Soy and its Isoflavones: A Review of their Effects on Bone Density

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Abstract

Menopausal hormone decline contributes significantly to the risk of osteoporosis. Therapies for treating osteoporosis, such as hormone replacement therapy (estrogen or combination estrogen-progestins), inhibit bone resorption. Both animal and human studies demonstrate phytoestrogenic soy isoflavones favorably impact bone health. The exact mechanism is still unclear. Additional research is needed to determine if isoflavones are an effective alternative to hormone replacement therapy for the prevention and treatment of osteoporosis. This paper reviews *in vitro*, animal, and human studies involving isoflavones and bone health.

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Osteoporosis – General Overview and Statistics

The menopausal transition is characterized by a rapid decline in ovarian function and a subsequent decline in circulating hormones, including estradiol. This hormone-deficient state contributes to significant risk for developing osteoporosis in postmenopausal women.[1] Osteoporosis poses a major health threat to more than 28 million Americans, 80 percent of whom are women. Currently, in the United States, 10 million people have osteoporosis, and 18 million more have low bone mass, placing them at an increased risk for osteoporosis.[2] One in two women and one in eight men over age 50 will experience an osteoporosis-related fracture in their lifetime.[2] Osteoporosis is responsible for more than 1.5 million fractures per year, including 300,000 hip fractures, 700,000 vertebral fractures, 250,000 wrist fractures, and 300,000 fractures at other sites.[2]

Risk factors for developing osteoporosis include the following: female gender, thin and /or small frame, advanced age, family history of osteoporosis, postmenopausal (including early or surgically-induced menopause), amenorrhea, anorexia nervosa or bulimia, a diet low in calcium, long-term use of medications such as corticosteroids and anticonvulsants, low testosterone levels in men, an inactive lifestyle, cigarette smoking, excessive use of alcohol, and Caucasian or Asian heritage (although African Americans and Hispanic Americans are at significant risk as well).[2]

Bone loss occurs most rapidly during the years immediately prior to and after menopause.[3] In the 5-7 years following menopause, women can lose up to 20 percent of their bone mass.[2] Several bone protective actions exerted by estrogen include increased synthesis of $1,25(OH)_2$ vitamin D, control of bone-resorbing cytokine production, and decreased bone sensitivity to parathyroid hormone.[4] Conventional therapies for treating osteoporosis in women have emphasized agents that inhibit bone resorption[5] and include hormone replacement therapies, either estrogen alone (ERT) or combination estrogen and progestogens (HRT), calcitonin, raloxifene, and bisphosphonates.[6] Despite its potential benefits, hormone replacement therapy has significant risks, including increased occurrence of thromboembolic events, endometrial cancer (with unopposed estrogen), gall bladder and liver disease, and breast and ovarian cancers. Long-term compliance is poor due to side effects and patient concern about risks.[1] It has also been noted that the protective effect of estrogen

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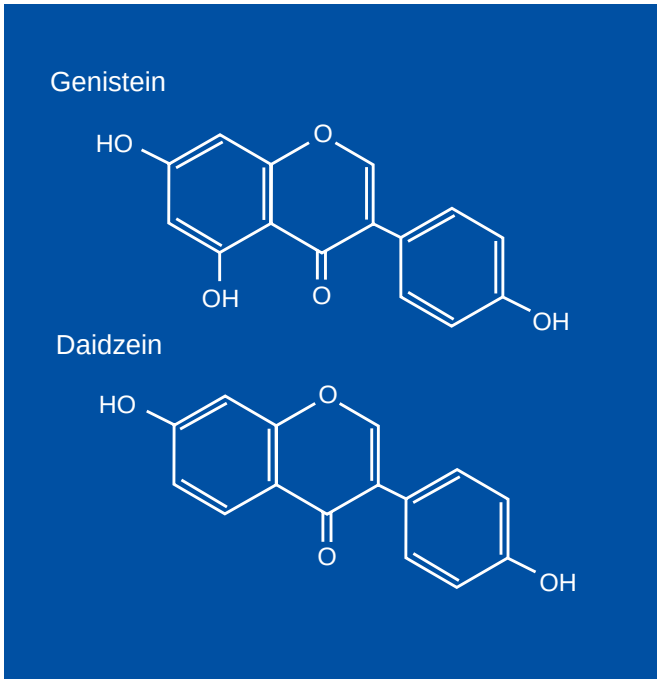
Figure 1. *Structures of Soy Isoflavones: Genistein and Daidzein*



on vertebral and hip fractures in women is diminished with prolonged use.[7] Therefore, there is a need for alternative treatments to HRT that provide benefits to bone without adverse effects. Data from animal studies suggest that soy isoflavones, possibly due to their structural similarity to estrogen, may protect against bone loss occurring as a result of estrogen deficiency.

Isoflavones – General Overview

Isoflavones are a subclass of flavonoids with a chemical structure similar to 17β-estradiol, the most potent, naturally occurring estrogen.[8,9] Isoflavones bind to estrogen receptors, affecting estrogen-regulated processes, and are therefore referred to as phytoestrogens (plant estrogens).[3,10] Many of their effects, however, may not involve the estrogen receptor.[9] Isoflavones are extremely limited in nature, found in nutritionally significant amounts only in soy.[1,3,11,12] The main isoflavones in soybeans are genistein and daidzein (Figure 1).[14] A third isoflavone, glycitein, is also present, although in much smaller amounts.[10]

Cells vary in their distribution of the classic estrogen receptor alpha (ERα) and the newly discovered ERβ, depending on the tissue.[11] For instance, reproductive cells, especially those of the uterus and breast, are abundant in ERα,[1,11,13] whereas, bone tissue has greater amounts of ERβ.[13] Genistein binds with a much greater affinity to ERβ than to ERα[1,10,11,13] The different tissue distribution of α- and β-receptors points to the possibility of tissue-selective effects of the isoflavones,[1] as they appear to have different effects in different tissues.[10] Isoflavones, therefore, could influence several biological processes controlled by estrogen, including bone metabolism.[14] The occupancy time and affinity of isoflavones for the ERα receptor is much less than that of estradiol.[15]

Food Content of Isoflavones

Isoflavone content and bioavailability vary among different soy products and are easily altered during extraction, processing, and cooking.[16-18] Soybeans contain 2-5 mg of isoflavones per gram of protein. Dehulling, flaking, and defatting to produce isolated soy protein results in lower isoflavone content. Textured soy protein and soy flour contain approximately 5 mg of isoflavone per gram of protein; soy milk and tofu contain approximately 2 mg of isoflavone per gram of protein. Non-fermented soy foods, such as roasted soybeans and soy beverage powders, have two to three times the total amount of isoflavones, compared to fermented soy foods (tempeh, miso, fermented bean curd).[19] Low-fat and nonfat soy products are significantly depleted of isoflavones, and alcohol-washed soy protein concentrates contain few isoflavones. On the other hand, baking of soy flour does not alter isoflavone content.[16]

Besides the isoflavone effects of soy, another manner in which ingestion of soy foods may favorably affect bone health is related to protein consumption and calcium excretion. A high-protein diet has been shown to increase urinary calcium excretion, thereby leading to osteoporosis.[20] The hypercalciuric effect of protein

Table 1. In vitro Studies of Soy Isoflavones and Bone Markers

Author, Year	Cell Types	Study Design	Outcome Measure	Intervention	Findings
Blair et al; 1996	Avian osteoclastic cells	*In vitro*	Protein synthesis in osteoclastic cells	Genistein	↓ Protein synthesis; inhibition of osteoclastic activity
Williams et al; 1998	Avian osteoclastic cells	*In vitro*	Osteoclastic HCl secretion	Genistein	↓ Acid secretion (osteoclast antagonism)
Sugimoto et al; 2000	Osteoblastic MC3T3-E1 cells	*In vitro*	Protein content, alkaline phosphatase activity, DNA content of osteoblastic cells	Daidzein	↑ Protein content, alkaline phosphatase activity, and DNA content

is primarily attributed to the metabolism of the sulfur-containing amino acids methionine and cysteine.[21,22] Because soybeans are relatively low in sulfur amino acids, this would suggest that soy protein is less hypercalciuric than animal protein. There may be other mechanisms, however, whereby a diet rich in animal protein may cause hypercalciuria.[20] The clinical significance, however, of substituting soy foods for animal foods on calcium balance and bone health depends on the relative level of intake.

In vitro Studies

Sugimoto et al[23] investigated the effect of daidzein on osteoblastic MC3T3-E1 cells *in vitro*. Osteoblastic MC3T3-E1 cells were cultured and placed in medium containing various concentrations of daidzein. The presence of 10^{-5} M daidzein caused a significant increase in protein content. This effect was prevented completely by the presence of the anti-estrogen tamoxifen and the protein synthesis inhibitor cyclohexamide. Osteoblastic MC3T3-E1 cells cultured in the presence of daidzein (10^{-7}-10^{-5} M) caused a significant increase in alkaline phosphatase activity, which is a marker enzyme in osteoblastic cell differentiation, a reflection of bone formation. Thus, daidzein may have a stimulatory effect on the proliferation and differentiation of osteoblastic MC3T3-E1 cells.[23]

The effect of genistein on alkaline phosphatase activity in osteoblastic MC3T3-E1 cells was also examined. Alkaline phosphatase activity in cells was increased significantly in the presence of genistein. Thus, the effect of genistein in osteoblastic cells appears to be the same as that of

daidzein. Also noted in this study was a significant increase in DNA content of osteoblastic cells in the presence of daidzein, suggesting isoflavones stimulate cell proliferation.[23]

Blair et al[24] studied the effects of genistein as a naturally occurring tyrosine kinase inhibitor, which in turn inhibits bone resorption. Osteoclast activity, an indicator of bone resorption or bone loss, is regulated by phosphorylation of cell membrane constituents, involving tyrosine kinases. The osteoclastic membrane includes the receptor $pp60^{c\text{-}src}$, which is essential for bone resorption.[25] The study examined the effects of genistein on avian osteoclasts. Genistein was found to inhibit osteoclastic bone resorption at concentrations consistent with tyrosine kinase inhibition. The mechanisms of genistein inhibition were compared to mechanisms of other osteoclast inhibitors that adsorb to bone.[24] They concluded that genistein inhibited osteoclastic activity directly, by a mechanism independent of cellular attachment, and at doses similar to those inhibiting tyrosine kinase autophosphorylation. The researchers concluded at least one of genistein's actions on bone was via tyrosine kinase inhibition.

Williams et al[26] cultured *in vitro* osteoclast membrane in the presence of genistein. They examined osteoclastic hydrochloric acid secretion, which is responsible for dissolving bone mineral, to determine whether this process is regulated by tyrosine kinase activity, and found genistein reduced acid secretion by osteoclastic membranes. See Table 1 for summary of *in vitro* studies.

Animal Studies

The effects of isoflavones on skeletal tissue of experimental animals have been highly consistent. The U.S. Food and Drug Administration has accepted the ovariectomized (OVX) rat model as a model for studying osteoporosis;[3] thus, most rodent experiments have employed ovariectomized animals to study the effects of soy isoflavones.

Arjmandi et al[5] conducted a randomized, controlled trial on thirty-two 95-day-old Sprague-Dawley rats in order to examine whether soy protein isolate was effective in preventing bone loss due to ovariectomy, and if so, whether it functioned in a similar manner to estrogen. Rats were randomly divided into four groups: sham-operated group, OVX group, OVX + soybean group, and OVX +17β-estradiol group. Right femurs and fourth lumbar vertebrae were analyzed for bone mineral density (BMD) utilizing Archimedes' principle. In this procedure each bone was put in an unstopped vial filled with deionized water and all air removed. Bones were then removed from the vials, blotted, weighed and returned to the vial. The bone was reweighed in water and the density was calculated via g/cm^2 bone volume. Results showed that ovariectomy caused atrophy of the uterus that was prevented by 17β-estradiol, but not soybean protein. OVX rats had significantly lower densities of the right femurs and fourth lumbar vertebra compared with the sham group. Rats treated with 17β-estradiol had either similar or significantly higher BMD than rats in the sham group or the OVX + soybean group. Rats fed the soybean diet had significantly higher mean bone densities of the right femur and fourth lumbar vertebra than rats in the OVX-only group. This study leaves unanswered whether the protective effect of soybean protein isolate is due to the protein itself or to the presence of isoflavones in soybeans.

Arjmandi et al[27] conducted a subsequent randomized, placebo-controlled trial, utilizing forty-eight 95-day-old female Sprague-Dawley rats, to determine whether the isoflavones in soy protein are responsible for soy's bone-sparing effects. Rats were divided into four groups: sham-operated fed a casein-based diet, ovariectomized fed a casein-based diet (OVX+casein), ovariectomized fed soy protein isolate with a moderate isoflavone content (OVX+soy), and ovariectomized fed soy protein isolate with a substantially reduced isoflavone content (OVX+soy-). The isoflavone content of the low-isoflavone soy group was approximately 10 percent that of the moderate-isoflavone diet (meaning approximately 90 percent of the isoflavones were removed from the isolate in the low-soy group).

At the end of the treatment period, the bone volume and density of the femurs were measured by Archimedes' principle. Alkaline phosphatase (an indicator of bone formation) was higher in all OVX groups as would be expected, and was significantly different from that of the sham group in those fed the soy diet containing moderate isoflavones. During the 35-day period post-surgery, the OVX groups had a significant reduction in femoral bone density of 4.8 percent compared to the sham group. The moderate, but not the low-soy, treatment prevented this decrease in bone density, indicating the isoflavone content made the difference in bone loss. The rate of bone formation was higher in the OVX groups than the sham group, and was not significantly different in either OVX plus soy groups, indicating that isoflavones did not slow the increased rate of bone turnover with ovariectomy in this study population. The authors speculated the positive effect of soy on bone may be partially due to increased intestinal calcium absorption. Part of this study looked at the *in vitro* uptake of calcium, which indicated that soy, with various isoflavone contents, stimulated duodenal calcium transport. The study also did not show any uterotrophic activity from the isoflavone intake. The research suggests that isoflavones may act similarly to tamoxifen, which is similar in structure to isoflavones and has anti-estrogenic effects on breast tissue and pro-estrogenic effects on bone.

In another study by Arjmandi et al,[28] seventy-two 95-day-old female rats were randomly divided into six groups of 12 rats each and were either sham-operated (2 groups) or ovariectomized (4 groups). Thirty-five days after surgery, one sham and one OVX group were sacrificed to verify bone loss. Of the remaining groups, one sham and one OVX group received a casein-based diet. The other two OVX groups received diets in which casein was replaced with soy protein isolate with either normal or reduced isoflavone content. The isoflavone content of the low-soy group was approximately 10-percent that of the moderate-soy diet. The treatment period was 65 days. BMD was assessed at the right femur and fourth lumbar vertebra utilizing Archimedes' principle.

This study did not show any significant effects of soy on bone density. There was, however, a significant increase in IGF-1 mRNA in the normal isoflavone- and the reduced isoflavone-treated groups in comparison with the OVX+casein-fed group. This effect was greater in the moderate isoflavone group than the reduced isoflavone group. IGF-1 stimulates bone formation. The authors concluded the lack of bone loss reversal may have been due to the study's short duration. They suggest once bone loss has occurred, long-term consumption of soy may be needed to produce an effect. This study also showed soy feeding did not inhibit ovariectomy-induced declines in serum 17β-estradiol concentrations or uterine atrophy, indicating an absence of true estrogenic activity of soy or its isoflavones.

Blair et al[24] tested the hypothesis that genistein may be an osteoclastic inhibitor at pharmacologically attainable levels. They used OVX rats fed a diet containing 44 micromol/day genistein. Controls were fed an identical diet with the isoflavones removed. Their results showed, with approximately 95-percent confidence, genistein was effective in reducing bone loss in OVX rats.

Ishimi et al[29] examined the effect of genistein on hemopoiesis and bone metabolism in OVX mice to determine whether genistein had an estrogenic effect on bone and bone marrow. Estrogen deficiency stimulates B-lymphopoiesis, which in turn stimulates bone resorption.[29] Eight-week-old female mice were either sham-operated or ovariectomized. OVX mice were administered either genistein (0.1-0.7 mg/day) or 17β-estradiol for 2-4 weeks. Control mice were treated with a vehicle solution. Bone marrow cells were analyzed from the tibiae; bone density of the femur was measured by dual-energy x-ray absorptiometry (DEXA). Microcomputed tomography (mCT) was used to analyze bone volume, trabecular thickness, and trabecular separation. Genistein restored the OVX-induced stimulation of bone marrow hemopoiesis, indicating that genistein, at the dose administered, regulated bone marrow hemopoiesis without exhibiting estrogenic action on the uterus.

The mineralized cancellous bone of the femur, as visualized on x-ray, was significantly decreased in the OVX mice. Treatment with genistein or estrogen significantly prevented the bone loss. BMD, as measured with DEXA, was significantly reduced in OVX mice and was significantly restored with genistein treatment. Confirmation of the bone mass was performed using mCT. Histological sections of the femur showed a significant increase in osteoclastic activity in OVX mice, levels restored with genistein treatment.

Table 2. *Animal Studies on Soy Isoflavones and Bone Density*

Author, Year	Population, Number	Study Design	Outcome Measure	Intervention, Study Length	Findings
Arjmandi et al; 1996	OVX rats (n=32)	Prospective RCT	BMD of femur and 4th lumbar vertebrae	Soybean protein isolate; 30 days	↑ Bone density
Blair et al; 1996	OVX rats	Prospective controlled trial	Femoral weight	44 micromol/day genistein; 30 days	↑ Bone mass
Ishimi et al; 1999	OVX rats	Prospective controlled trial	BMD; DEXA, microCT, x-ray; bone marrow B-lymphopoiesis	0.7 mg/day genistein; 4 weeks	↓ Bone loss; prevented ↑B lymphopoiesis
Fanti et al; 1998	OVX rats n=30	Prospective RCT	Uterine weight; BMD	1, 5, or 25 mcg genistein/gm body wt/day; 21 days	Uterine weight unchanged; 5 mcg genistein ↓ bone loss
Fanti et al; 1998	OVX rats n=40	Prospective RCT	Osteocalcin, TNF-alpha, tibia length and BMD; histomorphology; uterine weight	5 mcg genistein/gm body wt/day; 21 days	↑ Bone formation rate; 5 mcg genistein ↓ bone loss; blocked ↑ TNF-alpha
Arjmandi et al; 1998	OVX rats n=72	Prospective RCT	Femur and spinal BMD; underwater uterine weighing	Soy or no-soy diets; 65 days	↑ Femoral density; uterine atrophy
Arjmandi et al; 1998	OVX rats n=48	Prospective RCT	Femoral BMD; underwater weighing	Soy or no-soy diets; 35 days	↑ Femoral density with soy but not no-soy diet
Picherit et al; 2000	OVX rats n=60	Prospective RCT	DEXA; urinary DPD excretion	Isoflavones 0, 20, 40, or 80 mg/kg body wt/day	↓ Bone turnover; no reversal in BMD; ↓ urinary DPD excretion in 40 and 80 mg

The 1998 study of Fanti et al[30] was designed to clarify whether administration of genistein could prevent the rapid bone loss that occurs after ovariectomy in adult female rats. This study utilized thirty 60-day-old nulliparous female rats, randomized by weight into five groups of six animals each and either sham-operated (one group) or ovariectomized (four groups). The day before surgery, three OVX groups were given daily injections of 1, 5, or 25 mcg genistein per gram body weight, while the other groups were injected with vehicle.

BMD of the tibia was significantly lower in the OVX-vehicle than in the sham-vehicle rats. The rats fed 5 mcg genistein per gram body weight had a 50-percent reduction in post-ovariectomy loss of cancellous bone and whole tibia BMD, suggesting that the bone-sparing action of genistein is mostly due to its effects on cancellous bone. One mcg genistein did not prevent decrease in BMD and 25 mcg genistein did not add further to the effect of the 5 mcg dose.

In the Picherit et al[31] randomized control trial, seven-month-old female rats were studied to test whether there were any dose-dependent, bone-curative effects (ability to reverse established bone loss) of long-term daily soy isoflavone intake. On day 1, rats were either sham-operated or ovariectomized. On day 80, five rats were sacrificed to confirm ovariectomy-induced bone loss. The remaining OVX rats (n=36) were randomly assigned to one of four groups of nine rats each, fed isoflavones at 0 (OVX), 20, 40, or 80 mg/kg per day for 84 days. Body composition was estimated using DEXA. Urine samples were taken on days 1, 40, 80, 122, and 164 to measure urinary calcium excretion and/or deoxypyridinoline (DPD), a marker of bone resorption. Plasma markers of osteoblastic activity were measured and femur BMD assessed.

BMD was significantly lower in ovariectomized rats than in sham-operated, and isoflavone feeding did not affect BMD in this population. Uterine weights in OVX rats were no different in rats fed isoflavone than in OVX rats not fed isoflavones, showing no uterotrophic effect of the isoflavone diet. Urinary DPD excretion was higher in isoflavone-fed ovariectomized rats than in sham rats. However, at day 122, DPD concentrations in high isoflavone-fed rats (80 mg/kg/day) were significantly lower than in OVX rats fed no isoflavone. At days 122 through 164, DPD excretion in rats fed 40 mg/kg per day decreased. Therefore, urinary DPD excretion in both isoflavone-dose groups was significantly lower than that in the OVX (no isoflavone) group. This suggests the isoflavone-induced anti-osteoclastic activity occurred in a dose-dependent fashion. This study showed that daily isoflavone intake reduced bone turnover but failed to reverse established bone loss. It also appears the two highest intake levels were more effective in reducing the ovariectomy-induced increase in bone turnover. These results may suggest that soy isoflavone consumption may have more of a preventive rather than curative role on bone health in humans. See Table 2 for summary of animal studies.

Clinical Studies

The potential use of phytoestrogens to preserve bone tissue and delay or prevent the onset of osteoporosis has only recently been addressed, with few published reports on the skeletal effects of soy foods or soy protein on human subjects.

Potter et al[14] examined the effect of isoflavones on BMD in 66 hypercholesterolemic, postmenopausal women (ages were not provided) in a placebo-controlled, 24-week study. Subjects were randomly assigned to one of three treatment groups providing 40 g protein/day from one of the following: isolated soy protein containing moderate concentrations of isoflavones (ISP56=56 mg isoflavones/day), isolated soy protein containing higher concentrations of isoflavones (ISP90=90 mg isoflavone/day), or casein and nonfat dry milk.

Bone mineral content (BMC) and density of the lumbar spine, proximal femur, and total body were measured by DEXA at the beginning and after the 24-week intervention period. The lumbar spine BMC and BMD increased significantly at the end of the 24-week treatment period in the ISP90 group. There were no significant changes noted in density or mineral content in the total-body or other skeletal sites.

Table 3. Clinical Studies of Soy Isoflavones and Bone Density

Author, Year	Population, Number	Study Design	Outcome Measure	Intervention, Study Length	Findings
Potter et al; 1998	Post-menopausal women; n=66	Double-blind RCT	BMD via DEXA of lumber spine, femur, total body	Moderate isoflavone vs. high isoflavone diet; 26 weeks	Lumbar BMD ↑ in high isoflavone group
Alekel et al; 2000	Peri-menopausal women; n=69	Double-blind RCT	BMD via DEXA of lumbar spine	Isoflavone-rich vs isoflavone-poor diet; 24 weeks	Isoflavone-rich diet attenuated bone loss
Somekawa et al; 2001	Post-menopausal women; n=478	Case control	BMD via DEXA of lumbar spine	Estimated isoflavone intake	High isoflavone consumption ↑ bone mass

A double-blind, randomized, placebo-control, 24-week trial[6] examined the effect of isoflavones on 69 perimenopausal women (median age 50.6 years). The effects of an isoflavone-rich soy protein (80.4 mg aglycone components/day; n=24), an isoflavone-poor soy protein (4.4 mg aglycone components/day; n=24), or whey protein (control; n=21) on bone loss were examined. (Aglycone components are the unconjugated parent forms of the isoflavones.)

DEXA was used to assess body composition and lumbar spine BMD and BMC at baseline and post-treatment. Significant losses in BMD and BMC occurred in the control group but not in either of the soy groups. This study demonstrated a positive effect of soy isoflavone on bone mass. The results of this study showed that the control group had significant bone loss, whereas the isoflavone-rich treatment attenuated bone loss from the lumbar spine. After various contributing factors were accounted for, the isoflavone-rich treatment was shown to have a significantly positive effect on both BMD and BMC.

It should be noted that a confounder in isoflavone treatment studies is the variability in the bioavailability and metabolism of isoflavones among subjects. Subjects range from low to moderate to high metabolizers of soy isoflavone. Thus, although the same dose of isoflavone was given, variability in the responses can be expected. See Table 3 for a summary of clinical studies on soy isoflavones and bone density.

Protein Intake and Calcium Excretion

It has been suggested that soy protein consumption results in less urinary calcium excretion than eating animal protein.[20] Several human studies have shown this to be the case.

Breslau[20] studied subjects who were fed in random order for 12-day periods each of three different diets containing similar amounts of protein and calcium, but different sources of protein. The researchers found that subjects excreted 150, 121, and 103 mg of calcium/24 hours when on the animal protein, soy and animal protein, and soy protein diets, respectively. This may have possible clinical significance if soy foods are substituted for animal protein in the diet.

Discussion

The evidence from both animal and human studies that soy protein favorably affects bone health without apparent adverse effects on reproductive tissues is encouraging. Results from ovariectomized rat models have consistently demonstrated a beneficial effect of isoflavones on bone mass. These studies support consumption of a threshold dose of isoflavones for a sufficiently long period of time (months at minimum), before any quantifiable effect on bone mass and density can be detected. Some animal studies have shown that when higher doses of isoflavones are consumed, the effect was no greater than the minimal threshold dose, and the higher dose may even be less effective.

The exact mechanisms for the effect of isoflavones on bone and the components of soy responsible for the effects remain unclear. The potential mechanisms by which phytoestrogens may affect cellular activities have been summarized by Anderson et al[11] and divided into genomic and non-genomic effects. Genomic effects occur when phytoestrogen molecules bind to an estrogen receptor in the cell nucleus. Presumably, the estrogen receptor acts by altering the production of specific proteins within the cell by binding to regulatory sites on DNA molecules and increasing or decreasing the expression of specific genes. The binding of a phytoestrogen to the receptor may result in either partial activation of the receptor (agonistic effect) or the displacement of an estrogen molecule, thus reducing receptor activation (antagonistic or anti-estrogenic effect).

It also appears the beneficial effects of soy isoflavones on bone can result from increased bone formation by osteoblasts.[23] Estrogen receptors have been shown to be present in low numbers on osteoblastic cells, and genistein has been shown to bind to estrogen receptor β in osteoblastic cells.[23] In theory, phytoestrogens may act directly on osteoblasts by the genomic mechanism involving the activation or inhibition of the nuclear estrogen receptor, or via a variety of non-genomic mechanisms, including the inhibition of tyrosine kinase[24] or topoisomerase II.[11] These non-genomic effects of phytoestrogens might occur by alteration of cellular protein activity, such as inhibition of regulatory proteins like tyrosine kinases, which control the activity of other proteins, and topoisomerase II, which helps to regulate cell differentiation and the cell replication cycle.[11] These effects are theoretical and remain to be proven.

The inhibition of osteoclastic cells may also result from a non-genomic mechanism,[11] since mammalian osteoclasts lack estrogen receptors. Blair[24] showed that genistein inhibited osteoclastic activity via tyrosine kinase inhibitors. Williams[26] also showed that genistein decreased acid secretion by osteoclasts, thus decreasing bone dissolution.

The work of Arjmandi et al[23] raises the possibility that soy's protective effect on bone may be due to its positive effect on intestinal calcium absorption. Another Arjmandi[24] study showed there was a significant increase in IGF-1 mRNA in the isoflavone treated groups compared to the control groups. Since IGF-1 mRNA stimulates bone formation, this is another possible mechanism of isoflavone's positive role on bone health.

Ishimi's[29] study lends the possibility of an effect via genistein's ability to reduce OVX-induced increase of bone marrow hemopoiesis (which stimulates bone resorption), thereby preventing bone loss.

It has also been shown that high protein intake increases urinary calcium excretion, leading to osteoporosis. This hypercalciuric effect of protein is primarily attributed to the metabolism of the sulfur-containing amino acids methionine and cysteine. Breslau et al[20] demonstrated in a human study that those fed a soy protein versus an animal protein diet excreted less calcium.

Although this difference was attributed to the low sulfur content in soy, the mechanism by which animal protein induces hypercalciuria has not been clarified. Bresslau's results suggest that the major calciuric factor is bone resorption in response to the acid load created by the higher animal protein diet.

Caution should be exercised when applying results from animal studies to humans. These studies have been of short duration; some utilized rats of young age; most studies had small population sizes; and some studies did not incorporate confirmatory tests of ovariectomy-induced bone loss. Generalization to humans in regard to dosage for optimal bone protection cannot be extrapolated from these animal studies.

Alekel et al[6] showed that isoflavones attenuated bone loss from the lumbar spine in estrogen-deficient perimenopausal women. It is therefore possible that including isoflavone-containing soy products in the diets of perimenopausal women could decrease their risk for osteoporosis.

Potter et al[14] showed a significant increase in bone mineral content and density in the lumbar spine in postmenopausal women supplemented with 90 mg/day of isoflavones, an amount that may be difficult for many to obtain by diet alone.

Further research is also needed to identify any potential negative effects of soy consumption. There have been reports from *in vitro*, animal, and human studies showing that isoflavones may interfere with thyroid hormone production.[32,33]

Conclusion

The regulation of bone growth and repair is complex and multifactorial, involving signaling events in both osteoblasts and osteoclasts. Thus, there are multiple possibilities for isoflavones' effects on bone health.

The reviewed studies show evidence of a beneficial effect of soy isoflavones on bone health in peri- and postmenopausal women. Results from animal studies also show a positive role. The exact mechanisms involved still remain unclear, requiring further investigation. From *in vitro* and animal studies, isoflavones appear to stimulate osteoblastic bone formation and inhibit osteoclastic bone resorption. When high-isoflavone soy protein is incorporated in the diet as an alternative to animal protein, bone loss in the lumbar spine is inhibited. Animal studies and the few short-term human studies that have been completed yield some positive results, justifying the need for additional randomized, double-blind, clinical trials with a larger sample population, longer duration (at least two to three years), and examination of various dosages. Studies on humans looking at fracture prevention rates are also indicated.

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