Dietary Soy Phytoestrogens and Biomarkers of Osteoporosis

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Abstract

Osteoporosis, decreased bone strength increasing the risk of fractures, is the result of alterations in bone remodeling causing an imbalance between bone formation and resorption with a predominance of resorption. In postmenopausal women, bone loss increases due to lower levels of estrogen. One of the most common treatment strategies for osteoporosis after incidence of fractures is the use of antiresorptive agents to stimulate osteoblastic proliferation. Hormone replacement therapy (HRT) for the treatment of menopausal symptoms also reduces the risk of osteoporosis, although its adverse side effects have led researchers to investigate alternative treatments. Dietary soy phytoestrogens have gained considerable attention for exhibiting beneficial effects on bone metabolism and modulating related biomarkers of osteoporosis. Studies using cultured bone cells and postmenopausal rat models support a significant bone-sparing effect of soy phytoestrogens. These findings have initiated clinical studies for the evaluation of soy phytoestrogen effects on postmenopausal bone loss. Human clinical studies have shown both promising and conflicting results. Only few studies show that consumption of soy phytoestrogens increase bone mineral density in postmenopausal women, whereas most studies show no such effects. This short review focuses on the potential effects of soy-derived phytoestrogens on biomarkers (alkaline phosphatase, N-telopeptide of type 1 collagen) of osteoporosis by examining the evidence from in vitro cultured bone cells, in vivo animal models, and human clinical studies. These collective data suggest the bone-sparing effects of soy phytoestrogens.

Keywords

Osteoblast • Osteoclast • Ovariectomy • Orchidectomy • Genistein • Daidzein • Soy isoflavone • Bone mineral density • Ipriflavone • Postmenopause

Abbreviations

ALP	Alkaline phosphatase
AP-1	Activator protein 1
ASC	Adipose-derived stromal/stem cell
BAP	Bone-specific alkaline phosphatase
BMC	Bone marrow stromal osteoprogenitor cells
BMD	Bone mineral density
BMP	Bone morphogenetic protein
BMSC	Bone marrow-derived mesenchymal stem cell
BV/TV	Trabecular bone volume
Cbfa1	Core binding factor 1
Cd	Cadmium
$CdCl_2$	Cadmium chloride
CLO	Caged layer osteoporosis
Col I	Collagen type 1
DXA	Dual-energy X-ray absorptiometry

E2	17β-estradiol
E2B	Estradiol-3 benzoate
ER	Estrogen receptor
ER-PKCα	Estrogen receptor-protein kinase C alpha
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
FDA	Food and Drug Administration
FRAX®	Fracture Risk Assessment Tool
HOB	Trabecular bone osteoblasts
IGF	Insulin-like growth factor
IP	Ipriflavone
MAPK	Mitogen-activated protein kinase
MAR	Mineral apposition rate
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NTx	N-telopeptide of type 1 collagen
OCN	Osteocalcin
O-DMA	*o*-Desmethylangolensin
OPG	Osteoprotegerin
ORX	Orchidectomized
OVX	Ovariectomized
PTH	Parathyroid hormone
RANKL	Receptor activator of NF-kappa B ligand
ROI	Region of interest
SD	Standard deviation
SIE	Soy isoflavone extract
Tb.Sp	Trabecular separation
TGF-β	Tumor growth factor-beta
Th.N	Trabecular number
VDR	Vitamin D receptor
vitD3	Vitamin D3
VOI	Volume of interest
WHO	World Health Organization

Key Facts

Key Facts on In Vitro Effects of Soy Phytoestrogens on Bone Cell

- Soy phytoestrogens suppress the formation of osteoclast.
- Soy phytoestrogens promote proliferation and differentiation of osteoblast.
- Osteoblastic differentiations are induced by expressions of *BMP*, *Col I*, and *OCN* genes as well as p38 MAPK-Cbfa1 and estrogen receptor-protein kinase C alpha (ER-PKCα)-related signaling pathways.
- Osteoclastic differentiation is induced by suppression of NF-kB (RANKL).

Key Facts on In Vivo Effects of Soy Phytoestrogens in Ovariectomized (OVX) Rats

- Soy phytoestrogens prevent bone loss in OVX rats which represent the condition of postmenopausal estrogen deficiency.
- Soy phytoestrogens increase femoral mass as well as both tibia and femur BMD in OVX animals.
- The activity of soy phytoestrogen is enhanced in the presence of other supplements such as vitamin, soy extract, and soy yogurt.

Key Facts on In Vivo Effects of Soy Phytoestrogens in Intact and Orchidectomized (ORX) Rats

- Soy protein without isoflavone enhances bone quality in ORX rats.
- Soy isoflavones show effects on Tb.Sp, trabecular number, and BV/TV in ORX rats.
- Soy phytoestrogens exhibit positive effects on bone health in in utero and intact rats.
- Soy phytoestrogens show no effects on bone heath of intact rats as well as on the lactation period in female rats.

Key Facts on Effects of Soy Phytoestrogens in Postmenopausal Women

- Soy phytoestrogens exhibit conflicting results in human clinical studies.
- No effects on bone loss and bone turnover.
- Increases bone formation and reduces bone resorption in few studies.
- Shows positive effects on BMD in few studies.
- No effects on bone marker level on bone biomarkers such as bone-specific alkaline phosphatase (BAP) and N-telopeptide of type 1 collagen (NTx)/creatinine.

Key Facts on Effects of Ipriflavone, a Synthetic Isoflavone in In Vitro, In Vivo, and Human Studies

- IP is derived from soy isoflavone daidzein.
- IP increases bone formation and inhibits bone resorption in animal and human bone cells.
- IP maintains bone mineral content, restores bone mass, and increases bone or bone marrow percentage in animal models.
- IP prevents bone loss and promotes bone formation in postmenopausal women.

Introduction

Osteoporosis is defined as a condition of low mineral density resulting in fragile bones with increased risk of fracture (Bernabei et al. 2014). The World Health Organization (WHO 1994) defines osteoporosis as a bone mineral density less than 2.5 standard deviations (SD) below the standard reference for maximal bone mineral density of a young adult female. Women are more prone to develop osteoporosis as compared to men due to the decrease in estrogen level after menopause leading to the decline in bone formation and increase in bone resorption activity (Roush 2011). However, male osteoporosis is becoming an increasingly important public health problem (Gielen et al. 2011). One in three osteoporotic fractures occurs in men from age 50 onward and fracture-related morbidity and mortality are even higher than in women (Gielen et al. 2011). Hormone replacement therapy (HRT) is widely used in the prevention and treatment of osteoporosis. However, HRT has considerable side effects, such as increased risks of breast cancer, uterine cancer, and thromboembolism (Ferguson 2004). According to Women's Health Initiative studies, participants on HRT had slightly higher rates of breast cancer, ovarian cancer, heart attack, stroke, thromboembolism, and Alzheimer's disease compared to nonusers (Rossouw et al. 2002; Chlebowski et al. 2003; Shumaker et al. 2003). The problems associated with HRT lead to the development of alternative therapeutics in the management of osteoporosis incorporating phytoestrogens (Brink et al. 2008).

Phytoestrogens are polyphenolic compounds that structurally and functionally mimic the endogenous estrogen, 17β-estradiol (E2), which are broadly classified into three main groups, isoflavones, lignans, and coumestans (Dixon 2004). Soybean (*Glycine max*, Fabaceae) food contains macronutrients such as lipids, carbohydrates, and proteins and micronutrients such as isoflavones, phytate, saponins, phytosterol, vitamins, and minerals (Cederroth and Nef 2009). Soybeans are rich in isoflavones and have been widely used as a dietary source of phytoestrogens in animal and human studies (Cederroth and Nef 2009). The metabolism of isoflavones is complex. Two major isoflavones present in soybeans as β-D-glycosides, namely, genistin and daidzin (Fig. 1), are biologically inactive (Setchell 1998). Once ingested, these glycosides are hydrolyzed in the intestinal tract by bacterial β-glucosidases forming the corresponding bioactive aglycones, genistein, and daidzein, which are absorbed into the bloodstream. Daidzein can be further metabolized in the digestive tract to dihydrodaidzein, equol and *o*-desmethylangolensin (O-DMA), and genistein to *p*-ethyl phenol (Setchell 1998). Isoflavones in soybeans are tightly bound to proteins, which explains the variability of phytoestrogen contents in different soy products and therefore their availability for absorption in the digestive tract. Bhathena and Velasquez (2002) reported the soy protein contents in different soy products as follows: 0.1–5 mg isoflavones/g of soy protein in mature and roasted soybeans, 0.3 mg/g soy protein in green soybeans and tempeh, and 0.1–2 mg/g soy protein in tofu and selected soy milk preparations.

Genistein, daidzein, equol, and O-DMA are the major isoflavones detected in blood and urine of humans and animals (Setchell 1998). In rodents, equol is the

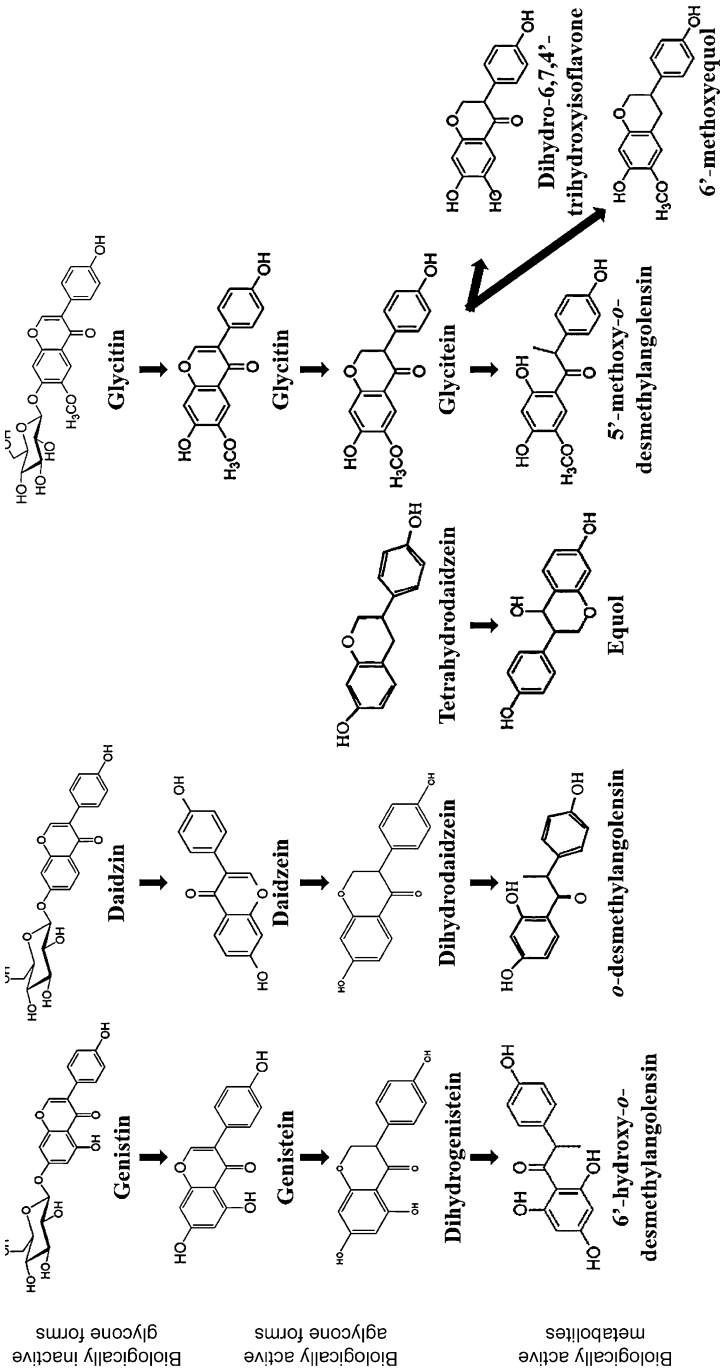

Fig. 1 Soy phytoestrogens and metabolism. The above diagram shows active forms of soy phytoestrogens and their metabolic derivatives

major circulating metabolite representing up to 70–90% of all circulating isoflavones. While all rodents are equol producers, only 30% of humans are able to metabolize daidzein into equol (Atkinson et al. 2005). Pharmacokinetic studies confirm that healthy adults absorb isoflavones rapidly and efficiently (Setchell et al. 2001). The average time for the aglycones in phytoestrogen-rich food to reach plasma concentrations after ingestion is 4–7 h. Hydrolysis of glycosidic moiety of β-glycosides in phytoestrogen-rich food is a rate-limiting step for absorption since it can delay absorption of aglycones to 8–11 h (Setchell et al. 2001).

This review aims to present a brief summary of the role of soy phytoestrogen and its synthetic derivative, ipriflavone (IP), on biomarkers of osteoporosis primarily based on studies using murine and human bone cells, experimental animal models, and human studies (Fig. 2).

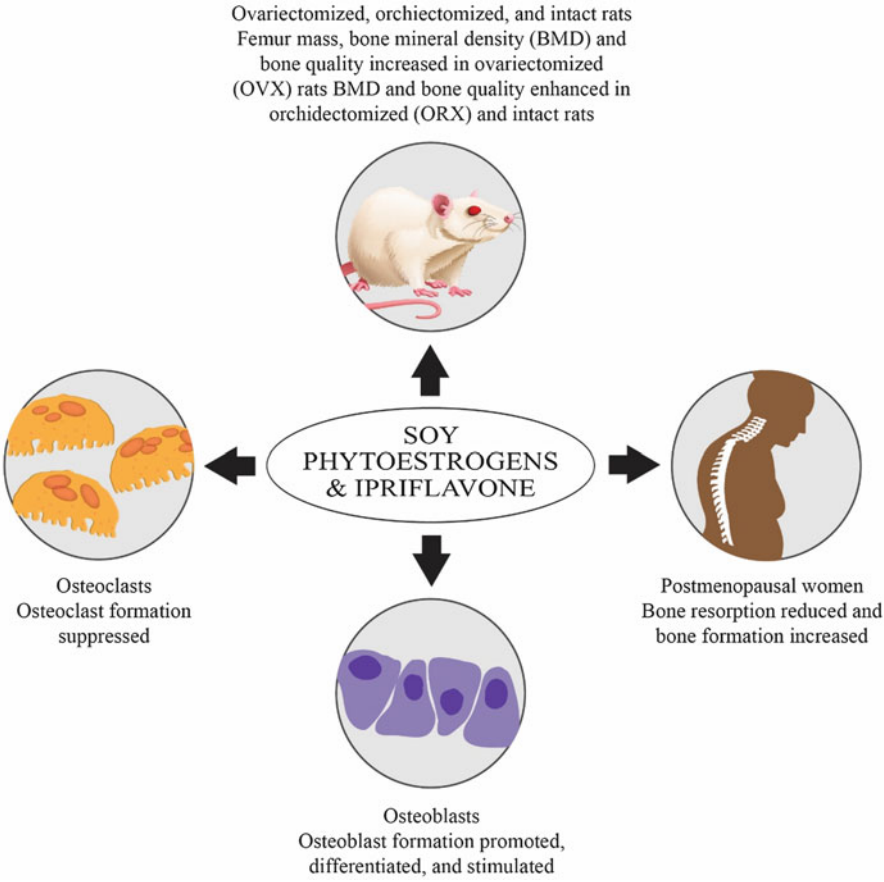

Fig. 2 Effects of soy phytoestrogens and ipriflavone on biomarkers of osteoporosis in experimental models and in humans. The above figure is a summary of the effects of soy phytoestrogens and ipriflavone on bone metabolism and biomarkers of osteoporosis using cultured murine or human bone cells, animals (intact, ovariectomized, orchidectomized), and humans (postmenopausal women)

In Vitro Effects of Soy Phytoestrogens on Bone Cell Metabolism and Biomarkers of Osteoporosis

Bone remodeling is defined as the removal of mineralized bone by osteoclasts followed by the formation of bone matrix by osteoblasts, which subsequently becomes mineralized (Hadjidakis and Androulakis 2006). The remodeling cycle consists of three consecutive phases: resorption, reversal, and formation. During resorption, partially differentiated mononuclear preosteoclasts migrate to the bone surface where they form multinucleated osteoclasts. After completion of osteoclastic resorption, in the reversal phase, mononuclear cells provide signals for osteoblast differentiation and migration and prepare the bone surface for new osteoblasts to begin bone formation. In the formation phase, osteoblasts lay down new bone completely replacing the resorbed bone (Hadjidakis and Androulakis 2006). At the end of the remodeling cycle, the bone surface is covered with flat lining cells and rests for a period of time before the next remodeling cycle (Hadjidakis and Androulakis 2006). Table 1 summarizes the effects of soy phytoestrogens on in vitro murine and human osteoblasts, osteoblast-like cells, osteoclasts, and bone marrow stromal osteoprogenitor cells (BMSCs). In general, the regulation of bone remodeling is both systemic and local. The major systemic regulators include parathyroid hormone (PTH), calcitriol, and other hormones such as growth hormone, glucocorticoids, thyroid hormones, and sex hormones. A large number of cytokines and growth factors that affect the bone cell function are attributed to the local regulation of bone remodeling (Hadjidakis and Androulakis 2006). Examples of growth actors are insulin-like growth factor (IGFs), prostaglandins, tumor growth factor-beta (TGF-β), and bone morphogenetic protein (BMP). Furthermore, through the receptor activator of NF-kappa B ligand/osteoprotegerin (RANKL/OPG) system, the processes of bone resorption and formation are tightly coupled, thus maintaining the skeletal integrity through the bone formation followed by each cycle of bone resorption (Hadjidakis and Androulakis 2006). Besides effects on systemic and local regulators, Table 1 also summarizes the effects of soy phytoestrogens through estrogen receptor (ER) and non-estrogen receptor (non-ER) pathways on bone remodeling. Studies by Chen and Wong 2006, Liao et al. 2014, Strong et al. 2014, and Wang et al. 2014 reported that osteoblastic differentiation in corresponding human and murine bone cells is mediated through the ER pathways. Human osteoblasts express both estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ), although the expression of ER subtypes varies during differentiation (Onoe et al. 1997; Bodine et al. 1998). The greatly increased expression of ERβ during bone mineralization (Arts et al. 1997) is particularly pertinent to the potential hormonal effects of phytoestrogens since compounds such as genistein show a much higher affinity for ERβ than for ERα (Kuiper et al. 1998; Morito et al. 2001). Osteoblastic differentiation is also mediated through mitogen-activated protein kinase-core binding factor 1 (MAPK-Cbfa1) and other non-ER pathways (Liao et al. 2007; Strong et al. 2014). Osteoblastic differentiation and inhibition of osteoclastic formation are modulated by prevention of nuclear factor kappa B (NF-kB) translocation (Lee et al. 2014).

Table 1 In vitro effects of soy phytoestrogens on bone cell metabolism and biomarkers of osteoporosis

Author, year	Bone cells	Phytoestrogen treatment	Significant outcomes
Suh et al. (2003)	Clonal murine MC3T3-E1 osteoblastic cells	Genistein (10^{-5} M) Daidzein (10^{-5} M)	Antiresorptive activity in the presence of TNF-α in both treatments
Chen and Wong (2006)	Human osteoblastic-like SaOS-2 cells	Genistein (10^{-8} M–10^{-6} M)	Stimulation of ER-dependent ALP activities Increase of OPG protein expression Suppression of expression of NF-kB (RANKL) Modulation of osteoclastogenesis
Ge et al. (2006)	MC3T3-E1, mouse calvaria osteoblast-like cell line	Daidzein (10^{-9} M–10^{-5} M)	Differentiation and mineralization, promotion of bone formation in early and late growing osteoblasts
Liao et al. (2007)	Mouse bone marrow-derived mesenchymal stem cell (BMSC)	Genistein (0.01–1 μmol/L)	Stimulation of osteoblastic differentiation through p38 MAPK-Cbfa1 pathway
Lee et al. (2014)	Murine macrophage RAW 264.7 cells	Genistein (1–20 μM)	Inhibition of osteoclast formation of receptor activator of RANKL-induced cells by preventing the translocation of NF-kB
Liao et al. (2014)	Mouse MC3T3-E1, primary osteoblasts	Genistein (0–100 μM)	Induction of ERα gene expression via activation of MAPK/ NF-kB/activator protein 1 (AP-1) Stimulation of osteoblast differentiation and maturation through ERα-dependent expressions of the *BMP-6*, *Col I*, and *OCN* genes
Strong et al. (2014)	Human BMSCs	Daidzein analog 2c (1 μM), 2 g (1 μM), or 2 l (1 μM)	Increase in calcium deposition Activation of distinct ER and non-ER pathways resulting in differentiation of BMSCs and adipose-derived stromal/stem cells (ASCs)
Tadaishi et al. (2014)	Mouse macrophage pre-osteoclast RAW 264 cells	Genistein (10 μM) or equol (10 μM) combined with β-carotene (0.1 μM)	Enhancement of suppressive effect on osteoclast formation

(*continued*)

Table 1 (continued)

Author, year	Bone cells	Phytoestrogen treatment	Significant outcomes
Wang et al. (2014)	Rat neonatal calvaria osteoblast	Equol (0.01–1 mM)	Promotion of proliferation and differentiation of osteoblasts through activating ER-PKCα-related signaling pathway

The above table is a summary of in vitro studies examining the effects of soy phytoestrogens on cultured human or murine bone cell metabolism and biomarkers of osteoporosis

In Vivo Effects of Soy Phytoestrogens on Bone Metabolism and Biomarkers in Ovariectomized (OVX) Rats

In vitro studies provide insight into the effects of soy phytoestrogens on individual cells, whereas in vivo studies provide the advantage of using intact systems that take into account coupling effects among osteoblasts, osteoclasts, their progenitor cells, and the effects of metabolic activities that influence the efficacy of a compound (Setchell and Lydeking-Olsen 2003). Table 2 summarizes the effects of soy phytoestrogens in ovariectomized (OVX) rats. Due to acute deficiency of ovarian estrogen that leads to loss of bone mass, the OVX rat represents a good postmenopausal osteoporosis model (Setchell and Lydeking-Olsen 2003). In rats, ovariectomy leads to a selective reduction in the number of vitamin D receptors (VDR) in jejunum (Chan et al. 1984). This reduction in VDR results in lower responsiveness of intestinal cells to vitamin D signaling and lower calcium absorption by the intestine. This leads to reduction in bioavailability of dietary calcium, an essential building block for new bone formation. Arjmandji et al. (1996) reported that soy protein isolate was as effective as estradiol in retarding bone loss following OVX in rat model. Devareddy et al. (2006) observed that treatment of OVX rats with soy isoflavones did not increase the tibial bone mineral density (BMD) up to the level of sham despite a small percentage (4.5%) of increase in BMD as compared to the OVX controls. The soy isoflavone treatments also did not show any beneficial effects on lumbar microarchitectural properties in OVX rats (Devareddy et al. 2006). Om and Shim (2007) and Rachon et al. (2007) reported the positive effects of purified soy phytoestrogens daidzein and equol on OVX rats. Daidzein increased the femoral mass in cadmium-induced OVX rats (Om and Shim 2007) and equol attenuated trabecular bone loss and increased the density of lumbar spine in OVX rats (Rachon et al. 2007). Other studies (Shiguemoto et al. 2007; Jeon et al. 2009; Zhang et al. 2009; Chang et al. 2013) reported that diet supplemented with isoflavone and vitamin D resulted in high BMD and alkaline phosphatase (ALP) activity and maintained the proper bone microarchitecture indicating the bone-sparing effects of soy phytoestrogens on OVX rats.

Table 2 In vivo effects of soy phytoestrogens on bone metabolism and biomarkers in ovariectomized (OVX) rats

Author, year	Rat strain	Phytoestrogen treatment	Significant outcomes
Devareddy et al. (2006)	Female Sprague–Dawley rats Age: 9 months N = 78	Sham (N = 13) versus OVX (N = 12–13) Five treatment groups [OVX (control): E2, 10 μg/kg body wt; soy protein without added isoflavones, 0.06 mg isoflavones/g protein; soy protein with normal isoflavones, 3.55 mg isoflavones/g protein; soy protein with enriched isoflavones, 7.10 mg isoflavones/g protein]	Soy proteins unable to restore bone loss Isoflavones in higher doses reversed loss of tibial microstructural properties
Om and Shim (2007)	Female Wistar rats Age: 4 weeks N = 45	Sham (N = 9) versus OVX (N = 9) Four treatment groups (experimental diet: $CaCl_2$, 50 ppm; $CaCl_2$, 50 ppm + daidzein, 10 μg per kg of body wt.; $CaCl_2$, 50 ppm + estradiol, 10 μg per kg of body wt.)	Daidzein increased femoral mass and inhibited fast bone turnover in Cd-exposed OVX rats
Rachon et al. (2007)	Female Sprague–Dawley rats Age: 3 months N = 28	OVX (N = 4–5) Three treatment groups (control group, soy-free diet only, N = 8; E2B group, soy-free diet + E2B, N = 10; equol group, soy-free diet + equol, N = 10)	E2B lowered OVX-induced BMD loss at proximal tibia Equol showed no effect Equol and E2B attenuated trabecular bone loss and increased density of lumbar spine
Shiguemoto et al. (2007)	Female Wistar rats Age: 13 weeks N = 56	Sham (N = 7) versus OVX (N = 21) Three treatment groups for each sham and OVX [soy yogurt (aqueous soy extract + 1% lactose + 2.5% nonfat dry milk + 0.7% soy oil + 7% sucrose + 0.3% gelatin + 0.2% stabilizer/emulsifier Recodan®) + sedentary; resistive exercise; soy yogurt + resistive exercise)	Isoflavone-supplemented soy yogurt increased tibia and femur BMD and activity of serum alkaline phosphatase in all treated groups
Jeon et al. (2009)	Female Sprague–Dawley rats Age: 6 weeks N = 30	Sham versus OVX Three treatment groups (non-isoflavone-enriched milk; isoflavone-enriched milk; isoflavone- and Ca-enriched milk + vitamin D and K)	Isoflavone-enriched milk showed partial preventive effect on bone loss Addition of vitamin D and K and Ca increased bone mass

(*continued*)

Table 2 (continued)

Author, year	Rat strain	Phytoestrogen treatment	Significant outcomes
Zhang et al. (2009)	Female C57BL/6 J mice Age: 12 weeks N = 56	Sham (N = 10) versus OVX Four treatment groups [control diet, N = 10; control diet + orally administered E2, 2 mg/kg, N = 12; genistein, 500 mg/kg diet, N = 12; Novasoy (40% 1.3:1:0.3 genistein/daidzein/glycitein + 7–12% protein + 4% ash + 6% moisture + 41% natural soy phytocomponents), 2500 mg/kg diet, N = 12]	Soy extract with genistein more effective than purified genistein in improving tibial trabecular bone quality
Chang et al. (2013)	Female Sprague–Dawley rats Age: 3 months N = 48	Sham (N = 8) versus OVX (N = 40) Four treatment groups [E2; vitD3; soy isoflavone extract (SIE); SIE + vitD3]	Soy isoflavone prevented bone loss Combination of isoflavone with vitD3 increased osterix expression and preosteoblast proliferation

The above table is a summary of in vivo studies examining the effects of soy phytoestrogens on bone metabolism and biomarkers of osteoporosis in OVX rats

In Vivo Effects of Soy Phytoestrogens on Bone Metabolism in Intact and Orchidectomized (ORX) Rats

Osteoporosis poses a great challenge to the aging population in the USA, and though largely manifested in women, men also exhibit risk factors of this degenerative condition (Khosla 2010). One-third of hip fractures and one-half of symptomatic vertebral fractures are reported in men (Johnell and Kanis 2006). One of the causes for male osteoporosis is hypogonadism with aging (Becker 2008; Szulc et al. 2001; Khalil et al. 2005; Soung et al. 2006). Table 3 summarizes the effects of soy phytoestrogens on orchidectomized (ORX) and intact rat models. Few studies (Khalil et al. 2005; Soung et al. 2006; Juma et al. 2012) have focused on the effects of soy phytoestrogens on ORX rats, and even fewer studies (James et al. 2002) have concentrated on the effects on soy phytoestrogens on young and peripubertal rats.

James et al. (2002) aimed to compare calcium metabolism and bone mineralization in young female rats after feeding them casein versus isoflavone-rich diets. Results indicated that compared to soy protein, casein either alone or with the addition of isoflavones showed positive effects on growth and bone mineralization in the peripubertal period when the growth rate was at its maximum. Results also indicated that calcium metabolism was higher in casein with isoflavone-treated rats compared to soy protein. Studies on intact rats yielded mixed results detailed in Table 2. For example, a pilot study by Peterson et al. (2009) demonstrated that bone formation in female Wistar rats during lactation was not effected by soy isoflavone

Table 3 In vivo effects of soy phytoestrogens on bone metabolism in intact and orchidectomized (ORX) rats

Author, year	Rat strain	Phytoestrogen treatment	Significant outcomes
James et al. (2002)	Female Sprague–Dawley rats Age: 3 weeks N = 25	N = 3 Three treatment groups [soy protein (12 %); casein; casein + isoflavone (0.046 %)]	No difference in growth and bone mineralization between casein and casein + isoflavone groups Calcium metabolism high in casein + isoflavone group than soy protein
Khalil et al. (2005)	Male Fisher 344 rats Age: 13 months N = 72	Sham (N = 12) versus ORX (N = 12) Five treatment groups (only AIN-93 M casein-based diet; casein-based diet + 600 mg/kg of isoflavones; casein-based diet +1200 mg/kg isoflavones; soy protein-based diet + 600 mg/kg isoflavones; soy protein-based diet +1200 mg/kg isoflavones)	Casein-based diet supplemented with isoflavones Decreased loss of whole body BMD but not significantly as compared to controls Induced higher bone volume and trabecular number Decreased Tb.Sp
Nakai et al. (2005)	Sprague–Dawley female rats Age: 3 months N = 50	Control group – 200 g casein Four treatment groups (low soy, 100 g soy protein; high soy, 200 g soy protein; low isoflavone extract, 17.2 g; high isoflavone extract, 34.4 g)	Soy isoflavones and soy protein had no effects on femur and lumbar BMD
Soung et al. (2006)	Male Fisher rats Age: 13 months N = 72	Sham (N = 12) versus ORX (N = 12) Five treatment groups (AIN-93 M casein-based control diet; 600 mg/kg of isoflavones; 1200 mg/kg of isoflavones; soy + 600 mg/kg of isoflavones; soy + 1200 mg/kg of isoflavones)	Soy protein diet supplemented with isoflavones reduced ORX-induced decrease of BV/TV and Th.N and increased Tb.Sp at femoral neck site
Ward and Piekarz (2007)	Female and male CD-1 mice Age = 4 months N = 12/group/age	Control (0.4 ml corn oil) Three treatment groups [genistein (3.75 mg) in 0.4 ml corn oil; daidzein (3.75 mg) in 0.4 ml corn oil; genistein + daidzein (3.75 mg each) in 0.4 ml corn oil from day 9 to day 21 of pregnancy through subcutaneous injection]	In utero isoflavone exposure shows no effect on bone health No significant effect on femur peak load

(*continued*)

Table 3 (continued)

Author, year	Rat strain	Phytoestrogen treatment	Significant outcomes
Peterson et al. (2009)	Female Wistar rats Age: 100 day N = 48	Control group – 0 mg aglycone isoflavones/g dietary protein Three treatment groups (2 mg aglycone isoflavones/g dietary protein; 4 mg aglycone isoflavones/g dietary protein; 8 mg aglycone isoflavones/g dietary protein)	Soy isoflavones showed no effects on bone metabolism during lactation
Gautam et al. (2011)	Female Sprague–Dawley rats Age = not reported N = not reported	Two treatment groups [methoxylated daidzein (cladrin), 10 mg/kg/ day dose; formononetin, 10 mg/ kg/ day dose, oral administration for 30 consecutive days]	Cladrin showed increase in MAR and bone formation rates Formononetin showed no effect
Juma et al. (2012)	Male Sprague–Dawley rats Age: 95 days N = 40	Sham (N = 10) versus ORX (N = 10) Three treatment groups (AIN-93 M casein-based diet; soy protein + isoflavone; soy protein without isoflavone)	Regardless of isoflavone content, soy protein was unable to prevent ORX-induced femoral decrease in bone density and mineral content Isoflavone enhanced bone quality by increasing yield force

The above table is a summary of in vivo studies examining the effects of soy phytoestrogens on bone metabolism and biomarkers of osteoporosis in intact and ORX rats

consumption. Another study by Nakai et al. (2005) showed that soy protein and isoflavones have no effects on femur and lumbar BMD of intact Sprague–Dawley female rats. The study by Ward and Piekarz (2007) indicated that genistein, daidzein, or their combinations have no effect in utero and femur peak load. The positive effects of cladrin were reported on female Sprague–Dawley rats (Gautam et al. 2011). Cladrin increased the mineral apposition (MAR) and bone formation rates compared to controls, whereas formononetin showed no effect on bone formation in vivo (Gautam et al. 2011).

Effects of Soy Phytoestrogens on Biomarkers of Osteoporosis in Postmenopausal Women

Postmenopausal estrogen deficiency results in increased bone resorption, which is the major contributing factor of osteoporosis (Leboime et al. 2010). Bisphosphonates, HRT, and other antiresorptive treatments are available for the

treatment and even prevention of postmenopausal osteoporosis. However, HRT has been associated with health problems such as coronary heart disease, pulmonary embolism, and stroke (Rossouw et al. 2002), whereas the use of bisphosphonate can lead to osteonecrosis of the jaw and atypical fractures (Arrain and Masud 2008). These adverse side effects have led to the identification and use of complementary and alternative treatments, which are considered safer and effective (Barnes et al. 2008). Phytoestrogens, especially isoflavones, have been used as dietary alternatives to HRT and Food and Drug Administration (FDA)-approved drugs (alendronate, risedronate, ibandronate, zoledronic acid, raloxifene, denosumab) (Pawlowski et al. 2015). Soybean isoflavones, components of dietary supplements, are genistein, daidzein, and glycitein. Setchell et al. (2002) reported that subjects who have gut microflora that can metabolize daidzein to equol showed greater activity to isoflavones than those who do not have the proper microflora. Table 4 summarizes the effects of soy isoflavones and soy food on postmenopausal bone loss. Results of the studies mentioned in Table 4 show variability regarding the efficacy of isoflavones in preventing postmenopausal bone loss. Some studies (Morabito et al. 2002; Chen et al. 2003; Marini et al. 2007; Pawlowski et al. 2015) show positive bone-sparing effects of phytoestrogens, whereas others (Brink et al. 2008; Alekel et al. 2010; Tai et al. 2012) show no effects on reducing bone loss. These result differences could be attributed to the differences in population under study, sample size, and study duration. In addition to human clinical studies, epidemiology also revealed the protective effects of soy phytoestrogens in women against osteoporosis (Somekawa et al. 2001; Zhang et al. 2005). Thus, while some studies are promising, further research is needed on the effects of whole soy foods, soy proteins, and purified isoflavones in larger trials to support the beneficial effect of soy phytoestrogens in osteoporosis.

Effects of Ipriflavone, a Synthetic Isoflavone on Bone Biomarkers in In Vitro, In Vivo, and Human Studies

Evaluation of phytoestrogens, mainly soy isoflavones, as candidates for bone loss treatment are also supported by results on the bone-sparing effects of ipriflavone (IP) (Brandi 1993). Ipriflavone, 7-isopropoxyisoflavone, is a synthetic isoflavone derived from daidzein in the 1930s (Sziklai et al. 1992; Head 1999) showing positive effects in the treatment and prevention of osteoporosis by suppressing bone resorption, increasing bone calcium retention and enhancing the beneficial action of estrogen on bone metabolism (Reginster 1993). Ipriflavone has been used as an alternative to HRT in the prevention of acute bone loss in postmenopausal women (Reginster 1993). Arjmandi et al. (2000) reported that IP prevents bone loss in postmenopausal women and OVX rats, and Ge et al. (2010) observed the significant effect of IP in increasing BMD, osteocalcin, and hydroxyproline contents in a dose-dependent manner in OVX rats. Ipriflavone is extensively metabolized in the liver and excreted in urine. In dogs and rats, seven metabolites were identified in the plasma. However, in humans, only MI, MII (daidzein), MIII, and MV seem to

Table 4 Effects of soy phytoestrogens on biomarkers of osteoporosis in postmenopausal women

Author, year	Study design	Subject characteristics	Phytoestrogen treatment	Significant outcomes
Morabito et al. (2002)	Randomized double-blind placebo-controlled study Duration: 1 year	Age: 47–57 years N = 90 BMD: femoral neck (<0.795 g/cm^2)	Genistein (54 mg/day) tablets versus placebo	Reduction in bone resorption Increase in bone formation
Chen et al. (2003)	Randomized double-blind, placebo-controlled trial Duration: 1 year	Age: 48–62 years N = 230 BMD: spine (0.6 %) and total hip (1.53 %)	Two treatments: [medium dose (0.5 g soy extracts + 40 mg isoflavones) capsules; high dose (1.0 g soy extracts + 80 mg isoflavones) capsules] versus placebo	Attenuation of bone marrow stromal osteoprogenitor cell (BMC) loss at trochanter, intertrochanter, and total hip
Marini et al. (2007)	Randomized, double-blind, placebo-controlled trial Duration: 1 year	Age: 49–67 years N = 389 BMD: < 0.795 g/cm^2 at the femoral neck	Genistein (54 mg) tablets versus placebo	Decreased levels of bone resorption markers and increased levels of markers of new bone formation Improved BMD and markers of bone turnover
Brink et al. (2008)	Randomized, double-blind, placebo-controlled, parallel, multicenter trial Duration: 1 year	Age: 53–56 years N = 237 BMD: exclusion < −2 *z* scores	Soy isoflavone-enriched biscuits and bars containing genistein (60-75 %), daidzein (25-35 %), and glycitein (1-5 %) versus placebo	No effects on preventing bone loss and on bone turnover
Alekel et al. (2010)	Randomized, double-blind, controlled trial Duration: 3 years	Age: 45.8–65 years N = 432 BMD: lumbar spine and/or proximal femur T scores – low (1.5 SD below young adult mean) or high (1.0 SD above mean)	Two treatments [soy isoflavones (80 and 120 mg/day)] versus placebo	No bone-sparing effects of soy isoflavones Modest effects at the femoral neck

(*continued*)

Table 4 (continued)

Author, year	Study design	Subject characteristics	Phytoestrogen treatment	Significant outcomes
Tai et al. (2012)	Randomized double-blind placebo-controlled trial Duration: 2 years	Age: 45–65 years N = 431 BMD: 1 SD below the young adult female mean value (T-score < −1)	Soy isoflavones capsules (50 mg) containing genistein (57.5 %) and daidzein (42.5 %) versus placebo	No effects on BMD in lumbar spine or total femur No changes in serum BAP and urinary NTx/creatinine
Pawlowski et al. (2015)	Randomized blinded, crossover intervention trial Duration: 4 years	Age: 50–68 years N = 24; equol producers (N = 8) and no equol producers (N = 16) BMD: not reported	Five mixed isoflavone treatments with oral supplements (tablets): [genistein low dose (52.85 mg isoflavones/day); genistein high dose (113.52 mg isoflavones/day); soy low dose (105.23 mg isoflavones/day); soy high dose (219.67 mg isoflavones/day); soy + genistein (161.07 mg isoflavone/day] versus risedronate as control	Mixed isoflavones effective as bone-preserving agent as compared to genistein-enriched isoflavones Capability of converting daidzein to equol showed no effect on suppressing bone resorption

The above table is a summary of the effects of soy phytoestrogens on biomarkers of osteoporosis in postmenopausal women

predominate. Out of these metabolites, MIII is the most potent than MII and MI and MV were least potent (Head 1999). Table 5 summarizes selected in vitro, in vivo, and human studies on the effects of ipriflavone on osteoporosis. Ipriflavone stimulated osteoblast and inhibited osteoclast formation in murine and human bone cells (Giossi et al. 1996; Yao et al. 2007; Civitelli 1997). In vivo effects of IP were observed in caged hens (Yao et al. 2007; Lv et al. 2014) where IP increased egg production while maintaining the bone mineral content and alleviated caged layer osteoporosis (CLO). Ipriflavone also increased bone formation and restored bone mass in male Japanese white rabbits and Sprague–Dawley rats, respectively (Minegishi et al. 2002; Deyhim et al. 2005). According to Zhang et al. (2010), ipriflavone exhibited positive effects in postmenopausal women by inhibiting bone resorption, whereas Alexandersen et al. (2001) and Katase et al. (2001) reported no effects of IP in postmenopausal bone loss and biochemical markers of bone metabolism.

Table 5 Effects of ipriflavone, a synthetic isoflavone on bone biomarkers in in vitro, in vivo, and human studies

Author, year	Bone cells/rat strain/ human subjects	IP treatment	Significant outcomes
Giossi et al. (1996)	Fetal rat long bone cells	IP metabolites [M1, M2, M3 (10 μM), and M5]	Inhibition of parathyroid-stimulated bone resorption
Alexandersen et al. (2001)	Human prospective, randomized, double-blind, placebo-controlled Duration: 4 years Age: 45–75 years N = 474 BMD of lumbar spine (L2–L4) below 0.86 g/cm^2	IP dose: 200 mg, three times/day (N = 234) Placebo (N = 240) All received 500 mg/day calcium	No effect on bone loss No effect on biochemical markers of bone metabolism Induction of lymphocytopenia
Katase et al. (2001)	Human, randomized, placebo-controlled Duration: 2 years Age: 45–75 years N = 89 (premenopausal bilateral OVX = 37 and menopaused or OVX for >3 years before the start of study) Early stage BMD (L2–L4) 1.138 ± 0.220 g/cm^2 Late stage BMD (L2–L4) 0.929 ± 0.077 g/cm^2	IP dose: 600 mg/day for 24 months Placebo for 24 months All received 600 mg/day calcium carbonate (approximate total of 240 mg calcium/day)	Prevention of bone loss compared to placebo No effect on acute bone loss in early stage following OVX
Minegishi et al. (2002)	Male Japanese white rabbits Age: not reported N = 5	IP in a collagen gel versus collagen gel alone	Bone formation increased at an early stage
Deyhim et al. (2005)	Sprague–Dawley rats Age: 90 day N = 72	Two treatment groups OVX + IP (100 mg[sol]/kg body wt./day); OVX + E2 (10 μg[sol]/kg body wt./day) OVX control	Increase in expression of IGF-I in the femur Restoration of bone mass
Civitelli (1997)	Human BMC and trabecular bone osteoblasts (HOB)	IP and its metabolites MI (10^{-6} M), MII, MIII (10^{-5} M), and MV	Bone sialoprotein, decorin, and type I collagen expressions stimulated Bone mineralized matrix deposited

(*continued*)

Table 5 (continued)

Author, year	Bone cells/rat strain/ human subjects	IP treatment	Significant outcomes
Yao et al. (2007)	Embryonic chick calvariae osteoblasts Chick tibias and humeri osteoclasts	IP (10^{-4} M–10^{-10} M)	Stimulation of osteoblasts Inhibition of osteoclasts
Yao et al. (2007)	Caged hens Age: 58 weeks N = 500	Five treatment groups (100 hens/group): 15, 25, 50, and 100 ppm IP Control group-base layer diet	Improvement and increase in egg production while maintaining bone mineral content
Zhang et al. (2010)	Human, randomized, and double-blind Duration: 3 years Age: 45–75 years N = 60 BMD of lumbar vertebrae (L1–L4) below 1 SD in same age group	Two treatment groups [1000 mg/day compound calcium acid chelate + 1 tablet/d vit AD guttate; 1000 mg/d compound calcium acid chelate + 1 tablet/day vit AD guttate + 200 mg (3 times)/day IP] Placebo 1000 mg/d calcium acid chelate	Inhibition of bone resorption Promotion of bone formation
Lv et al. (2014)	Hy-Line Brown laying hens Age: 24 weeks N = 200	Three treatment groups [low-calcium diet CaL; low-calcium diet CaL + 8 mg/kg IP; low-calcium diet CaL + 20 mg/kg IP Control standard diet CaN	Increased in trabecular bone area and bone quality Alleviation of CLO

The above table is a summary of the effects of ipriflavone, a synthetic isoflavone on bone biomarkers in in vitro, in vivo, and human studies

Potential Applications to Prognosis, Other Diseases, or Conditions

As the human population ages, osteoporotic fractures are increasingly recognized as a common and serious health problem that significantly compromise quality of life in elderly people. Osteoporosis and its consequence of fragility fractures are characterized by highly complex phenotypes, which include BMD, bone strength, bone turnover markers, and nonskeletal traits, as reviewed earlier in this chapter. Thus, the early identification of bone biomarkers that are associated with osteoporosis phenotypes or response to therapy can eventually help individualize the prognosis, treatment, and prevention of fractures and their adverse outcomes. Bone density assessment has been identified as a clinically useful and

cost-effective tool in the prognosis and treatment of osteoporosis in older adults (Schousboe et al. 2005, 2007). Although BMD is well established as a predictor of future fracture risk and several prospective studies have demonstrated a 1.5- to 2.5-fold increased risk of fracture for every 1 SD decrease in BMD, this biomarker alone displays poor sensitivity in predicting future fractures. Thus, fracture risk assessment scores are better used in the prognosis of osteoporosis. One such example is the WHO Fracture Risk Assessment Tool (FRAX®), which combines age and sex with clinical risk factors to provide an estimate of the 5- or 10-year probability of fracture for an individual (Kanis et al. 2008). A clear advantage of fracture prediction tools is that they provide an estimate of absolute risk, in that if a 55-year-old woman has osteoporosis according to dual-energy X-ray absorptiometry (DXA), for example, she can still have a low 10-year risk of fracture that might not indicate the need for pharmacological treatment. The estrogenic effects of isoflavones have led researchers to view soy foods and isoflavone supplements as alternatives to conventional hormone therapy. However, as described earlier in this chapter, the evidence that isoflavones reduce bone loss in postmenopausal women is quite conflicting and can be largely explained by the heterogeneity in the study sample and dosing and overall limited clinical studies in this area. Based on the anabolic effects of soy phytoestrogens on bone formation in preclinical animal models of osteoporosis, the inclusion of whole soy foods and beverages may be considered a positive health choice in the older population. Further studies must identify the effects of soy phytoestrogens on novel bone biomarkers, such as those related to genomics, epigenomics, and metabolomics, as well as composite fracture risk scores in the prognosis and management of osteoporosis.

Summary Points

- Soy phytoestrogens promote osteoblastic differentiation through the expressions of biomarkers such as: *BMP* (participates in matrix differentiation and bone formation), collagen type 1(*Col I*) (stimulates osteoblast adhesion and differentiation), and osteocalcin (*OCN*) genes (control osteoblast function).
- Soy phytoestrogens suppress osteoclast formation by inhibiting translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (transcription factor and essential receptor activator for RANKL-induced osteoclast formation) to the nucleus.
- In postmenopausal (OVX) rat models, soy phytoestrogens increase femoral mass and BMD in the tibia and femur.
- In male osteoporosis represented by ORX rats, soy isoflavones show effects on trabecular separation (Tb.Sp), trabecular number, and trabecular bone volume (BV/TV).
- Soy isoflavones increase bone formation, reduce bone resorption, and exhibit positive effects in studies in postmenopausal women, whereas in few studies soy

isoflavones show no effects on bone biomarkers such as bone-specific alkaline phosphatase (BAP) and N-telopeptide of type 1 collagen (NTx)/creatinine.
- Ipriflavone, the synthetic isoflavone, shows positive effects in cultured bone cells, animals, and postmenopausal women.

Definitions of Words and Terms

Alkaline phosphatase (ALP)	An enzyme that hydrolyzes phosphate esters and liberates inorganic phosphate with an optimal pH of about 10.0 serum ALP activity increases in bone diseases such as bone cancer, hyperparathyroidism, and osteitis deformans
Bone marrow-derived mesenchymal stem cell (BMSC)	Postnatal stem/progenitor cells capable of self-renewing and differentiating into osteoblasts, chondrocytes, adipocytes, and neural cells
Bone mineral density (BMD)	Measurement of calcium in the bone which indicates the strength of bone
Bone morphogenetic protein (BMP)	30–38-kD homodimeric family of protein involved in the formation of bone and cartilage and provides morphogenetic signals guiding normal tissue architecture
Lymphocytopenia	A condition with abnormally low levels of blood lymphocytes
Orchidectomized rat	Male rats with one or both testicles removed
Ovariectomized rat	Female rats with one or both ovaries removed
Peripubertal	Early stages of puberty
Postmenopausal	Time period after which a woman undergoes a lack of menstruation for twelve consecutive months
Trabecular bone volume (BV/TV)	The fraction of a given volume of interest (VOI) occupied by mineralized bone. It is reported as a % value and is also used to evaluate a bone volume density following a given treatment
Trabecular number (Th.N)	Quantification of relative number of individual trabeculae within 3-D region of interest (ROI). It is also one of the bone microstructural indices
Trabecular separation (Tb.Sp)	Quantification of relative spacing between individual trabeculae within 3-D ROI. It is one of the bone microstructural indices

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