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REVIEW ARTICLE

Soy isoflavones in the management of postmenopausal osteoporosis

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Abstract

This is a review article designed to address the effects of soy isoflavones on bone metabolism in postmenopausal women and their place in the prevention and treatment of postmenopausal osteoporosis. Soy isoflavones are natural products that could be used as an alternative to menopausal hormone therapy because they are structurally and functionally related to 17β-estradiol. In vitro and animal studies have shown that they act in multiple ways to exert their bone-supporting effects. They act on both osteoblasts and osteoclasts through genomic and nongenomic pathways. Epidemiological studies and clinical trials suggest that soy isoflavones have beneficial effects on bone mineral density, bone turnover markers, and bone mechanical strength in postmenopausal women. However, there are conflicting results related to differences in study design, estrogen status of the body, metabolism of isoflavones among individuals, and other dietary factors. The long-term safety of soy isoflavone supplements remains to be demonstrated.

Key Words: Soy isoflavones – Menopause – Osteoporosis – Phytoestrogens – Bone mineral density.

Osteoporosis is a progressive systemic skeletal disorder characterized by reduced bone mass and poor bone quality leading to increased bone fragility and fracture risk.[1] Because of increasing life expectancy and consequently the proportion of older individuals in the general population, the prevalence of osteoporosis is expected to increase as well, and it is considered to be a worldwide major health problem with increasing treatment costs.[2] Osteoporosis-related fractures are one of the major causes of morbidity and mortality in postmenopausal women.[3] The mortality of older men and women with hip fractures has increased by 15% to 20%. For white women, lifetime risks of hip, vertebral, and distal radius fractures are 19%, 15.6%, and 16%, respectively, and for women older than 50 years of age, the lifetime fracture risk for any skeletal region is 40%.[2,3] In addition, there are other dire consequences to fractures, including adverse effects on quality of life for many years after the fracture.[4-6]

Risk factors for osteoporosis include female gender, advanced age, family history of osteoporosis, thin body frame, postmenopausal estrogen deficiency, testosterone deficiency in men, cigarette smoking, excessive alcohol consumption, diet low in calcium, sedentary lifestyle, long-term use of drugs such as glucocorticoids and anticonvulsants, anorexia nervosa or bulimia, and white race.[7] Bone loss occurs most rapidly during the years after menopause. Women may lose up to 20% of their bone mass in 5 years after menopause.[7] However, this may be an overestimate as recent studies report a 5.6% bone loss in the 4 years after menopause.[8] Therapies for the prevention and treatment of postmenopausal osteoporosis include agents that inhibit bone resorption (menopausal hormone therapy [MHT] with estrogen alone or a combination of estrogen and progestins, bisphosphonates, raloxifene, and calcitonin) and agents that stimulate bone formation (only teriparatide to date).

Before the publication of Women's Health Initiative (WHI) trial findings, the combination of estrogen with or without progestin therapy was a first-line therapy for the prevention of early postmenopausal bone loss and for the treatment of osteoporosis. The WHI study did not directly address early postmenopausal bone loss. The WHI trial demonstrated a 24% decreased risk of fractures and a 37% decreased risk of colon cancer, but a 26% increased risk of breast cancer and 22% increased risk of total cardiovascular outcomes after an average 5.2 years of use of the estrogen and progestin combination.[9] The trial with an estrogen-alone arm in hysterectomized women demonstrated an increased risk of cerebrovascular events.[10] Even before the WHI trial, compliance to MHT by many women was very low due to the concerns about the risks of therapy. The results of WHI trial only added more uncertainty for both postmenopausal

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TABLE 1. *Observational studies of soy isoflavone intake and bone in humans*

Ref.	Population	Method	Outcome	Findings
[69]	Pre- and perimenopausal women (African American, white, Chinese, Hispanic, and Japanese), n = 3,000	Food frequency questionnaire	BMD of spine, femoral neck, and total hip	Highest genistein intake in Chinese and Japanese women. Positive relationship between BMD and genistein intake in premenopausal Japanese women. No association in other groups
[68]	Pre- and postmenopausal Chinese women, n = 650	Food frequency questionnaire	BMD of lumbar spine and femur	Premenopausal: no association between isoflavone intake and BMD. Postmenopausal: women with highest tertile of isoflavone intake had highest BMD, lowest osteocalcin, N-telopeptide, and PTH
[70]	Postmenopausal Japanese women, n = 85	Food records	BMD of lumbar spine	Soy protein intake positively related with BMD and negatively related with bone resorption
[71]	Postmenopausal white women, n = 208	Standardized questionnaire	BMD of lumbar spine and hip	No significant associations between isoflavone intake and BMD and bone turnover markers
[85]	Postmenopausal Chinese women, n = 24,403	Food frequency questionnaire, prospective cohort of 4.5 y (five quintiles of soy protein intake)	Risk of fracture	Correlation between higher soy protein and isoflavone intake and lower risk of fracture (especially in early menopause)

BMD, bone mineral density; PTH, parathyroid hormone.

women and their physicians. Therefore, research has begun to focus on pharmaceutical and natural alternatives to MHT that could provide the beneficial effects of estrogen on bone, cognitive function, and menopausal symptoms without adverse effects on the breast, uterus, and cardiovascular system.

Selective estrogen-receptor modulators (SERMs) were developed for this reason, and raloxifene has been approved in many countries as the first SERM for the prevention and treatment of postmenopausal osteoporosis. However, raloxifene treatment is not without risk. Besides having as much risk of thromboembolic events as estrogen, it may exacerbate menopausal symptoms. Therefore, both women and physicians remain concerned about the adverse effects of estrogen and are looking to natural products that have the beneficial effects of estrogen. Recent reports suggest that soy phytoestrogens (plant estrogens), namely isoflavones, act like natural SERMs.[11] This review focuses on soy isoflavones, their effects on bone, and their place in the prevention and treatment of postmenopausal osteoporosis. The aim of this review is to explore the mechanism of action of soy isoflavones as reported in in vitro and animal studies, to discuss the results and controversies of observational and interventional studies, and to discuss the place of soy isoflavones in the management of postmenopausal osteoporosis. No attempt has been made to

TABLE 2. *Interventional clinical studies of dietary soy intake and bone*

Ref.	Population	Design/duration	Intervention	Outcome	Findings
[81]	Premenopausal, n = 27	Randomized/12 mo	90 mg/d isoflavone-containing diet or control diet	BMD and BMC of spine and hip	No effects of isoflavones on BMD and BMC
[65]	Postmenopausal white women, n = 65	Randomized, double-blind/ 9 mo (additional 6 mo of follow-up)	Soy with 96 mg/d isoflavones, soy with 2 mg/d isoflavones or soy without isoflavones	BMD of spine and hip	No significant effect of soy isoflavones on BMD
[66]	Postmenopausal white women, n = 202	Randomized, double-blind/ 12 mo	25.6 g soy protein containing 99 mg isoflavones or total milk protein	BMD of spine and hip	No difference in BMD between the groups
[67]	Postmenopausal white women, n = 89	Randomized, placebo-controlled/2 y	Soymilk containing isoflavones, transdermal progesterone; soymilk containing isoflavones and progesterone or placebo	BMD and BMC of spine and hip	No change in BMD and BMC in soymilk containing isoflavones and progesterone groups, but bone loss in placebo and combined treatment groups
[74]	Postmenopausal women, n = 187	Randomized/6 mo	Diet rich in soy, MHT, or control	BMD and BTMs	Decrease in BMD in control but not in intervention groups; increase in osteocalcin in diet group
[76]	Postmenopausal Chinese women, n = 203	Randomized, double-blind, placebo-controlled/1 y	1 g soy + 80 mg isoflavones, 0.5 g soy + 40 mg isoflavones or placebo	BMC and BMD of whole body, spine, and hip	High-dose group had higher increase in hip BMC
[78]	Postmenopausal white women, n = 106	Randomized, double-blind, placebo-controlled/3 mo	Dietary soy supplementation or placebo	Pyridinoline and deoxypyridinoline	No effect of soy on bone resorption

BMC, bone mineral content; BMD, bone mineral density; BTMs, bone turnover markers; MHT, hormone therapy.

rate the quality of studies. A formal systematic review or meta-analysis was not performed because of the many differences in study populations, design, duration, route and dose of administration of soy proteins and/or isoflavones, and outcome variables (Tables 1, 2, and 3). In this review, we have included relevant reports published in the English literature listed in PubMed. After briefly reviewing the food sources of isoflavones and their structure and metabolism, we review preclinical (in vitro and animal) and human (observational and interventional) studies under separate headings. We also list the observational (Table 1) and interventional studies in separate tables (Tables 2 and 3).

FOOD CONTENT, STRUCTURE, AND METABOLISM OF SOY ISOFLAVONES

Isoflavones are a class of plant estrogens (phytoestrogens) found predominantly in legumes. Other classes of phytoestrogens include lignans and coumestans.[12] Soy foods are the most significant source of dietary isoflavones, followed by lentils, kidney beans, lima beans, broad beans, and chickpeas. Isoflavone content and bioavailability of soy products differ and are altered during extraction, processing, and cooking. Defatting, fermentation, and ethanol extraction result in lower isoflavone content.[13,14] Nonfermented soy foods, such as roasted soybeans and soy powder, have two to three times more isoflavones than fermented foods such as miso and tempeh. Low-fat and nonfat soymilk contains significantly lower amounts of isoflavones. However, the baking of soy flour does not alter soy content. Soy flour contains 5 mg isoflavone per gram of protein, whereas tofu and soymilk contain 2 mg isoflavone per gram of protein due to fermentation and defatting processes.[14]

Soy isoflavones are nonsteroidal molecules structurally and functionally related to 17β-estradiol. Three major isoflavones found in soybeans are genistein, daidzein, and glycitein. When they bind to estrogen receptors α and β via their phenolic rings, they may exert agonistic, antagonistic, or partially agonistic/antagonistic effects at the receptor. Isoflavones occur in soybeans as glucosides. When they are consumed, they undergo metabolic changes in the gastrointestinal system. A sugar moiety is removed, and metabolically active aglycons form.[12] One third of aglycon is absorbed as free isoflavone, and two thirds are converted to metabolites such as equol and p-ethylphenol by the intestinal microflora, which are also absorbed. Both free and metabolized isoflavones are excreted by the kidney within 24 hours.[15]

Recent data suggest that equol binds with a greater affinity to estrogen receptors than daidzein from which it is derived.[16] There are large interindividual differences in metabolism of isoflavones. Equol production is largely dependent on intestinal microflora. Some people can produce larger amounts of equol than others and are referred to as "equol producers." Metabolism of isoflavones is also influenced by other components of the diet. A diet rich in carbohydrates results in extensive biotransformation of isoflavones and leads to greater amounts of equol. In the presence of antibiotics that alter intestinal microflora, the metabolism of isoflavones is reduced.[12]

TABLE 3. *Interventional clinical studies of soy isoflavone intake and bone*

Ref.	Population	Design/duration	Intervention	Outcome	Findings
80	Pre- and postmenopausal women, n = 31	Randomized, double-blind, crossover/3 mo	8, 65 or 130 mg/d soy isoflavones	BTM	Premenopausal: increase in IGF-I and IGFBP-3 with low isoflavone diet. Postmenopausal: decrease in B-ALP, no change in IGF-I or IGFBP-3
63	Perimenopausal Japanese women, n = 23	Randomized, placebo-controlled/4 wk	61.8 mg soy isoflavone extract or placebo capsules	Urinary pyridinoline and deoxypyridinoline	Decrease in urinary pyridinoline and deoxypyridinoline
64	Postmenopausal Chinese women, n = 203	Randomized/1 y	Placebo mid-dose (40 mg) or high-dose (80 mg) isoflavones	BMD and BMC at whole body, spine, and hip	Favorable effects of isoflavones on total hip and trochanter BMC
72	Postmenopausal white women, n = 66	Randomized, double-blind, placebo-controlled/6 mo	56 mg/d isoflavones plus casein 90 mg/d isoflavones plus casein or casein only	BMD of lumbar spine femur and total body	Increase in lumbar spine BMD with 90 mg/d isoflavone group
73	Early postmenopausal women, n = 90	Randomized, placebo-controlled/1 y	Genistein 54 mg/d MHT or placebo	BMD of lumbar spine and femur BTM	Increase in BMD in genistein and MHT groups. Decrease in resorption markers in both groups decrease in formation markers in MHT and increase in formation markers in genistein groups
75	Postmenopausal Japanese women, n = 40	Randomized, placebo-controlled/10 wk	37.3 mg/d soy isoflavone or placebo	Bone resorption markers	Decrease in markers in isoflavone group
77	Postmenopausal women, n = 19	Randomized, placebo-controlled, crossover/ 6 mo	Soy isoflavone 110 mg/d or placebo	BTM, BMD, and BMC of spine and hip	Decrease in bone resorption increase in spine BMD in isoflavone group

B-ALP, bone alkaline phosphatase; BMC, bone mineral content; BMD, bone mineral density; BTM, bone turnover markers; MHT, hormone therapy; IGF-I, insulinlike growth factor I; IGFBP-3. insulinlike growth factor binding protein-3.

MECHANISM OF ACTION OF SOY ISOFLAVONES

In vitro studies

Bone remodeling is mediated by two different cell lines: osteoclasts and osteoblasts. Osteoclasts, which are derived from hematopoietic precursors, are responsible for bone resorption, osteoblasts are responsible for bone formation. Osteoblasts respond to changes in the activity of osteoclasts. The actions of the two cell lines are dependent on many hormones, cytokines, and growth factors. Estrogen alters the function of both osteoblasts and osteoclasts. However, its main mechanism of action is suppressing osteoclast life span and function through induction of apoptosis. When estrogen depletion occurs, as in menopause, osteoclast activity exceeds osteoblast activity, leading to a rapid loss of bone mineral and matrix and an increased risk of fractures.

The exact mechanism by which isoflavones alter bone remodeling is incompletely understood. In vitro studies suggest that this effect may be due in part to their estrogenic activity because they act like SERMs. They exert estrogen agonistic, antagonistic, and agonistic/antagonistic effects, depending on conditions at the estrogen receptor. Two estrogen receptors have been identified to date. The α subtype is found in the uterus, ovaries, testes, hypothalamus, kidney, and bone. The β subtype is found in bone, prostate, heart, vessels, ovaries, brain, and bladder. Isoflavones bind to both estrogen receptors but with a greater affinity for β subtypes.[17] In addition to estrogen receptors, isoflavones also bind to androgen receptors, progesterone receptors, oxytocin receptors, and peroxisome proliferator activator receptors.

Studies on the mechanism of action of isoflavones show that they act on both osteoblasts and osteoclasts.[18-20] Sugimoto and Yamaguchi[18] investigated the effects of daidzein in MC3T3-E1 osteoblastic cells. They found that daidzein caused significant increases in DNA content, protein content, and alkaline phosphatase activity, suggesting a stimulatory effect on osteoblasts. Osteoclast activity is regulated by phosphorylation of cell membrane constituents, involving tyrosine kinases.[14] Genistein is one of the naturally occurring tyrosine kinase inhibitors.[21] In a study by Blair et al,[19] the effects of genistein on avian osteoclasts were found to be achieved through inhibition of osteoclastic bone resorption at concentrations that inhibit tyrosine kinase. They could not document similar effects with daidzein. Therefore, they concluded that tyrosine kinase inhibition is a feature of genistein but not daidzein, and it is possibly one of the mechanisms by which genistein inhibits bone resorption. These findings were supported by a separate study in which genistein but not daidzein decreased the secretion of hydrochloric acid, a contributor of bone resorption.[20] This action of genistein was also found to occur via tyrosine kinase inhibition.

Estrogen deficiency, such as that occurs during menopause, results in an increase in the levels of tumor necrosis factor α (TNF-α), a cytokine that is involved in many changes of aging including bone resorption. The discovery of new members of the TNF super family led to increased understanding of what happens during estrogen-repleted and estrogen-depleted states. Osteoblasts express the receptor activator of nuclear factor-κB ligand (RANKL). RANKL is involved in osteoclast differentiation in a paracrine manner by binding to its receptor, which is located on the osteoclast membrane. Osteoblasts also secrete osteoprotogerin (OPG), a decoy factor that neutralizes the actions of RANKL. Estrogen increases the production of OPG and hence decreases the activity of RANKL. Recently, genistein has been found to increase the production of OPG from osteoblasts, providing a further mechanism for the bone-sparing effects of isoflavones.[22,23]

Interleukin (IL)-6 is a proinflammatory cytokine that has been shown to increase in chronic inflammation, diabetes mellitus, cardiovascular disease, rheumatoid arthritis, Alzheimer's disease, osteoporosis, aging, and some cancers. The expression of IL-6 is thought to occur via the transcription factor nuclear factor κB (NF-κB).[24] Isoflavones have been shown to inhibit NF-κB activation and indirectly inhibit IL-6 production in osteoporosis,[23] arthritis,[25] and some cancers such as breast, prostate, lymphoma, and pancreas.[23,25-30]

In addition to coupled osteoblast-osteoclast activities, isoflavones seem to act independently on osteoclasts via non-estrogenic mechanisms because there are no estrogen receptors in the nuclei of osteoclasts. Isoflavones act on osteoclasts by inhibition of tyrosine kinase as previously mentioned,[19,20] inhibition of topoisomerase I and II,[22,31] and induction of apoptosis.[32] Other postulated mechanisms of action for isoflavones include inhibition of angiogenesis,[33] inhibition of free radical formation,[34] stimulation of antioxidant enzymes,[35] reduction of lipid peroxidation,[36] and inhibition of aromatase,[37] 5α-reductase,[38] and 17β-hydroxysteroid dehydrogenase enzymes.[39]

Although the mechanisms of isoflavones are still not completely known, evidence from these in vitro studies suggests that they act in multiple ways, via genomic and

TABLE 4. *Potential mechanisms of soy isoflavone action on bone*

Bind to ERs, with greater affinity to ER-β than ER-α
Have selective estrogen-receptor modulator–like actions on ER
Bind progesterone, androgen, oxytocin, and peroxisome proliferator activator receptors
Alter RANK-RANKL-OPG pathway (increase OPG and decrease RANKL)
Inhibit nuclear factor-κB activation
Inhibit production of TNF-α, IL-1, and IL-6
Inhibit protein tyrosine kinases (genistein)
Inhibit topoisomerase I and II
Inhibit aromatase, 5α-reductase, and 17β-hydroxysteroid dehydrogenase
Increase the activity of antioxidant enzymes
Inhibit free radical formation and lipid peroxidation
Induce apoptosis of osteoclasts
Inhibit angiogenesis
Increase intestinal calcium absorption
Modulate IGF-I and IGFBP-3 levels

ER, estrogen receptor; IGF-I, insulinlike growth factor I; IGFBP-3, insulin-like growth factor binding protein-3; IL, interleukin; OPG, osteoprotogerin; RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator of nuclear factor-κB ligand; TNF-α, tumor necrosis factor α.

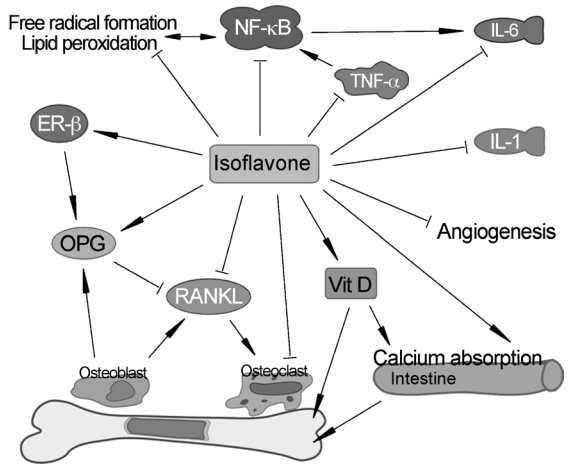

FIG. 1. Mechanisms of action for soy isoflavones.

nongenomic pathways and via both osteoblasts and osteoclasts, to maintain bone mass. Table 4 and Figure 1 summarize the mechanisms of action of isoflavones.

Animal studies

While in vitro studies provide clues to the effects of isoflavones on individual osteoblasts and osteoclasts, in vivo studies provide knowledge about the effects of isoflavones on an intact system. However, as discussed previously, the intestinal metabolism of isoflavones varies considerably among individuals with regard to equol production.[15,16] Therefore, one might expect differences in results of animal and human studies. The ovariectomized rat is a good model for studying postmenopausal osteoporosis. For this reason, most animal studies are performed on ovariectomized rats, and limited data exist for pigs and monkeys. End points of these studies are bone mineral density (BMD), mechanical strength, markers of bone turnover, and uterine weight. The actual design differs among individual studies; they use either pure isoflavones (such as genistein and daidzein) or soy proteins with or without isoflavones, and comparisons are made with casein, semipurified diets, or conjugated estrogen. The routes of administration are also different: by subcutaneous injection, gavage, or orally.

Arjmandi et al[40] compared soy protein isolate with either casein or 17β-estradiol and found that soy protein improved BMD by 15% compared with control and was as effective as estradiol. However, they could not demonstrate whether this improvement was due to the protein itself or the presence of soy isoflavones. Isoflavone content made the difference in improving BMD, and the bone-sparing effect might be due to increased intestinal calcium absorption.[41] In another study by Arjmandi et al,[42] there was no effect of soy on bone mass in ovariectomized rats, which may have been due to the short duration of the study. However, femoral mRNA expression of insulinlike growth factor I (IGF-I), which is a marker of bone formation, was found to be increased with soy.

Fanti et al[43] reported that genistein prevented both trabecular and cortical bone loss in ovariectomized rats. Genistein increased bone formation, osteoblast number, and osteocalcin level and blocked the increased production of TNF-α, which might be responsible for bone-sparing effects of genistein. Another possible mechanism of genistein is thought to be through the regulation of B lymphopoiesis. B lymphocytes increase after ovariectomy and secrete TNF-α, IL-1, and IL-6, which are involved in bone loss related to estrogen deficiency. Ishimi et al[44] found that the increased lymphopoiesis after ovariectomy was reversed by both genistein and 17β-estradiol. They also documented that genistein prevented trabecular bone loss. The findings of Fanti et al[43] and Ishimi et al[44] suggest an anti-inflammatory effect of genistein in preventing ovariectomy-induced bone loss. Ishimi et al[44] also reported a dose-response effect for pure isoflavones. However, Anderson et al[45] reported that a lower dose of genistein was more effective on bone than a high dose.

The bone-preserving effects of soy isoflavones are also confirmed by other studies performed on ovariectomized rats[46] and mice.[47,48] Soy isoflavones have been found to prevent cadmium-induced bone loss when given alone[49] or together with n-3 polyunsaturated fatty acids in ovariectomized rats.[50] Ovariectomy significantly decreased intestinal calcium absorption, which was prevented by soy protein with normal isoflavone content.[51] In a study by Fonseca and Ward,[52] the combination of daidzein and a high-calcium diet preserved femur and vertebra BMD and mechanical strength; however, much of this effect was mediated by the high-calcium diet. In a separate study, they compared high calcium plus soy protein with soy or high calcium alone. The only outcome that significantly benefited from the combination diet was lumbar vertebra BMD.[53] In contrast, some studies showed that soy isoflavones had minimal or no effect on bone loss in animal models.[54-58] Bahr et al[54] found that soy protein and isoflavones had no effects on BMD, deoxypyridinoline levels, and the reproductive tract of ovariectomized Sprague-Dawley rats except for statistically significant changes in bone histomorphometric parameters in a high isoflavone extract group. Picherit et al[55] found that soy isoflavones reduced bone turnover but failed to reverse established bone loss. They concluded that isoflavones might have a preventive rather than curative role in the management of osteoporosis. However, Nakai et al[56] found no significant beneficial effect of soy isoflavones on femur and lumbar vertebra BMD and deoxypyridinoline levels in the presence of estrogen. There was a negative effect on the uterus. However, the negative effect could not be confirmed on intact female Fischer 344 rats, known as high responders to estrogenic stimuli.[57] These results raise the question of whether there are species-specific differences in the effects of isoflavones. A 3-year longitudinal study in ovariectomized female monkeys revealed no beneficial effects of soy isoflavones.[58] However, most animal studies support in vitro findings that soy isoflavones modulate bone turnover and prevent or reverse bone loss in estrogen depletion. There may be species-specific differences in the effects of isoflavones,

and the action of isoflavones may vary according to estrogen status of the body.

OBSERVATIONAL AND INTERVENTIONAL HUMAN STUDIES

Interest in investigating potential benefits of isoflavones on bone mass came from the data on bone-sparing effects of a synthetic isoflavone, ipriflavone, which has been shown in several studies to suppress bone resorption, increase calcium retention in bone, and augment the effects of estrogen on bone.[59,60] Ipriflavone is structurally similar to soy isoflavones. One of its metabolites, daidzein, is responsible for an estimated 10% of the actions of ipriflavone. Therefore, ipriflavone in doses between 600 and 1,200 mg/d seemed an alternative to MHT for postmenopausal women. However, a recent 3-year multicenter study established that it is no better than placebo in preventing bone loss and bone fractures in postmenopausal women, and some women developed lymphocytopenia during ipriflavone treatment.[61]

The lower rate of hip fractures among Japanese women in comparison to US women, despite having similar or lower BMD measurements, is thought to be mainly due to dietary factors. Japanese women seem to consume more soy products than US women, and this observation led researchers to perform epidemiological and interventional studies with soy isoflavones in different ethnic populations. However, factors other than diet, such as shorter hip axis length of Japanese women and less tendency to fall, seem to play a role in lower rates of hip fractures among Japanese women.[62]

During the past few years, numerous studies evaluating the effect of soy protein–containing isoflavones or pure isoflavones have been published. The results vary greatly due to the types of study design used and whether they are epidemiology-based or randomized, controlled clinical trials. As discussed previously, the estrogen status is also important. Even years since menopause is a confounding factor because bone turnover is markedly elevated during the early years of menopause.[63-66] Type, dose, and metabolism of individual isoflavones may also differ, making it difficult to compare the results from different studies. As mentioned previously, the ability to produce equol also differs among individuals.[12,15,16] Those who are equol producers may benefit most from isoflavones. To date, there is only one clinical trial that distinguished the equol producers who were demonstrated to have a greater BMD and bone mineral content in a 2-year intervention of soymilk.[67] Lack of dietary control and confounders in the diet that may affect bone mass—such as calcium, vitamin D, and protein—make it difficult to interpret the results of these studies.

Observational studies of dietary soy intake and osteoporosis

Mei et al[68] used a food frequency questionnaire to assess phytoestrogen intake in 650 Chinese women between the ages of 19 and 86 years. Lumbar spine and femoral BMDs were compared according to tertiles of phytoestrogen intake. Among postmenopausal women, those with the highest tertile (53.3 mg/d) had significantly higher BMDs and lower osteocalcin, urinary N-telopeptide, and parathyroid hormone levels than women with lowest or medium tertiles. In premenopausal women, no association was found between BMD values and phytoestrogen intake. This study shows the SERM-like actions of phytoestrogens because they act like estrogen agonists in that endogenous estrogen is depleted such as in postmenopausal women and as estrogen antagonists in that endogenous estrogen is present such as in premenopausal women. Another possible explanation is that, in premenopausal women, estrogen receptors are saturated and additional phytoestrogens have no further benefit.

One of the most comprehensive studies that assessed dietary soy intake is the Study of Women's Health Across the Nation (SWAN).[69] This is an National Institute of Health sponsored multicenter, nationwide study in the United States that included women who had menstrual periods within the past 3 months and who were not on any MHT. Women were 42 to 52 years of age and of five ethnic origins: African American, white, Chinese, Hispanic, and Japanese. Dietary isoflavone consumption was assessed by a food frequency questionnaire, and BMD of the spine, femoral neck, and total hip was measured. African American and white women reported either no or very low amounts of soy intake. Therefore, they were excluded from the analysis. The highest intake of genistein was among Chinese and Japanese women, with the latter being higher. There was a positive dose-response relationship between BMD and genistein intake for premenopausal Japanese women, but not for Chinese women or perimenopausal Japanese women. This study emphasizes the importance of ethnicity and menopause status on the effects of genistein. Japanese and Chinese women had different BMD responses despite similar intake of dietary genistein. The authors propose that Japanese women could have a higher number of osteoblast estrogen receptors, their diet might contain more readily absorbed forms of genistein, or they might metabolize genistein more efficiently. Although a food frequency questionnaire has limitations, this study found a positive dose-response relationship between BMD and genistein intake in premenopausal Japanese women.

Another study performed on 85 postmenopausal Japanese women revealed that soy protein intake was a significant determinant of BMD and a major contributor to suppression of bone resorption.[70] In contrast to the Japanese women, data regarding the soy consumption among US women differ. The Soy Health Effects study evaluated 208 postmenopausal women aged 45 to 74 living in southern California.[71] The authors found no significant association between soy intake or bone turnover markers and BMD.

Interventional studies of soy diet and soy isoflavone supplements

There are many published reports of randomized, controlled studies on humans investigating the effects of soy isoflavones on bone. These studies usually included only

postmenopausal women. One of the earliest reports was a 6-month study by Potter et al[72] who reported that 90 mg/d isoflavones significantly increased BMD of the lumbar spine. Women receiving 56 mg/d or casein had no significant increases in lumbar spine BMD. There were no changes in proximal femur and total body BMD in any of the groups. However, this was a short-term study with a small number of subjects. Morabito et al[73] evaluated the effects of isolated genistein in early postmenopausal women aged 47 to 57 years in a randomized, placebo-controlled trial of 1-year duration. The women were assigned to MHT, genistein (54 mg/d), or placebo. Urinary pyridinoline and deoxypyridinoline levels decreased significantly in the genistein group, and the reductions were comparable with those of the MHT group. There were significant increases in bone alkaline phosphatase and osteocalcin at 6 months in the genistein group, and their levels remained elevated throughout the study. However, bone alkaline phosphatase and osteocalcin decreased significantly in the MHT group. Both the genistein and the MHT group had increases in lumbar spine and femoral neck BMD. This study highlighted the difference of estrogen and genistein in bone metabolism. Both estrogen and genistein decreased bone resorption markers. However, increases in bone formation markers were observed only with genistein. This effect of genistein is consistent with the results of in vitro studies suggesting an anabolic role of soy isoflavones on osteoblasts.[18,22,23]

More recent studies continue to show conflicting results of soy isoflavones in postmenopausal women, most being in favor of soy[63,64,66,67,74-77] and a few showing no beneficial effect of soy on bone.[65,78] The results were affected by factors such as the study design and duration, initial BMD, years since menopause, body weight, and calcium intake. In one double-blind pilot study of 15-month duration, no effect of soy protein isolate was found in early postmenopausal women.[65] The authors supplemented soy protein for 9 months and then discontinued. BMD was measured 6 months after discontinuation. This study raises the question of whether continuous supplementation of soy protein is necessary for beneficial effects on bone. In a 3-month study, dietary soy supplementation had no effect on bone turnover markers but favorable effects on lipid profiles in postmenopausal women.[78] The authors suggested that dietary soy supplementation in postmenopausal women might have estrogenic effects on lipid profile but might not have estrogenic effects on bone resorption. In a study by Huang et al,[79] 12 postmenopausal women were given soymilk containing isoflavones daily for 16 weeks. Blood levels of TNF-α, IL-1, and IL-6 were measured before, during, and after soymilk consumption. TNF-α was decreased by 66.7% after 10 weeks and returned to prediet levels 4 weeks after the termination of soy consumption. IL-1 levels and monocyte count decreased by 56.6% and 14.4%, respectively. However, there were no significant changes in IL-6 levels. In cultures of monocytes from these women, soy isoflavones decreased lipopolysaccharide-induced TNF-α production by 55%. These results are consistent with the results of in vitro and animal studies that suggest a role for TNF-α and other inflammatory cytokines in the pathogenesis of age-related diseases such as osteoporosis.[22,23,43,44] Although the effects of soy isoflavones on intermediate markers of bone and lipid metabolism and inflammatory cytokines are interesting, the effects on hard end points such as endometrial hyperplasia, breast cancer, cardiovascular events, and bone fractures are lacking.

There are fewer studies performed in premenopausal women. If soy isoflavones increased BMD in premenopausal women, we might expect that they would increase peak bone mass. However, as discussed previously, there are some differences in the effects of soy isoflavones due to endogenous estrogen levels. In a 3-month dietary intervention trial, three soy protein isolates containing 8, 65, and 130 mg/d isoflavones were given to pre- and postmenopausal women. IGF-I and IGF binding protein-3 levels were increased in premenopausal women with a low isoflavone diet. Levels of deoxypyridinoline were higher in the early follicular phase than mid-follicular or periovulatory phases with both low- and high-isoflavone diets. In postmenopausal women, only bone alkaline phosphatase levels were reduced significantly. Osteocalcin, IGF-I, and IGF binding protein-3 decreased only to a minor and nonsignificant extent.[80] In another study, 12 months' intake of soy protein enriched with isoflavones of approximately 90 mg/d was compared with soy protein that is isoflavone deficient in healthy premenopausal women aged 21 to 25 years with normal menses.[81] No changes were noted in bone mineral content and BMD of spine and hip in either group at the end of 12 months, supporting the results of previous epidemiological studies that demonstrated no beneficial effects of isoflavones on bone in premenopausal women when the endogenous estrogen level is high enough to saturate estrogen receptors. The results from epidemiological and randomized clinical trials suggest that early intervention with soy isoflavones to augment peak bone mass during the premenopausal period may not have beneficial effects on bone.

Animal studies have shown that one of the beneficial effects of isoflavones on bone is its positive effect on intestinal calcium absorption,[51] and several human studies on the relationship between soy consumption and calcium metabolism have been reported. Breslau et al[82] reported that participants excreted 150, 121, and 103 mg calcium per day with animal protein, soy and animal protein, and soy protein diets, respectively. They concluded that it was due to a lower content of sulfur-containing amino acids in soy protein. In a study by Spence et al,[83] urinary calcium excretion was significantly lower with a soy protein diet than with casein-whey control diet. However, they could not demonstrate any difference in total fecal calcium excretion, calcium retention, and bone deposition and resorption. In another study comparing soy and meat protein, no differences among calcium retention, urinary calcium excretion, and bone turnover were noted among the treatment groups.[84] Therefore, when evaluating the effects of soy isoflavones on

calcium metabolism, all aspects of calcium metabolism must be taken into consideration.

To date, there is only one study examining the association between soy intake and the risk of fracture. The Shangai Women's Health Study is a population-based, prospective cohort study of 75,000 Chinese women aged 40 to 70 years.[85] Only 24,403 postmenopausal women who had never used MHT and reported no history of fractures and cancer were included in the analysis. Dietary intake of soy and its isoflavones was assessed by a food frequency questionnaire at baseline and during biennial visits. After a mean follow-up of 4.5 years, only 1.3% of women were lost to follow-up, and 1,770 incident fractures were identified. The women were divided into five quintiles of soy protein intake. Results revealed that a higher soy protein and isoflavone intake was associated with a lower risk of fracture. The inverse association was more pronounced among women with early menopause. This finding further confounded the knowledge about the timing of soy isoflavones to prevent postmenopausal bone loss. A limitation of the study is that it was based on self-reports. Certain fractures, such as vertebral fractures, might have been underestimated, whereas others, such as high-impact trauma fractures, might have been overestimated. Although the sample size was large enough to evaluate fracture incidence, it lacked adequate power to evaluate site-specific fractures. Nevertheless, it has a population-based, prospective design with large sample size and high participation rate and provides the first evidence of an inverse relationship between soy food consumption and fracture risk.

SAFETY

Because soy isoflavones have estrogen-like activity, clinicians are often confounded about their safety. There are studies reporting infertility in sheep and cheetahs that were fed high-phytoestrogen diets, compromised follicular development and shortened luteal phase in ewes, cystic ovaries in cattle, and reduced breeding in California quails.[86] In a randomized, double-blind, placebo-controlled study in 376 postmenopausal healthy women, Unfer et al[87] reported an increased occurrence (3.37% vs 0%) of endometrial hyperplasia in women who received 150 mg soy isoflavones daily for 5 years compared with women who received placebo. Data about the effects of soy products on breast tissue are also inconsistent. Other potential problems may be alterations in the sex steroid pathway[37,39] and thyroid hormone production.[88,89] Most products of phytoestrogens do not seem to have harmful effects in humans in short-term studies. Supplementation of soy formula for infants has been used for decades without toxic effects and growth retardation.[90] There is evidence that consuming soy foods has many benefits and no adverse effects among people who consume large amounts of soy products throughout their lives in Asia. However, consuming soy isoflavone supplements is not the same as eating soy foods. Therefore, long-term studies are needed to determine the safety of soy isoflavone supplements in humans.

CONCLUSION

Soy foods and soy isoflavone supplements are consumed by many women to prevent osteoporosis and other age-related diseases. Soy isoflavone supplements are regarded as natural phytoestrogenic products that can be used as an alternative to MHT. In vitro studies have shown that they have antiresorptive and anabolic effects on bone. Most animal studies have documented that they can prevent bone loss in ovariectomized rats, a good model for postmenopausal osteoporosis. Epidemiologic and randomized clinical trials in humans have conflicting results due to differences in study designs, estrogen status of the body, metabolism of the isoflavone used, and other dietary factors. Most clinical studies in humans demonstrate that they have favorable effects on BMD and bone turnover markers in postmenopausal women. However, the effects on bone fracture and the safety of long-term soy isoflavone supplement use need to be established. Based on the available literature, it is impossible to make an accurate estimate of a treatment effect and to make treatment recommendations at this time. Further clinical studies are needed to assess the role of soy isoflavones in the prevention and treatment of postmenopausal osteoporosis.

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