Dietary phytoestrogens and their effect on bone: evidence from in vitro and in vivo, human observational, and dietary intervention studies[1–3]

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ABSTRACT Impressive data from the many studies on cultured bone cells and rat models of postmenopausal osteoporosis support a significant bone-sparing effect of the soy isoflavones genistein and daidzein. Translating this research to the clinic has been more challenging, and thus far only a few clinical studies have attempted to tease out the influence of phytoestrogens on bone from the many other components of the diet. Human studies have shown promising although variable results. Studies have been mostly of short duration and with relatively small sample sizes, making it difficult to observe significant and accurate changes in bone. Levels of intake of the soy protein and isoflavones are varied, and the optimal isoflavone intake for bone-sparing effects remains to be determined. Clinical studies thus far performed can be broadly divided into those that have assessed biochemical evidence of reduced bone turnover from measurement of surrogate markers of osteoblast and osteoclast activity, and those that have examined changes in bone mineral density. There are no studies examining effects on fracture rate. This review focuses specifically on the potential influence of phytoestrogens on bone by examining the evidence from 17 in vitro studies of cultured bone cells, 24 in vivo studies of animal models for postmenopausal osteoporosis, 15 human observational/epidemiologic studies, and 17 dietary intervention studies. On balance, the collective data suggest that diets rich in phytoestrogens have bone-sparing effects in the long term, although the magnitude of the effect and the exact mechanism(s) of action are presently elusive or speculative. *Am J Clin Nutr* 2003;78(suppl):593S–609S.

KEY WORDS Phytoestrogens, bone, isoflavones, soy, osteoporosis

INTRODUCTION

Osteoporosis is now a major public health threat, and its prevalence is expected to rise dramatically in the coming decades. Figures from the National Osteoporosis Foundation indicate that about 44 million Americans are at risk for the disease by virtue of having low bone mineral densities. Presently 10 million adults have osteoporosis, and while the majority of these patients are women, it is not a sex-exclusive disease. Nationally, the direct expenditure on treating the 1.5 million fractures that occur each year associated with osteoporosis runs at approximately $47 million every day and, alarmingly, almost a quarter of the patients over the age of 50 y die within 1 y of their hip fracture.

Estrogen deficiency is generally not listed as one of the main risk factors for osteoporosis, but it is indirectly and strongly associated with the many recognized risk factors: being female, being thin, being of advanced age, being postmenopausal, having amenorrhea, and using alcohol excessively. In the 1940s, Fuller Albright first highlighted the importance of estrogen with clinical descriptions of osteoporosis in ovariectomized women and how estrogen improved calcium status (1–3). However, it was not until the 1970s and only after it became possible to directly measure bone density that the full impact of estrogen was realized (4, 5). Although adequate dietary calcium is important in the prevention of osteoporosis (6, 7), acute ovarian deficiency accounts for the loss of $\approx 20\%$ of bone mass in the first 5–7 y of the postmenopausal period (8). Innumerable studies attest to the importance of estrogen in bone remodeling, evident from the fact that hormone replacement therapy (HRT) administered in a dose-dependent manner effectively prevents bone loss in postmenopausal women (5, 9) and reduces the incidence of fractures (10–13). Unfortunately, few women are likely to reap the bone-sparing benefits of HRT long term because of poor compliance due partly to the fear of increased risk for breast and endometrial cancers (14, 15) and because of unwanted side effects associated with these powerful steroids. Recent results from the Women's Health Initiative Study showing an unexpected lack of cardioprotective effects of HRT (14) will undoubtedly serve to increase the search for alternative and natural strategies for menopausal estrogen deficiency, including ways of managing the prevention of osteoporosis with aging. Of all the natural alternatives currently under investigation, phytoestrogens appear to offer the most potential for the prevention of bone loss. Investigations of the bone-conserving properties of isoflavones have been justified by the following lines of tantalizing circumstantial evidence:

1) In vitro studies of cultured bone cells showing isoflavones modulate their activity (16, 17);

2) In vivo beneficial effects of phytoestrogens in animal models of postmenopausal estrogen deficiency, detailed below;

3) Bone-conserving effects in animal models of the synthetic isoflavone ipriflavone, which was later approved for clinical use for the prevention of osteoporosis (18–20) [a recent large multicenter study (21) has since found it to be ineffective, however];

4) Epidemiologic evidence of reduced rates of hip fractures in Asians consuming soy protein despite the lower calcium intakes in this population (22–26);

5) The finding of the estrogen receptors ERα and ERβ in bone (27, 28);

6) The positive effects of selective estrogen receptor modulators such as raloxifene (Evista) in animals (29) and humans (30) and the fact that phytoestrogens such as genistein, by virtue of their similarity to raloxifene in conformational binding to estrogen receptors (31), might be expected to have selective actions in bone (32); and

7) Human dietary intervention studies showing effects of isoflavone-rich soy protein diets on surrogate markers of bone turnover and on reducing bone loss as measured from bone mineral density (BMD) and content (33, 34).

IN VITRO STUDIES OF PHYTOESTROGENS ON BONE CELLS

Bone remodeling is the function of the activity of 2 different cell lines. Osteoblasts, responsible for bone formation, respond to changes in the activity of osteoclasts, the bone resorbing cells. Many hormones, growth factors, and cytokines play a regulatory role in maintaining bone homeostasis (35–38) by their effects on these 2 cell lines, and estrogen in particular is responsible for suppressing osteoclast activity and thereby preventing bone resorption. However, in acute ovarian estrogen deficiency, as occurs in surgical or natural menopause, the rate of bone resorption due to increased osteoclast activity exceeds the rate at which osteoblasts are capable of forming new bone. The net result is depletion of calcium, collagen, and protein from bone, and increased porosity and accompanying risk for fracture. Estrogen receptors ERα and ERβ are both found in human osteoblasts, although the expression of these subtypes varies considerably during differentiation (28, 39). The greatly increased expression of ERβ during bone mineralization (27) is particularly pertinent to the potential hormonal effects of phytoestrogens because compounds such as genistein show a much higher affinity for ERβ than for ERα (40, 41). For example, genistein at physiologic concentrations is a relatively good "estrogen" where ERβ is concerned, and its transcriptional activity is actually almost twice that of estradiol on ERα and ERβ (40).

The first in vitro studies of the action of a number of phytoestrogen classes predated any clinical studies of the actions of phytoestrogens on bone. One of the earliest studies of phytoestrogens found that the coumestan, coumestrol, not only inhibited bone resorption of 9-d-old chick embryo femur explants (42) but also negated the bone resorption effects of parathyroid hormone, vitamin D, and prostaglandin at doses that were 10^{-5} mol/L (43). Some years later, it was reported that the potent antiestrogen tamoxifen blocked the inhibitory actions of genistein on parathyroid-induced

bone resorption in tissue culture (44). Numerous in vitro studies with human and animal osteoblasts or osteoblast-like cell lines, and with osteoclasts, have been carried out, with consistent observations of direct effects of phytoestrogens and related compounds on both cell types (42, 45–59). These are summarized in **Table 1**. Daidzein and genistein have been found to have a stimulatory effect on protein synthesis and on alkaline phosphatase release by various types of osteoblast cells in vitro (60–62). This effect is blocked by the addition of actinomycin or cycloheximide, suggesting that these isoflavones influence transcriptional or translational events. Osteoprotegerin (OPG), a member of the tumor necrosis factor receptor superfamily, prevents bone resorption by a paracrine mechanism (63). It is now apparent that osteoclast activity is modulated through osteoblasts via OPG. The cytokine receptor/activator of nuclear factor-K (RANKL) (64) stimulates osteoclast differentiation and function with higher levels of RANKL expression leading to increased bone resorption. OPG is a ligand for this cytokine and blocks its expression. Ovariectomy, or the pure antiestrogen ICI 182 780 decreases, and estrogen increases expression of OPG mRNA and protein by human fetal osteoblastic cell line (hFOB/ER-9) transfected with ERα (56). More recently, genistein has been found to stimulate the production of osteoprotegerin by human paracrine osteoblasts, providing a further mechanism for the bone-sparing effects of soy isoflavones. It is apparent that in addition to osteoblast and osteoclast activities being coupled, the actions of isoflavones on osteoclasts could also be independent of their effects on osteoblasts because estrogen receptors appear not to be present in the nucleus of these cells. Genistein and daidzein both suppress osteoclast activity by a number of possible mechanisms, including induction of apoptosis, activation of protein tyrosine phosphatase, inhibition of cytokines, changes in intracellular Ca^{++}, and membrane depolarization (45, 46, 51, 65, 66), further highlighting the level of complexity in mechanism of estrogens and phytoestrogens in bone turnover.

While the mechanism of action for isoflavones remains elusive, it is evident from the many lines of evidence that there are probably multiple pathways, genomic and nongenomic, that conserve the integrity and activity of these 2 cell lines to maintain stable bone mass in adults. Certainly the presence of estrogen receptors in bone (27, 28) and the wide-ranging biological properties of these nonsteroidal dietary estrogens (67–70) provide good rationale for thinking that dietary phytoestrogens should play a role in bone remodeling.

IN VIVO EFFECTS OF PHYTOESTROGENS IN ANIMAL MODELS OF POSTMENOPAUSAL BONE LOSS

While in vitro studies provide useful insight into possible actions of isoflavones on individual bone cells, in vivo studies offer the advantage of an intact system that takes account of any coupling effects between osteoblasts and osteoclasts and their progenitor or precursor cells, while also allowing for metabolic events that might influence the efficacy of a candidate compound. For phytoestrogens and most phytochemicals, intestinal metabolism plays a crucial role in their bioavailability and biological activity (71).

Most of the bone studies of phytoestrogens have been performed in rodents that have been ovariectomized, although limited data exist for nonhuman primate species (72) and for pigs (53). The models are accepted models for postmenopausal osteoporosis in that acute ovarian estrogen deficiency leads to rapid

TABLE 1
In vitro studies on phytoestrogens and bone effects[1]

Reference	Model	Treatment	Findings
Soy/isoflavones			
Tsutsumi (42)	Chick embryo femur	Coumestrol or E2—response to resorption induced by PTH, 1,25(OH)D, or PGE$_2$	Both coumestrol and E2 inhibit the induced bone resorption. Coumestrol stimulates bone mineralization. Optimal range of coumestrol $\approx 10^{-5}$ mol/L.
Osteoclasts			
Blair et al (45)	Avian osteoclasts	Genistein or daidzein	Inhibition of bone resorption by genistein but not daidzein, possibly because of genistein's inhibiting effect on tyrosine kinase C.
Williams et al (46)	Avian osteoclasts	Genistein or daidzein effects on tyrosine kinase inhibition and osteoclast HCl transport	Tyrosine kinase inhibition directly inhibits osteoclast HCl transport. Genistein but not daidzein inhibits osteoclast HCl membrane transport and thus inhibits bone resorption.
Rassi et al (50)	Osteoclasts from young female piglets	1,25(OH)D$_3$ compared with E2 compared with daidzein compared with combinations (all 10 nmol/L)	Number of osteoclast-like cells was decreased 51%[2] by daidzein + 1,25(OH)D$_3$ and 33%[2] by E2 + 1,25(OH)D$_3$ compared with 1,25(OH)D$_3$ alone. Number of osteoclast progenitor cells was decreased 54%[2] by daidzein + 1,25(OH)D$_3$ and 50%[2] by E2 + 1,25(OH)D$_3$ compared with 1,25(OH)D$_3$ alone. Apoptosis was induced via caspase-3 activation, and synthesis of both ERα and ERβ receptors increased.
Okamoto et al (51)	Rat osteoclasts	Genistein compared with daidzein effects on inward rectifier (K$^+$) current, I(Kir)	Genistein inhibited I(Kir), independent of PTK inhibition, thus causing membrane depolarization, elevation of Ca^{++}, and inhibition of osteoclastic bone resorption. Daidzein had a similar but weaker effect, whereas the glycoside form genistin did not affect this process.
Osteoblasts			
Anderson et al (49)	MC3T3-E1 (osteoblast-like)	Genistein or E2 effects on IL-6, alkaline phosphatase, mRNA, and collagen type I	No significant difference between E2 and genistein with regard to ALP production, mRNA expression of osteocalcin, and collagen type I. Genistein stimulation on d 4–6 results in higher expression of ER and synthesis of IL-6 compared with that on d 0–2. The reverse was found for E2: higher expression of ER and IL-6 synthesis if stimulated on d 0–2 compared with d 4–6.
Viereck et al (56)	hOB (mature human osteoblasts)	Genistein effects on OPG production	Genistein up-regulated OPG production 2–6-fold in a dose- and time-dependent manner (thus neutralizing RANKL).
Chen and Anderson (57)	MCB T3-E1 (osteoblast-like) hFOB/ER9 (human fetal osteoblasts)	Isoflavone-rich soy product in either glycoside or aglycone form compared with E2, genistein, daidzein, or both genistein and daidzein on OPG, RANKL, mRNA, and IL-6	The aglycone isoflavone product, genistein, daidzein, or both, as well as E2, doubled the OPG/RANKL ratio (thus down-regulating osteoclastic bone resorption), increased osteoblast proliferation 20–30%, and down-regulated Il-6 secretion 30–40% compared with the control or the glycoside form of the isoflavones.
Dang et al (58)	Mouse and cell-line KS483 (osteoblast and adipocyte) progenitor cells	Genistein or daidzein	Genistein and daidzein stimulate osteogenesis as measured by ALP activity, osteocalcin, PTH, and mesenchymal PTH-related protein receptors, as well as by decreasing adipocyte numbers, in an ER-dependent way.
Cho et al (59)	MG63 osteoblastic cells	Genistein, daidzein, or ethanol extracts of soy or black bean (*Rhynchosia molubilis*) compared with E2 and control	Increased cell proliferation with E2 63%, genistein 74%, daidzein 57%, soybean extract 68%, and black bean extract 76%. Black bean extract increased insulin-like growth factor I mRNA expression and estrogen response element plasmid.
Cusack et al (52)	SaOS$_2$ (osteoblast-like)	Genistein compared with daidzein compared with E2 on cell viability and expression of markers	No changes on viability or expression of ALP by any treatment. E2 increased osteocalcin expression slightly. ERβ expression increased by 1 nmol E2/L as well as by daidzein and genistein in a concentration-dependent manner (physiologic range).
De Wilde et al (53)	Osteoblasts from young female piglets	Daidzein (1 nmol/L) compared with control for 15 d	No effect on osteoblast proliferation but an earlier increase in ALP activity (7 d compared with 14 d). Daidzein increased both ERα and ERβ and vitamin D receptor synthesis as well as increased the responsiveness to PTH.
Other phytoestrogens and related compounds			
Mizutani et al (47)	MC3T3-E1 cells (osteoblast-like)	Resveratrol (a phenolic compound found in grape skin, red wine)	Dose-dependent increase of cell proliferation and differentiation as well as DNA synthesis. Increased ALP activity and decreased PGE$_2$ activity. Tamoxifen eliminated proliferation and ALP activity induced by resveratrol.

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TABLE 1 (*Continued*)

Reference	Model	Treatment	Findings
Tamir et al (48)	MC F-7 cells (human breast cancer cells)	Glabridin (an isoflavane from licorice root)	Weak binding to the estrogen receptor. Biphasic dose-response curve. Low levels stimulate breast cancer cell growth (10 nmol/L–10 μmol/L); high levels inhibit both ER+ and ER− cells (>10 μmol/L).
Wattel et al (55)	Mature osteoclasts from young rabbits	Quercetin and kaempferol (flavonols found in a variety of fruit and vegetables)	Both kaempferol and quercetin inhibited bone resorption in a dose-dependent manner (0.1–50 μmol/L). IC_{50} was 0.8 μmol/L for kaempferol and 5.7 μmol/L for quercetin compared with 1.3 μmol/L for genistein and 7.4 μmol/L for daidzein. Apoptosis of osteoclasts was doubled by genistein and daidzein and quadrupled by quercetin. Quercetin maintained this effect in ER-blocked conditions (tamoxifen) and had a potent antioxidant activity in osteoclasts.
Prouillet et al (54)	MG63 osteoblastic cells	Quercetin compared with kaempferol with fisetin on ALP activity	Significant increases in ALP activity with kaempferol: 85% at 50 μmol/L and 40% at 10 μmol/L in 24 h. Similar with quercetin and fisetin, but after 48 h. Signalling pathways possibly involved in mediating the action of flavonoids on ALP is suggested to be a MAP-kinase, especially an extracellular regulated pathway, ERK. Quercetin and fisetin but not kaempferol dramatically inhibited ROS production. All 3 flavonols decreased the activity of AP-1 and NFκB, which are oxidative stress–sensitive transcription factors.

[1] E2, 17β-estradiol; 1,25(OH)D, 1,25-hydroxyvitamin D; PTH, parathyroid hormone; PGE_2, prostaglandin E_2; ALP, alkaline phosphatase; PTK, protein kinase; ER, estrogen receptor; IL-6, interleukin 6; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor kappa B ligand; ROS, reactive oxygen species; I(Kir), inwardly rectified K^+ current; MAP-kinase, mitogen-activated protein kinase; ERK, extracellular regulated kinase; AP-1, proto-oncogene product AP-1 protein; NFκB, nuclear factor kappa B.
[2] $P < 0.01$.

and measurable loss of bone mass; however, they are often highly stressed with regard to calcium requirement. With few exceptions (73–76), soy isoflavones have mainly been investigated. Armandji et al first reported that soy protein isolate was as effective as estradiol in retarding bone loss following ovariectomy (77). The earliest studies examined the effects of soy milk (78) and of soy protein isolate (77, 79) compared with casein, and all found BMD in rats to be highest in the soy-fed animals relative to controls. In total, we can document 22 rodent studies on phytoestrogens and bone (45, 72, 80–99), mostly isoflavones; the study designs, specific models used, and the main findings of each are summarized in **Table 2**. Not all of these studies have been published in detail or subjected to peer review publication, and in some cases data have been taken from published abstracts from recent presentations at national and international congresses. Collectively the study designs are in principle similar, but among individual studies there is high variability. For example, subcutaneous injection, gavage, or oral feeding has been the chosen route of administration of phytoestrogens; the source has been usually isoflavones, either pure compounds (mainly genistein) or soy proteins, with or without their isoflavones; and the control comparisons have been with casein or semipurified diets. In a number of the studies, the effect of phytoestrogens has been compared with conjugated estrogen (Premarin) or estradiol. Primary endpoints generally measured have been bone mass of trabecular and/or cortical bone after ashing, BMD, and mechanical strength, and secondary measures often included surrogate markers of bone turnover and effects on uterine weight. The latter is aimed at addressing the estrogenic effectiveness of the treatment and drawing some conclusions on possible negative effects on the uterus.

As with the findings on ipriflavone, almost without exception phytoestrogens universally influence both trabecular and/or cortical bone in ovariectomized rodents (Table 2). Interestingly, the one exception, a study by Draper et al, found no effect of a red clover isoflavone supplement on BMD of ovariectomized rats (74), yet a recent clinical study reported that a commercial red clover isoflavone supplement increased BMD in postmenopausal women by a surprising (given the physiologically slow rate of bone turnover) 4.1% in 6 mo with no observed dose-response effect (100). Single studies of ovariectomized monkeys (72) and growing pigs (53) have also found no effects of soy isoflavones on bone, so there may be species differences in responsiveness to soy isoflavones. Teasing out the specific component(s) of soy that may be responsible for the bone-sparing effects observed in these animal models is not simple or straightforward, as it is evident that where whole foods or soy protein extracts are concerned there must be multiple effects that collectively contribute. For example, the controversial role of the protein on bone (101–103) should not be discounted because vegetable protein, such as soy, is less hypercalciuric than animal protein (104, 105) and some soy foods, such as soy milk, can reduce calcium bioavailability in adults (106). It should be pointed out that many soymilks are, however, fortified with calcium, and overall the effect of soy protein foods is to reduce urinary calcium excretion and enhance net calcium retention, independent of isoflavones (107). Nevertheless, there is ample evidence from the animal model studies for the effectiveness of isoflavones in conserving bone. To our knowledge, Blair et al (45) were the first to test pure genistein added to the diet, as opposed to an isoflavone-rich soy protein, in ovariectomized Sprague-Dawley rats and found that it increased BMD by 12%

TABLE 2
Animal research regarding soy, isoflavones, and bone effects[1]

Reference	Model	Treatment	Findings
Soy/isoflavones			
Omi et al (78)	OVX rats, BMD, bone markers	High- or low-molecular-weight soy milk compared with control	Significantly higher BMD and mechanical strength in soy milk groups compared with control. Soy milk increased intestinal calcium absorption.
Arjmandi et al (77)	OVX rats, BMD	Natural soy diet compared with casein $\pm$ E2	Soy diet improved BMD 15% compared with control.
Anderson et al (80, 81)	Lactating OVX rats, femur ash weight	Semipurified genistein-rich isoflavone preparation compared with Premarin compared with vehicle	Isoflavones more effective than Premarin in increasing femur ash weight +15% compared with +3% ($P < 0.05$). Biphasic effect on bone retention—lower dosages improve retention; higher dosages are less beneficial.
Blair et al (45)	OVX rats, femur ash weight	Purified genistein compared with genistin compared with control	Genistein improved femur ash weight 12% ($P = 0.07$).
Jayo et al (72)	OVX monkey	Isoflavone-enriched soy (28 mg/d) for 2 y	No effect on bone. Single, relatively small dose.
Harrison et al (79)	OVX rats 10 wk old, BMD, bone markers	4 Groups: soy protein 22%, casein + E2 injection, casein, sham treatments started 2 wk after ovariectomy for 4 wk	Soy, sham, and E2 groups had significantly higher BMD and tibial calcium content than casein group. No uterotrophic effect of soy.
Arjmandi et al (83)	OVX rats 3 mo old, BMD	Casein, soy+ or soy− diet for 65 d starting 35 d after ovariectomy	Slight reversal of cortical bone loss, both for soy+ and soy−, possibly related to higher expression of femoral mRNA transcription of insulin-like growth factor I. No effect on uterus, resorptive bone markers or increase in E2 with soy+.
Arjmandi et al (84)	OVX rats 3 mo old, BMD	Casein, soy+, or soy− diet for 35 d starting at ovariectomy	Ovariectomy-induced bone loss prevented in the soy+ group but not in casein or soy−, compared with sham.
Fanti et al (85)	OVX rats 2 mo old, BMD	Genistein by subcutaneous injection at 1, 5, or 25 µg/g bw starting at ovariectomy, for 21 d	Genistein (5 µg/g bw) prevented bone loss, trabecular and cortical. Genistein associated with higher bone formation as measured by serum osteocalcin and osteoblast cell number. Resorption parameters not affected by genistein. Ex vitro: ovariectomy increased tumor necrosis factor α markedly, and this was blocked by genistein.
Ishida et al (86)	OVX rats, BMD, ash weight from ovariectomy, for 4 wk	Genistein or daidzein (orally 50 mg · kg^{-1} · d^{-1}) compared with estrone 7.5 µg/d subcutaneous	Ovariectomy lowered BMD and diminished mechanical strength, ash weight, and calcium/phosphorous content.[2] These changes were largely prevented by daidzein or genistein. Uterine atrophy and increased uDpy and uPy excretion were prevented by estrone and daidzein but not genistein.
Ishimi et al (87)	OVX mice, BMD	Genistein (0.7 mg/d) or E2 (0.03 mg/d) by subcutaneous injection immediately after ovariectomy	Trabecular bone loss was partially prevented by genistein and completely prevented by E2. Uterine atrophy was prevented by E2, but not by genistein. Increased B-lymphophoiesis was completely restored by both E2 and genistein.
Toda et al (98)	OVX rats, BMD	6-O-acylated-daidzein or -genistein 50 mg/kg, 4 wk, positive controls daidzein and genistein	These new isoflavone glycosides, occurring in fermented soybean (Japanese natto), prevented bone loss, as did daidzein and genistein.
Ishimi et al (88)	OVX mice, BMD	Genistein by subcutaneous injection at 0.7, 2, or 5 mg/d starting at ovariectomy for 4 wk	0.7 mg/d prevented bone loss without uterine hypertrophy (serum level 1.25 nmol/L). 5 mg/d caused uterine hypertrophy in both intact and OVX mice (serum level 20.4 nmol/L).
Picherit et al (89)	OVX rats 12 mo old, BMD	Genistein (10 µg/g bw), daidzein (10 µg/g bw), E2 by oral route starting at ovariectomy for 3 mo	Daidzein and E2 prevented bone loss in trabecular and cortical bone. Genistein prevented bone loss only in cortical bone.
Ishida et al (90)	OVX rats 11 wk old, BMD	Oral genistein, daidzein, or glycitein (50 mg/kg) or E2 (7.5 mg/kg) subcutaneous from ovariectomy and for 4 wk	Daidzein, genistein, glycitein, and E2 prevented femoral bone loss and maintained mechanical strength. Uterine atrophy was prevented by daidzein, glyticein, and E2 but not by genistein. High-dose study.
Jeffery et al (91)	OVX rats 4 mo old, BMD	Casein, soy protein 10% or 20%, or casein + isoflavones 0.4 or 0.8% diets; ovariectomy-negative and E2-positive controls, starting from ovariectomy and for 2 mo	Casein + 0.8% isoflavones prevented bone loss with BMD not different from intact or E2 animals.

(Continued)

TABLE 2 (*Continued*)

Reference	Model	Treatment	Findings
Cai et al (93)	OVX rats 6 mo old, BMD	Casein ± isoflavones, soy protein ± isoflavones, or both diets compared with E2 implants ± isoflavones (9 groups); starting from ovariectomy, 2 mo	E2 prevented bone loss, but none of the diets prevented trabecular bone loss. Soy protein reduced urinary calcium excretion regardless of isoflavone level.
Nakajima (92)	OVX rats 3 wk old	Genistein 12 mg/kg bw compared with resistance exercise compared with combined compared with control. Adaptation and treatment periods of 2 × 4 wk	Uterine weight decreased in all groups except sham.[2] Genistein and exercise increased BMD 5%,[3] with a synergistic effect in the combined group at +8%,[2] compared with the OVX group. Bone loss was prevented in the combined group and slowed in the other.
Wu et al (99)	OVX mice 7 wk old	6 Groups: sham, OVX, OVX + genistein 0.4 mg/d subcutaneous, OVX + exercise 30 min/d, OVX + genistein + exercise, and OVX + E2 0.03 mg/d, for 4 wk	BMD was higher in the genistein and exercise groups and significantly higher in the combined group. Bone volume was restored completely in the combined and E2 groups. Moderate exercise and genistein showed a synergistic effect in preventing bone loss.
De Wilde et al (53)	Growing pigs 6 wk old	Isoflavone-enriched diet at 0, 0.7, 1.4, and 2.8 mg isoflavones · kg bw^{-1} · d^{-1} until slaughter; in vivo and ex vivo assays	No changes in bone growth rate, mineralization, length, density, or osteoclast/blast surfaces. In the 1.4-mg/kg group there were some architectural changes in trabecular bone but no changes in bone volume. In the 2.8-mg/kg group, ovary weight and no. of follicles were increased. OB precursors were stimulated at 1.4 and 2.8 mg/kg.
Uehara et al (94)	OVX mice 3 wk old, male GX rats, BMD	Semipurified diet ± 0.2% isoflavones ± 5% FOS (4 groups), starting immediately after ovariectomy or gastrectomy	In mice, both isoflavones and FOS prevented bone loss, and there was a synergistic effect between them. In rats, bone loss was prevented in the combined group. In both mice and rats, FOS increased equol production from daidzein, which indicated that bifidobacteria are important.
Chanteranne et al (95)	OVX rats 7 mo old	Semipurified diet ± isoflavone extract at 0, 4, 8, and 16 mg · kg bw^{-1} · d^{-1} starting at ovariectomy, for 3 mo	The 16-mg isoflavone dose prevented bone loss, but bone strength was not maintained. No uterotrophic effect was seen.
Arjmandi et al (96)	OVX rats, intestinal calcium transport	Casein or soy ± isoflavones, starting at ovariectomy and for 35 d	Ovariectomy decreased intestinal calcium transport and soy+ prevented this. No changes were seen in 1,25(OH)D or insulin-like growth factor 1 levels.
Fernandes et al (97)	OVX mice	Casein or soy protein with either corn or fish oil for 2 mo before ovariectomy; after 4 mo, BMD and RANKL expression were measured	The most pronounced bone loss (20%) was found in casein + corn oil, which also had a high RANKL expression. Casein + fish oil lost 10%, whereas soy + corn oil lost 13% and soy + fish oil lost 4%. There appears to be a synergistic effect of soy protein and fish oil in preventing bone loss.
Other phytoestrogens and related compounds			
Tamir et al (48)	Female rats	Injected test compound glabridin 2.5, 5, 25, and 250 µg/animal or estradiol 5 µg. Sacrifice at 24 h; organs analyzed for creatine kinase activity	Increased creatine kinase activity in rat tissue similar to or exceeding E2: uterus, dia- and epiphysis, aorta, pituitary, and left ventricle.
Miyamoto et al (75)	OVX rats	8-isopentyl naringenin 30 mg · kg^{-1} · d^{-1} or E2 10 µg · kg^{-1} · d^{-1} injected daily for 2 wk; effect on uterus and bone assessed by bone markers and DXA	Ovariectomy resulted in increased excretion of bone resorption markers and a decrease in BMD and uterine atrophy. Both 8-isopentenyl naringenin and E2 prevented these changes.
Draper et al (74)	OVX rats, femur BMD	Injected coumestrol twice weekly compared with estradiol compared with control	Coumestrol and estradiol improved BMD 5% and 10%, respectively ($P < 0.05$). No effect of diet supplemented with clover.
Horcajada et al (76)	OVX rats 3 mo old	10% Flaxseed diet compared with control	Lignans did not prevent bone loss, but femur mechanical strength increased significantly in the lignan group compared with both OVX and sham groups.[3] Lignans decreased urinary calcium excretion compared with ovariectomy,[4] and bone markers showed a pattern of decreased resorption. Lignans may beneficially affect microarchitecture rather than mineralization.

(Continued)

TABLE 2 (*Continued*)

[1] OVX, ovariectomized; BMD, bone mineral density; E2, 17-β estradiol; OC, osteocalcin; uDpy, urinary deoxypyridinoline; uPy, urinary pyridinoline; OB, osteoblast; bw, body weight; FOS, fructooligosaccharide; RANKL, receptor activtor of nuclear factor kappa B ligand; DXA, dual-energy X-ray absorptiometry.
[2] $P < 0.001$.
[3] $P < 0.01$.
[4] $P = 0.05$.

over a 30-d period following surgery. This observation was subsequently confirmed by others working with the pure isoflavones, and dose-response effects were noted for daidzin and genistin (87), including a biphasic response reported by Anderson et al (81) in which a low dose of genistein (0.5 mg/d) was considerably more effective than higher doses (> 1.6 mg/d) and comparable to Premarin's effects on bone in a lactating, ovariectomized, and calcium-stressed rat model. Also of interest was the finding that delaying administration of genistein until long after ovariectomy was less effective in conserving bone than if it was given immediately on loss of ovarian estrogen (84). This isolated observation may have implications for humans because what is not yet known is whether the timing of administration of isoflavones affects the ultimate outcome for bone. For example, can having early intakes better prevent postmenopausal osteoporosis rather than waiting for menopausal bone loss to be initiated? Overall, the animal studies on phytoestrogens convincingly support in vitro studies showing that isoflavones modulate bone turnover and retard bone loss in acute estrogen deficiency.

CLINICAL AND DIETARY EFFECTS OF PHYTOESTROGENS ON BONE

Short-term human studies of surrogate markers of bone turnover

A number of observational and dietary intervention studies (**Table 3**) confirm the general findings from the in vitro effects of phytoestrogens on bone cells in culture. Thus far, 9 observational or epidemiologic studies (108–116) and 9 dietary intervention studies (117–125) have shown significant relationships between phytoestrogens and surrogate markers for bone turnover that are indirectly consistent with reduced bone turnover (**Table 4**). Markers indicative of osteoblast and osteoclast activity that have been measured include urinary calcium, magnesium and phosphorous, hydroxyproline, and collagen cross-links, while serum measures have included bone-specific alkaline phosphatase, tartarate-resistant acid phosphatase, osteocalcin, insulin-like growth factor I (IGF-I), and interleukin 6. One advantage of using these sensitive markers is that biochemical events occurring in bone can be detected long before significant changes in BMD or bone mineral content (BMC) can be measured, or fractures occur.

Most of the observational studies on bone markers have been performed in women living in countries where the indigenous population have a relatively high phytoestrogen intake, largely because of the consumption of isoflavones in soy protein foods. Typical intakes, estimated at 15–50 mg/d (125–129), however, never approach the high levels currently being adopted in most clinical intervention studies being performed in the Western countries, or for that matter advocated for the prevention of osteoporosis. Notwithstanding the limitations of using bone markers, observational studies have consistently found a significant inverse correlation between isoflavone intake or urinary excretion and the excretion of the bone resorption markers pyridinoline and deoxypyridinoline cross-links for postmenopausal women living in Japan, Korea, and China (109–111, 114), while one study of whites in the United States found urinary NTx to be 18% lower in women with the highest intake of dietary genistein (114).

The acute effects of phytoestrogen-rich diets on bone markers is also revealed in a number of intervention studies summarized in Table 4. There is little consistency in the design among these

TABLE 3
Human observational studies regarding usual soy/phytoestrogen intake, bone and bone metabolism[1]

Reference	Population	Method for assessing soy and isoflavone intake	Site and method for assessing bone	Findings
Soy/isoflavones: postmenopausal women				
Kaardinaal et al (108)	European, $n = 67$	Urine excretion, GC-MS	BMD in radius, annually for 5 y by SPA	No difference in isoflavone excretion between the group with <0.5% compared with those with >2.5% annual loss in this unsupplemented low-intake group.
Guthrie et al (135)	Australian, $n = 354$, age 51–61 y	FFQ with isoflavone-rich foods	Femoral neck by DXA	51 women consumed >40 mg isoflavones/d. Compared with the rest, they had higher intakes of fruit, energy, protein, vitamins, and minerals; were less likely to smoke and more likely to exercise; had lower BMI and negative mood scores; and had higher femoral neck BMD (all $P < 0.05$).
Fukui et al (109)	Japanese in Japan, $n = 39$, age 54.8 y; Japanese in Hawaii, $n = 48$, age 74.4 y	24-h urine excretion, HPLC	Bone stiffness value by ultrasound divided into high-, medium-, and low-stiffness groups	In both populations, the high-stiffness group had significantly higher isoflavone excretion than did the low-stiffness group (20.0 ± 12.8 compared with 8.6 ± 7.7 μmol/d, $P < 0.05$). Significant inverse correlation was found between isoflavone excretion and bone resorption markers pyridinoline and deoxypyridinoline ($r = -0.338$, $P < 0.05$ and $r = -0.387$, $P < 0.05$, respectively).
Horiuchi et al (110)	Japanese, $n = 85$, age 66.9 ± 7.4 y	3-d weighed food records	Lumbar spine BMD by DXA	Soy protein intake ($\bar{x}$: 12.6 g/d; range: 2.8–32.9) significantly associated with higher bone mass relative to age (z score BMD; $r = 0.23$, $P = 0.04$) and with less bone resorption (uDPy; $\beta = -0.08$; $P = 0.03$)
Sung et al (111)	Korean, $n = 160$, 60 high-intake, age 47–85 y	24-h dietary recall, spot urine excretion, HPLC	DXA, lumbar spine and femoral neck	uDPy inversely associated with urinary genistein and femoral neck BMD. Significant inverse association between isoflavone excretion and uDPy ($P < 0.01$).
Rice et al (112)	Japanese American, $n = 267$, age 65–93 y	FFQ with 14-item soy questionnaire categorizing current and lifetime soy isoflavone intake	DXA, lumbar spine and hip	Femoral neck BMD significantly higher in women with a high compared with women with a low lifetime soy intake (0.680 g/cm² compared with 0.628 g/cm², $P \le 0.03$). Lumbar spine BMD higher in women using fiber supplements and having a high current isoflavone intake compared with that in women using fiber and having a low isoflavone intake (0.968 g/cm² compared with 0.843 g/cm², $P = 0.01$). ERT users who were high soy consumers had highest BMD at all sites.
Mei et al (114)	Southern Chinese, $n = 357$, age 63 ± 8.3 y	FFQ and interview with 33 items including 9 soy foods	DXA, lumbar spine	Women in the highest tertile of isoflavone intake (mean 53.3 mg/d) had significantly higher lumbar spine BMD ($P = 0.02$), Wards triangle T-score ($P \le 0.001$), and total hip T-score ($P = 0.02$) than did women in the low- ($\bar{x}$ intake: 2.1 mg/d) or mid-intake ($\bar{x}$ intake: 10.4 mg/d) groups. High isoflavone intake was associated with lower levels of PTH (19.38 compared with 26.56 pg/mL, $P = 0.03$) helping reverse the secondary E2 withdrawal–associated hyperparathyroidism. Compared with the low-intake group, women in the high-intake group had lower osteocalcin (4.95 compared with 6.69 mg/L, $P = 0.05$) and urinary N-telopeptide (34.18 compared with 49.66 nmol bone collagen eq/mmol creatinine, $P < 0.05$).
Somekawa et al (113)	Japanese, $n = 478$ (269 elderly and 209 late menopausal)	FFQ interview with 8 soy foods; urinary excretion of isoflavones by HPLC in a subset	DXA, lumbar spine	Both early and late postmenopausal women in the 2 highest quartile groups of isoflavone intake (50–65 mg/d and +65 mg/d had 7–9% higher BMD than women in the 2 lowest quartile groups (<35 mg/d and 35–50 mg/d; $P < 0.001$ early PMG and $P = 0.01$ late PMG). Absolute values, 0.933 compared with 0.865 g/cm² early PMG and 0.877 compared with 0.806 g/cm² late PMG for highest- compared with lowest-intake groups.
Kritz-Silverstein et al (115)	US white, $n = 208$, age 45–74 y	Standardized questionnaire covering the past year	DXA, lumbar spine and hip	Women in the highest isoflavone intake group had better lumbar spine BMD ($P < 0.10$) after adjustment for all covariates. Urinary NT_X was 18% lower in women in the highest daily genistein intake group compared with women with no genistein intake ($\bar{x}$: 37.29 compared with 45.44, $P = 0.01$).

(Continued)

TABLE 3 (*Continued*)

Reference	Population	Method for assessing soy and isoflavone intake	Site and method for assessing bone	Findings
Kim et al (116)	Korean, $n = 75$, $\bar{x}$ age 58 y	24-h urine excretion by GC-MS; 24-h dietary recall	DXA, lumbar spine and hip	No relation was found between levels of daidzein, genistein, equol, or enterolactone and BMD at any site when subjects were classified as osteoporotic, osteopenic, or normal, based on T-scores, WHO definition. Enterolactone correlated positively with lumbar spine ($r = 0.388$, $P < 0.01$), femoral neck ($r = 0.271$, $P < 0.05$) BMDs. Apigenin correlated negatively with femoral neck and Wards triangle BMD ($r = -0.412$, $P < 0.01$ and $r = -0.395$, $P < 0.01$).
Soy/isoflavones: premenopausal women				
Mei et al (114)	Southern Chinese, $n = 293$, age 37.5 ± 9.4 y	FFQ and interview with 33 items including 9 soy foods	DXA, lumbar spine and hip	No association was found between BMD values and PE intake in women with high endogenous E2 levels.
Ho (132)	Chinese, $n = 116$, age 30–40 y	FFQ, including soy foods	DXA, lumbar spine for 3 y (baseline, 3 y, and in between)	Significantly positive effect of soy isoflavones between women in the first and fourth quartile of soy intake after adjustment for age, body size, lean mass, physical activity, calcium intake, and follow-up time. Soy had significant effect on the maintenance of spinal BMD in women aged 30–40 y.
Song and Paik (133)	Korean, $n = 34$, mean age 22 y	24-h recall, 8 times during 2 y	DXA, lumbar spine and femoral neck (twice at 2-y intervals)	Mean soy intake 36.3 g/d. High- compared with low-intake groups showed a 5.55% compared with 1.37% increase in Wards triangle BMD (adjusted $P = 0.07$), a 2.52% compared with 2.21% increase in lumbar spine BMD (NS), and a 1.31% compared with -0.91% change in femoral neck BMD ($P = $ NS).
Di Leo et al (131)	Italian, $n = 30$, median age 36 y	Interview	Peripheral quantitative computed tomography, distal radius	50% Lactoovovegetarians with PE intake -50% on Mediterranean diet. Total and trabecular bone density were higher in the PE group, although not significantly so.
Other phytoestrogens and related compounds				
Wu et al (151)	Epidemiological study: $n = 497$ men and 540 women, aged ≥ 30 y		Examining relation between tea intake and BMD by DXA	Long-term ($+10$ y) moderate tea consumption (350–550 mL/d) as oolong or green tea is associated with higher BMD in both men and women compared with nontea drinkers: whole body, $+2.1$% ($P = 0.006$); lumbar spine, $+4.3$% ($P = 0.006$); femoral neck, $+4.7$% ($P = 0.002$); Wards triangle, $+6.2$% ($P = 0.003$).

[1] SPA, single photon absorptiometry; GC-MS, gas chromatography–mass spectrometry; HPLC, high-pressure liquid chromatography; FFQ, food-frequency questionnaire; ERT, estrogen replacement therapy; DXA, dual-energy X-ray absorptiometry; PE, phytoestrogen; BMD, bone mineral density; uDpy, urinary deoxypyridinoline; PTH, parathyroid hormone; E2, 17β-estradiol; PMG, postmenopausal group; NT_x, crosslinked N-terminal telopeptides of type I collagen.

TABLE 4
Human intervention studies with phytoestrogen and bone in peri- and postmenopausal women[1]

Reference	Subjects	Intervention (protein type and daily isoflavone level)	Study length	Findings
Soy/isoflavones, BMD endpoint by DXA				
Dalais et al (140)	Postmenopausal, $n = 45$, 4 groups—crossover	Soy grits 45 g compared with wheat kibble 45 g compared with flaxseed 45 g	3 mo	No change was seen in whole-body BMD in any group. All 3 groups had increases in BMC: soy group, 5.2% ($P = 0.03$) and the flax and control groups, 5.2% and 4.0% (NS), respectively.
Potter et al (137)	Postmenopausal, $n = 66$, 3 groups; $\bar{x}$ age 60.8 y (range: 41–83 y)	Casein 40 g/d compared with soy 40 g/56 mg compared with soy 40 g/90 mg; Ca supplemented	6 mo	+2.2% increase in lumbar spine BMD in soy 90 mg group compared with casein −0.6% ($P < 0.05$). The 56-mg isoflavone group remained unchanged at −0.2%. No significant changes occurred in other skeletal sites.
Alekel et al (139)	Perimenopausal, $n = 69$, 3 groups; $\bar{x}$ age 50.6 y (range: 42–62 y)	Whey 40 g/d compared with soy 40 g/4.4 mg compared with soy 40 g/80 mg; Ca supplemented	6 mo	Lumbar spine BMD and BMC remained unchanged in the soy 80-mg isoflavone group at −0.2% and 0.6%, respectively. The whey control group lost significantly: −1.3% ($P = 0.01$) and −1.7% ($P = 0.01$) for BMD and BMC. Minimal nonsignificant loss occurred in the low-isoflavone group (−0.7% and −0.6% for BMD and BMC). Regression analysis revealed that 80 mg soy isoflavones had a positive effect on changes in BMD (5.6%; $P = 0.02$) and BMC (10.1%; $P = 0.003$).
Gallagher et al (141)	Early postmenopausal, $n = 65$, 3 groups; $\bar{x}$ age 55 y	Soy 40 g/0 mg compared with soy 40 g/52 mg compared with soy 40 g/96 mg; Ca not mentioned	9 mo	No significant changes among the three groups at lumbar spine or femoral neck; all groups lost bone in the lumbar spine: −0.8%, −2.8%, and −1.7% with increasing isoflavone intakes from 0 to 96 mg. Femoral neck results were −0.3%, −1.3%, and −1.1%, respectively.
Clifton-Bligh et al (100)	Postmenopausal, $n = 46$, 3 groups; $\bar{x}$ age 56.7 y	Clover-derived isoflavone tablets; doses of 28.5, 57, and 85.5 mg	6 mo	BMD at proximal radius and ulna increased 4.1% with 57 mg/d ($P = 0.002$) and 3.0% with 85.5 mg/d ($P = 0.02$). The 18.5-mg isoflavone group remained unchanged at +2.8%. No increase in endometrial thickness was seen in any group.
Morabito et al (142)	Early postmenopausal, $n = 90$, 3 groups	Genistein 54 mg/d compared with E2 1 mg/d + norethisterone 0.5 mg/d compared with placebo	12 mo	BMD increased significantly in both active groups—genistein group +3.6% femoral neck and +3.0% lumbar spine similar to HRT group at +2.4% and 3.8%, respectively. Control group lost 0.65% in femoral neck and 1.6% in lumbar spine. uDpy and uPy decreased in active groups. Serum BAP and serum OC increased in the genistein group and decreased in the HRT group, respectively.
Vitolins et al (144)	Peri- and postmenopausal, $n = 172$, 3 groups	Soy 25 g/<5 mg compared with soy 25 g/42 mg compared with soy 25 g/58 mg	24 mo	Total-body BMD did not differ between groups. Minimal nonsignificant loss of −0.5%, −0.3%, and −0.9% with increasing soy isoflavone intakes, indicating bone-sparing effect on cortical bone of soy protein regardless of isoflavone level.
Lydeking-Olsen et al (145)	Postmenopausal, $n = 89$, 4 groups; $\bar{x}$ age 58 y (range: 41–75 y)	Soy milk 500 mL·18 g^{-1}·< 1 mg^{-1} compared with soy milk 500 mL·18 g^{-1}·85 mg^{-1} compared with progesterone compared with combined; Ca supplemented	24 mo	Prevention of bone loss in the lumbar spine by soy with 85 mg isoflavones. Nonsignificant increase of 1.1% and 2.2% in BMD and BMC, respectively, with soy containing 85 mg isoflavones compared with losses of 4.2% and 4.3%, respectively, in control group. Negative interaction between 85 mg soy isoflavone and progesterone groups. Minimal ($P = $ NS) change in femoral neck BMD and BMC among the groups.
Anderson et al (143)	Young, healthy adult women, $n = 27$, age 21–25 y	Isoflavone-rich diet, 90 mg compared with control diet	12 mo	No effect of soy diet on BMD or BMC in healthy, menstruating women.
Bone marker endpoints				
Wong (119)	Postmenopausal, $n = 6$, open pilot study; $\bar{x}$ age 55.4 y	Soy isoflavones, 160 mg/d	6 wk	Resorption markers: uDpy, −7 ± 41%; urinary Ca concentration, −12 ± 46%; sPTH, +33 ± 60%; urinary Ca excretion, −5 ± 29%. Bone formation markers: serum OC, 9 ± 15%; serum BAP, −16 ± 23%; serum IGF-I, −8 ± 27%. Changes are not significant because of small sample size and large interindividual variation. Changes are of similar magnitude to those reported for ERT.

(Continued)

TABLE 4 (*Continued*)

Reference	Subjects	Intervention (protein type and daily isoflavone level)	Study length	Findings
Wangen et al (118)	Premenopausal, $n = 14$; postmenopausal, $n = 17$ (double-blind crossover)	Soy protein with 8 (control), 65 (low), or 130 (high) mg isoflavones/d	3 mo	Resorption markers: uDpy, serum ICTP. Formation markers: serum BAP, serum IGF-I, serum IGFBP3. In premenopausal women, serum IGF-I and IGFBP were increased by the 65-mg diet, and uDpy increased in both 65-mg and 130-mg isoflavone diets. In postmenopausal women, BAP was decreased by both diets, and a trend toward decreased OC, IGF-I, and IGFBP was seen with increasing isoflavone intakes. Changes were of small magnitude.
Pansini et al (117)	Postmenopausal, nonosteoporotic, $n = 40$	60 mg soy protein isolate compared with casein	3 mo	Women taking soy ($n = 17$) had a significant reduction in resorption markers: uDpy, 10% ($P < 0.05$) and urinary Nt_x, 24% ($P < 0.001$) compared with placebo.
Khalil et al (121)	Men, $n = 64$, $\bar{x}$ age 55.4 y: $n = 21$, age <65 y; $n = 43$, age >65 y	40 g casein/d compared with 40 g soy protein/d	3 mo	Soy protein diet increased significantly serum IGF-I, 104% and 45% in young and old men, respectively, compared with corresponding 41% and 17% for casein in the young men. No changes in total and bone-specific ALP, or in urinary excretion of Dpy, OH-Py, or Mg, Ca, and P.
Arjmandi et al (120)	Middle-aged and older women: $n = 71$, age 59.2 y; $n = 16$, age <65 y; $n = 55$, age >65 y	40 g soy protein/d compared with 40 g casein/d	3 mo	Soy protein significantly increased serum IGF-I, 109% and 99% for soy compared with 33% and 60% for casein in the younger and older women, respectively; soy protein reduced uDpy excretion significantly, regardless of age group. No changes were seen in total or BAP, OH-Py, Ca, Mg, or P excretion.
Scheiber et al (122)	Postmenopausal, $n = 42$; $\bar{x}$ age 55.5 y; open pilot study	Whole soy foods, 60 mg isoflavones/d; no Ca supplementation	3 mo	Resorption markers: serum ALP unchanged, urinary NT_x decreased 13.9% ($P < 0.02$). Formation markers: serum OC increased 10.3% ($P < 0.02$).
Teramoto et al (123)	Postmenopausal, $n = 26$, age 55.6 y (range: 41–69 y); Single-blind crossover	Isoflavone-rich test drink, 40 mg glycosides compared with placebo drink	2 wk with 3 wk wash-out	Resorption markers: uPy and uDpy excretion reduced slightly ($P = 0.09$) and significantly ($P = 0.05$), respectively.
Lu et al (124)	Postmenopausal, $n = 12$, age range: 49–66 y	Soy milk 1.08 L/d containing $\approx$112 mg isoflavones	4 mo	Increase in uDpy 21%, increase in serum BAP 18%, and increase in OC 34%. Values returned to prediet level 4 mo after diet. No estrogenic effect on the uterus. No changes in serum levels of PTH, follicle-stimulating hormone, estradiol, or testosterone.
Other phytoestrogens and related compounds				
Cook and Pennington (125)	Single-blind, noncontrolled intervention study in early postmenopausal women, $n = 21$, age 53.4 y	Multivitamin-mineral combined with a herbal blend consisting of 6 herbs: *Angelica sinensis, Glycyrrhiza glabra* root extract, *Vitex agnus castus, Cimicifuga racemosa, Helonias opulus,* and *Foeniculum vulgare* extract, 2 y; bone assessed by DXA at lumbar spine, hip, and radius		No protective effect from bone loss associated with menopause. Six of the 21 women withdrew because of excessive losses, 1 because of hyperparathyroidism.

^{1} DXA, dual-energy X-ray absorptiometry; uDpy, urinary deoxypyridinoline; uPy, urinary pyridinoline; PTH, parathyroid hormone; BAP, bone-specific alkaline phosphatase; IGF-I, insulin-like growth factor I; ERT, estrogen replacement therapy; ICTP, carboxy-terminal telopeptide type I; IGFBP3, insulin-like growth factor binding protein type 3; NT_x, crosslinked N-telopeptides of type I collagen; OC, osteocalcin; HRT, hormone replacement therapy; BMD, bone mineral density; BMC, bone mineral content; E2, 17β-estradiol; Ca, calcium; PTH, parathyroid hormone; OH-Py, hydroxypyridinoline; Mg, magnesium; P, phosphorus; ALP, alkaline phosphatase.

human studies, and a variety of soy foods have been tested with differing levels of isoflavones. Nevertheless, most studies have found that when soy foods containing substantial levels of isoflavones are substituted in the diet of postmenopausal women, urinary pyridinoline cross-links are reduced (117–120, 122–124) consistent with reduced bone resorption. One study of 12 postmenopausal women fed about 1 L of soymilk each day for 4 mo actually observed an increase in deoxypyridinoline cross-links (124). To our knowledge, only one study in men has been described; this found that 40 g/d of soy protein as compared with casein fed over a 3-mo period increased serum IGF-I (121), a marker associated with bone formation, but found no changes in urinary hydroxyproline, deoxypyridinoline magnesium, calcium, or phosphorus were detected. Overall, the encouraging findings from the short-term bone marker studies and the vast amount of positive data from in vitro and in vivo studies have given sufficient justification for longer-term clinical studies investigating more fully the role of phytoestrogens on bone.

Epidemiologic and dietary intervention studies of phytoestrogens and bone

Much of the early justification for investigating phytoestrogens, and particularly soy isoflavones, as candidates for preventing bone loss came from the wealth of positive data on the bone-sparing effects of the synthetic isoflavone ipriflavone (19). This pharmacologic OTC agent was found to suppress bone resorption, increase Ca^{++} retention in bone, and augment the action of estrogen on bone and was deemed a rational alternative to HRT in preventing bone loss in acute and ovarian-deficient states and in postmenopausal women. One of its metabolites, coincidentally, is the soy isoflavone daidzein (18, 130). The drug became approved in a number of countries, but a recent large 3-y multicenter clinical study found it to be no better than placebo in preventing bone loss, and reports of lymphocytopenia have raised concerns about its use (21).

Human studies to elucidate the role of phytoestrogens in preventing bone loss can be broadly separated into epidemiologic studies and dietary intervention trials. It is outside the scope of this article to review all the factors that pertain to differences in BMD and fracture rates among Asian and Western populations, but it is evident that these are multifactorial. Even within Asian populations, several observational studies now show that postmenopausal women consuming the highest amounts of soy foods, and hence isoflavones, have the highest femoral and/or lumbar spine BMD (109, 113, 114), an observation also confirmed in 2 studies of Japanese-Americans (109, 112) (Table 3). With regard to premenopausal women, only 4 studies have been reported, and it is not possible to draw conclusions regarding the impact of phytoestrogens on bone earlier in life (114, 131–133). Interestingly, a recent study of Chinese women found that those who consumed the most soy foods as adolescents had the lowest risk for breast cancer as adults; soy food intake as adolescents was assessed from dietary recall questionnaires administered to the study subjects, and the accuracy of recall was confirmed by their surviving mothers (134). Whether this type of early exposure effect could also occur in relation to osteoporosis risk is uncertain, as no prospective studies of soy and BMD, or fracture rates, have been performed to date. Data are limited, so it is difficult to draw conclusions on the relationship between phytoestrogen intake and bone density or fracture rate in adults living in Western countries (108, 115, 135), especially given that phytoestrogen intake is generally negligible in such countries.

Overall, it is difficult to discern whether it is the intake of phytoestrogens or other components of the diet, including lifestyle, that account for what appear to be positive associations between soy food or isoflavones and bone density, but the data are tantalizing enough to warrant clinical investigations. In this regard, only a few dietary intervention studies have been completed thus far (Table 4), and the results have been variable and conflicting (136). Perhaps the biggest problem with these studies is that they are all of different design and of relatively too short a duration to accurately detect significant changes in BMD given the slow rate of bone turnover. The landmark publication of Potter et al (137), which found a significant bone-sparing effect (BMD increased 2.2%) at the lumbar spine of a soy protein diet with an intake of 90 mg/d isoflavones over a 6-mo period but not with 45 mg/d, set the benchmark for the choice of "dosing" in subsequent studies. It should be noted that an intake of 45 mg/d of isoflavones from soy foods had previously been shown to have endocrine-modulating effects on the menstrual cycle of healthy premenopausal women (138). On the issue of dosing, it is not always clear how the isoflavone intake is calculated because the absolute level of isoflavone is considerably higher if expressed as total isoflavones (inclusion of the glycoside portion) as compared with aglycons only. Note that 90 mg of total isoflavone is really equivalent to only 50–55 mg of isoflavones after removal of the glycoside moiety by intestinal bacteria (71), and this is the true maximal bioavailable fraction of the molecule. Nevertheless, there does appear from the few dietary intervention studies thus far performed to be a threshold level of intake below which changes in BMD have been undetectable in the short term. Whether this implies that there are no effects of low doses of isoflavones in the diet, or whether it is a case of low doses taking a very long time to be effective in preventing bone loss, remains to be determined. Certainly, the typical isoflavone intake of Japanese and Chinese women consuming traditional diets [estimated at 15–50 mg/d (126–129)] does not approach the levels being tested in clinical intervention studies. This again poses the question of how important early exposure to phytoestrogens might be in the longer term for bone health. This question could be answered only by long-term intervention studies of premenopausal women.

Since the work of Potter et al (137), there have been 3 dietary intervention studies in postmenopausal women that were of 9-mo duration or less with soy (139–141), and one study that used a red clover supplement rather than soy foods as the source of isoflavones (100). Of these, 2 studies showed no changes in BMD with soy foods containing isoflavones when compared with a placebo or control diets (140, 141), one showed a bone-sparing effect where BMD remained unchanged whereas the control group consuming whey protein significantly lost bone (139), and one showed a surprising 4.1% increase in BMD measured at the proximal radius and ulna (100) with a dose of 57 mg/d of red clover isoflavones. The magnitude of this increase over 6 mo in the latter study seems improbable given the slow physiologic rate of bone gain, while the lack of a dose-response effect is also difficult to reconcile if isoflavones have bone-sparing effects (100). Problems seem apparent with the study by Dalais et al (140), which indicated a 5.2% increase in BMC over a 3-mo period with various phytoestrogen-rich diets yet no change in BMD. This change in whole-body BMC would by our calculations imply the equivalent of a gain of 115–125 g in BMC—seemingly improbable over a 3-mo period.

Only 2 long-term studies in postmenopausal women have been reported to date, both of 2-y duration; these also revealed somewhat conflicting data with regard to isoflavones (144, 145). In one study, the Food and Drug Administration–approved level of 25 g of soy protein for heart health was used, and it was varied with regard to its isoflavone content; 3 different levels of isoflavones— 5, 42, and 58 mg/d—were tested. Total-body BMD did not differ among the 3 groups, and only minimal bone loss was observed, suggesting that soy protein had some bone-sparing effects independent of isoflavones (144). By contrast, a 2-y study from our group of 108 postmenopausal women consuming 500 mL of soy milk (18 g soy protein) containing 85 mg isoflavones (aglycon equivalents) as compared with the same amount of soy milk with < 1 mg/d isoflavones prevented bone loss in the lumbar spine, with only minimal change in the femur regardless of isoflavone level (145). BMD and BMC showed 1.1% and 2.2% increases, respectively, and this change was not significantly different from baseline values in those women consuming soy milk with isoflavones, while women who consumed the same amount of soy protein lacking isoflavones lost 4.2% and 4.3%, respectively, in lumbar spine BMD and BMC ($P < 0.01$ for both measures). This magnitude of change is typical of the usual physiologic loss of bone anticipated in the first 2 y of menopause in the absence of any therapeutic intervention, and it should be mentioned that the bone-sparing effect was unrelated to dietary calcium or protein composition, which was identical in all women. Interestingly, interim analysis of the study data failed to find any significant effects of soy isoflavones on BMD after 1 y as measured by dual-energy X-ray absorptiometry, emphasizing our contention that more long-term studies are needed before definitive conclusions can be reached regarding the effectiveness of phytoestrogens on bone.

More interestingly, we have found that the extent of intestinal metabolism of isoflavones may be the single most important clue to the clinical efficacy of soy foods in preventing bone loss (146). Equol, a specific bacterial metabolite of daidzein (147), and an isoflavone not found in soy, was formed in only 45% of the postmenopausal women studied (145), but in those capable of making equol, referred to as "equol producers," lumbar spine BMD increased by 2.4% ($P < 0.001$ compared with control group), while there was no significant change in BMD in the "non-equol producers." Equol has a much higher affinity for the estrogen receptor than daidzein, its precursor phytoestrogen, and of all the isoflavones it has the highest antioxidant capacity (146), factors that could account for the greater effects observed in equol producers in this bone study (145). The ability to "bacteriotype" individuals for their ability to produce equol now seems crucial in the design of future clinical studies of soy foods, and the failure to do this in all of the previously reported studies may explain the variances in reported findings on phytoestrogens and bone. It is evident there are 2 distinct subpopulations for which soy isoflavones may show different efficacy (146). In some ways, this could be considered analogous to the differences in responses to calcium seen by genotyping for the vitamin D receptor (*VDR*). Although the finding is controversial, calcium supplementation may have greater benefit on BMD in women with the BB genotype rather than the bb or Bb alleles for the *VDR* (148, 149). The role of the many other components of the diet, including but not restricted to vitamin K and vitamin D, and the possibility of nutrient interactions need also to be considered along with lifestyle issues that orchestrate the quality of bone earlier in life. For example, moderate consumption of tea, which contains flavonoids closely related to soy isoflavones, has been found to be associated with higher BMD in men and women (150, 151) and lower rates of fracture (152, 153), while caffeine intake has opposing effects in certain *VDR* genotypes (154). It is not always clear whether such factors are considered in the dietary intervention studies of soy isoflavones or in the observational studies of soy intake and bone in the Asian studies.

SUMMARY

As Erdman et al (136) stated recently, the results from the few studies of phytoestrogens, in particular soy isoflavones and bone health, are provocative and too few to draw definitive conclusions. A wealth of supporting data from many in vitro mechanistic studies of bone cell lines and in vivo studies of models of osteoporosis convincingly shows bone-sparing effects from dietary phytoestrogens. The data are, however, sufficiently tantalizing to justify large-scale clinical dietary intervention studies of phytoestrogens. The timing of intervention, however, is an important consideration because it is possible that soy isoflavones may offer the maximum benefit for prevention rather than treatment of osteoporosis. Ultimately, a prospective study of the impact of phytoestrogen-rich diets on fracture rate would provide definitive answers to the efficacy of phytoestrogen-rich diets and their value as a possible alternative to pharmacologic treatments of what will likely become a disease of epidemic proportions in the future.

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