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Age-Related Male Osteoporosis, and Soy, Its Alternative Therapy

- Review -

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Abstract

As the population of the elderly grows, the prevalence of osteoporosis and its related fractures will increase in both men and women. The etiology, preventive and curative strategies of male osteoporosis are relatively unknown and understudied in comparison with those of female osteoporosis. Even currently approved therapies, e.g. bisphophonates, parathyroid hormone, and testosterone for male osteoporosis are in need of further investigation to test their safety and efficacy. Isoflavones which are found in soy have been shown to positively affect bone by stimulating bone formation while concurrently slowing down bone resorption. These observations mainly come from studies that have employed women or female animal models of osteoporosis. Therefore, there is a need to explore the role of soy and its isoflavones in preventing bone loss or rebuilding bone utilizing men or animal models of male osteoporosis. From the review of existing literature it is too early to state the extent to which men with osteoporosis can benefit from consumption of soy or its isoflavones. In this review, the efficacy of soy and its isoflavones as alternative and/or adjunctive treatment for male osteoporosis will be discussed.

Key words: male, osteoporosis, soy, isoflavones

INTRODUCTION

Osteoporosis is a metabolic bone disease which progresses silently and becomes evident only after a period of long latency. It produces physiological changes in which the amount of normally mineralized bone has been reduced to a level where the risk of fracture in the absence of trauma is increased, or such fractures have already occurred (1). Specifically, osteoporosis can be defined as bone mineral density (BMD) values of 2.5 standard deviations below that of the average BMD of young adult reference group (2,3).

Although osteoporosis is common among elderly women, men also have an increased risk of osteoporosis primarily due to aging (2-4). This paper reviews the incidence of male osteoporosis and its related bone fractures. It also discusses the available and potential therapies for male osteoporosis including the role of soy and its isoflavones on prevention or reversal of bone loss in men and animal models of male osteoporosis.

MALE OSTEOPOROSIS

Prevalence and incidence

Male osteoporosis is increasingly recognized as a debilitating condition that not only affects men in western societies, but also those living in Asian countries. For example, the prevalence of distal radius osteoporosis was 8.0, 19.8, and 42.9% of men aged 60 to 69, 70 to 79, and over 80, respectively in the Korean male population (2). The Rotterdam study which was conducted in Netherlands (3) also found a similar pattern to the Korean study. In that study, about 5, 12, 12, 18, 22, and 35% of men in the age categories of 60 to 64, 65 to 69, 70 to 74, 75 to 79, 80 to 84, and over 85 years, respectively had osteoporosis. These increase in male osteoporosis is particularly noticeable in nursing homes. Elliott and her colleagues (4) reported that 50% of men between the ages of 65 to 74, 62% of men between the ages of 75 to 84, and 81% of men over the age 85 displayed signs of osteoporosis. Thus, these reports suggest that worldwide male osteoporosis is a serious public health concern in the elderly population, especially among the elderly men who reside in nursing homes.

Osteoporotic fractures in men

A negative correlation between bone density and fracture has been previously reported in men by a number of studies (6-13). Melton and his colleagues (14) estimated that osteoporosis may attribute to 30 to 85% of hip fractures, 55 to 90% of vertebral fractures, 20 to 45% of distal forearm fracture, and 15 to 45% of fractures

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at other skeletal sites in men over the age of 45 years. Additionally, in that study the investigators found that osteoporosis attributed more to fractures in old men than young men (14).

The findings of a study by Gehlbach et al. (15) has shown that men accounted for about 23% of all hip fractures in people aged over 45 years. Hip fractures threaten quality of life more than any other osteoporotic fractures and attribute to substantial mortality due to complications within one year (16-18). Mortality after hip fracture was reported to be twice as high in men compared with age-matched women (17,18). In terms of vertebral fractures, about one fourth of fractures occur in men according to US hospital data (15). The findings of a Swedish group of investigators (16) indicated that about 22% of all vertebral fractures occur in men. In that particular study, they reported that the death rate due to vertebral fractures was similar in both men and women within one year (16).

The cost of osteoporosis-related fractures in men is a huge economic burden (15). The worldwide medical cost of only hip fractures among men has been predicted to exceed $14.2 billion by year 2050 (19). Since fractures lead to lack of autonomy in the elderly, this economic burden continues until the elderly people have completely recovered from fractures. In addition, osteoporotic fractures impair physical, social, and emotional well-being of individuals (20-23). Thus, osteoporosis-related fracture, in addition to being an immense economic burden, also jeopardizes quality of life in elderly men.

Etiology

The lower incidence of osteoporosis and its related fractures in males in comparison with females has been explained by factors such as the higher peak bone mass, shorter life expectancy, and the absence of a distinct menopausal stage (24-26). For these reasons, the etiology of male osteoporosis has been relatively understudied and underdiagnosed in comparison with that of female osteoporosis.

Male osteoporosis can be either primary (i.e. aging and idiopathic), or secondary (26,27). Osteoporosis in the elderly men has been mainly explained by age-related senescence of skeletal and disorders of endocrinal, renal, and gastrointestinal systems (27). These senescent tissues lead to irregular function of bone cells, disruption of hormone balance (e.g. hypogonadal conditions; testosterone and/or estrogen deficiency), and an imbalance of serum calcium, vitamin D, and parathyroid hormone (PTH), resulting in loss of bone mass in aging men (28-32). There is another distinct type of osteoporosis known as idiopathic osteoporosis which occurs without any known cause (33). However, there have been studies indicating the role of insulin-like growth factor (IGF)-I and estrogen in idiopathic osteoporosis (34,35).

Secondary osteoporosis in men has many causes such as certain medications, alcohol abuse, cigarette smoking, and immobility (27). Additionally, chronic conditions such as rheumatoid arthritis, gastrointestinal, and cardiovascular diseases are considered independent secondary risk factors for male osteoporosis (36-38). Glucocorticoid therapy is widely used for the treatment of certain diseases (e.g. bronchial asthma and lupus) and is well known to cause bone loss (39). This is due to the fact that glucocorticoid suppresses the release of gonadal hormones, reduces muscle mass and strength, and induces secondary hyperparathyroidism (39).

Expanding our knowledge about the etiology of osteoporosis is an important step towards finding new agents that can either prevent or treat male osteoporosis and its related fractures.

AVAILABLE AND POSSIBLE THERAPIES FOR MALE OSTEOPOROSIS

Several drugs have been approved by the United States (US) Food and Drug Administration (FDA) for female osteoporosis as a result of extensive studies. However, currently few investigations (40-45) have been conducted in men to examine the efficiency of drug therapies. A brief review of available and possible therapies for male osteoporosis is presented here.

Bisphosphonates

Bisphosphonates, which are classified as synthetic analogs of pyrophosphate, include alendronate, risedronate, and etidronate. Among these bisphosphonates, alendronate has been approved by the US FDA as the first antiresorptive agent for treatment of male osteoporosis.

Orwoll et al. (41) recruited men with osteoporosis and 33 percent of these men had low serum free testosterone concentrations and no secondary causes for osteoporosis. They observed that treatment with 10 mg alendronate daily plus calcium (500 mg) and vitamin D (400 IU ~ 450 IU) supplements increased the BMD of the lumbar spine by 7.1% and the femoral neck by 2.5% (41). As a result, the incidence of vertebral fractures was decreased by 88.7% in their two-year double-blind trial. In a follow-up study by Drake et al. (46), the beneficial effects of alendronate on male skeletal health were found unrelated to age, body mass index, testosterone, estrogen, IGF-I, IGF-binding protein (IGFBP)-3, and prior fractures. These investigators (46) concluded that alendronate must act through other mechanisms to improve male bone mass. Ringe et al. (43) conducted a 2-year, open-labeled, prospective and comparative controlled

clinical trial in which the effects of alendronate were tested in men with primary osteoporosis due to aging. They reported that oral treatment with alendronate (10 mg daily) along with 500 mg of calcium significantly increased BMD of the lumbar spine and the femoral neck by 10.1% and 5.2%, respectively (43). This study (43) was extended for one more year to confirm the appreciable effect of 10 mg alendronate then results showed further increases in BMD of the lumbar spine (11.5%) and the femoral neck (5.8%) (44). During this time, new vertebral fractures occurred in only 10.3% of the alendronate group as compared to 24.2% of alfacalcidol which is structurally and functionally similar to calcitriol (vitamin D3) (44). In addition to these two studies, Weber and Drezner (47) measured the bone protective effects of alendronate (10 mg daily average of 1.9 and 2.7 years) in men with idiopathic osteoporosis. Their findings indicated that alendronate treatment increased BMD of spine, trochanter, and total hip by 4.5, 6.4, and 4.7%, respectively. The other bisphosphonates, i.e. risedronate and etidronate, have also been studied for treatment of male osteoporosis. Unlike alendronate, both risedronate and etidronate have been used in men with oral corticosteroid-induced osteoporosis (48,49). Wallach and his colleagues (49) conducted a one-year, double-blind, placebo-controlled clinical trial using men and women who were on oral corticosteroid therapy (7.5 mg prednisone daily or greater). Among the 518 participants of that study, 184 were men. Their data for men and women showed that 5 mg of risedronate increased or maintained BMD of the lumbar spine, femoral neck, trochanter, and distal radius while these parameters were decreased in the placebo group. Furthermore, Reid et al. (48) reanalyzed the data from the Wallach et al. (49) using only male subjects and showed that men who took either 2.5 mg or 5 mg risedronate had a vertebral fracture rate of 5%, whereas the placebo group had a vertebral fracture rate of 24%. Etidronate therapy was also studied in a 3-year prospective randomized trial using men who took more than 7.5 mg of prednisone daily for more than 3 months (50). In that study (50), they found that etidronate intake resulted in an increase of 3.7% in lumbar spine BMD and participants did not experience any new vertebral fractures.

Bisphosphonate therapy is effective in treating male osteoporosis, but it has side effects such as nausea, dyspepsia, esophagitis/gastritis, abdominal pain, constipation, and diarrhea (48,49). Therefore, there is a need to explore new analogues of bisphosphonates for osteoporosis without or with less side effects.

Parathyroid hormone (PTH)

PTH is a major calcium-regulating hormone in humans (51). PTH maintains serum calcium by stimulating bone resorption, regulating renal calcium excretion, and indirectly affecting intestinal calcium absorption (51). PTH level is increased as a result of aging, which has been implicated in the pathogenesis of bone loss (29). Ironically, recombinant 1~34 region of the 84-amino acid peptide of PTH (52) has been shown to increase the rate of bone formation (42,53-57) Based on PTH's anabolic effects, teriparatide, a human PTH (1-34), was approved by the FDA in the US for treatment of severe osteoporosis in both women and men.

The first clinical trial using PTH (1-34) in men was conducted by Slovik et al. (57). In a one-year study (57), it was found that PTH improved intestinal calcium and phosphorus absorption and increased BMD of the lumbar spine. Another randomized, controlled trial using PTH (400 IU/day) was carried out in 23 men with idiopathic osteoporosis (30~68 years old) (55). Men treated with PTH had a significant increase (13.5%) in lumbar spine BMD while there was no BMD change in placebo-treated subjects at the end of the 18-month trial. Orwoll et al. (42) recruited osteoporotic men and randomly assigned them to three groups; placebo, 20 μg, or 40 μg of teriparatide. After 11 months of treatment, the participants' mean spinal BMD was increased by 5.9% and 9.5% in the 20 μg and 40 μg treatment groups, respectively. Furthermore, the paired bone biopsies from before and after PTH treatment for 18~36 months verified the bone anabolic properties of PTH by observing that PTH stimulated the formation of new bone matrix (56).

Nonetheless, teriparatide therapy should be reserved as a last option because of cost and possible risk associated with its use. In the US, teriparatide currently costs $600 for a month supply and must be administered daily via subcutaneous injection for a median of 19 months to see an overall beneficial effect on bone. The onset of undesirable adverse events such as marrow fibrosis, tunneling resorption, nausea, and headache have been encountered in clinical trials (42,54). Hence, teriparatide should be kept as a last resort for treatment of osteoporosis.

Androgen and estrogen

Male osteoporosis has, in part, been linked to testosterone deficiency (32,58). Several reports (40,45,59) indicate that bone loss related to hypogonadism can be reversed by testosterone therapy. A three-year study examining the effect of testosterone therapy on bone

(scrotal patch, 6 mg testosterone/day) in hypogonadal men showed a maximum increase in BMD of the lumbar spine (7.7%) and trochanter (4.0%) after two years of treatment (59). The findings of a recent study (40) confirmed that three-year testosterone therapy (200 mg intramuscular injection every 2 week) increased BMD of the intertrochanteric and trochanteric regions of femur in hypogonadal men 65 years of age or older. The bone effects of testosterone therapy have been found to be dose-dependent (45). On the other hand, dihydrotestosterone (DHT), a final metabolite of testosterone, has not been shown to have any beneficial effects in either men with age-related hypogonadism (40) or men with benign prostatic hyperplasia (60). Nonetheless, DHT has been shown to improve bone mass in rodent models of male hypogonadism (61,62). Although testosterone exerts beneficial effects on the male skeleton, there is still controversy regarding its use as a therapy because of its adverse effects on the benign prostatic hyperplasia and prostate cancer.

In addition to compromised testosterone levels, accumulated data suggest that decreased bioavailable estrogens is also involved in the pathogenesis of male osteoporosis (30,64-67). This notion is supported by observations that there is a positive relationship between bioavailable estrogens and bone mass in men (30,64-67). Short-term use (i.e. 3 weeks) of estrogen replacement therapy in normal elderly men (30) and in hypogonadal men of various ages (66) has been shown to significantly reduce markers of bone resorption. A study conducted by Falahati-Nini et al. (30) reported that estrogen replacement therapy was more effective than testosterone. Additionally, estrogen has been shown to be a predictor of bone density (62,68) more than DHT in orchidectomized rodents (62). From these data, it can be suggested that estrogen is important for skeletal health in males. However, the long-term effects of estrogen replacement therapy in men are unknown. Therefore, studies are needed to evaluate the safety and efficacy of estrogen replacement therapy in men.

Fluoride

Fluoride, a bone anabolic agent, is used for the treatment of osteoporosis in Germany (69). Ringe et al. (69) conducted a study in which men with idiopathic osteoporosis received intermittent (3 months on, 1 month off) dose of 114 mg monofluorophosphate (15 mg fluoride ions) and about 1000 mg calcium supplement daily. Their findings (69) showed increases in BMD of the lumbar spine, radius, and femoral neck. Meanwhile, the control group which only received calcium showed decreases in BMD of all regions. Ringe and Rovati (70) also observed that combination of 20 mg sodium fluoride and 400 mg etidronate in established primary male osteoporosis significantly increased BMD when compared to sodium fluoride or etidronate alone after 12 and 24 months of therapy.

The use of fluoride therapy for osteoporosis is extensively reviewed in a paper by Ringe and Rovati (70). Although fluoride therapy is economically feasible, it is associated with undesirable side effects such as the risk of new vertebral fractures and gastric bleeding. In this review, Ringe and Rovati (70) have indicated that there is a need for conducting large size clinical trials in order to confirm the efficacy of fluoride therapy.

Thiazide diuretics

Thiazide diuretics decrease urinary calcium and therefore, can potentially influence bone and calcium metabolism. An epidemiological study (71) reported that among 7983 participants (3071 men and 4820 women aged 55 years and older), thiazide diuretic users experienced progressive reductions in the rate of hip fractures with increasing duration of treatment period. In contrast, a randomized, double-blind, placebo-controlled trial (72) with a study period of three-years showed that hydrochlorothiazide treatment (12.5 mg and 25 mg per day) had no bone protective effect. Another study by Legroux-Gerot et al. (73), reported that though 18-month thiazide diuretic treatment reduced urinary calcium excretion by 45.9% in hypercalciuretic osteoporotic men, it was unable to modulate BMD. Furthermore, thiazide therapy has been reported to cause elevated serum levels of triglycerides, blood glucose, and uric acid and reduced levels of serum potassium and sodium (72,73). Therefore, because of their controversial bone beneficial effects and undesirable side effects, thiazide therapy is doubtful to be an option for osteoporosis treatment in men.

Calcitonin

Calcitonin is a polypeptide hormone made in the C-cells of thyroid gland (74). Pharmacologic doses of calcitonin have been shown to potently inhibit bone-resorption through the contraction of the ruffled borders of the osteoclast cell membrane (75).

Erlacher et al. (76) demonstrated that men with osteoporosis, when intermittently (i.e. 3 months on, 3 months off) given 100 units of salmon calcitonin (i.e. subcutaneous injection, three times per week) along with 1000 mg of a calcium supplement had significantly higher vertebral and femoral BMD after one year compared to baseline. Another study by Trovas et al. (77) reported that men with idiopathic osteoporosis who were prescribed nasal salmon calcitonin (200 IU) daily along with 500 mg

calcium had significantly higher lumbar BMD values compared with their baseline BMD. However, these two clinical trials were conducted with nine and fifteen subjects, respectively for 1 year. Further research using larger number of subjects and longer treatment periods are necessary to verify whether calcitonin treatment is efficacious in treating male osteoporosis.

In conclusion, an appropriate approach for the evaluation of therapies for osteoporosis in men has not been systematically developed. Possible therapies for men are either associated with side effects and/or are not yet proven safe or efficacious in improving bone quality or decreasing susceptibility to osteoporosis-related fractures. Therefore, it would be desirable to find safe, naturally occurring compounds, such as those found in soy, which can improve bone quality and reduce the risk of osteoporosis in men.

SOY AND BONE

Soybeans or more specifically isolated soy proteins are rich sources of the glycosylated isoflavones, genistin and daidzin. The glycosylated isoflavones are converted to aglycone forms of genistein and daidzein, respectively by microflora in the intestine (78,79). Due to their structural similarities to estrogen, there has been a rising interest in soybeans' health benefits not only on sex hormone metabolism, but also on other biological activities including cholesterol-lowering properties (80,81) and anti-carcinogenic effects (82). Some studies have indicated that soy protein and/or its major isoflavones could positively affect bone (83-91). The beneficial effects of soy isoflavones have been mostly tested in animal models of postmenopausal osteoporosis and clinical trials involving women. To date, there have few studies in which the role of soy and/or its isoflavones in male osteoporosis has been investigated. Despite the lack of research performed with regards to the topic, soy and its isoflavones are expected to have similar impact on male skeletal health based on the following reasons.

Epidemiological studies indicate that osteoporosis related fractures are less common among Asian men as compared to their counterparts in most Western countries (92,93). This lower fracture incidence may be, in part, due to their higher intakes of soy foods and vegetables. In a recent double-blind, randomized, controlled, parallel design clinical study (94), healthy men (59.2 ± 17.6 y) supplemented with 40 g of soy protein for three months, had significantly greater circulating IGF-I levels than those consuming milk-based protein. In addition to epidemiological studies (92,93) and a short-term human clinical trial (94), the bone protective properties of isoflavones were tested in orchidectomized rodents (68,95). Their results showed that isoflavones improved bone mass and quality without any androgenic properties (68, 95). The modes of action by which soy isoflavones exert their bone protective effects are not clear. Several studies have been done investigating their mechanism of action and are discussed in the following section.

Numerous *in vitro* (96-113), animal studies (114-117), and clinical trials (94,118-120) have been undertaken to clarify the possible mechanisms by which soy or its isoflavones influence bone. One hypothesis is that soy and its isoflavones increase bone formation while concurrently inhibiting bone resorption.

Proposed bone forming mechanisms of action of soy and its isoflavones

In terms of mode of action, several studies suggest that soy or its isoflavones have bone anabolic properties based on at least three lines of evidence: 1) induction of proliferation, differentiation, and activity of osteoblast lineages (97,98,103,104,106-108, 111-113); 2) protection of osteoblasts from apoptosis (97,104,105) and 3) enhancement of bone formation rate (115,116).

Histomorphometric data support the bone forming abilities of soy isoflavones. Fanti et al. (116) found that genistein was associated with a higher rate of bone formation and a greater number of osteoblasts. Furthermore, Blum et al. (115) confirmed that soy protein significantly stimulated bone formation in both cancellous and cortical bone. Together these studies indicate that the beneficial effects of soy protein and its isoflavones on bone may be due to the enhanced bone formation.

Prolonging the lifespan of osteoblasts is an important aspect in bone formation and mineralization. The lifespan of osteoblasts ranges from a few to 100 days (121). Under certain conditions such as inflammation and sex hormone deficiency (e.g. estrogen and testosterone), the rate of apoptosis is accelerated (122-124). Delaying the process of apoptosis can result in increased number of osteoblasts that may enhance the rate of bone formation. Choi et al. (97) reported that soy isoflavones were able to hinder the apoptosis of osteoblasts and their findings were confirmed by a study conducted by Sik et al. (106). In that study, the investigators showed that the major soy isoflavones, genistein and daidzein, were able to prevent tumor necrosis factor (TNF)-induced apoptosis of osteoblastic cells (106). However, these observations should be considered preliminary and require further investigation.

The cellular events involved in bone formation include the proliferation and differentiation of osteoblast precursors. During differentiation *in vitro*, osteoblast phenotypic markers appear in the order of: 1) accumulation

of a collageneous matrix, 2) expression of alkaline phosphatase (ALP) and secretion of osteocalcin (OC) and, finally, 3) mineralization of bone nodules (125). Soy isoflavones have been shown to stimulate DNA synthesis and ALP activity in osteoblasts (97). The individual soy isoflavones, genistein and daidzein, have also been shown to induce proliferation and differentiation of osteoblasts in MC3T3-E1 cells (104,106-108,112), osteoblasts isolated from rat calvaria (103), tissue culture using rat femoral diaphysis and metaphysis (98,111), and normal human bone cells. Morabito et al. (120) in a clinical trial confirmed that genistein increased circulating bone-specific ALP activity and OC, indicative of higher rate of bone formation.

The synthesis of bone matrix proteins from osteoblastic proliferation and differentiation has been reported to be mediated by the transcription factors, Runx2 (126, 127), which is activated by the osteogenic growth factors, IGF-I, transforming growth factor (TGF)-β, and bone morphogenetic protein (BMP) (127-130). Runx2 has several target genes including collagen type I (COL), OC, osteopontin (OP), bone sialoprotein, and fibronectin (126). Recent reports suggest that IGF-I, TGFβ and BMP are upregulated by soy or its isoflavones (94,102,103,114, 118). For instance, using a rat model of female osteopenia, soy increased the gene expression of IGF-I as indicated by higher femoral mRNA levels (131,132). Furthermore, soy protein supplementation has been shown to significantly increase serum IGF-I levels in both men (94) and women (118). TGFβ1 and BMP were also overexpressed in osteoblast cells isolated from newborn Wistar rats in the presence of genistein and daidzein (102,103). Therefore, the mechanism of action of isoflavones on bone matrix proteins synthesis may be through the upregulation of Runx2 under the control of IGF-I, TGFβ and/or BMP (Fig. 1).

Proposed anti-bone resorptive mechanisms of action of soy and its isoflavones

Both soy and its isoflavones have also been observed to have anti-bone resorptive properties. For example, *in vitro* and *in vivo* studies have shown that genistein and daidzein effectively lower the number of osteoclasts (68,99,100,105,110).

The reduction in osteoclast numbers due to isoflavones has been explained by two possible scenarios (Fig. 2A ~ 2C). The first is that genistein and daidzein induce apoptosis of osteoclasts (Fig. 2A), which might be mediated through the pathway of intracellular calcium signaling (100), upregulation of caspases 3 and 8 (105), and/or inhibition of protein kinase and activation of protein tyrosine phosphatase (101). Another possible scenario is that isoflavones can reduce the number of osteoclasts by suppressing osteoclastogenesis (Fig. 2B, 2C). Osteoclastogenesis occurs when receptor activator of nuclear factor-kappaB ligand (RANKL), synthesized by osteoblasts, bind with RANK on the surface of osteoclast progenitors. However, osteoprotegerin (OPG), another product of osteoblasts, binds with RANK instead of RANKL, which then inhibits RANK/RANKL signaling. Thus, a lower level of RANKL and a higher level of OPG might inhibit osteoclastogenesis (Fig. 2B). In line with this concept, it has been reported that genistein increases the mRNA level of OPG, while decreasing RANKL in co-cultures of mouse spleen cells and clonal osteogenic stromal ST2 cells (110). Genistein and dai-

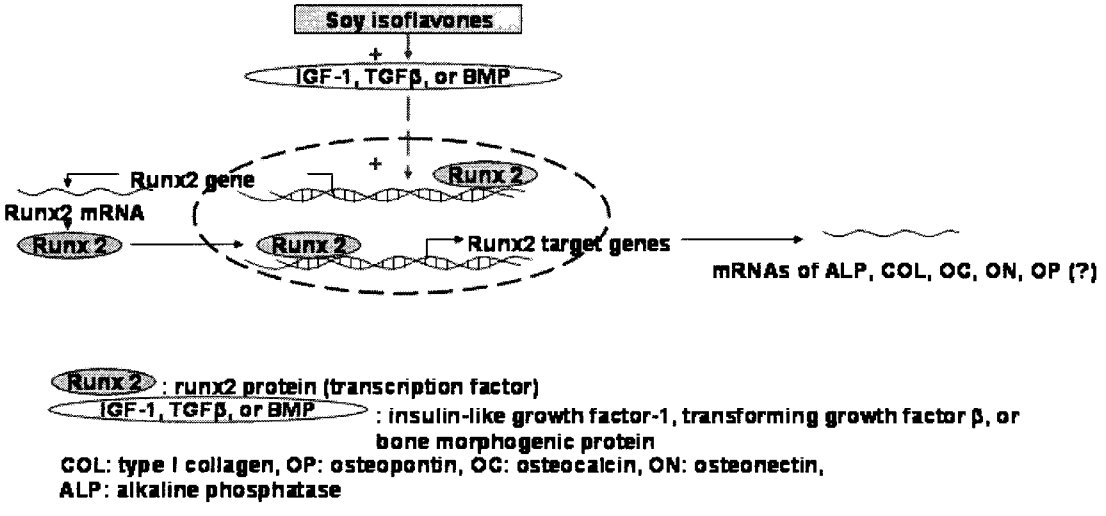

Fig. 1. A proposed mechanisms of action of soy isoflavones on bone formation through the activation of Runx2 gene expression under the control of IGF-I, TGFβ or BMP.

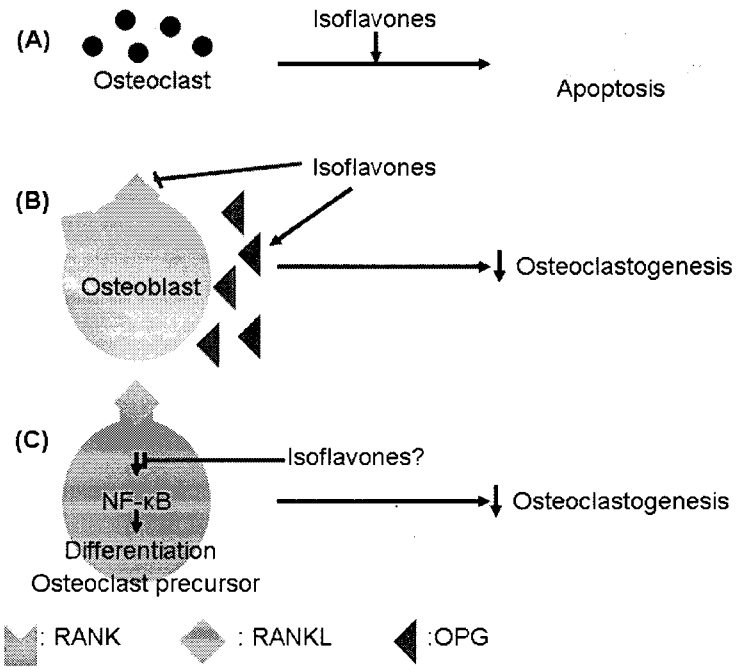

Fig. 2. A proposed anti-bone resroptive mechanism of action of soy-isoflavones. There are two possible scenarios that isoflavones may reduce osteoclast numbers. (A) isoflavones, genistein and daidzein, induce apotosis of ostoeclasts; (B) isoflavones may interrupt the pathway of osteoclastogenesis by modulating the expression of receptor activator of nuclear factor-kappaB ligand (RANKL)/ osteoprotegerin (OPG); (C) by directly inhibiting activation of nuclear factor-kappaB (NF-κB).

dzein have also been shown to increase mRNA level of OPG in primary human trabecular osteoblasts (96, 102,109). Additionally, one-year supplementation of genistein (54 mg/day) has been reported to lower serum RANKL/OPG ratio in healthy postmenopausal women as compared to placebo (119). This lowered RANKL/ OPG ratio may be also associated with increased femoral neck BMD (119). Currently, the upregulation of OPG pathway by isoflavones has been reported to involve estrogen receptors (96,109). On the other hand, Yamagishi et al. (110) reported that the inhibition of topoisomerase II activity by genistein might increase OPG levels. Further downstream in osteoclastogenesis, a heterodimer of RANK with RANKL can induce the signal of nuclear factor kappa B (NF-κB), a transcription factor (133,134). The relationship between isoflavones and NF-κB is not known in osteoclasts, however, genistein has been reported to inhibit the NF-κB in RAW 264.7 macrophage cells (135). Thus, isoflavones might interrupt the pathway of osteoclastogenesis by directly inhibiting activation of NF-κB (Fig. 2C) as well as modulating the expression of RANKL/OPG (Fig. 2B).

Soy and its isoflavones could increase bone formation and decrease bone resorption through several pathways. Nevertheless, this deduction is drawn from the studies that solely have employed women or female animal models. Furthermore, many of these conclusions on the mechanisms of action of soy and its isoflavones on bone have been drawn using *in vitro* systems that may not necessarily correspond to *in vivo* conditions. Thus, the role of soy and its isoflavones on bone anabolic and anti-bone resorptive properties should be verified in an animal model of male osteoporosis and men.

FUTURE DIRECTIONS FOR MALE OSTEOPOROSIS AND ITS THERAPIES

The incidence of male osteoporosis and its related fractures is increasing and unlike women, this silent disease has not been extensively studied in men. There are numerous paths that can be taken to reduce osteoporosis and its related fractures including: 1) epidemiology studies to observe the prevalence and incidence of male osteoporosis; 2) investigational experiments to identify the pathogenesis and risk factors for male osteoporosis; and 3) basic and clinical studies to test the efficacy of diverse synthetic and natural agents' abilities in preventing or reversing bone loss in males. To my knowledge, there have been only few osteoporosis studies conducted in Korean men. For example, Rowe et al. (136) and Shin et al. (2) investigated the epidemiology and prevalence of male osteoporosis, respectively. Only two studies (137,138) evaluated the etiological factors for osteoporosis in middle and aged Korean men. Moreover, the research on intervention therapies using either

drugs or functional foods has not been explored in Korean men with osteoporosis. In spite of the similarities between men and women in terms of bone biology and metabolism, the pathogenesis of male osteoporosis is far more complex than that of female osteoporosis. Hence, there is an urgent need to explore the underlying mechanism by which male osteoporosis occurs as well as searching for treatment options.

Bisphosphonate, teriparatide, and testosterone are approved therapeutic agents for osteoporosis in men, however, these therapies may be associated with undesirable and adverse events. Furthermore, the role of estrogen, fluoride, thiazide diuretics, and calcitonin for male osteoporosis in terms of safety and efficacy of these agents in improving bone quality or decreasing susceptibility to osteoporosis-related fractures require further investigation. Therefore, it would be desirable to find safe, naturally occurring compounds, such as those found in soy, which can improve bone quality and reduce the risk of osteoporosis in men.

Many of the conclusions regarding the role of soy and its isoflavones on bone have been drawn from *in vitro* studies that may not necessarily correspond to *in vivo* conditions. Furthermore, most studies dealing with soy and its isoflavones have been conducted in women or female animal models of osteoporosis. While it is conceivable that soy and its isoflavones exert similar effects on the male skeleton, this would require further investigation. In terms of animal models, rat's skeleton anatomically and morphologically differ from those of humans. Because of atypical arrangement and organization of human bone structures in comparison with rat, verification of the pathology and the effectiveness of treatments, i.e. isoflavones in prevention of bone loss need to be tested in clinical trials employing men.

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