Comparison of Natural Products for Effects on Bone Balance

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Abstract

Dietary compounds from natural products are the subject of investigation for their beneficial effects on bone. Natural products may be safer and better tolerated by consumers than current therapies for treatment of osteoporosis. Soy isoflavones have been the most studied but results are mixed. Whole soy food consumption in Asian women is associated with reduced fracture incidence in observational studies. However, purified isolated soy isoflavones in randomized controlled trials in postmenopausal Western women are not protective of bone loss. Polyphenolic compounds in plum and berries have both anabolic effects and the ability to suppress bone resorption. These effects occur through antioxidation and anti-inflammatory cell signaling pathways. Rapid screening approaches using urinary excretion of calcium tracers from labeled bone can be used to compare doses and types of natural products for their effect on bone calcium balance.

Keywords

Natural products • Soy isoflavones • Plum • Berry • Bone turnover

Introduction

Loss of estrogen at menopause is a major contributing factor to bone loss in women. Up to 20 % of bone density may be lost in the 5–7 years following menopause [1]. Estrogen replacement therapy is effective at reducing bone loss [2], but has fallen out of favor following the discovery of potential adverse effects in the Women's Health Initiative study [3]. Many natural products, mostly from plant sources, have been investigated as potential alternative therapies. Isoflavones have been the most studied natural product for their effect on menopausal bone loss. Higher fruit and vegetable intake has been associated with greater bone mass in postmenopausal Chinese women [4] and elderly US men and women [5]. Our laboratory has been investigating the efficacy of some plant-derived constituents for their

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ability to improve bone balance following estrogen deficiency associated with menopause.

Measuring Effects on Bone

The most common traditional method for assessing efficacy of interventions on preventing bone loss associated with estrogen deficiency is by measuring bone mineral density (BMD) changes over time measured by dual energy X-ray absorptiometry (DXA). The ovariectomized (OVX) rodent is an approved model of bone loss following menopause. BMD is the most used method because it is predictive of fracture. Fracture studies are not feasible for dietary studies.

Bone turnover rates are also predictive of fracture. Urinary excretion of bone-seeking tracers can be used to monitor bone turnover in relatively short intervention periods and, thus, are useful to compare several interventions in a crossover design. When the intervention is an antiresorptive agent, reduction in excretion of tracer indicates the extent of the antiresorptive agent. Reduction in excretion of tracer indicates the extent of the antiresorptive capacity of the intervention. In a comparison of urinary excretion of a bone-seeking tracer compared to full calcium kinetics in postmenopausal women, it best predicted net bone balance [6].

Muhlbauer et al. [7] surveyed a number of foods for their antiresorptive capacity using tritiated tetracycline (^{3}H-TC) as a bone-seeking label. Rats were given ^{3}H-TC to pre-label their bones and were then randomized to diets with a specific fruit, vegetable, or other food component. Urinary ^{3}H-TC excretion in response to these diets was compared to urinary tetracycline excretion in rats fed a control diet to determine the antiresorptive capacity of the foods. Using this method, Muhlbauer's group found that several vegetables, mushrooms, fruits, and red wine reduced bone resorption.

We have developed a similar method utilizing ^{45}Ca as a bone-seeking label. ^{45}Ca incorporates into bone with higher efficiency than ^{3}H-TC [8]. We previously compared ^{3}H-TC and ^{45}Ca kinetics simultaneously in OVX rats. We developed a 9-compartment model to fit both tracers simultaneously, including two bone compartments with different turnover rates. Bone resorption rates from both bone compartments did not differ between ^{3}H-TC and ^{45}Ca, suggesting that the two tracers may be used interchangeably to measure bone resorption [9]. Therefore, ^{45}Ca is preferred over ^{3}H-TC in animal studies because ^{3}H-TC is more expensive and is a surrogate for calcium in bone mineral matrix.

In humans, neither ^{3}H-TC and ^{45}Ca are practical choices for a bone-seeking label because of safety and the short half-life of ^{45}Ca. ^{41}Ca, a long-lived radioisotope ($t_{1/2}$ = ~10^5 year), can be used as alternative tracer. The long half-life of ^{41}Ca allows it to behave more like a stable isotope, minimizing radiation exposure for subjects and allowing long-term monitoring of bone turnover. ^{41}Ca can be measured in urine of dosed subjects by accelerator mass spectrometry (AMS). The sensitivity of this instrument allows ^{41}Ca excretion to be determined for years following the initial dose. This methodology can be used to screen multiple therapies in a crossover design in the same subject [10]. Figure 14.1 illustrates how changes in urinary ^{41}Ca can be used to determine the efficacy of an intervention. ^{41}Ca correlates with traditional biomarkers of bone turnover including serum

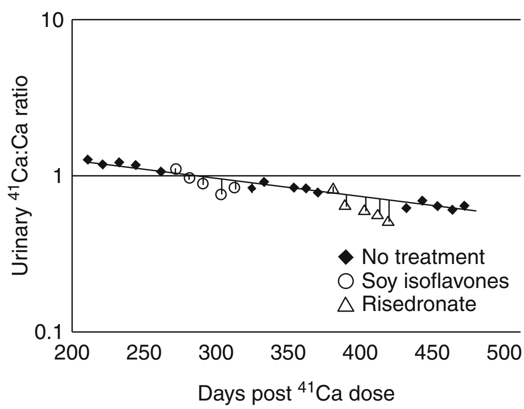

Fig. 14.1 Urinary ^{41}Ca for one subject over multiple treatments. The baseline and washout periods, where no treatment occurs, are used to generate a prediction equation. The difference between this prediction line and the observed ^{41}Ca during a treatment, which is represented by the *vertical lines*, is used to calculate the effect of treatment on net bone turnover

osteocalcin and urinary NTx [11], but is more specific to bone mineral matrix, is more sensitive, and has a greater precision than biomarkers, allowing for reduced sample size.

Soy Isoflavones

Isoflavones have been the most studied natural product. Isoflavones, also known as phytoestrogens, are naturally occurring plant compounds that bind to estrogen receptors in humans and animals. The predominant source of isoflavones is soy, but they are also found in red clover and kudzu. The primary isoflavones found in soy are genistein, daidzein, and glycitein [12].

Epidemiological evidence suggests a link between soy consumption and reduced risk of fracture. In the Shanghai Women's Health Study, a cohort of 75,000 Chinese women aged 40–70 years, there was an inverse relationship between soy isoflavone intake, adjusted for age, calorie intake, and other covariates, and risk of fracture [13]. The Singapore Chinese Health Study, a prospective cohort study of over 63,000 Chinese men and women, showed a significant association between soy intake and hip fracture risk in women but not in men. After adjusting for covariates, women in the second through fourth quartiles of soy intake had 21–36 % lower risk of hip fracture than those in the lowest quartile [14].

The relationship between soy isoflavone intake and fracture risk has not been clearly established in non-Asian populations, largely due to low consumption of isoflavones. Greendale et al. [15]. used data from the Study of Women's Health Across the Nation, an ethnically diverse US community-based cohort, to examine the relationship between genistein intake and bone mineral density. Higher genistein intake was associated with higher spine and femoral neck BMD in premenopausal Japanese women. However, no relationship between genistein intake and BMD was observed in postmenopausal Japanese women or in Chinese women, and genistein intakes in Caucasian and African-American women were too low to pursue analyses. These epidemiological studies are summarized in Table 14.1.

In contrast to epidemiological evidence for postmenopausal women consuming whole soy foods in Asian countries, randomized controlled trials focusing on the effect of isoflavone supplementation on bone mineral density have had largely negative results (Table 14.1). The soy isoflavones for reducing bone loss (SIRBL) study, 3-year randomized, placebo-controlled trial of two doses of isoflavones, 80 and 120 mg/day, found no protective effect on BMD with the exception of a modest effect at the femoral neck [16]. Similarly, Tai et al. observed no effect on BMD following 2 years of supplementation with 300 mg/day isoflavone in Taiwanese

Table 14.1 Epidemiological studies of soy isoflavone consumption and bone health

Reference	Location	Population *n*, characteristics	Findings
Zhang et al. [13]	Shanghai, China	$n=24,403$ Chinese women aged 40–70 years	Soy protein and soy isoflavone consumption was associated with reduced risk of fracture; effect was strongest in women within 10 years of menopause.
Koh et al. [14]	Singapore	$n=63,257$ Chinese men and women aged 45–74 years	Soy intake associated with reduced risk of fracture among women, but not men.
Greendale et al. [15]	USA	$n=2,413$ African-American, Caucasian, Chinese, and Japanese women aged 42–52 years	Higher genistein intake associated with higher spine and femoral neck BMD in premenopausal Japanese women, but not in Chinese women or postmenopausal Japanese women. African-American and Caucasian women had too low of genistein intakes to pursue analysis.

Table 14.2 Randomized, controlled trials of soy isoflavones for postmenopausal bone loss

Reference	Population *n*, average age	Intervention	Duration	Primary outcomes	Effect of isoflavone intervention
Alekel et al. [16]	*n*=255, 54 years	80 or 120 mg/day soy isoflavones or placebo	3 years	Lumbar spine, proximal femur, and total body BMD	No effect except a modest benefit at the femoral neck with 120 mg
Levis et al. [18]	*n*=248, 53 years	200 mg/day soy isoflavones or placebo	2 years	Lumbar spine, total hip, femoral neck BMD	No effect
Tai et al. [17]	*n*=431, 56 years	300 mg/day soy isoflavones or placebo	2 years	Lumbar spine, proximal femur BMD	No effect
Wong et al. [19]	*n*=403, 55 years	80 or 120 mg/day soy hypocotyls aglycone isoflavones or placebo	2 years	Whole body, lumbar spine, total hip, femoral neck, and trochanter BMD and BMC	120 mg/day reduced whole-body bone loss but no effect at regional sites

women [17]. Levis et al. [18]. observed no effect on BMD or menopausal symptoms following 2 years of supplementation with 200 mg/day isoflavones. In a 2-year multicenter trial, Wong et al. found 120 mg/day, but not 80 mg/day, soy hypocotyl aglycone isoflavones exerted a small protective effect on total body BMD, but did not prevent bone loss at common fracture sites [19]. These recent clinical trials are summarized in Table 14.2.

One limitation of the large clinical trials that have been conducted to date is that most studies have focused only on BMD. As previously mentioned, BMD is a major predictor of fracture risk, but is not the only contributor. In a 5-year longitudinal study, Wainwright et al. found that 54 % of subjects who suffered a hip fracture would not be classified as osteoporotic based on their baseline BMD scores [20]. Additionally, changes in BMD cannot fully account for the reduction in fracture risk seen in patients using antiresorptive therapies [21]. Thus, other factors in addition to BMD are important in determining overall bone strength and resistance to fracture. Such factors may include rate of bone turnover, bone shape, size, microarchitecture, and material properties at the tissue level. There is some evidence to suggest that soy isoflavones may impact these factors instead of, or independently of, changes in BMD. Indices of bone turnover have been recognized as BMD-independent predictors of mechanical competence of bone [22]. Using ^{41}Ca methodology, we have tested several isoflavone preparations for their ability to suppress bone turnover. In one study, we investigated the ability of isoflavones from several different plant sources to reduce bone resorption [23]. In this randomized, crossover trial, 11 postmenopausal women were given a dose of ^{41}Ca and, following an equilibration period of 100 days or more, were then assigned to 50-day interventions in a randomized order. The interventions consisted of four botanical supplements from different plant sources, including soy cotyledon, soy germ, red clover, and kudzu, and a positive control treatment of either estrogen or risedronate. Urinary $^{41}Ca:Ca$ during pre-intervention and intervention periods was used to determine suppression of bone turnover. The positive controls, estrogen and alendronate, reduced bone resorption by 22 % and 24 %, respectively. The soy cotyledon and soy germ interventions significantly reduced net bone resorption by 9 and 5 %, respectively, while the red clover and kudzu interventions did not have a significant effect. Although the soy isoflavones were not as effective as the drugs, they may provide some benefit to protecting against bone loss for long periods in non-osteoporotic women without the serious side effects of the drugs.

Evidence from animal studies suggests that isoflavones may affect bone microarchitecture.

In OVX rats, Devareddy et al. found that an isoflavone-enriched soy protein diet restored tibial trabecular number and separation to levels seen in sham rats, but did not restore BMD or BMC [24]. Only one study has looked at the effects of soy isoflavones on bone geometry in humans. Subjects in the SIRBL study underwent pQCT scanning at baseline and at 12, 24, and 36 months of isoflavone supplementation. Scans were taken at the femoral midshaft and the distal tibia. The authors found that soy isoflavone treatment had no significant effects on geometry or volumetric BMD (vBMD) at the distal tibia and only modest effects on femoral midshaft vBMD and stress–strain index (SSI) [25]. However, pQCT is limited to peripheral sites, so whether isoflavone treatment affects bone geometry at clinically relevant fracture sites such as the hip and spine remains unknown. Isoflavones may also improve bone material properties. Vertebrae from OVX rats treated with 5 mg/kg/day genistein for 15 weeks had lower microcrack density and microcrack length, and higher maximum load, than untreated OVX controls. However, BMD and BMC did not significantly differ between the two groups [26]. A novel technology, reference point indentation, has recently made it possible to assess bone material properties at the tissue level in vivo in humans [27]. Future studies with soy isoflavones and other natural products should utilize novel technologies to assess the effect of these treatments on bone material properties, geometry, microarchitecture, and bone turnover.

One potential explanation for the inconsistent results of isoflavone intervention studies is variation in the ability to produce the isoflavone metabolite equol. Equol is a product of bacterial metabolism of daidzein in the intestine. S-equol, the naturally occurring enantiomer, has approximately 80-fold greater estrogen receptor-β binding affinity than daidzein [28], suggesting that it may be a more potent antiresorptive. Approximately 30–50 % of humans have the capacity to produce equol [29]. In a 1-year double-blind trial of 75 mg/day isoflavones in Japanese women, Wu et al. [30]. found that the capacity to produce equol significantly enhanced the effect of isoflavones. Among the women randomized to isoflavone treatment, equol producers experienced BMD changes of −0.46 % at the total hip and −0.04 % at the intertrochanteric region, while non-equol producers experienced changes of −2.28 and −2.61 % at these sites. To determine the effect of equol-producing status on the efficacy of an isoflavone intervention, we prescreened subjects for equol-producing status prior to enrollment in an isoflavone intervention trial. Subjects were categorized as equol producers or non-equol producers based on equol excretion in urine following consumption of 1 soy bar/day for 3 days. Subjects were classified as equol producers if urinary equol was greater than 10,000 nM. Nineteen subjects, including 7 equol producers and 12 non-producers, were dosed with ^{41}Ca and participated in a 50-day intervention with a commercial soy isoflavone product (Novasoy 50, ADM) containing 105 mg total isoflavones, including 46 mg genistein, 44 mg daidzein, and 15 mg glycitein. Net bone turnover decreased by 8 % with the soy treatment, with no significant difference between equol producers and non-producers, suggesting that equol-producing status did not affect the efficacy of the soy intervention [31].

Equol can also be given as a supplement itself. In OVX rats, dietary racemic equol increased femoral calcium content but also had modest uterotropic effects [32]. Tousen et al. [33]. found that supplementation with *S*-equol reduced bone resorption in non-equol-producing menopausal women. Following 12 months of supplementation with 10 mg/day equol, subjects had significantly greater whole-body BMD (but not for regional sites) as well as significantly lower urinary DPD than subjects who received the placebo.

Dried Plum

Dried plum (*Prunus domestica L.*) has been shown to suppress bone resorption, prevent and reverse bone loss, and prevent loss of mechanical strength in sex steroid deficiency female and male animal models of osteopenia [7, 34–36]. In the pre-labeled bone rat model of Mühlbauer described above, dried plum was the most effective fruit source tested. In the 9-month-old orchidectomized, male rat model with established bone loss, feeding dried plum at 25 % of the diet for 90 days increased vertebral and femoral BMD by ~11, 50 % as effective as PTH with about 60 % of the effect of PTH on biomechanical properties [37]. Trabecular microarchitecture was restored (not observed with other dietary interventions) and cortical bone increased through periosteal expansion. Bone resorption was reduced dose-dependently by up to 60 % as determined by urinary resorption markers which is of similar magnitude to bisphosphonates [36]. Feeding dried plum to 6-month- and 18-month-old male mice increased trabecular bone at dietary levels of 25 % plum and bone gain in the younger adult male mice at dietary levels of 15 % [38]. Improvements in bone measures have been associated with a dose-dependent increase in serum IGF-1 in female [34] and male [35] rats and humans [39] which suggests one mechanism of action may be through stimulating this anabolic hormone.

The bioactive constituent(s) in dried plum is uncertain. The mechanism of action of plum on bone differs from classical estrogens, because it has no uterotrophic activity [40]. Dried plums contain high amounts of polyphenols relative to many other fruits and vegetables at 184 mg/100 g [41]. The predominant phenolic compounds are neochlorogenic and chlorogenic acids. These and other phenolics may inhibit bone resorption due to their antioxidant and inflammatory properties. In fact, dried plums have higher oxygen radical absorbance capacity than most fruits and vegetables [42]. However, it may be that specific polyphenolic compounds have potent bone resorption inhibiting potential. For example, dried plums contain 3.3 ng/100 g rutin [41]. Rutin was identified as the bioactive ingredient in onion that makes it one of the most effective plant food or ingredient tested on bone resorption [43]. However, this group later identified Γ-L-glutamyl-trans-S-1-propenyl-L-cysteine sulfoxide as the likely bioactive component of onion [44]. Still, rutin, in purified form, increased BMD in estrogen-deficient osteopenic rats [45]. Although Bu et al. [37]. claimed that the effect of dried plum was greater than the effect of rutin alone, there is no report of a direct comparison. Rutin is hydrolyzed to its aglycone, quercetin, prior to absorption and can be converted to glucuro- or sulfoconjugates during absorption. Quercetin has antioxidant activity, but also binds to ER_{β} [46]. Quercetin dose-dependently inhibited osteoclast-like cell formation, inhibited RANKL, induced tartrate-resistant acid phosphatase of preosteoclasts, and disrupted the actin rings of these precursor cells [47]. This could explain how quercetin-like compounds may reduce bone resorption. In addition to polyphenols, dried plum also contains high levels of potassium (745 mg/100 g), vitamin K (assumed to be high as fresh plums have 8 μg K_1/100 g), and boron (2.2 mg/100 g) [41]. Each of these nutrients has been associated with positive effects on bone [48–50], but they are unlikely to play a major role in reducing postmenopausal bone loss at dietary concentrations [43, 51–53].

Clinical research on dried plums and bone health is minimal. Feeding 100 g/day of dried plums for 3 months increased serum IGF-1 by 17 % and a biochemical marker of bone formation, bone-specific alkaline phosphatase, by 5.8 % in postmenopausal women, while feeding 100 g/day dried apples did not [54]. A 1-year trial of the same treatments in 160 postmenopausal women resulted in positive changes from both fruits in ulna, spine, femoral neck, total hip, and whole-body BMD with more pronounced effects with plum on spine and ulna [55]. These changes were associated with decreased markers of bone turnover.

Blueberries

Recent studies have shown an anabolic action on bone of blueberry supplemented diets. Blueberry powder (5 % w/w) prevented OVX-induced whole-body BMD loss in 6-month-old Sprague–Dawley rats, but BMD of tibia, femur, and vertebrae were not significantly different [56]. Osteoblastogenesis and mineral apposition rate were increased in vivo associated with increased expression of Runx2 in bone following activation of Wnt-β catenin signaling and increased phosphorylation of MAP kinase p38 [57].

Blueberry extracts have the highest antioxidant capacity of fruits [58], and blueberry juice was surpassed only by the lingonberry juice for total oxidant scavenging capacity among 14 juices [59]. Blueberries are rich in phenolic compounds with established antioxidant activity. Total polyphenolics ranged from 399 to 556 mg/100 g of Georgia blueberries [60]. They include phenolic acids (≤259 mg/100 g gallic acid, ≤104 mg/100 g p-hydroxybenzoic acid, ≤16 mg/100 p-coumaric, ≤6.3 mg/100 g caffeic, ≤17 mg/100 g ferulic, and ≤6.7 mg/100 g ellagic acids) and flavonoids (≤114 mg/100 g anthocyanins, including delphinidin and malvidin glucosides and galactosides, ≤387 mg/100 g catechin, ≤130 mg/100 g epicatechin, ≤15 mg/100 g quercetin, ≤3.7 mg/100 g kaempferol, and ≤10 mg/100 g myricetin). Antioxidant activity as measured by Trolox equivalent antioxidant capacity (TEAC) ranged from 8.11 to 38.29 μM TEAC/g in the Georgia blueberries which correlated with phenolic content ($r^2=0.98$) and, to a lesser degree, anthocyanin content ($r^2=0.60$). A mixture of the phenolic acid metabolites in serum after feeding blueberries (hippuric acid, phenylacetic and hydroxybenzoic acids) induced the same effects on Wnt signaling and osteoblastogenesis as blueberries [60]. The effect of blueberries on bone health in humans has not been reported.

Future Research

There is great promise for plant bioactives to help protect against bone loss associated with menopause. Most of the work has focused on in vitro or preclinical models. We likely need to move beyond BMD as the main outcome measure to better understand their impact on bone strength through influencing bone turnover and bone quality. Better understanding of the mechanisms of action of bioactive compounds in the diet can provide insights for what to measure.

The pathogenesis of osteoporosis has moved from an estrogen-centric to a perspective of aging and oxidative stress [61], which extends the potential role of diet in ameliorating bone loss beyond compounds that interact with estrogen receptors. Antioxidants retard reactive oxygen species (ROS) which influences cells involved in bone turnover. The interaction of redox systems with sex steroids via nonnuclear MAP kinase regulated pathways may be the molecular targets for nutritional interventions. MAP kinase activation results in downstream actions on a number of cellular redox systems to increase antioxidant capacity and inhibit formation of ROS. ROS production in mesenchymal stem cells inhibits osteoblastogenesis. Estrogens, and presumably phytochemicals, antagonize ROS actions in bone cells via upregulation of glutathione reductase and a number of antioxidant systems. Plant sources of bioactives that merit more research for bone health include plum berries, grapes, oranges, mushrooms, and many herbs.

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