growth factor-ß. Mol Cell Biochem 1994 Jul 27;136(2):163–9.
35. Yamaguchi M, Matsui T. Stimulatory effect of zinc-chelating dipeptide on deoxyribonucleic acid synthesis in osteoblastic MC3T3-E1 cells. Peptides 1996;17(7):1207–11.
36. Yamaguchi M, Goto M, Uchiyama S, Nakagawa T. Effect of zinc on gene expression in osteoblastic MC3T3-E1 cells: enhancement of Runx2, OPG, and regucalcin mRNA expressions. Mol Cell Biochem 2008 May;312(1-2):157–66.
37. Kishi S, Yamaguchi M. Inhibitory effect of zinc compounds on osteoclast-like cell formation in mouse marrow cultures. Biochem Pharmacol 1994 Sep 15;48(6):1225–30.
38. Yamaguchi M, Kishi S. Zinc compounds inhibit osteoclast-like cell formation at the earlier stage of rat marrow culture but not osteoclast function. Mol Cell Biochem 1996 May 24;158(2):171–7.
39. Yamaguchi M, Kishi S. Zinc compounds inhibit osteoclast-like cell formation at the earlier stage of rat marrow culture but not osteoclast function. Mol Cell Biochem 1996 May 24;158(2):171–7.
40. Yamaguchi M, Uchiyma S. Receptor activator of NF-kappaB ligand-stimulated osteoclastogenesis in mouse marrow culture is suppressed by zinc in vitro. Int J Mol Med 2004 Jul;14(1):81–5.
41. Eihorn TA. The cell and molecular biology of fracture healing. Clin Orthop Relat Res 1998 Oct;(355 Suppl):S7–21.
42. Igarashi A, Yamaguchi M. Increase in bone protein components with healing rat fractures: Enhancement by zinc treatment. Int J Mol Med 1999 Dec;4(6):615–20.
43. Igarashi A, Yamaguchi M. Increase in bone growth factors with healing rat fractures: The enhancing effect of zinc. Int J Mol Med 2001 Oct;8(4):433–8.
44. Yamaguchi M, Uchiyama S. Preventive effect of zinc acexamate administration in streptozotocin-diabetic rats: Restoration of bone loss. Int J Mol Med 2003 Nov;12(5):755–61.
45. Yamaguchi M, Sugimoto E. Stimulatory effect of genistein and daizein on protein synthesis in osteoblastic MC3T3-E1 cells: Activation of aminoacyl-tRNA synthetase. Mol Cell Biochem 2000 Nov;214(1-2):97–102.
46. Gao YH, Yamaguchi M. Inhibitory effect of genistein on osteoclast-like cell formation in mouse marrow

cultures. Biochem Pharmacol 1999 Sep 1;58(5):767–2.
47. Yamaguchi M. Isoflavone and bone metabolism: Its cellular mechanism and preventive role in bone loss. J Health Sci 2002;48:209–22.
48. Yamaguchi M. Nutritional factors and bone homeostasis: Synergistic effect of zinc and genistein osteogenesis. Mol Cell Biochem 2012 Jul;366(1-2):201–21.
49. MaZJ,ShimanukiS,IgarashiA,KawasakiY,Yamaguchi M. Preventive effect of dietary fermented soybean on bone loss in ovariectomized rats: Enhancement with isoflavone and zinc supplementation. J Health Sci 2000;46:263–8.
50. Yamaguchi M, Igarashi A, Sakai M, Degawa H, Ozawa Y. Prolonged intake of dietary fermented isoflavone-rich soybean reinforced with zinc affects circulating bone biochemical markers in aged individuals. J Health Sci 2005;51:191–6.
51. Price PA. Vitamin K-dependent formation of bone Gla protein (osteocalcin) and its function. Vitam Horm 1985;42:65–108.
52. Delmas PD. Biochemical markers of bone turnover. I: Theoretical considerations and clinical use in osteoporosis. Am J Med 1993 Nov 30;95(5A):11S–6S.
53. van Summeren MJ, Braam LA, Lilien MR, Schurgers LJ, Kuis W, Vermeer C. The effect of menaquinone-7 (vitamin K2) supplementation on osteocalcin carboxylation in healthy prepubertal children. Br J Nutr 2009 Oct;102(8):1171–8.
54. Hodges SJ, Akesson K, Vergnaud P, Obrant K, Delmas PD. Circulating levels of vitamin K1 and K2 decreased in elderly woman with hip fracture. J Bone Miner Res 1993 Oct;8(10):1241–5.
55. Binkley NC, Krueger DC, Engelke JA, Foley AL, Suttie JW. Vitamin K supplementation reduces serum concentrations of under-gamma-carboxylated osteocalcin in healthy young and elderly adults. Am J Clin Nutr 2000 Dec;72(6):1523–8.
56. Schurgers LJ, Vermeer C. Determination of phyloquinone and menaquinones in food: Effect of food matrix on circulating vitamin K concentrations. Haemostasis 2000 Nov-Dec;30(6):298–307.
57. Shearer MJ. Vitamin K in parenteral nutrition. Gastroenterology 2009 Nov;137(5 Suppl):S105–18.
58. Ehara Y, Takahashi H, Hanahisa Y, Yamaguchi M. Effect of vitamin K2 (menaquinone-7) on bone metabolism in the femoral-metaphyseal tissues of normal and skeletal-unloaded rats: Enhancement with zinc. Res Exp Med (Berl) 1996;196(3):171–8.
59. Yamaguchi M, Uchiyama S, Tsukamoto Y. Stimulatory effect of menaquinone-7 on bone formation in elderly female rat femoral tissues in vitro: Prevention of bone deterioration with aging. Int J Mol Med 2002 Dec;10(6):729–33.
60. Yamaguchi M, Sugimoto E, Hachiya S. Stimulatory effect of menaquinone-7 (vitamin K2) on osteoblastic bone formation in vitro. Mol Cell Biochem 2001 Jul;223(1-2):131–7.
61. Yamaguchi M, Ma ZJ. Inhibitory effect of menaquinone-7 (vitamin K2) on osteoclast-like cell formation and osteoclastic bone resorption in rat bone tissues in vitro. Mol Cell Biochem 2001 Dec;228(1-2):39–47.
62. Yamaguchi M, Weitzmann MN. Vitamin K2 stimulates osteoblastogenesis and suppresses osteoclastogenesis by suppressing NF-κB activation. Int J Mol Med 2011 Jan;27(1):3–14.
63. Tabb MM, Sun A, Zhou C, et al. Vitamin K2 regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. J Biol Chem 2003 Nov 7;278(45):43919–27.
64. Yamaguchi M, Taguchi H, Gao YH, Igarashi A, Tsukamoto Y. Effect of vitamin K2 (menaquinone-7) in fermented soybean (natto) on bone loss in ovariectomized rats. J Bone Miner Metab 1999;17(1):23–9.
65. Yamaguchi M, Kakuda H, Gao YH, Tsukamoto Y. Prolonged intake of fermented soybean (natto) diets containing vitamin K2 (menaquinone-7) prevents bone loss in ovariectomized rats. J Bone Miner Metab 2000;18(2):71–6.
66. Tsukamoto Y, Ichise H, Yamaguchi M. Prolonged intake of dietary fermented soybeans (natto) with the reinforced vitamin K2 (menaquinone-7) enhances circulating γ-carboxylated osteocalcin concentration in normal individuals. J Health Sci 2000;46:317–21.
67. Tsukamoto Y, Ichise H, Kakuda H, Yamaguchi M. Intake of fermented soybean (natto) increases circulating vitamin K2 (menaquinone-7) and γ-carboxylated osteocalcin concentration in normal individuals. J Bone Miner Metab 2000;18(4):216–22.
68. Ma ZJ, Igarashi A, Yamakawa K, Yamaguchi M. Enhancing effect of zinc and vitamin K2 (menaquinone-7) on bone components in the femoral tissues of female elderly rats. J Health Sci 2001;47:40–5.