Symposium: Relative Bioactivity of Functional Foods and Related Dietary Supplements

Soy Isoflavones and Bone Health: The Relationship Is Still Unclear[1,2]

Connie M. Weaver[3] and Jennifer M. K. Cheong

Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana

ABSTRACT Evidence of the effect of purified soy isoflavones and soy protein isolates containing isoflavones on bone health in rats and in humans is inconsistent. Differences may be because of synergies or antagonisms among the isoflavones, threshold or biphasic dose effects, life stage of animals or human subjects, estrogen status, and environment–genetic interactions, including the ability to produce metabolites upon ingestion of isoflavones. At this time, the benefits of soy protein and isoflavones on bone health are inconclusive. This overview will summarize these discrepancies and will suggest future studies to clarify the conditions under which these dietary substances can be helpful for bones.   J. Nutr. 135: 1243–1247, 2005.

KEY WORDS: • soy isoflavones • bone • estrogen status • equol

Osteoporosis prevention

The prevalence of osteoporosis in people aged 50 y and over is projected to increase from 10,100,000 in 2002 to 12,000,000 and 13,900,000 by 2010 and 2020, respectively (1). Consequences of osteoporosis can be serious; 24% of hip fracture in patients aged 50 y and over die in the year after the fracture (1).

Existing measures for osteoporosis prevention include adequate calcium and vitamin D intake, hormone therapy (HT)[4], and selective estrogen receptor modulators. Although most gynecologists prescribed HT for their menopausal patients before 2002, only 35 to 40% of women ever start HT and many discontinue it, because of perceived side effects and discomfort. HT will likely not be regularly prescribed in the future given the recommendations by several groups against long-term use for chronic diseases after the release of the findings of the Women's Health Initiative (2). According to this study, women on estrogen plus progestin who were fol-lowed up for an average of 5.2 y showed greater evidence for breast-cancer harm and some increase in coronary heart disease, stroke, and pulmonary embolism, which outweighed the evidence of benefit for hip, clinical vertebral, and other osteoporotic fractures, and for colorectal cancer. The number of women who had a substantial and a clinically important decline in the Modified Mini-Mental State Examination was greater in the estrogen plus progestin group than in the placebo group (3). Subsequently, the estrogen-alone arm was stopped after an average of a 7-y follow-up, because it increased the risk of stroke and was not effective in reducing heart disease, although it reduced hip fractures. Consequently, many postmenopausal women are turning to botanical dietary supplements containing isoflavones as an alternative to HT, but evidence of protection against postmenopausal bone loss is limited.

Isoflavones and bone health

Isoflavones belong to a class of plant compounds called phytoestrogens, which have both estrogenic and antiestrogenic properties (e.g., reduction of circulating estradiol or reduction of estradiol availability by increasing sex-hormone-binding globulin synthesis) in in vivo and in vitro models (4). The 3 main classes of phytoestrogens are isoflavones, lignans (component of plant-cell walls), and coumestans. The main dietary sources of isoflavones, lignans, and coumestans are soybeans, oilseed (e.g., flaxseed), and clover and alfalfa sprouts, respectively. Isoflavones are being marketed as dietary supplements, for which there are no approved health claims, even though numerous claims are being made pertaining to heart, bone, and general menopausal health benefits. The evidence for a positive effect of isoflavones on bone health is mixed.

There are numerous studies in the literature over the last decade trying to address the efficacy of soy protein containing

[4] Abbreviations used: BMC, bone mineral content; BMD, bone mineral density; HT, hormone therapy.

isoflavones or purified isoflavones on bone health in animal models and in humans. Explanations for why some are positive and others show no effect have not been clearly determined. In this review, some of the possible reasons for differential efficacy outlined in **Table 1** will be addressed. Inadequacies of study design and of choice of animal models likely play a large role in our lack of understanding of the relation between isoflavones and health, which will also be addressed throughout.

Life-stage–independent effects of isoflavones on bone

To the extent that isoflavones work by estrogenic action, it would seem that isoflavones might be effective when estrogen is deficient, i.e., during menopause, and is not effective when estrogen is in sufficient supply. Indeed, cross-sectional analysis showed that high dietary isoflavones was associated with higher bone mineral density (BMD) of the spine and the hip in postmenopausal, but not premenopausal, Chinese women (5). Furthermore, soy protein reduced a biochemical marker of bone resorption and increased serum insulin-like growth factor-1, which is thought to increase bone formation, more than milk-based protein in postmenopausal women, and the effect was more pronounced in women not on HT than in those who were taking HT (6). However, studies in animal models generally show a benefit in younger, growing animals than in older, ovariectomized models.

One of the earliest studies reporting a benefit of soy protein and bone was in a young, ovariectomized rat model (7). Suppression of ovariectomized-induced femoral bone loss was comparable between soy protein isolate and 17β-estradiol. Similar positive effects of purified genistein have been observed in young rats (8,9). In contrast, studies in older, adult animal models have generally found no benefit of soy isoflavones in reversing osteopenia (10). Dietary isoflavones had no benefit on BMD or bone resorption markers in 6-mo-old ovariectomized rats (11). Similarly, soy protein had no protective effect on bone turnover or BMD in ovariectomized monkeys (12,13).

It is important to select skeletally mature animal models to appropriately interpret benefits for postmenopausal women, because human postmenopausal bone loss starts after skeletal maturity. Rats younger than 3 mo of age are still undergoing bone modeling. To introduce accelerated bone remodeling through ovariectomy on top of bone modeling because of growth confounds interpretation of results.

The few clinical studies on the effect of isoflavones and bone health are in older, generally postmenopausal, women. According to Potter et al. (14), lumbar-spine bone mineral content and BMD increased significantly in the subjects who received 40 g/d protein from isolated soy protein containing

TABLE 1

Possible explanation for variable results in studies of phytoestrogens on bone

Life-stage dependent, estrogen dependent.

Compound dependent, dose dependent, interactions—synergistic or opposing?

Equol vs. nonequol producers.

Lack of dietary control.

2.25 mg isoflavones per gram protein (i.e., 90 mg isoflavones) during a 6-mo study. In another similar human study, perimenopausal subjects who received isoflavone-rich soy diet (80.4 mg/d aglycone isoflavones) experienced an attenuation of bone loss from the lumbar spine, whereas the control group had significant loss (15). Soy extracts containing 80 mg/d isoflavones, but not 40 mg/d, significantly increased bone mineral content (BMC), but not BMD, in postmenopausal Chinese women, at the trochanter after 1 y, but only in those women with low initial bone mass (16). In contrast, Hsu et al. (17) did not find significant changes in calcaneus BMD of postmenopausal women after 6 mo of isoflavone supplementation at 150 mg/d. This study is difficult to interpret without a control group. In a small study of young women, isoflavone-enriched (90 mg/d) soy protein fed for 1 y had no effect on BMC or BMD (18). Nor was a dose–response effect found after 9 mo (19). A large multisite trial found a synthetic isoflavone, ipriflavone, was not effective in reducing bone loss in postmenopausal women (20), whereas, studies using purified genistein (21) and red clover isoflavone (22) found these supplements effective on bone.

Clinical studies of small sample size or of short duration cannot adequately assess benefits to bone quality, a slowly responding organ. The length of a human remodeling cycle is ~120 d. Ideally, evaluation of treatments on bone properties should occur over several remodeling cycles. Bone turnover can be evaluated more quickly, but biochemical markers of bone turnover are highly variable and require large sample sizes. Calcium isotopic tracer studies to assess bone turnover, and specifically bone resorption, have not shown benefits of soy isoflavones (unpublished data from our laboratory). The one study that was adequately powered and of sufficient duration to assess effectiveness of isoflavones on bone mass was the European multisite trial of ipriflavone (20). Postmenopausal women ($n = 475$) were randomized to either ipriflavone (600 mg/d) or placebo for 3 y in a double-blind trial. Neither bone loss nor biochemical markers of bone turnover were affected by treatment. Longer (2 and 3 y) multisite trials using soy isoflavones are underway in the United States.

Compound dependent interactions and dose effects

The mixed results of the effect of isoflavones and health may be because of differential actions of individual isoflavones and their effective doses. It is likely that isoflavones differ in their bioactivity for various target tissues. Effects of individual isoflavones may be dose dependent, have a threshold effect, exhibit a biphasic response, or have no effect at all. Various isoflavones may interact in synergistic or antagonistic ways or have no interaction.

Genistein is one of the most abundant and estrogenic isoflavones in soy. The amount of genistein in most of the soy-food materials ranges from 0.2 to 1 mg/g as various forms of glycosidic conjugates. Morabito et al. (21) reported that administration of 54 mg/d genistein was as effective as HT in protecting against bone loss in postmenopausal women. Genistein increased femur and spine BMD (**Fig. 1**); reduced bone resorption, as assessed by a decrease in urinary pyridinium cross-links; and increased bone formation, as assessed by serum-bone-specific alkaline phosphatase and osteocalcin in postmenopausal women. This strong result may be because of the high dose of a single bioactive isoflavone comparable with the total isoflavone dose in other studies that used isolated soy protein (14,15), or it may be because genistein was unopposed by antagonistic components in soy, e.g., daidzein.
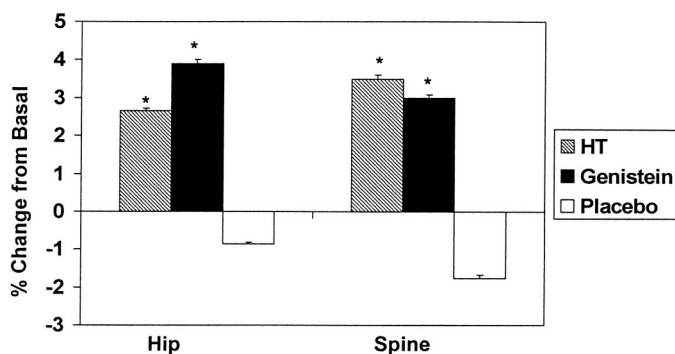
FIGURE 1 Change in femoral neck BMD in postmenopausal women given 54 mg/d genistein (n = 30), HT (n = 30), or placebo (n = 30) for 1 y. *Treatment differences were significant at $P < 0.01$ compared with the placebo group (32).

A comparison of the results of the study by Morabito et al. (21) (Fig. 1) and by that of another randomized, placebo-controlled trial (22) in 177 slightly younger pre-, peri-, and postmenopausal women aged 49–65 y is informative. The Atkinson et al. (22) study tested a red clover derived isoflavone supplement that provided a daily dose of 26 mg biochanin A, 16 mg formononetin, 1 mg genistein, and 0.5 mg daidzein. Thus, the total daily dose of isoflavones was similar [54 mg in the study by Morabito et al. (21) vs. 45 mg in the study by Atkinson et al. (22)]. The intervention duration of 1 y was identical between the 2 studies. Although the isoflavone treatment was effective in both, the nature of the differences is striking. In the study by Atkinson et al. (22) (**Fig. 2**), women were losing bone, and the effect of dietary isoflavones was to suppress bone loss in the spine, but benefits to the hip did not reach significance. In contrast, in the study by Morabito et al. (21) (Fig. 1), both isoflavones and HT increased BMD of the spine and the femoral neck (by 3.5 ± 3%), despite a smaller number of subjects per group. Typically, short-term benefits of diet are seen in areas of largely trabecular bone, e.g., the spine, and the benefits are usually to suppress bone loss rather than to induce bone gain. Estrogen replacement suppresses bone loss.

Several animal studies have also shown positive effects of genistein on bone. Daily injection of genistein in 2-mo-old female rats at 5 μg/g body weight resulted in reduction by >50% of the early postovariectomy BMD loss via bone-for-mation enhancement, without any effect on bone resorption, significantly higher serum osteocalcin levels, both within the sham-operated and ovariectomized rats, and prevention of increased production of TNFα, a potent inhibitor of bone formation (8). It was suggested that the bone-sparing action of genistein may be primarily because of the effects on cancellous bone. In another study, subcutaneous genistein treatment at 0.4–0.8 mg/d for 3 wk in orchidectomized male mice significantly prevented orchidectomized-induced bone loss (23). According to Albertazzi (4), 0.5–0.7 mg/d genistein is effective in preventing ovariectomy-induced bone loss without uterine stimulation in animals. Specifically, subcutaneous administration of genistein at 0.7 mg/d prevented trabecular bone loss in ovariectomized mice without hypertrophic effects on the uterus, whereas 5 mg/d induced uterine hypertrophy (24), and 0.5 mg/d had a similar effect to that of conjugated equine estrogens on retention of trabecular bone tissue (4). In an unusual animal model, an ovariectomized lactating rat, a biphasic response to genistein doses was observed, with an

intermediate dose being most beneficial (9). The authors suggested an estrogen agonistic effect at lower doses and an antagonistic effect at higher doses.

There are numerous proposed mechanisms by which genistein improves bone health. These include genistein's positive estrogen-like effect on bones via a mechanism that involves ER-β. It has been demonstrated that antiestrogen tamoxifen inhibits the anabolic effect of pure genistein in culture medium at 10^{-5} M concentration on bone compartments in the femoral–metaphyseal tissues from elderly female rats (25). Genistein's positive effect on bones might also be because of its strong inhibitory effect on protein tyrosine kinase (26). The effects of low genistein concentration are likely to be ER-mediated, whereas that of high concentration (10 μM) is tyrosine kinase inhibition. Also, genistein is an osteoclast inhibitor in in vitro studies, with genistein-treated osteoclasts in bone-tissue cultures losing their bone degradation potency at relatively low genistein concentrations of between 0.1 to 10 μmol/L. Genistein inhibits the production of acid in the osteoclast (27). The inhibition of osteoclast-like cell formation could also occur via an adenosine monophosphate signaling pathway (4). It has also been suggested that genistein may modulate the production of nitric oxide (a substance that is believed to be an important modulator in bone formation) in bone and hence decrease bone loss via a nitric oxide mediated mechanism. Genistein stimulates protein synthesis in osteoblast cell lines in vitro and exerts an anabolic effect on bone in some animal studies (4). Regardless of the mechanism of genistein's effect in bones, there is not one single target protein or receptor that could mediate and explain genistein's action inside the cell.

Purified daidzein has not been studied directly for its effect on bones in humans. It is present in just slightly lower amounts than genistein in soybeans. Daidzein is one-fourth as estrogenic as genistein, as assessed by uterine weight (28). The relative binding affinities of daidzein to ER-α is 0.2 and 1% for ER-β of that of estradiol. Unlike genistein, daidzein does not inhibit protein tyrosine kinase activity (26) nor suppress mammary tumor development (29). Purified daidzein was more effective than genistein at 10 μg/g body weight in preventing ovariectomy-induced bone loss from cancellous bone after 3 mo in 12-mo-old rats (30). A combination of genistin (159 mg), daidzin (156 mg), and glycitin (33 mg) did not prevent trabecular bone loss in 7-mo-old rats for 12 wk at 0, 20, 40, or 80 mg/kg body weight/d (10). Collectively, this suggests that the 2 isoflavones may be antagonistic.
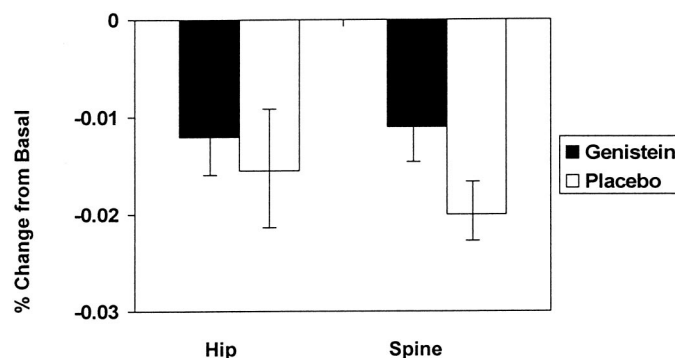

FIGURE 2 Changes in spine and hip BMD in women aged 49–65 y receiving red clover isoflavones (n = 77) or placebo (n = 81) for 1 y. *Treatment effects were significantly different compared with the placebo for the spine ($P = 0.03$) but not the hip (33).

Equol vs. nonequol producers

Equol [7-hydroxy-3-(4′-hydroxyphenyl)-chroman] is the end product of intestinal bacterial metabolism of daidzein (31). It is a mammalian isoflavone rather than of plant origin. The general structure of equol is similar to estrogen. However, equol lacks the lipophilic moiety that is found in estrogen. Equol is excreted in the urine after consumption of soy foods that contain daidzin and daidzein. Only 30–50% of adults excrete equol in the urine (32). The ability to produce equol likely depends on the profile of intestinal microflora, because it can be manipulated by polysaccharide substrates for the bacteria. Equol producers are characterized by higher consumption of energy as carbohydrates and fiber (32).

Setchell et al. (31) put forward a hypothesis that equol production is the key to clinical effectiveness of isoflavones in various tissues, including heart and bone. There are several reasons for this. Equol has affinity for both ER-α and ER-β of similar magnitude to genistein and greater than daidzein but is the strongest isoflavone inducer of transcription, especially with ER-α, in in vitro systems (33). Nearly half of equol in the serum is unbound, which is more than daidzein (18.7%) or estradiol (4.6%), and makes it more available for ER binding (33). It has the highest antioxidant activity of the isoflavones.

The ability of subjects to metabolize equol may predict their ability to benefit from isoflavone-containing interventions. However, equol producers have only been distinguished in one study of bone (34). Increases greater in BMD and BMC were observed in a 2-y intervention of soy milk in the equol producers.

Lack of dietary control

A number of dietary conditions influences bone loss, including calcium, vitamin D, protein, and sodium. Not controlling for diet and other factors that influence bone loss could obscure effects of isoflavone treatment. Estrogen is more effective if calcium intakes approach the recommended intakes of 1200 mg/d in postmenopausal women (35).

Animal chow diets have high levels of isoflavones, which would confound treatment effects. Use of semipurified diets provides constant dietary components, but some of these studies have used calcium-deficient diets.

Recommendations for future research

To clarify the effect of isoflavones on bone health, appropriately powered and controlled studies in humans or in relevant animal models of good design are needed. Among the many questions that still need to be addressed include:
- What is the dose–response effect of the natural complement of soy isoflavones and individual isoflavones?
- Are there agonistic or antagonistic interactions among various isoflavones?
- What are the mechanisms of action of isoflavones on bone health?
- What role does metabolite production of isoflavones play in benefiting bone?
- What is the effect of isoflavones on bone architecture beyond bone density?

The next few years will bring answers to many, if not all, of these questions. There is presently a large federal commitment to determining whether a dietary intervention as simple as soy isoflavones can replace estrogen for postmenopausal women without its adverse effects.

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