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Soy isoflavones' osteoprotective role in postmenopausal women: mechanism of action

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Abstract

Ovarian hormone deficiency is a major risk factor for osteoporosis. Current therapies emphasize the use of antiresorptive agents, such as estrogen, calcitonin, and bisphosphonates. These therapies are associated with certain risks and side effects making compliance a major obstacle. Recent findings suggest that a class of synthetic and naturally occurring compounds, selective estrogen receptor modulators, e.g. raloxifene and soy isoflavones can offer attractive alternatives. Evidence for bone-sparing effects of isoflavones relies mainly on animal findings supported by a limited number of human studies. These observations suggest that isoflavones exert their effects on bone by stimulating bone formation and at the same time suppressing bone resorption. However, the precise osteoprotective mechanism of isoflavones remains uncertain and awaiting further clarification. From a clinical point of view, larger and longer duration studies are warranted to enable us to draw clear conclusions in regards to the role of isoflavones on bone.

Keywords: Osteoporosis; Menopause; Isoflavones; Soy; Selective estrogen receptor modulators (SERMs)

1. Introduction

The prevalence of age-related bone loss is greater in women than in men, and in 25 to 30% of aging women this loss results in major orthopedic problems [1,2]. Natural or surgical menopause results in an initial phase of rapid bone loss followed by a period of slower deterioration of the skeleton [3,4]. This rapid phase of bone loss occurs within the first 10 years following the cessation of menses or surgical removal of the ovaries. The ovarian hormone deficiency associated with menopause results in increased rate of bone turnover and causes an imbalance between resorption and formation, and thereby accelerates bone loss [4].

Although the optimal treatment of osteoporosis remains controversial, as suggested by Verhaeghe et al. [5], the most logical approach is to combine an antiresorptive agent to reverse the increased bone remodeling and an agent that stimulates osteoblastic proliferation so that bone formation accrues more rapidly. Among the antiresorptive agents available today, hormone replacement therapy (HRT) is perhaps the most effective treatment, as it has been shown to both reduce the rate of bone loss [6] and decrease the risk of fracture, including hip fracture [7]. However, not all the patients are willing to initiate this treatment due to a number of undesirable side effects and increased risk of endometrial and breast cancer [8–11] associated with prolonged use of estrogen therapy. Recent surveys have indicated that only 3 to 8% of women with natural menopause are receiving HRT [12,13].

Other agents such as bisphosphonate family and calcitonin appear to induce small incremental increases in bone mass over one or two years of treatment [14,15]. Like HRT, these agents decrease bone turnover but do not correct the imbalance between the total amount of bone resorbed and bone formed.

Recent introduction of raloxifine, which has been shown to be effective in preventing bone loss or increasing bone mass [16,17], has refocused interest in the treatment of osteoporosis. Raloxifene is among a group of compounds that are collectively known as selective estrogen receptor modulators (SERMs) [18,19]. SERMs are a group of chemically diverse non-steroidal compounds that bind to and interact with the estrogen receptors. Lately, certain estrogen-like compounds of plant origin, such as soy isoflavones,

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have been characterized as naturally occurring SERMs with similar beneficial effects to raloxifene on bone [18,20,21]. Hence, similar to synthetic SERMs, soy isoflavones have been suggested to exert the beneficial effects of estrogen without its side effects [20]. However, it is premature to state whether the bone protective effects of soy are derived solely from its isoflavones, soy protein itself, or their combination. The focus of this review will be the efficacy of soy or its isoflavones on bone in ovarian hormone deficiency. This review includes findings from both animal and human studies with a brief overview of mechanisms of action and some related safety issues.

2. Bone-modulating effects

Until the turn of the twentieth century it was assumed that estrogens were exclusively produced by animals. However, the principle that plants can also produce estrogen-like molecules was established by 1966 [22]. Now, it is recognized that certain plants and plant products contain these phytoestrogens. One group of such compounds reported to have estrogenic activity is the flavonoids [23]. This group of compounds includes isoflavones, which are found in a limited number of plants and plant products. Soybeans, or more specifically isolated soy proteins, are considered a rich source of the isoflavones genistin and daidzin [24,25] which are converted to genistein and daidzein by the gut microflora [24]. In recent years, there has been rising interest regarding the soy isoflavones and their influence not only on sex hormone metabolism, but also other biological activities including cholesterol-lowering properties [26,27], anti-carcinogenic effects [28], and more recently their protective role in bone health (Table 1) [29–39].

The protective role of soy protein, its isoflavones, or their combination on bone in ovarian hormone deficient models of osteoporosis is uncertain (Table 2) [40–47]. In these studies, the rate of bone formation and bone resorption, as assessed by biochemical markers are unchanged, decreased, or increased. Analogous to animal findings, the effect of soy and its isoflavones on bone in humans are also inconsistent (Table 1). Furthermore, as mentioned earlier, it is not clear whether the bone protective effect of soy protein is due to its amino acid composition [48], non-protein constituents such as isoflavones [41,42], or a combination of these factors [35]. Our recent animal studies using the ovariectomized rat model have shown that the soy diet with isoflavones [41] was more effective in preventing ovariectomy-induced loss of bone density than either the casein or the soy protein, depleted of its isofavones, diet. Using an osteopenic rat model, we have also reported [42] a slight reversal of the ovarian hormone deficiency-induced loss of bone with soy protein diets with either normal or reduced isoflavone content. Although our animal findings [40–42], so far, indicate the importance of isoflavones in preserving bone, it is still not clear whether isoflavones can exert similar bone protective effects independently of soy protein.

3. Is it soy protein or its isoflavones?

Currently, both soy protein and its isolated isoflavones are being marketed to postmenopausal women, hence it is important to identify the bone protective components of soy necessary to guide the consumers in making appropriate choices. The recent findings of Picherit and colleagues [43] support the bone protective role of isoflavones independent of soy protein. They [43] reported that isoflavones dose-dependently prevented ovariectomy-induced bone loss in a rat model. However, the same group of investigators did not find similar beneficial effects of isoflavones in reversing bone loss in ovariectomized osteopenic rats [44], even though the rate of bone turnover was reduced. Hence, one can speculate that a longer treatment period with isoflavones would have reversed bone loss. Whether the magnitude of effects of isoflavones on bone in these animal studies by Picherit [43,44] would have been greater had isoflavones been given in conjunction with soy protein still remains to be answered.

On the other hand, studies investigating the effects of individual soy isoflavones, genistin and daidzin, support the important role of these naturally occurring compounds in bone health regardless of the dietary protein source. For instance, two weeks of a genistin-rich treatment (1.0 mg/day) in lactating ovariectomized rats was effective in maintaining trabecular bone tissue in comparison with ovariectomized control animals [45]. Furthermore, in the same report, genistin stimulated alkaline phosphatase activity of an osteoblast-like cell line, suggesting a positive effect on bone formation. In another study, Fanti et al. [47] reported that genistein (5 mg/kg body weight) maintained both cortical and trabecular bones in ovariectomized rats, and the bone-sparing effect of genistein appeared to be biphasic. Although it is believed that genistin is the most potent of all the soy isoflavones, a recent study by Picherit et al. [46] reported that daidzin, is more efficient than genistin in preventing the ovariectomy-induced increase in bone turnover and decrease in bone mineral density. Clearly, this demonstrates that there are uncertainties as to which isoflavone plays a more important role in skeletal health. Use of a single isoflavone may not necessarily be the approach to be taken and future studies should address whether the combination of isoflavones exerts a more pronounced effect on bone.

4. Mechanism of action

From a mechanistic point of view, there is some evidence [34–36,39,43,44,47] suggesting that soy or its isoflavones, while suppressing the rate of bone resorption, concomi-

Table 1
Representative clinical studies examining the effects of soy protein with various doses of isoflavones (Iso) on bone mineral density (BMD), bone mineral content (BMC), and/or biomarkers of bone formation and bone resorption

Author(s)/ reference no.	Study design	No. of subjects	Follow-up time	BMD	Bone markers
Potter et al./29	Prospective; postmenopausal women received either 40 g milk protein or 40 g soy protein containing 45 or 90 mg Iso/daily	66	6 months	2.5% ↑ lumbar spine BMD in 90 mg Iso group	Not assessed
Dalais et al./30	Prospective/cross-over; postmenopausal women received 45 g of either soy grits containing 52 mg Iso, wheat flour, or flaxseed daily	44	3 months	No change in BMD 5.2% ↑ in BMC	Not assessed
Alekel et al./31	Prospective; perimenopausal women received 40 g milk protein or 40 g soy protein containing 40 or 80 mg Iso/daily	69	6 months	No change in lumbar spine BMD of 80 mg Iso group but ↓ in control and 40 mg Iso groups	No change
HSU et al./32	Prospective; postmenopausal Chinese women received 150 mg Iso daily	37	6 months	No change in calcaneous BMD	Not assessed
HO et al./33	Prospective/cross-sectional; women age 30–40 years were divided into 4 quartiles based on Iso intake	132	3 years	↑ Lumbar spine BMD in the 4th Iso quartile	Not assessed
Horiuchi et al./34	Cross-sectional; postmenopausal Japanese women's BMD were correlated with soy intake	85	Not assessed	Positive correlation between soy intake and lumbar spine BMD	Soy intake ↓ urinary [†]Dpd
Wangen et al./35	Prospective/cross-over; postmenopausal women received 40 g soy protein containing 8, 65 or 130 mg Iso daily	17	3 months	Not assessed	All doses ↑ serum [§]OC, [¶]IGF-I, [£]BAP; no change in urinary Dpd
Scheiber et al./36	Pospective/single open-group; postmenopausal women received 60 mg Iso from consuming whole soy foods daily	42	3 months	Not assessed	↓ urinary [‡]NTX; no change in serum BAP
Anderson et al./37	Prospective; young adult women received either 40 g of soy protein or milk protein daily	48	1 year	Ward's triangle 4.1% ↑	NA
Gallagher et al./38	Prospective; postmenopausal women received 40 g soy protein depleted of Iso or containing 52 or 96 mg Iso daily	65	9 months	No change in lumbar spine or femoral neck BMD	No change
Arjmandi et al./39	Prospective; postmenopausal women on HRT or not on HRT received 40 g milk protein or soy protein containing 90 mg Iso daily	44	3 months	Not assessed	↓ Urinary Dpd and ↑ serum IGF-I in women not on HRT

[†] Dpd, deoxypyridinoline.
[§] OC, osteocalcin.
[¶] IGF-I, insulin-like growth factor-I.
[£] BAP, bone-specific alkaline phosphatase.
[‡] NTX, N-terminal cross-linked peptide.

tantly enhance the rate of bone formation. In ovariectomized rats, soy isoflavones have been shown [43,44] to reduce the urinary excretion of deoxypyridinoline (Dpd), a specific marker of bone resorption. Similarly, at least three human studies have clearly demonstrated the anti-resorptive properties of soy or its isoflavones. One of these studies was a cross-sectional study by Horiuchi et al. [34], in which soy protein intake in Japanese postmenopausal women was associated with lower urinary Dpd excretion. In the second study, postmenopausal women who were assigned to a soy milk regimen, providing 60–70 mg of isoflavones, experienced a significant decrease in urinary N-terminal cross-linked peptide, another specific marker of bone resorption [36]. We have also recently reported [39] that the daily consumption of 40 g soy protein, but not milk protein, for three months by postmenopausal women who were not on HRT significantly reduced the urinary excretion of Dpd.

It is reasonable to suggest that soy or its isoflavones enhance bone formation based on at least two lines of evidence: 1) soy isoflavones stimulate osteoablastic activity through activation of estrogen receptors [49], and 2) soy or its isoflavones promote insulin-like growth factor-I (IGF-I) production [42]. Using a rat model of osteopenia, we have shown that soy protein increased the gene expression of IGF-I as indicated by higher femoral mRNA levels (Figs. 1 and 2) [42]. In that study, incorporation of soy protein with normal isoflavone content (2.3 mg/g protein) had a greater effect on femoral IGF-I mRNA than the isoflavone-depleted soy protein-based diet (approximately 0.1 mg/g protein). This finding indicates that isoflavones may have a role in

Table 2
Representative animal studies examining the effects of soy protein and soy isoflavones (Iso) on bone mineral density (BMD) and biomarkers of bone formation and bone resorption

Author(s)/ reference no.	No. of groups	Treatment(s)	Treatment duration	BMD	Bone markers
Isoflavones in the context of soy protein					
Arjmandi et al./40	4	Ovx rats received either casein- or soy protein-based diet	35 days after ovx	↑ Femoral and 4th lumbar bone density in animals receiving soy-based diet	No change
Arjmandi et al./41	4	Ovx rats received either casein or soy protein with or without Iso as a source of protein	35 days after ovx	↑ Femoral BMD in group receiving soy with isoflavones	No change
Arjmandi et al./42	4	Osteopenic ovx rats received either casein or soy protein with or without Iso as a source of protein	65 days	Slightly ↑ femoral but not 4th lumbar BMD	(Soy–Iso) had higher urinary excretion of hydroxyproline than all the other ovx groups
Total isoflavones in the context of non-soy protein					
Picherit et al./43	4	Ovx rats received casein-based diets with 4 levels of Iso: 0, 20, 40, or 80 mg per kg body wt.	91 days after ovx	All doses of Iso ↑ total femoral BMD	All Iso levels maintained ovx-induced higher serum §OC; Iso 40 and 80 ↓ †Dpd
Picherit et al./44	4	Osteopenic ovx rats received casein-based diets with 4 levels of Iso: 0, 20, 40, or 80 mg per kg body weight	84 days	No change in BMD	Isoflavones dose-dependently ↓ serum OC and urinary Dpd
Individual isoflavones in the context of non-soy protein					
Anderson et al./45	4	Ovx-lactating rats received genistein-rich Iso at three doses: 0.5, 1.6, or 5.0 mg day	14 days	Genistein dose at 0.5 mg ↑ femoral ash	Not assessed
Picherit et al./46	4	Ovx rats received either casein-based diet with genistein or daidzein both at 10 μg/g body weight daily	90 days	Only daidzein prevented the ↓ in L2-L5 and femur BMD	Daidzein ↓ OC and urinary Dpd
Fanti et al./47	5	Ovx rats were injected with genistein at 1, 5, or 25 μg/kg body weight daily	21 days	Genistein at 5 and 25 μg/g body wt. had similar tibial BMD as sham	Genistein at μg/g body wt. ↑ serum OC; no change in resorption markers

§ OC, osteocalcin.
† Dpd, deoxypyridinoline.

enhancing the synthesis of IGF-I and more importantly at the bone level. Similarly, we observed [39] that soy protein supplementation also significantly increased serum IGF-I levels, confirming the animal findings. It is well recognized that IGF-I enhances osteoblastic activity in humans [50] and IGF-I concentrations have been reported to correlate positively with bone mass in pre- [51], peri- [52], and post- [53] menopausal women. Nonetheless, these indirect observations should be confirmed using in vitro and in vivo models including long-term human studies in which more definitive techniques such as bone histology and bone histomorphometry are assessed.

5. Dose

Before isoflavones are to be considered as an alternative or adjunctive treatment for skeletal health, an efficacious dose needs to be established on the basis of the model of osteoporosis and the type of isoflavone being used. Recent efforts to identify such a dose, found that daily intake of approximately 90 mg isoflavones in conjunction with 40 g soy protein for six months, increased both lumbar spine bone mineral density and bone mineral content in post-menopausal, not on HRT [29], and perimenopausal [31] women. Soy intake has also been shown to be beneficial in the maintenance of peak bone mass in Chinese women who habitually consume soy products [33]. Recent findings of a three-year study by Ho et al. [33] indicated that the higher intake of soy was able to maintain the spinal bone mineral density (SBMD) of Chinese women aged 30–40 years. In that study [33], the investigators followed 132 women for three years and their soy isoflavone intakes were assessed using a food frequency questionnaire. They observed that the loss of SBMD in women belonging to the fourth quartile (15.16 ± 9.59 mg of soy isoflavones/day) was significantly lower than those in the first quartile (1.40 ± 1.21 mg of soy isoflavones/day). However, controlled, long-term, and dose-response studies are necessary to evaluate whether whole soy, soy protein, or its isoflavones are effective in preventing bone loss or restoring bone mineral density in women. Furthermore, to establish the role of soy protein in bone

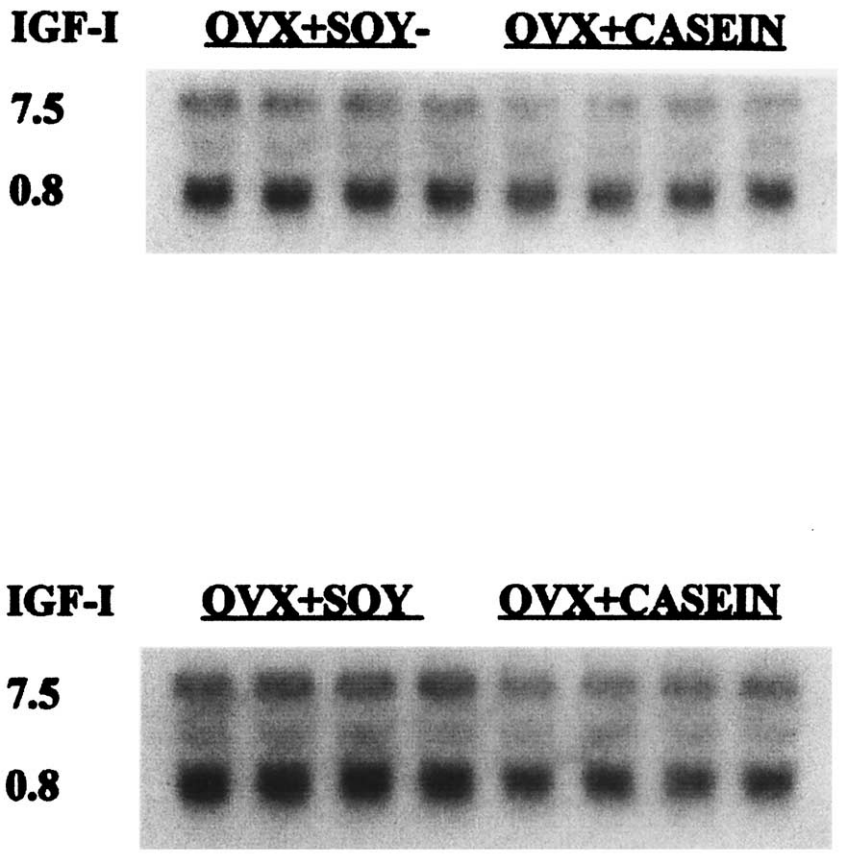

Fig. 1. Effects of ovx, soy protein with normal (SOY; 2.3 mg total isoflavones/g protein) or depleted (SOY-; 0.1 mg total isoflavones/g protein) isoflavone content on Northern blots of femur total cellular RNA probed with ^{32}P-labeled cDNA for rat insulin-like growth factor-I (IGF-I) RNA (0.8 and 7.5 kb). IGF-I mRNA was quantitated by phosphorimaging of the membranes and normalized to 28S ribosomal RNA to correct for differences in total RNA. Each lane represents RNA extracted from a femur of one animal (n = 4 per treatment group).

formation, levels of soy and isoflavones that are practical, safe and effective for inclusion in a daily diet regimen (or supplement) should be considered. The potential effect of soy isoflavones on bone health has immense implications should this dietary source of phytoestrogens be demonstrated effective in exerting beneficial effects on biomarkers of bone metabolism and maintaining or increasing bone mass in postmenopausal women.

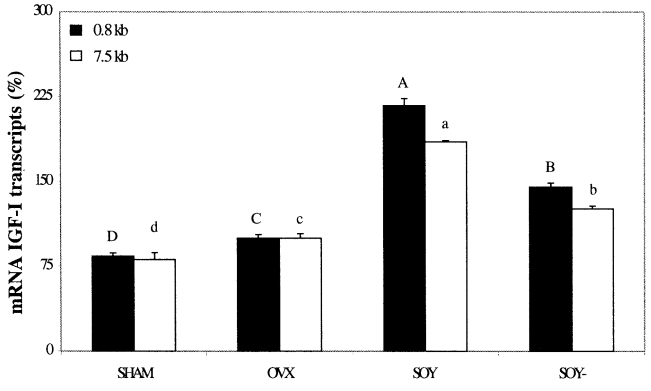

Fig. 2. Graphical presentation of IGF-I mRNA transcripts expressed as percent change in 0.8 kb (white bars) and 7.5 kb (black bars). Bars are mean + SE; $n = 4$ animals per treatment group. For each subtype of IGF-I bars with different letters are significantly ($P < 0.05$) different from each other.

6. Safety

The safety of consuming isoflavone-rich soy compounds is of concern. The existing epidemiological observations in Asian women, who consume high amounts of soy foods, indicate low rates of breast [54] and endometrial [55] cancer. However, these observations are based on whole food consumption and not soy protein or its isolated nonprotein constituents such as isoflavones. From the breast cancer point of view, there two opposing lines of research regarding soy consumption. On the one hand soy or its isoflavones are protective against breast cancer while on the other hand soy has been shown to promote breast cancer. The overall conclusion that can be made at this time based on the existing data is that the consumption of soy should neither be encouraged or discouraged with respect to breast cancer. For an extensive review of the literature related to soy and

breast cancer, readers are referred to a recently published review paper by Messina and Loprinzi [56].

Another health concern that has been raised is the relationship between soy or its isoflavone consumption and fertility. A classic example that has been used is where Australian sheep breeders noticed a sharp decrease in fertility in animals grazing on red clover. It would be unreasonable to extrapolate such observations to human fertility at least for two reasons. First, these sheep by ingesting red clover received vast quantities of isoflavones, raising their blood levels far beyond those conceivably attainable in humans [24]. Second, metabolism of isoflavones may differ considerably among species. To illustrate this point, it has been reported that many feline species lack the enzyme necessary to conjugate isoflavones, making them extremely sensitive to these compounds [57]. By comparison, rodents are usually exposed to very high doses of isoflavones from the soymeal added to commercial feed, and yet these extreme levels have not resulted in noticeable breeding problems [20].

In accordance with the epidemiological reports, our animal studies [40,41] and those of other investigators [58,59] indicate that, unlike estrogens, animals fed soy protein with isoflavones or individual isoflavones produce no uterotrophic response. From a human standpoint, though there are limited controlled investigations, the message concerning the estrogenicity of soy components are unclear. For instance, a short-term dietary study of soy intake in premenopausal women with benign and malignant breast conditions was shown to stimulate breast tissue proliferation [60]. In comparison, a four-week study by Baird et al. [61] of whole soy or texturized soy protein consumption by postmenopausal women did not induce any clear estrogenic response. The safety aspects of isolated compounds from soy require more scrutiny by long-term controlled clinical trials. These types of studies should address questions such as whether long-term intake of these compounds from soy in women with benign, malignant, or no evidence of breast conditions has stimulatory or inhibitory effects on breast tissue proliferation.

7. Summary

Soy protein may have a modest beneficial effect on bone. However, from the review of existing literature it is too early to state whether soy protein or its isoflavones can be substituted for estrogen in preventing the bone loss induced by ovarian hormone deficiency. Future studies are needed to address numerous questions including but not limited to whether: 1) isoflavones independent of soy protein can prevent ovarian hormone deficiency-associated bone loss; 2) consumption of soy containing food or intake of isoflavones on a daily basis is necessary to observe the expected beneficial effects on bone or simply intermittent use will produce the same results; 3) the effect of soy protein or its isoflavones on bone is transitory; and 4) the combination of soy isoflavones and lower doses of estrogens can prevent postmenopausal bone mineral loss and at the same time lower the estrogen-associated risks. As these and other questions are answered, the efficacy of soy protein and its isoflavones as alternative and/or adjunctive treatments for postmenopausal osteoporosis can be determined.

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