Osteoporosis Treatment with New Osteogenic Factors

Masayoshi Yamaguchi*

Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, Georgia, USA

Abstract

Bone homeostasis is maintained through a delicate balance between osteoblastic bone formation and osteoclastic bone resorption. Bone loss may be due to decreased osteoblastic bone formation and increased osteoclastic bone resorption. Osteoporosis is induced with its accompanying decrease in bone mass. Nutrition and functional food factors may play a role in the prevention of bone loss. This is worthy of notice in the treatment of osteoporosis. It has been shown that functional food factors including zinc, genistein and vitamin K$_7$ (menaquinone-7) have potential osteogenic effects in vitro and in vivo. These factors have been shown to have stimulatory effects on osteoblastic bone formation and suppressive effects on osteoclastic bone resorption. Moreover, the osteogenic effects of genistein, menaquinone-7, and vitamin D$_3$ have been found to be synergistically enhanced with combination of zinc, which plays an essential role in protein synthesis at translational process and gene expression related to zinc finger transcription factors. Intake with their combination has been shown to have potential effects in the treatment of bone loss in animal models of osteoporosis and human subjects. Supplemental intake with these compositions may have potential effects on osteoporosis treatment.

Keywords: Osteoporosis; Osteoblastic bone formation; Osteoclastic bone resorption; Zinc; Genistein; Vitamin K$_7$; Menaquinone-7; Vitamin D$_3$

Introduction

Bone homeostasis is maintained through a delicate balance between osteoblastic bone formation and 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 leading to osteoporosis, a devastating bone disease that is widely recognized as a major public health threat [1,2]. Osteoporosis is induced with decrease in bone mass. Postmenopausal osteoporosis, a consequence of ovarian hormone deficiency, is the archetypal osteoporotic condition in women after menopause and leads to bone destruction though complex and diverse metabolic and biochemical changes. The most dramatic expression of the disease is represented by bone fractures.

Reduction in bone mass is induced 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 [3]. 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 [4]. Zinc, genistein, and vitamin K$_7$ (menaquinone-7) has 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 [4,5]. Interestingly, their combination with zinc has been found to have potential synergetic effects on osteogenesis [4,5]. This review will discuss new development on osteoporosis treatment with the combination of biomedical food factors.
Zinc plays an important role in the regulation of bone homeostasis. Zinc finger transcription factors, which are zinc-binding protein, play a pivotal role in differentiation of osteoblastic and osteoclastic cells. Zinc transporter has been shown to locate in osteoblastic cells [9] and osteoclastic cells [10]. Zinc transporter may be a role in the uptake, intracellular sequestration or efflux of zinc. A novel zinc finger-containing transcription factor, called Osterix (Osx), has been found in osteoblastic cells [11]. Oxs is required for osteoblast differentiation and bone formation and it acts downstream of Runx2/Cbfal [12]. Cas-interacting zinc finger protein (CIZ) has been found to be a novel type inhibitor of bone morphogenetic protein (BMP)/Smad signaling in the modulation of BMP2-induced osteoblastic cell differentiation [13]. Moreover, Schnurri-3 (Shn3) is an essential regulator of adult bone formation [14]. A novel TIZ (TRAP6-inhibitory zinc finger protein) has been shown to inhibit osteoclastogenesis and the function of tumor necrosis factor receptor-associated factor 6 [15]. Nutritional zinc state may influence on function of zinc finger proteins.

Zinc is required for the growth, development, and maintenance of healthy bones. The retardation of bone growth is a common finding in various conditions associated with zinc deficiency [16,17]. 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 [18]. Zinc deficiency is associated with many kinds of skeletal abnormalities in fetal and postnatal development. Nutritional zinc may play 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 [19].

Thus, zinc plays a pivotal role in the regulation of bone homeostasis.

Zinc stimulates osteoblastic bone formation: Zinc stimulates bone formation in in vitro and in vivo [20,21]. Bone calcium content, alkaline phosphatase activity and collagen content have been shown to increase after culture with zinc, and these increases were depressed in the presence of an inhibitor of protein synthesis. Zinc has direct stimulatory effects on bone mineralization in vitro, and that bone protein synthesis is a necessary component of this response [22]. Endogenous zinc in the bone tissues has been shown to play an essential role in bone protein synthesis by using dipicolinate, a chelator of zinc [23].

Zinc has been shown to stimulate differentiation and proliferation in osteoblastic MC3T3-E1 cells [24,25]. The stimulatory effect of zinc on protein synthesis in osteoblastic MC3T3-E1 cells [26] in vitro has been demonstrated using [3H]-leucine. The activity of [3H]-leucyl-tRNA synthetase in the 105,000 g supernatant fraction (cytosol) of the bone homogenate was significantly increased about twofold after culture with zinc (10⁻⁴ M) [22,26]. Zinc is found to activate [3H]-leucyl-tRNA synthetase in the homogenate of osteoblastic cells [26]. Zinc has been shown to have direct stimulatory effects on protein synthesis at translational process. Endogenous zinc in the bone tissues is needed the stimulation of protein synthesis at the translational process in osteoblastic cells.

Zinc has been shown to increase various protein components including osteocalcin, IGF-I, or TGF-β1 in osteoblastic MC3T3-E1 cells [27]. The stimulatory effects of IGF-I on alkaline phosphatase activity, protein, DNA, and cell number in the cells were markedly enhanced in the presence of zinc sulfate (10⁻⁷ M) [28]. Such an effect was not seen in case of both insulin and zinc. The enhancing effect of zinc on the effect of IGF-I may be mediated through signaling pathway of protein kinase C and protein phosphatase in osteoblastic cells [28]. Zinc increases protein tyrosine phosphatase activity in osteoblastic cells [29]. The effect of IGF-I in increasing this enzyme activity was enhanced after culture with zinc sulfate [29]. Zinc modulates anabolic effect of IGF-I on protein tyrosine phosphatase activity and cell proliferation.

Zinc has also been to stimulate DNA synthesis in the homogenate of osteoblastic cells in vitro [30]. Moreover, zinc has been found to stimulate the mRNA expression of Runx2, a transcription factor, which is related to the differentiation from mesenchymal stem cells to preosteoblastic cells [31].

Thus, zinc stimulates cell differentiation, cell proliferation, and mineralization in osteoblasts, thereby promoting bone formation.

Zinc suppresses osteoclastic bone resorption: Zinc has a suppressive effect on bone resorption in tissue culture in vitro [32]. Calvaria were removed from weanling rats and 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)]. Culture with these factors caused a significant decrease in bone calcium content. Such decreases were completely inhibited in the presence of zinc (10⁻⁴ to 10⁻⁵ M). Also, zinc suppressed PTH- or IL-1α-induced increases in medium glucose consumption and lactic acid production by bone tissues [32]. Thus, zinc has been demonstrated to have suppressive effects on bone resorption in bone tissue culture system in vitro.

Osteoclasts, bone-resorbing cells, are formed by differentiation of bone marrow cells. Zinc has suppressive effects on osteoclast-like cell formation in mouse marrow culture in vitro [33,34]. Presence of 1, 25-dihydroxyvitamin D₃, PTH, IL-1α, or PGE₂ induced a remarkable increase in osteoclast-like multinucleated cells [33]. These increases were suppressed in the presence of zinc [33]. Suppressive effects of zinc were equal in comparison with the effect of other anti-bone resorbing agents (calcitonin, 17β-estradiol, or acetazolamide) on osteoclast-like cell formation in mouse bone marrow culture [33]. In addition, culture with zinc caused apoptotic cell death of mature osteoclast-like cells isolated from rat femoral tissues [34]. Zinc has suppressive effects on osteoclastogenesis and osteoclastic cell death.

Stimulatory effects of PTH on osteoclast-like cell formation were weakened (about 50%) in the presence of Ca²⁺ chelator or dibucaine, a regulatory factor of intracellular Ca²⁺ signaling [35]. Phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C, stimulated osteoclast-like cell formation [35]. This effect of PMA was inhibited in the presence of zinc [36]. These findings support the view that zinc inhibits PTH-stimulated osteoclast-like cell formation mediated through Ca²⁺-dependent activation of protein kinase C.

The receptor activator of NF-κB ligand (RANKL) plays a pivotal role in the development of osteoclasts from preosteoclasts [37,38]. RANKL is secreted from osteoblasts. RANKL is a member of the tumor necrosis factor (TNF) superfamily, which was originally identified as T-cell-derived immunomodulatory cytokines [37]. RANKL/RANK pathway is essential for osteoclast differentiation [37,38]. Expression of RANKL is increased in osteoblastic cells and bone marrow stromal cells in response to osteotropic factors such as PTH, 1, 25(OH)₂D₃, or PGE₂. The effect of RANKL is abrogated by osteoprotegerin (OPG), a natural antagonist of RANKL, [37,38]. OPG is produced in osteoblastic
cells. 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-kB and mitogen-activated protein kinase (MAPK) activation [37,38].

Zinc has been shown to have suppressive effects on RANKL-induced osteoclast-like cell formation in mouse marrow culture in the presence of M-CSF [39]. Also, zinc inhibited TNF-α-induced osteoclastogenesis [39]. 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 [31]. The mechanism by which zinc suppresses osteoclastogenesis may also be related to production of OPG in osteoblastic cells.

As described above, zinc has been shown to have stimulatory effects on osteoblastic bone resorption and suppressive effects on osteoclastic bone resorption, thereby increasing bone mass. Zinc plays a pivotal in the regulation of osteoblasts and osteoclasts. This may be involved in many zinc-related proteins, which are identified in bone cells as shown in Table 1.

Zinc supplementation prevents bone loss in various pathophysiological states: Zinc has been shown to have preventive effects on bone loss in various pathophysiologic 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 [40]. These processes may occur simultaneously during fracture repair. During fracture healing, a number of growth factors, cytokines, and their cognate receptors are present at elevated levels in and around the fracture site [40].

The role of zinc in fracture healing has been examined using by the diaphyseal tissues obtained at 7 or 14 days after the fracture of femoral diaphysis of rats [41,42]. Oral administration of zinc acexamate (100 mg Zn/kg) for 28 days prevented the decrease in zinc and other bone components induced in streptozotocin-induced diabetic rats [50,51], indicating that zinc has a restorative effect on insulin-dependent diabetic conditions.

In addition, citrated zinc bisglycinate has been developed as a zinc supplement. This compound has been shown to increase bioavailability in intestinal absorption of zinc, and it is used as a zinc supplementation.

Supplemental intake with zinc, which has stimulatory effects on osteoblastic bone formation and mineralization and suppressive effects on osteoclastic bone resorption, is a useful tool in the prevention and treatment of osteoporosis.

### Role of genistein in bone homeostasis

Genistein stimulates osteoblastic bone formation: 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. The anabolic effects of genistein on bone metabolism have been firstly demonstrated in tissue culture using the femoral metaphyseal tissues of rats [52]. Oral administration of zinc acexamate (25 mg Zn/kg body weight) for 14 or 21 days with once daily has been found to have restorative effects on the increase in serum glucose and triglyceride levels and the reduction of bone components induced in streptozotocin-induced diabetic rats [50,51], indicating that zinc has a restorative effect on insulin-dependent diabetic conditions.

Increasing age. Zinc content in the cellular components but not the matrix is lowered in the femoral diaphysis of elderly rats (30 weeks old) as compared with that of weanling rats (3 weeks old) [44]. Bone protein synthesis was most likely deteriorated with increasing age [44]. Oral administration of zinc sulfate (5 to 20 mg Zn/kg) for 3 days restored the rate of [3H]-leucine incorporation [44]. Bone endogenous zinc may play a physiological role in the development of bone loss with increasing age. In aging, supplementation of zinc may be important in the prevention of bone loss with aging.

Zinc content is decreased in the femoral-metaphyseal tissues of rats with skeletal unloading, which is involved in the alteration of bone metabolism [45]. 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. Animals were fed for 4 days with the unloading. Unloading induced a decrease in metaphyseal zinc content [46]. Zinc accumulation in the metaphyseal tissues after a single oral administration of zinc sulfate (200 mg Zn/kg) was depressed with unloading [46].

β-Alanyl-histidinato zinc (AHZ) has been shown to have a potent effect on osteogenesis as compared with that of zinc sulfate [47]. Oral administration of AHZ has been shown to have a preventive effect on bone loss, which is caused with aging [48], inflammation [36], and ovariectomy [49].

Zinc acexamate has been found to have a potent effect on bone formation as compared with that of AHZ in vitro. Oral administration of zinc acexamate (25 mg Zn/kg body weight) for 14 or 21 days with once daily has been found to have restorative effects on the increase in serum glucose and triglyceride levels and the reduction of bone components induced in streptozotocin-induced diabetic rats [50,51], indicating that zinc has a restorative effect on insulin-dependent diabetic conditions.

In addition, citrated zinc bisglycinate has been developed as a zinc supplement. This compound has been shown to increase bioavailability in intestinal absorption of zinc, and it is used as a zinc supplementation.

Supplemental intake with zinc, which has stimulatory effects on osteoblastic bone formation and mineralization and suppressive effects on osteoclastic bone resorption, is a useful tool in the prevention and treatment of osteoporosis.

### Table 1: Zinc-related protein molecules in bone cells.

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Bone cells</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc finger transcription factors</td>
<td>Osteoblast</td>
<td>11</td>
</tr>
<tr>
<td>Osterix</td>
<td>Osteoblast</td>
<td>13</td>
</tr>
<tr>
<td>Schnurri-3 (Shn3)</td>
<td>Osteoblast</td>
<td>14</td>
</tr>
<tr>
<td>TRAP6-inhibitory zinc finger protein</td>
<td>Osteoblast</td>
<td>15</td>
</tr>
<tr>
<td>Zinc transporter Zn25</td>
<td>Osteoblast</td>
<td>9</td>
</tr>
<tr>
<td>Zinc transporter ZIP1</td>
<td>Osteoclast</td>
<td>10</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Osteoblast</td>
<td>22</td>
</tr>
<tr>
<td>Aminoacyl-RNA synthetase</td>
<td>Osteoblast</td>
<td>24, 25</td>
</tr>
</tbody>
</table>

### Increase in gene expression

- Runx2
- a1 (1) collagen
- Osteocalcin
- Osteoprotegerin (OPG)
- IG-F-1
- TGF-β1

### Decrease in gene expression

- Caspase-3
- Cathepsin K
- Tartrate resistant acid phosphatase

This 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 [31]. The mechanism by which zinc suppresses osteoclastogenesis may also be related to production of OPG in osteoblastic cells.

The role of zinc in fracture healing has been examined using by the diaphyseal tissues obtained at 7 or 14 days after the fracture of femoral diaphysis of rats [41,42]. Oral administration of zinc acexamate (100 mg Zn/kg) for 28 days prevented the decrease in zinc and other bone components induced in femoral-diaphyseal tissues of rats with fracture healing [43]. Zinc supplementation 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. Zinc content in the cellular components but not the matrix is lowered in the femoral diaphysis of elderly rats (30 weeks old) as compared with that of weanling rats (3 weeks old) [44]. Bone protein synthesis was most likely deteriorated with increasing age [44]. Oral administration of zinc sulfate (5 to 20 mg Zn/kg) for 3 days restored the rate of [3H]-leucine incorporation [44]. Bone endogenous zinc may play a physiological role in the development of bone loss with increasing age. In aging, supplementation of zinc may be important in the prevention of bone loss with aging.

Zinc content is decreased in the femoral-metaphyseal tissues of rats with skeletal unloading, which is involved in the alteration of bone metabolism [45]. 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. Animals were fed for 4 days with the unloading. Unloading induced a decrease in metaphyseal zinc content [46]. Zinc accumulation in the metaphyseal tissues after a single oral administration of zinc sulfate (200 mg Zn/kg) was depressed with unloading [46].

β-Alanyl-histidinato zinc (AHZ) has been shown to have a potent effect on osteogenesis as compared with that of zinc sulfate [47]. Oral administration of AHZ has been shown to have a preventive effect on bone loss, which is caused with aging [48], inflammation [36], and ovariectomy [49].

Zinc acexamate has been found to have a potent effect on bone formation as compared with that of AHZ in vitro. Oral administration of zinc acexamate (25 mg Zn/kg body weight) for 14 or 21 days with once daily has been found to have restorative effects on the increase in serum glucose and triglyceride levels and the reduction of bone components induced in streptozotocin-induced diabetic rats [50,51], indicating that zinc has a restorative effect on insulin-dependent diabetic conditions.

In addition, citrated zinc bisglycinate has been developed as a zinc supplement. This compound has been shown to increase bioavailability in intestinal absorption of zinc, and it is used as a zinc supplementation.

Supplemental intake with zinc, which has stimulatory effects on osteoblastic bone formation and mineralization and suppressive effects on osteoclastic bone resorption, is a useful tool in the prevention and treatment of osteoporosis.
The anabolic effect of genistein on osteoblastic cells in vitro has been demonstrated [54-57]. Culture with genistein (10⁻⁴ or 10⁻⁵ M) or daidzein (10⁻⁴ or 10⁻⁵ M) caused a significant increase in protein content, alkaline phosphatase activity and DNA content in the cells [55]. The ability of genistein or daidzein to increase biochemical components in the cells was not seen in the presence of an inhibitor of protein synthesis, suggesting that the effects of isoflavone result from newly synthesized protein components [55]. The effects of 17β-estradiol in increasing protein content and alkaline phosphatase activity in osteoblastic cells were not enhanced in the presence of genistein [55]. Cellular protein content was additively increased with 17β-estradiol and daidzein, but their effects on alkaline phosphatase activity were not additively [56]. Genistein has been shown to bind to estrogen receptor β in osteoblastic cells, and daidzein cannot bind to the receptor [53]. The effect of genistein may partly mediated through estrogen receptor β, but not α, in osteoblastic cells. Genistein and daidzein may have stimulatory effects on the proliferation and differentiation of osteoblastic MC3T3-E1 cells.

The cellular mechanism by which isoflavones stimulate osteoblastic bone formation has been studied in relation to protein synthesis. Culture with genistein or daidzein stimulated protein synthesis in osteoblastic MC3T3-E1 cells in vitro [57]. Moreover, genistein or daidzein has been shown to increase [³H]-leucyl-tRNA synthetase activity in the cytosol fraction of osteoblastic cell homogenate [57]. Genistein had a greater effect than daidzein [57]. Genistein can directly activate leucyl-tRNA synthetase, a rate-limiting enzyme in the translational process of protein synthesis. The possibility that genistein acts on the transcription process in osteoblastic MC3T3-E1 cells cannot be excluded.

Estrogenic compound 17β-estradiol and genistein mediate very different actions on osteoblastic cells [58], although genistein can bind to estrogen receptor-β. While 17β-estradiol may stimulate bone anabolism, in part, by antagonizing TNF-α-induced NF-κB activation, genistein does not only fail to prevent the cytokin-induced NF-κB activation, but directly promotes NF-κB activation in MC3T3 cells [58]. These observations suggest mechanistic differences in the mechanisms by which 17β-estradiol and genistein promote osteoblast differentiation [58].

Genistein suppresses osteoclastic bone resorption: PTH, PGE₂, and LPS have stimulatory effects on bone resorption in in vitro culture system. Culture with PTH, PGE₂, or LPS clearly stimulated bone resorption in the femoral-metaphyseal tissues cultured for 48 hours, when bone resorption was estimated with a decrease in calcium content and production of lactic acid in the bone tissues [59]. The effects of bone-resorbing factors were completely suppressed in the presence of genistein [59]. Thus, genistein has been found to have suppressive effects on bone resorption.

Culture with PTH, PGE₂, 1,25(OH)₂D₃, or LPS induced a remarkable increase in the formation of osteoclast-like multinucleated cells from mouse bone marrow cells [60]. These increases were suppressed in the presence of genistein [60]. Suppressive effects of genistein (10⁻⁴ M) were equal to effects of other anti-bone-resorbing agents (calcitonin, 17β-estradiol, and zinc sulfate) on osteoclast-like cell formation in mouse marrow culture [60]. Suppressive effects of genistein on osteoclast-like MNC formation in mouse marrow culture were greater than that of daidzein [60]. The cellular mechanism by which genistein suppresses osteoclast-like cell formation from marrow cells has been shown to be involved in cyclic AMP signaling [60].

Genistein has been found to induce cell death (apoptosis) of osteoclasts isolated from rat femoral tissues [61]. Suppressive effects of genistein on mature osteoclasts are partly mediated through the pathway of Ca²⁺ signaling [61]. Genistein may stimulate Ca²⁺ entry into osteoclasts. Daidzein has also been shown to suppress the number of mature osteoclasts, although daidzein did not have greater suppressive effects than genistein [61].

Culture with genistein has been found to have an inhibitory effect on protein tyrosine kinase activity in mature osteoclasts [62]. Genistein may partly induce apoptosis of osteoclasts through a mechanism that inhibits protein tyrosine kinases in the cells, since tyrosine kinase Src is implicated in the process of osteoclast-induced bone resorption in vitro and in vivo [63].

Culture with genistein has also been found to cause a significant increase in protein tyrosine phosphatase activity in mature osteoclasts [62]. This effect was also seen after the addition of genistein to the enzyme reaction mixture in vitro. Genistein can directly activate protein tyrosine phosphatase in mature osteoclasts. Protein tyrosine phosphatase (Src homology 2 domain-containing tyrosine phosphatase) is a negative regulator of osteoclastogenesis and osteoclast-resorbing activity in mutant mice [63]. Suppressive effects of genistein on mature osteoclasts may partly be mediated through the activation of protein tyrosine phosphatase in the cells. Genistein did not have an effect on β-glucuronidase activity in osteoclasts [62]. The effects of genistein in inhibition of osteoclastic bone resorption may not be implicated in the activity of lysosomal enzymes in the cells. As described above, suppressive effects of genistein on mature osteoclasts may be involved in the induction of apoptosis mediated through Ca²⁺-signaling mechanism, inhibition of protein kinase, and activation of protein tyrosine phosphatase in the cells.

Combination with zinc and genistein has potential synergistic effects on osteogenesis

Synergistic effects of zinc and genistein in osteoblastic bone formation: The effect of zinc sulfate (5.5 mg/kg body weight) on increasing DNA and calcium contents in the femoral tissues has been found to synergistically enhance after simultaneous administration of genistein (100 μg/kg body weight) in vivo [64-66]. Oral administration of genistein (100 and 300 μg/kg) or zinc sulfate (1 and 5 mg Zn/kg) to female rats for 3 days caused a significant increase in alkaline phosphatase activity, DNA, and calcium content in the femoral-metaphyseal tissues in vivo. Combination of zinc and genistein may be a useful tool in the prevention and therapy of osteoporosis.

The cellular and molecular mechanisms by which zinc enhances anabolic effect of genistein on bone components have been examined. Combination of zinc (10⁻⁴ or 10⁻⁵ M) and genistein (10⁻⁴ or 10⁻⁵ M) was found to have synergistic effects on protein content in osteoblastic MC3T3-E1 cells in vitro [67]. This effect may involve in the activation of aminoacyl-tRNA synthase by zinc and genistein, since these factors can increase the enzyme activity in the cytosol of osteoblastic cells in vitro [67].

Alkaline phosphatase is an enzyme marker of osteoblasts, and the enzyme participates in bone mineralization [68]. α1 (I) collagen is a matrix protein that is related to bone formation and mineralization in osteoblast lineage cells [69]. Osteocalcin is a bone matrix protein containing γ-carboxyglutaminate acid, which is synthesized in osteoblasts and is the protein involved in mineralization [69]. The expressions of alkaline phosphatase, α1 (I) collagen, and osteocalcin mRNAs in osteoblastic cells were synergistically enhanced after culture with the
combination of zinc ($10^{-5}$ or $10^{-7}$ M) and genistein ($10^{-6}$ or $10^{-5}$ M) as compared to the effect of each factor [67]. This finding demonstrates that the combination of zinc and genistein has synergistic effects on gene expression in osteoblastic cells.

The effect of combination of zinc ($10^{-4}$ M) and genistein ($10^{-5}$ M) on the gene expression in osteoblastic cells was completely prevented in the presence of an inhibitor of protein synthesis or an inhibitor of transcriptional activity [67]. This suggests that the combination of genistein and zinc stimulates the transcriptional process in osteoblastic cells. Zinc or genistein has stimulatory effects on protein synthesis at the transcriptional process. Presumably, combination of zinc and genistein stimulates both transcriptional and translational activities in osteoblastic cells. Each factor activates aminoacyl-tRNA synthetase at the transcriptional process in osteoblastic cells. Zinc or genistein may have an effect on the binding of transcriptional factor(s), which is synthesized through translational process, to the nuclear DNA in osteoblastic cells.

Culture with zinc ($10^{-4}$ M) or genistein ($10^{-5}$ M) has stimulatory effects on the mineralization in osteoblastic cells [67]. Combination of zinc and genistein synergistically enhanced mineralization in osteoblastic cells. This finding was supported the view that the combination of zinc and genistein can effectively enhance bone mineralization, thereby increasing bone mass [67].

Synergistic effects of zinc and genistein in osteoclastic bone resorption: Moreover, combination of zinc ($10^{-5}$ M) and genistein ($10^{-4}$ M) has been shown to have potential suppressive effects on osteoclastic cells, which are generated in mouse marrow culture in the presence of M-CSF and RANKL in vitro [70]. The number of mature osteoclastic cells was synergistically decreased with culture of zinc ($10^{-5}$ M) plus genistein ($10^{-4}$ M) as compared with the value of each factor. Combination of zinc and genistein had potential suppressive effects on mature osteoclastic cells [70]. This effect was remarkable in the presence of M-CSF and RANKL, which are cytokines that stimulate osteoclastogenesis [70]. Culture with zinc or genistein caused a synergistical increase in DNA fragmentation in osteoclastic cells [70], indicating that both factors stimulate apoptotic cell death. In addition, zinc plus genistein-induced cell death was significantly prevented in the presence of caspase-3 inhibitor [70]. The stimulation of apoptotic cell death with the combination of zinc and genistein may be partly mediated through activation of caspase-3.

Zinc ($10^{-5}$ M) increases the expression of caspase-3 mRNA in osteoclastic cells cultured with or without M-CSF and RANKL, while genistein ($10^{-4}$ M) alone did not have an effect [70]. The effect of zinc was significantly enhanced with the combination of genistein. Such effect was completely inhibited after culture with an inhibitor of protein synthesis or an inhibitor of transcriptional activity in osteoclastic cells [70], suggesting that the effect with the combination of zinc and genistein is involved in protein synthesis and transcription activation in osteoclastic cells.

Activation of tartrate-resistant acid phosphatase (TRACP) or cathepsin K plays a role in the promotion of osteoclastic bone resorption by stimulating the decomposition of bone matrix [36,37]. The expression of TRACP and cathepsin K mRNAs was markedly decreased in the presence of genistein plus zinc in osteoclastic cells cultured with M-CSF and RANKL [70]. Combination of zinc and genistein has potential suppressive effects on bone-resorbing activity.

Thus, combination of zinc and genistein has been found to have additive or synergistic effects in the suppression of osteoclastogenesis, mature osteoclast cell death, and bone resorption-related gene expression in osteoclastic cells.

Supplemental intake of zinc and genistein has potential effects against bone loss: As mentioned above, combination of zinc and genistein has been demonstrated to have potential synergistic effects on both stimulation of osteoblastic bone formation and suppression of osteoclastic bone resorption. Combination of zinc and genistein may have potential effects in the prevention and treatment of bone loss with various pathophysiologic states.

Zinc and genistein largely contain in fermented soybeans (natto). The effects of experimental diets with fermented soybeans containing zinc and genistein on OVX-induced bone loss has been demonstrated [71]. 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, genistin, 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 [71]. These reductions were prevented with feeding a natto diet. Such effect was significantly enhanced in OVX rats fed a natto diet supplemented with zinc and isoflavone of more amounts. Prolonged intake of dietary natto supplemented with zinc and isoflavone has a preventive effect on OVX-induced bone loss, suggesting that it may have a role in the prevention and treatment of osteoporosis.

Change in circulating biochemical markers of bone metabolism in aged individuals with the intake of fermented soybean (natto), which was made from isoflavone-rich soybean, supplemented with zinc has been examined [72]. Thirty-six 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. As serum bone markers, bone-specific alkaline phosphatase, γ-carboxylated osteocalcin, bone tartrate-resistant acid phosphatase (TRACP), and N-telopeptide of type I collagen were assayed. The intake of regular natto for 4 or 8 weeks in men or women persons caused a significant increase in γ-carboxylated osteocalcin, a marker of bone formation, and a significant decrease in serum bone N-telopeptide of type I collagen, a marker of bone resorption, as compared with the value before intake [72]. Moreover, the 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 [72].

This study have demonstrated that the intake of regular natto with genistein-rich soybean has 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 has been shown to have synergistic effects on the stimulation of osteoblastic bone formation and the suppression of osteoclastic bone resorption. Supplementation with composition of zinc compound and pure genistein may be potential tool in the prevention and therapy of osteoporosis with various pathophysiologic conditions.
Synergistic effects of zinc and menaquinone-7 on osteogenesis

Role of vitamin K in bone metabolism: 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, synthesized by osteoblasts, which are converted into gamma-carboxy glutamate (Gla) by γ-carboxylase [73]. These Gla residues form calcium-binding sites that are essential for the activity of the proteins.

There are three types of vitamin K: vitamin K₁ (phylloquinone), vitamin K₂ (menaquinone), and vitamin K₃ (menadione). Vitamin K₁ is a sole compound, but vitamin K₂ is a series of vitamins with multisoprene 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 [73]. MK-4 has been shown to inhibit bone loss, which may be related to its side chain, in ovariectomized rats [74]. Natural menaquinone-7 (MK-7; vitamin K₃) with seven isoprene units is very abundant in the fermented soybean (natto). It has been shown that serum MK-7 concentration in women living in Tokyo, where the fermented soybean is consumed, is about ten times higher than that of those living in Europe [75]. These differences may result from the intake of fermented soybean.

There is growing evidence for the roles of vitamin K₃ in bone health in human subjects [76,77]. Clinically, vitamin K₃ maintains lumbar bone mineral density (BMD) and prevents osteoporotic fractures in patients with osteoporosis. Osteocalcin, which is newly synthesized by osteoblasts, is released into circulation. For this reason, the circulating levels of osteocalcin are considered sensitive markers of bone formation [78]. A poor vitamin K status will lead to production of undercarboxylated (inactive) osteocalcin (unOC) [79]. In postmenopausal women, a clear association between elevated unOC and increased fracture risk have been found [80]. Significantly lower levels of vitamin K₁ and vitamin K₃ have been found in the serum obtained from elderly patients within a few hours after a hip fracture [80]. A daily vitamin K₁ supplement of 80 μg seems to be necessary to reach a premenopausal carboxylated osteocalcin/total osteocalcin ratio [81]. An adult daily intake of about 100 μg of vitamin K₁ is recommended for the maintenance of hemostasis [82].

Common risk factors for vitamin K deficiency in the hospitalized patient include inadequate dietary intakes, malabsorption syndromes (especially owing to cholestatic liver disease), antibiotic therapy, and renal insufficiency [83]. Pregnant women and their newborns present a special risk category because of poor placental transport and low concentrations of vitamin K in breast milk [83]. Since 2000, the Food and Drug Administration (FDA) has mandated that adult parenteral preparations should provide a supplemental amount of 150 μg vitamin K₁ per day in addition to that present naturally [83]. Although this supplemental daily amount is probably beneficial in preventing vitamin K deficiency, it may be excessive for patients taking vitamin K antagonists, such as warfarin, and jeopardize their anticoagulation control. Natural forms of vitamin K have no proven toxicity.

MK-7 stimulates osteoblastic bone formation: 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 [84]. 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. MK-7 may have an important role in the regulation of bone metabolism. Culture with MK-7 (10⁻⁴ or 10⁻³ M) caused a significant increase in biochemical components (alkaline phosphatase activity, DNA and calcium contents) in the femoral-diaphyseal (cortical bone) and -metaphyseal (trabecular bone) tissues obtained from aged rats in vitro [85]. The effect of MK-7 was significantly enhanced in the presence of genistein (10⁻⁴ or 10⁻³ M), suggesting that the mode of action of MK-7 differs from that of genistein [85]. The effect of MK-7 in increasing bone components in the femoral tissues was completely depressed in the presence of cycloheximide, an inhibitor of protein synthesis in vitro [85]. Thus, MK-7 has a stimulatory effect on bone formation in vitro.

Culture with MK-7 (10⁻⁴ or 10⁻³ M) caused a significant increase in alkaline phosphatase activity, protein and DNA contents in osteoblastic cells [86]. Such effects of MK-7 were completely depressed in the presence of cycloheximide. MK-7 has a stimulatory effect on osteoblastic bone formation due to increasing protein synthesis including osteocalcin.

MK-7 suppresses osteoclast-like cell formation: MK-7 has been shown to have suppressive effects on osteoclastic bone resorption in vitro [87]. Culture with bone-resorbing factors, PTH and PGE₂, caused a significant decrease in calcium content and lactic acid production in the femoral-metaphyseal tissues obtained from young and aged rats in vitro [87]. Such effects of bone-resorbing factors were completely suppressed after culture with MK-7 (10⁻⁷-10⁻⁵ M), indicating that MK-7 has an inhibitory effect on bone resorption in bone tissue culture [87].

Osteoclast-like cells are formed from bone marrow cells in the presence of bone-resorbing factors (PTH or PGE₂) [87]. PTH- or PGE₂-induced increase in osteoclast-like cell formation was significantly suppressed after culture with MK-7 [87]. MK-7 had potential inhibitory effects at the later stage of differentiation of marrow cells. Osteoclast-like cell formation was stimulated when dibutyryl cyclic AMP (DcAMP) or PMA, an activator of protein kinase C, was added to the culture medium. MK-7 significantly suppressed the effect of PMA on osteoclast-like cell formation, although it did not have an inhibitory effect on DcAMP-induced increase in osteoclast-like cell formation [87]. These observations suggest that the suppressive effect of MK-7 is partly involved in protein kinase C signaling [87].

The effect of MK-7 on mature osteoclasts isolated from rat femoral tissues has been examined [87]. Culture with MK-7 caused a significant decrease in the number of mature osteoclasts. Such a decrease was also seen in the presence of calcitonin, DcAMP, or calcium chloride [87]. The effect of MK-7 in decreasing the number of osteoclasts was completely abolished in the presence of dibucaine or staurosporine, which are inhibitors of Ca²⁺-dependent protein kinases. Suppressive effects of MK-7 on osteoclasts may be partly mediated through the pathway of Ca²⁺- and cyclic AMP-dependent signalings [87].

Mechanism of MK-7 action: MK-7 has been shown to stimulate osteoblastic bone formation and osteoclastic bone resorption, thereby increasing bone mass. 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 [86]. 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. By contrast, NF-κB signaling potently antagonizes osteoblast differentiation and function. MK-7 action on osteoblast and osteoclast formation and activity is
accomplished by downregulating basal and cytokine-induced NF-κB activation, by increasing k堐mRNA in a γ-carboxylation-independent manner [88]. MK-7 prevented repression by TNF-α of Smad signaling induced by either TGF-β or BMP-2 [87]. MK-7 further antagonized receptor activator of NF-κB (RANK) ligand (RANKL)-induced NF-κB activation in osteoclast precursors [87]. These findings provide a novel mechanism to explain the dual pro-anabolic and anti-catabolic activities of vitamin K₅. Moreover, the suppressive effect of MK-7 on mature osteoclasts may be partly mediated through the pathway of Ca²⁺- and cyclic AMP-dependent signaling [87].

Vitamin K₅ 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 [89].

**Intake of MK-7 prevents bone loss in animal model for osteoporosis:** MK-7 has been shown to have preventive effects on osteoporosis. The preventive effect of dietary MK-7 on bone loss in ovariectomized (OVX) rats has been examined [90]. OVX rats were given experimental diets containing MK-4 (12 mg/100 g diet) or MK-7 (18.1 mg/100 g diet) for 24 days; MK-4 and MK-7 were equal in molar concentration [90]. This feeding caused a remarkable increase in MK-4 and MK-7 concentrations in the serum and femur of OVX rats. OVX-induced decrease in the femoral dry weight and femoral calcium content were prevented after feeding with dietary MK-4 or MK-7 [90].

In separate experiments, OVX rats were given experimental diets containing fermented soybeans (natto including MK-7, 9.4 μg/100 g diet) with or without added MK-7 (37.6 μg/100 g diet) for 77 days [90]. Feeding produced a significant elevation of MK-4 and MK-7 concentrations in the serum of OVX rats. The decreases in the femoral dry weight and femoral calcium content induced by OVX were significantly prevented after feeding with diets containing natto with MK-7 added (37.6 μg/100 g diet) [90]. Supplementation of MK-7 was found to have a preventive effect on OVX-induced bone loss. This effect may be partly contributed to MK-4 that is formed after degradation of MK-7 in body.

Moreover, the effect of prolonged intake of dietary MK-7 on bone loss in OVX rats has been shown [91]. 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 [91]. Feeding produced a significant elevation of the serum MK-7 concentration of OVX rats [91]. 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) [91]. OVX caused a significant decrease in femoral dry weight, femoral calcium content, and mineral density. These decreases were significantly prevented after supplementation of MK-7 (total, 18.8 μg/100 g diet) [91]. Co-relationship with dietary MK-7 intake and bone formation markers in OVX rats showed a good co-relationship [91].

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.

**Dietary MK-7 intake increases osteocalcin production in human subjects:** The change in circulating MK-7 and γ-carboxylated osteocalcin (Gla osteocalcin) concentrations in normal individuals with the intake of fermented soybean has been examined [92,93]. 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 [92]. 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 [93]. Serum γ-carboxylated osteocalcin concentration was significantly 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 [93]. The intake of reinforced natto that contains more MK-7 than regular natto may play a role in the prevention of age-related bone loss.

The effect of low-dose MK-7 supplementation on bone health has been examined [94]. Healthy postmenopausal women (n=244) received for 3 years placebo or MK-7 (180 μg MK-7/day) capsules [94]. Bone mineral density (BMD) of lumbar spine, total hip, and femoral neck was measured by DXA; bone strength indices of the femoral neck were calculated. Vertebral fracture assessment was performed by DXA and used as measure for vertebral fractures. Circulating uncarboxylated osteocalcin (ucOC) and carboxylated OC (cOC) were measured; the ucOC/cOC ratio served as marker of vitamin K status [94]. Measurements occurred at baseline and after 1, 2, and 3 years of treatment. MK-7 intake significantly improved vitamin K status and decreased the age-related decline in bone mineral content and BMD at the lumbar spine and femoral neck, but not at the total hip [94]. Bone strength was also favorably affected by MK-7. MK-7 significantly decreased the loss in vertebral height of the lower thoracic region at the mid-site of the vertebrae [94]. MK-7 supplements may help postmenopausal women to prevent bone loss.

**Synergistic effects of zinc and MK-7 in osteoporosis treatment:** Zinc has been shown to synergistically enhance the effect of MK-7 in increasing bone calcium content *in vitro* [84] and *in vivo* [95]. Rats were orally administered either 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 [95]. Femoral dry weight was significantly 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 [95]. Calcium content in the femoral-diaphyseal and metaphyseal tissues was significantly increased after zinc administration [95]. 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 [95]. Alkaline phosphatase activity and DNA content in the diaphyseal and metaphyseal tissues were significantly increased by administration of zinc or MK-7 alone; these increases were additively enhanced by both zinc and MK-7 [95]. 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 [95]. Thus, supplemental intake with the combination of MK-7 and zinc may be useful in the prevention and treatment of osteoporosis.

**Synergistic effects of zinc and vitamin D₃ on osteogenesis**

**Role of vitamin D₃ in bone metabolism:** Calcium, phosphorus and vitamin D, which are nutrients, were noticed in maintaining bone health with preservation of bone mineral components. Calcium and phosphorus are essential elements in bone composition and are regulated through calcium-regulating hormones including PTH,
1, 25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$) and calcitonin. Nutrient vitamin D is converted to hydroxyvitamin D$_3$ (25(OH)D$_3$) in the liver and then 1, 25(OH)$_2$D$_3$ in the kidney which are its active metabolite. These forms are hormone recognized to play a critical function in bone metabolism. This is evidenced by a formation of poorly mineralized bone during vitamin D deficiency leading to rickets in children and osteomalacia in adults.

This is largely a consequence of the necessity for vitamin D$_3$ to promote efficient calcium absorption in the small intestine. Any decline in serum calcium concentrations due to inadequate calcium absorption leads to a secondary hyperparathyroidism that catabolizes the skeleton to maintain a physiological level of calcium necessary for normal cellular metabolism [96].

Although vitamin D supplementation is commonly used to combat osteoporosis, currently the optimal dose of vitamin D required for fracture prevention is contentious. While a minimum of 10 ng/mL of osteoporosis, currently the optimal dose of vitamin D required for normal cellular metabolism [96].

The doses of vitamin D is needed to prevent rickets and osteomalacia [97], recent studies have demonstrated that a minimum threshold 25(OH)D$_3$, level of 29.7 ng/mL is necessary for protection from fracture [98]. However, there is a paucity of data as to the optimal vitamin D$_3$ concentration for fracture prevention and to complicate matters it is now appreciated that vitamin D$_3$ plays a number of extra-skeletal roles including promotion of innate and adaptive immune function, prevention of cancers, and prevention of hypertension [97,98]. The doses of vitamin D is needed to achieve these extra-skeletal actions may be considerable higher than that needed to effect its actions on the skeleton [99]. Recent meta-analysis has suggested that supplementation of greater than 400 IU of vitamin D may reduce fractures [100], however the mechanism is unclear and may be associated in part with decreased risk of falling as a consequence of improved neuromuscular function [101]. In a clinical study of bedridden older patients with chronic secondary hyperparathyroidism, low dose (400 IU/d) vitamin D supplementation led to a significant increase in amino-terminal propeptide of type I procollagen, a marker of in vivo bone formation. These gains were complete negated by high dose (1200 IU/d) vitamin D supplementation, while indices of bone resorption did not significantly change with either regimen [102]. In another study, wintertime vitamin D supplementation of healthy men led to a significant dose-dependent decline in bone specific alkaline phosphatase, a marker of in vivo mineralization [103].

As the vast majority of studies involve vitamin D supplementation in the context of antiresorptive therapy, typically a bisphosphonate, it becomes extremely difficult to assess and effects of vitamin D alone on bone turnover given that antiresorptive agents themselves potently suppress bone formation as a consequence of coupling. Furthermore, the amelioration of secondary hyperparathyroidism by vitamin D supplementation is often associated with a decline in bone turnover [104]. This may be a consequence of reduced parathyroid hormone (PTH)-driven bone resorption leading to reduced bone formation as a consequence of coupling.

Vitamin D further appears to have hallmarks of an anti-inflammatory agent as vitamin D deficiency has been linked to a number of different inflammatory conditions including inflammatory bowel disease and rheumatoid arthritis, which induce bone loss. In a population-based prospective cohort, vitamin D intake was inversely correlated with risk of rheumatoid arthritis [96], an inflammatory autoimmune disease. Furthermore, vitamin D insufficiency promotes the development of autoimmune in animal models of inflammatory bowel disease [96].

Zinc synergistically enhances osteogenic effects of vitamin D$_3$. The synergistic effects with the combination of vitamin D$_3$ and zinc on osteogenesis have been found in the femur of weanling rats [105] and aged rats [106]. Oral administration of vitamin D$_3$ (10 μg/kg body weight) did not cause any increase in zinc accumulation in the femoral tissue following treatment with zinc sulphate (10 mg Zn/kg) [105]. Administration of vitamin D$_3$ or zinc caused significant increases in alkaline phosphatase activity and DNA content of the femoral diaphysis [105]. The increase in bone alkaline phosphatase activity was additionally enhanced by simultaneous administration of vitamin D$_3$ and zinc [105]. Moreover, bone DNA content was synergistically enhanced (about 4 times) after the administration with combination of vitamin D$_3$ and zinc [105]. The synergistic effect was also seen with combination of vitamin D$_3$ (5 μg/kg) and zinc (10 mg/kg) [105]. Such synergistic effects on bone alkaline phosphatase activity were suppressed after treatment with cycloheximide or actinomycin D [105]. Moreover, aging has been shown to cause decreases in calcium content, alkaline phosphatase activity and DNA content in the femoral tissues of rats [106]. Such decreases were found to restore after the administration of vitamin D$_3$, and this steroid effect was synergistically enhanced with the combination of zinc [106].

Whether the synergistic effects with the combination of zinc and vitamin D$_3$ on osteogenesis are specific for this steroid has been examined using other calcium-regulating hormones [107]. Administration of 1, 25(OH)$_2$D$_3$ (1.50 μg/kg BW) or PTH (1-34) (100 U/kg) produced significant increases in alkaline phosphatase activity and DNA content in the femoral diaphysis of rats in vivo, while calcitonin (10 μg/kg) did not have a significant effect [107]. Among these hormones, both 1, 25(OH)$_2$D$_3$ and zinc caused synergistic increases in bone alkaline phosphatase activity and DNA content [107]. Thus, synergistic effect with combination of zinc and 1, 25(OH)$_2$D$_3$ on osteogenesis was unique among calcium-regulating hormones.

Synergistic effects with 1, 25(OH)$_2$D$_3$ and zinc on osteogenesis has also shown in bone tissue culture in vitro [108]. Calvaria were

![Figure 1: Functional food factors including zinc, genistein, menquinone-7 (MK-7), and vitamin D$_3$ stimulates osteoblastic bone formation and suppresses osteoclastic bone resorption, thereby increasing bone mass. These factors stimulate cell differentiation, cell proliferation, and mineralization in osteoblasts and suppress osteoclastogenesis that is induced by variables bone-resorbing factors in bone marrow culture. These factors regulate protein synthesis and gene expression of various proteins, which are related to functions of osteoblasts and osteoclasts. Zinc synergistically enhances stimulatory effects of genistein, MK-7 and vitamin D$_3$ on osteogenesis.](image-url)
removed from weanling rats. Bone calcium content and alkaline phosphatase activity were significantly increased after culture with 1, 25(OH)2D3 (10-8 - 10-7 M), while bone acid phosphatase activity was not significantly changed [108]. [3H]-Leucine incorporation by the bone tissues and bone DNA content were significantly increased after culture with 1, 25(OH)2D3 (10-8 and 10-7 M). Such effects of 1, 25(OH)2D3 on osteogenesis were found to synergistically enhance with the combination of zinc in bone tissue culture in vitro.

The receptors for 1, 25(OH)2D3, an active hormonal type, have been shown to have two zinc fingers at the site of interaction with DNA [109]. Possible mechanism by which zinc has a synergistic effect on vitamin D-stimulated osteogenesis may be to enhance the interaction of 1, 25(OH)2D3-receptor complexes with DNA at that site. In addition, zinc, which stimulates protein synthesis in osteoblastic cells, may increase receptor proteins for 1, 25(OH)2D3 in the cells.

Conclusion

Osteoporosis is induced by alteration in balance of bone homeostasis with physiological aging, postmenopausal with ovarian hormone deficiency, inflammatory condition, obesity, diabetes and cancer bone metastasis. Bone loss with osteoporosis induces bone fracture. Functional food factors may help to prevent and treat bone loss and osteolysis. Among many functional food factors, essential trace element zinc, isoflavone genistein and vitamin K2 (menaquinon-7) has been shown to have stimulatory effects on osteoblastic bone formation and suppressive effects on osteoclastic bone resorption, thereby increasing bone mass.

Combination with zinc and other factors (including genistein, menaquinon-7, and vitamin D3) has been found to have synergistical osteogenic effects, as summarized in Figure 1. Supplemental intake with the combination of zinc and these factors may have potential effects in the prevention and treatment of bone loss with various pathophysiologic states.

Drugs, which are clinically used in the treatment of osteoporosis, are based on the action of osteoclastic bone resorption. Clinical compound that stimulates bone formation is very few. An intensive effort has been made to identify new anabolic agents capable of rebuilding lost bone mineral density. At present, teriparatide, a fragment of human parathyroid hormone, is the only United State Food and Drug Administration (FDA) approved anabolic agent currently available. This agent represents a significant leap forward but as a biologic based on the action of osteoclastic bone resorption, thereby increasing bone mass.

Combination with zinc and other factors (including genistein, menaquinon-7, and vitamin D3) has been found to have synergistical osteogenic effects, as summarized in Figure 1. Supplemental intake with the combination of zinc and these factors may have potential effects in the prevention and treatment of bone loss with various pathophysiologic states.

Supplementation with ingredients (including zinc, genistein, menaquinon-7 and vitamin D3), which has synergistical osteogenic effects, may be a potential tool in the therapy of osteoporosis. Development of new drug is expected using chemically pure ingredients as biomedical food factors.

Author Contribution and Disclosures

The author contributed to the design and conduct of the study, collection, analysis, and interpretation of data, and manuscript writing. Author has no conflicts of interest.

Acknowledgements

The author was partly supported by Awards of the Sato Memorial Fundation (Japan), the Mishima Kaiun Memorial Foundation (Japan), the Senji Miyata Fundation (Japan), and the Japan Society for Biomedical Research on Trace Elements.

References


