Health Beneficial Effects of Food Factors Can Be Applicable to Humans?

Isoflavone metabolism and bone-sparing effects of daidzein-metabolites

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Several dietary phytochemicals exhibit anti-oxidative, anti-inflammatory and anti-osteoporotic activities relevant to prevention of chronic diseases, including lifestyle-related diseases. Soybean isoflavones are similar in structure to estrogen and have received considerable attention as potential alternatives to hormone replacement therapy. Daidzein, a major isoflavone found in soybean, is metabolized to equol by intestinal microflora; this metabolite exhibits stronger estrogenic activity than daidzein. Recent studies suggest that the clinical effectiveness of isoflavones might be due to their ability to produce equol in the gut. This review focused on the metabolic pathway of equol and possible bioactivities of equol and *O*-desmethylangolensin, another metabolite of daidzein, with regard to bone metabolism and the status of intestinal microflora. Furthermore, we considered risk-benefit analyses of isoflavones and their metabolites.

I soflavones found in soybeans are nonsteroidal phytoestrogenic and antioxidative diphenolic compounds with potential roles in the prevention of chronic diseases, including hormone-dependent cancer, cardiovascular disease, osteoporosis and post-menopausal syndrome.[1–5] Almost all isoflavones in food exist as glycosides; hence it becomes necessary to hydrolyze the glycosidic bonds for intestinal absorption to enable physiological activities.[6,7] These glycosidic bonds are hydrolyzed by glycosidase produced by intestinal microflora such as *Lactobacilli*, *Bacteroides* and *Bifidobacteria*.[8] Furthermore, intestinal microflora affect metabolism wherein isoflavones are transformed into metabolites such as equol, or *O*-desmethylangolensin (*O*-DMA) from their precursor daidzein (Fig. 1). Recent studies suggest that the clinical effectiveness of equol (7-hydroxy-3-(4'-hydroxyphenyl)-chroman) in the gut.[9–11] Equol is metabolized from daidzein by intestinal bacteria. To date, several bacterial strains that transform daidzein into equol have been isolated. The biotransformation mechanism has not been fully understood. This review summarizes the possible biotransformation from daidzein to equol and the bioactivities of equol and *O*-DMA with regard to bone metabolism. Furthermore, risk-benefit analyses of isoflavones and their metabolites are discussed.

Metabolism of Isoflavones

Isoflavones are present mainly as glycosides in soybeans,[6,7] and the hydrolyzed products, isoflavone aglycones, often occur in fermented soy foods such as *miso* and *natto*.[12] After ingestion, the main isoflavone glycosides, genistin, daidzin and glycitin, are either hydrolyzed by enzymes of intestinal microflora to the aglycones genistein, daidzein and glycitein, respectively,[6] or partly absorbed in their glycosidic forms.[13] The aglycones are then absorbed and appear in the blood primarily as glucuronide and sulfate conjugates.[6,7] Murota *et al.*[14] demonstrated that isoflavone aglycones were taken up easily as intact aglycone compared with flavonoid aglycones (quercetin, kaempferol, luteolin and apigenin) by using Caco-2 cell monolayers as a model of the human intestinal epithelium. Absorbed isoflavones are not glucuronidated or sulfated in the intestinal wall, and instead pass via the portal vein to the liver, where the majority of these isoflavones are conjugated. A fraction of these isoflavones may also be hydroxylated and subsequently methylated to form a number of metabolites.[15] These conjugates are partitioned between urine and bile for excretion. The conjugates enter the bowel in the bile and are deconjugated by fecal bacteria to release the aglycones, which may again be metabolized or absorbed (enterohepatic recirculation).

Recent studies suggest that the clinical effectiveness of isoflavones might be due to their ability to produce metabolites such as dihydrodaidzein (DHD), tetrahydrodaidzein (THD), equol and *O*-DMA in the gut.[16] In particular, equol, a metabolite of daidzein, has recently received considerable attention, because its biological activities differ from those of its precursor.[9–11] Metabolites of genistein and glycitein are also primarily found in human urine (genistein: dihydrogenistein, 6'-OH-*O*-DMA,[17] 4-hydroxyphenyl-2- propionic acid and phloroglucinol; glycitein: dihydroglycitein, 5'-methoxy-*O*-DMA and 6-methoxy-equol).[18,19] The physiological activities of these metabolites remain unclear.

Biochemistry and Metabolic Pathway of Equol

In comparison to daidzein, equol has higher estrogenicity, stronger anti-oxidative efficacy, and exhibits anti-androgenic properties.[9,10] Moreover, equol is a chiral molecule, that exists as

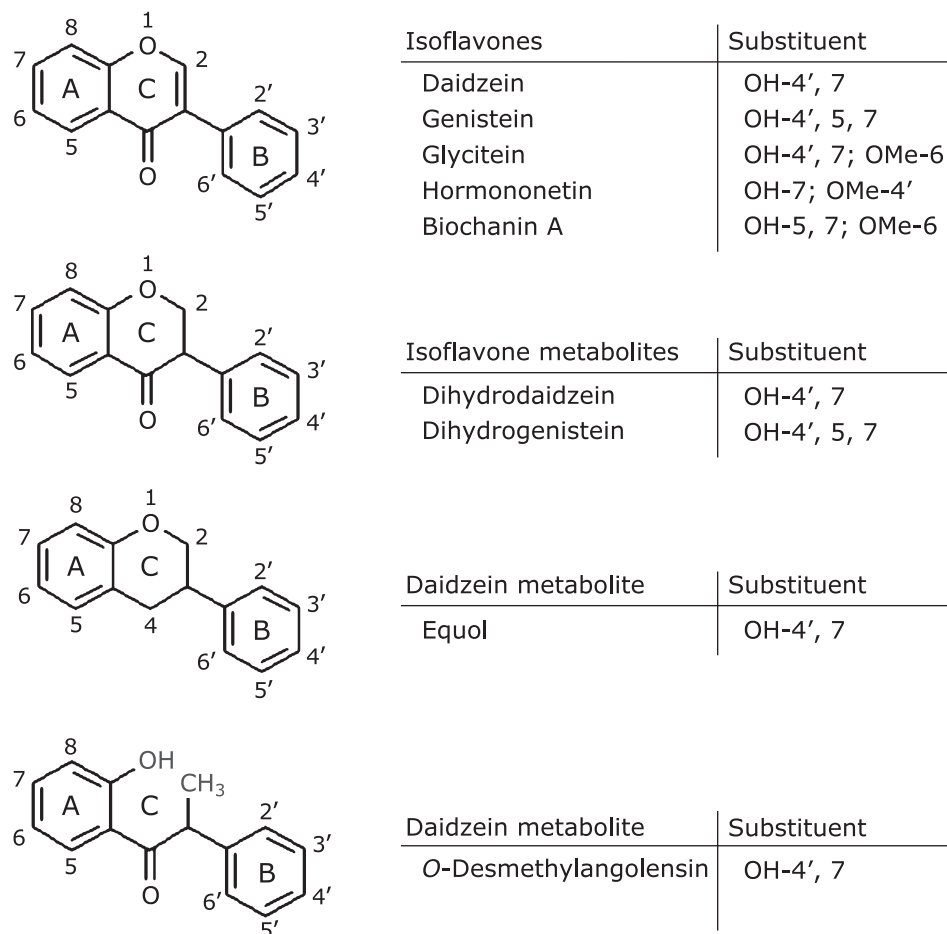*To whom corresponding should be addressed.
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Isoflavones	Substituent
Daidzein	OH-4′, 7
Genistein	OH-4′, 5, 7
Glycitein	OH-4′, 7; OMe-6
Hormononetin	OH-7; OMe-4′
Biochanin A	OH-5, 7; OMe-6

Isoflavone metabolites	Substituent
Dihydrodaidzein	OH-4′, 7
Dihydrogenistein	OH-4′, 5, 7

Daidzein metabolite	Substituent
Equol	OH-4′, 7

Daidzein metabolite	Substituent
O-Desmethylangolensin	OH-4′, 7

Fig. 1. Molecular structure of isoflavones and their metabolites

the enantiomers (*R*)(+)-equol and (*S*)(−)-equol.[20] Setchell *et al.*[21] established (*S*)-equol as the exclusive product of human intestinal microfloral synthesis using daidzein and showed that both enantiomers were bioavailable. The 2 chiral forms have been shown to differ in binding affinities and preferences for estrogen receptor α (ERα) and ERβ; (*S*)-equol has a high binding affinity for and preferentially binds to ERβ, whereas (*R*)-equol binds preferentially to ERα.[22] In humans, metabolism of daidzein to equol results in the production of only (*S*)-equol.[21] Interindividual variability in equol production may be unique to humans; all animals including rats, mice, and chimpanzees tested systematically excrete equol.[10,21,23–26] Although *O*-DMA was found in 80–90% of the human population, and equol was found in 30–50% of the population.[27–29] Interindividual variation in the ability to produce equol is a consequence of difference in gut microbial community. Intestinal microbiota responsible for equol production might differ across individuals.[7,26–29] Since some reports have indicated a lower disease risk for equol producers than for non-producers,[9–11] several candidate bacteria responsible for daidzein metabolism have been suggested; for example, a *Clostridium sp.* and *Eubacterium ramulus* metabolized daidzein to *O*-DMA *in vitro*.[30–32] It is suggested that other bacteria, including *Escherichia coli*, *Bacteroides ovatus*, *Ruminococcus productus*, and *Streptococcus intermedius*,[33] are involved in daidzein metabolism. It is known that equol is produced from TM-40, *Clostridium*-like bacterium that can produce DHD but not equol from both daidzein and daidzin.[34] To date, only 1 lactic acid bacterium [*Lactococcus* (*Lc.*) 20–92 homologous to *Lc. garvieae*] has been identified

that transforms equol directly from daidzein without producing *O*-DMA.[35] Interestingly, the strain *Lc.* 20–92 can also cleave glycosidic bonds of daidzin; Uchiyama *et al.*[36] detected *Lc. garvieae* in the Italian cheese Toma Piemontese. Wang *et al.*[37] reported 2 bacterial strains, namely, *Eggerthella* sp. Julong 732 and *Lactobacillus sp.* Niu-O16, which can transform DHD to (*S*)-equol and daidzein to DHD. In *Eggerthella sp.* Julong 732, DHD is converted to equol via the production of *cis/trans* THD as an intermediate metabolite. These studies have therefore suggested that daidzein is converted to equol via DHD and *cis/trans* THD. Furthermore, other bacterial strains that are capable of transforming daidzein to DHD or equol have been isolated.[38–43] Shimada *et al.*[44] purified a novel NADP(H)-dependent daidzein reductase (L-DZNR) from *Lc.* 20–92 and clarified that recombinant histidine-tagged L-DZNR converted daidzein to (*S*)-DHD with enantioselectivity. The same authors identified 3 other enzymes, DHD reductase (L-DHDR), THD reductase (L-THDR) and DHD racemase (L-DDRC) and elucidated that daidzein is first converted into (*R*)-DHD by L-DZNR and then into (*S*)-DHD by L-DDRC (Fig. 2).[45] Furthermore, the authors demonstrate that the final product, (*S*)-equol, is generated from (*S*)-*trans*-THD by L-THDR following the conversion of (*S*)-DHD into (*S*)-*trans*-THD (Fig. 2).[46] Tsuji *et al.* also identified *Slackia sp.* strain NATTS from healthy human feces, which has potent daidzein-to-equol conversion ability,[47] and 2 enzymes (ORF-1 and ORF-2) that catalyze *cis/trans*-THD to equol and DHD-to *cis/trans*-THD conversion reactions and their genes.[48] From a human fecal sample, Decroos *et al.*[49] have isolated a stable, mixed microbial
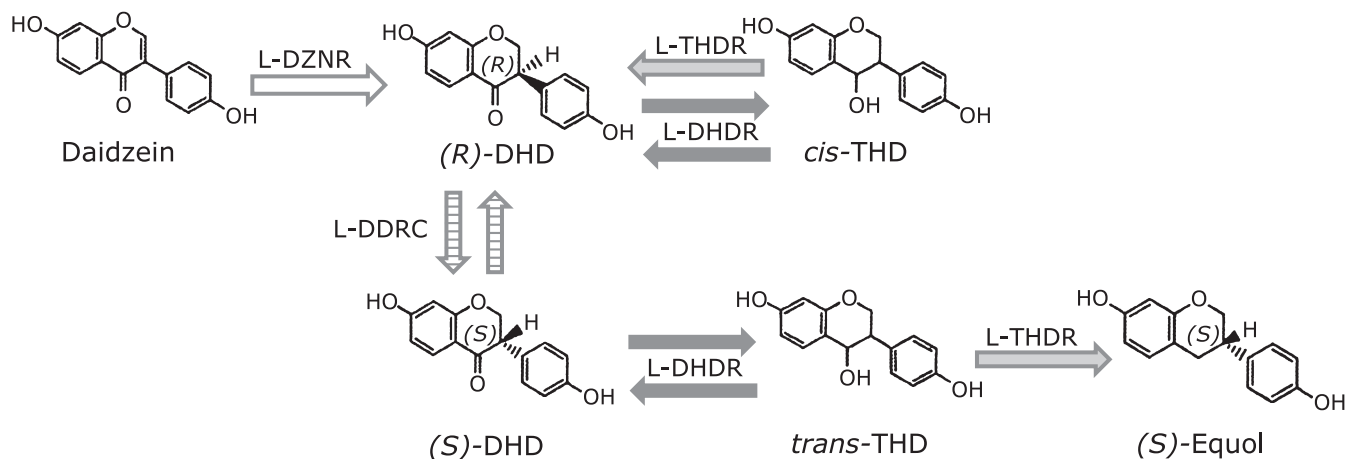
Fig. 2. Model of the equol biosynthetic pathway starting from daidzein in *Lactococcus* (*Lc.*) strain 20–92. L-DZNR, NADP(H)-dependent daidzein reductase from *Lc.* 20–92; L-DHDR, dihydrodaidzein reductase from *Lc.* 20–92; L-THDR, tetrahydrodaidzein reductase from *Lc.* 20–92; L-DDRC: dihydrodaidzein rasemase from *Lc.* 20–92 (reference (46) with permission and modifications).

Table 1. Time course changes of equol in central venous blood in rats fed the control diet or the 5% fructooligosaccharide (FOS) diet after a single dose of isoflavone glycosides

	Serum equol (nM/L)						
	1 h	3 h	6 h	12 h	24 h	48 h	72 h
Control	271.8 ± 49.6	144.7 ± 17.5	282.2 ± 59.1	277.8 ± 75.5	1185.3 ± 280.8	861.5 ± 81.1	507.6 ± 0.41
FOS	198.9 ± 55.4	214.8 ± 41.5	371.4 ± 67.2	1048.5 ± 648.5	2361.0 ± 966.5	2172.6 ± 494.0*	1211.0 ± 1.1*

Values are Means ± SEM, *n* = 5–7. *Significantly different from the control at a particular time point, *p*<0.05.

culture comprising 4 species (*Lactobacillus mucosae, Enterococcus faecium, Finegoldia magna,* and *Veillonella sp.*) that are capable of transforming daidzein into equol. The authors also investigated the influence of some environmental conditions in the colon on equol production.[49] They suggest that equol production is largely stimulated by hydrogen gas, which might be acting as an electron donor in the biotransformation reaction from daidzein to equol. Moreover, increased equol production is also found in the presence of short-chain fatty acids such as propionate and butyrate, suggesting that a carbohydrate-rich diet stimulates equol production. Interestingly, they indicate that short-chain fatty acids are also related to the production of hydrogen gas.[49]

Food Factors with Regard to Equol Production

Several food factors contribute to the ability to produce equol. Contradictory results, including association studies, also provided evidence. For example, Adlercreutz *et al.*[50] reported a positive association between urinary equol concentration and intake of fat and meat in a Japanese population, whereas in a Western population, Rowland *et al.*[51] found that equol producers consumed significantly less energy as fat and significantly more energy as carbohydrate than equol non-producers. In another cross-sectional study, equol-producing women had, on average, a higher intake of dietary fiber than non-producers. However, in a feeding study, it was not possible to induce equol production by supplementing the diets of non-producers with high-fiber wheat bran cereal or soy protein.[52] Bolca *et al.*[53] suggested that persons with a higher polyunsaturated fatty acid (PUFA) and alcohol intake were more likely to be strong equol producers, and no differences were found in the intake of dietary fiber or the use of pre-, pro- or symbiotic preparations among 100 healthy post-

menopausal women.

Fructooligosaccharides (FOS), a mixture of indigestible and fermentable sugars, are known to be prebiotics and enhance calcium, magnesium and iron absorption in the large intestine.[54,55] Uehara *et al.*[56] reported that FOS improve bioavailabilies of daidzein and genistein by stimulating enterohepatic circulation of these isoflavones in rats administered isoflavone glycoside conjugates (a single dose of 100 mg/kg BW via stomach tube). In a similar study, the equol concentration started to increase in the central venous blood at 12 h after the administration of isoflavone glycoside conjugates with FOS feeding and was significantly higher in the FOS-fed group than in the control group at 48 h and 72 h (Table 1). Furthermore, in ovariectomized (OVX) mice, preventing post-OVX bone loss has been most effectively achieved with a diet containing both FOS and isoflavone glycoside conjugates, thus correlating with increased equol production.[57] Non-digestible sugars such as FOS, polydextrose, and raffinose as well as resistant starch (non-digestible starch) could enhance equol production and inhibit femoral or tibial bone loss in OVX mice.[58,59] In an *in vitro* study, however, FOS inhibited equol production.[49] There is a discrepancy between results from the *in vivo* and *in vitro* studies. It seems to be good for equol production that non-digestible saccharides and starch can be fermented in the colon. In French postmenopausal women, FOS did not increase urinary equol production.[60] Racial differences might exist with regard to isoflavone metabolism. Therefore, further human studies involving Asian subjects will be required. Several factors such as animal species, human race, sex, age, and genetic background, including individual variation in intestinal microflora and diet, should be considered with regard to isoflavone metabolism and metabolite production.

Isoflavones as SERMs

Osteoporosis is a skeletal disorder in which bone strength is compromised by the loss of bone density and bone quality. It is the leading cause of increased morbidity and functional loss in the elderly people. Osteoporosis is more prevalent in postmenopausal women since bone loss is part of post-menopausal syndrome.[61] Although standard treatment for post-menopausal osteoporosis is hormone replacement therapy, reported side effects of hormone dependability, such as development of hormone-dependent breast and uterine cancers have prompted the use of alternative therapies.[62,63] Isoflavones are candidate chemicals of natural selective estrogen (E_2) receptor modulators (SERMs). SERMs are estrogen receptor (ER) ligands that act as estrogens in some tissues, while blocking estrogen action in other tissues.[63] SERMs require a plural phenolic ring and hydroxyl at 4'-position, and have the ability to bind to ERs in a manner similar to anti-estrogens such as tamoxifen and raloxifen that are used successfully to treat breast cancer.[64] Isoflavones show a relatively higher affinity for binding to the ERβ receptor, i.e., approximately 6- to 8-fold.[65] It is possible that isoflavones exert the beneficial effects of estrogen on bone without the negative side effects, particularly in tissues such as the endometrium and breast.

Bone-Sparing Effects of Isoflavones and Their Metabolites

In post-menopausal women, estrogen deficiency is associated with increased bone turnover and acceleration of bone loss, which lead to an increased susceptibility to bone fracture.[61,66] Isoflavones can reduce the rate of bone turnover and in particular inhibit bone resorption.[67]

Selective Hormonal Effects. Ishimi *et al.*[67] determined selective effects of genistein on B-lymphopoiesis and bone loss caused by estrogen deficiency in OVX mice. In their study, genistein (0.7 mg/day s.c.) inhibited bone loss due to OVX without any uterine hypertrophy, which has been thought to be a side effect of estrogen administration, suggesting that genistein may be a natural SERM.[68] Similarly, equol might inhibit bone loss due to OVX. Fujioka *et al.*[68] reported that administration of (±) equol (0.5 mg/day s.c.) inhibited reduced bone mineral density (BMD) of the whole body and femur in OVX mice. Although E_2 administration (0.03 μg/day s.c.) prevented OVX-induced bone loss from all regions, uterine hypertrophy occurred in E_2-administered OVX mice.[69] These results suggest that similar to SERMs, equol inhibits bone loss apparently without estrogenic activity in the reproductive organs of OVX mice. Futhermore, we compared the effects of the S(–)-equol and racemic forms (±) of equol on bone in OVX mice by the similar way in the previous study,[68] and observed higher activity of (S)-equol on bone fragility in the femora of OVX mice.[69] To clarify this difference in efficacy for bone, we measured the (S)- and (R)-enentiomer of equol in serum and urine using HPLC with chiral column.[69] The (S)-equol concentrations were much higher in the (S)-equol administered mice than in the racemic-equol administered mice. These high (S)-equol values in serum and urine might be associated with stronger inhibitory effects on bone fragility in the (S)-equol -administered OVX mice. However, (R)-equol concentrations were approximately 25% of (S)-equol concentrations in the racemic equol administered mice even though the racemic form (S:R = 50:50) was administered to the mice. We speculated that (R)-equol glucuronides might be less deconjugated enzymatically than (S)-equol glucuronides during enterohepatic recirculation after racemic mixture administration.[69] Further studies should attempt to compare the activities of (S)- and (R)-equol directly on their interactive metabolism.

Beyond hormonal effects. One of the main mechanisms by which estrogen deficiency causes bone loss is the stimulation of osteoclast formation,[70] a process enhanced by several inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL1-β). Once released in the bone microenvironment, TNF-α stimulates osteoclast formation in part by inducing the production of macrophage colony-stimulating factor by bone marrow stromal cells.[71] Transgenic mice expressing soluble tumor necrosis factor receptor are protected against bone loss caused by estrogen deficiency.[72] Therefore, these studies suggest that TNF-α is a key regulator of bone resorption in estrogen-deficient mice. Several phytoestrogens have been reported to exert other beyond hormonal effects, including inhibition of tyrosine kinase,[73] DNA topoisomerase I and II,[74] and anti-angiogenesis and antioxidant activity.[75,76] Equol was shown to have superior antioxidant activity compared to other phyto-estrogens and also inhibit lipoprotein oxidation *in vitro*.[9,77]

Mundy *et al.*[78] reported that statins, cholesterol-lowering agents that inhibit the activity of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, induce bone formation and inhibit bone resorption both *in vitro* and *in vivo*. Chiba *et al.*[79] showed that hesperidin, a citrus bioflavonoid, also regulates hepatic cholesterol synthesis and acts on the bone by using the same mechanism as that of statins. Genistein, daidzein and glycitein also inhibited HMG-CoA reductase in an *in vitro* study.[80] This finding suggests that isoflavones may prevent bone loss via inhibition of cholesterol synthesis.

In case of gastrectomized (GX) rats, mineral absorption disorder is occurred.[81] Therefore, isoflavone alone did not inhibit post GX osteopenia. The combination of isoflavone and FOS improved femoral trabecular bone loss by increasing calcium absorption and equol production (Fig. 3).[82]

Epidemiological Studies on Bone Health and Isoflavones Including Their Metabolites

As mentioned above, evidences from animal studies consistently showed that appropriate dose of isoflavone inhibited bone loss in osteoporotic animal models without exhibiting adverse effects on reproductive organs.[67–69,83] Numerous observational studies on the relationship of soy intake of pre- and postmenopausal women with BMD have generally reported significant positive associations, with some studies reporting the absence of such an association.[84–88] In addition, some intervention trials have found positive effects of soy and soy isoflavones specifically on BMD and/or biomarkers of bone metabolism, whereas other trials have reported no significant effects or effects that have any clinical relevance (Table 2).[88–104] Wu *et al.*[94] reported that a 1-year isoflavone intervention (75 mg of isoflavone conjugates/day) significantly decreased bone loss only at Ward's triangle and reduced the fat mass in the trunk region. Furthermore, the effect of combined intervention of isoflavone and walking exercise on the BMD of the total hip and Ward's triangle was greater than that of each administered alone in postmenopausal Japanese women.[95] Subsequently, Wu and coworkers classified the subjects based on their equol-producing phenotype.[96] In the isoflavone group, annualized changes in the BMD of the total hip and inter-trochanteric regions in equol producers were –0.46% and –0.04%, respectively, and in non-producers were –2.28% and –2.61%, respectively; these values differed significantly between equol producers and equol non-producers ($p < 0.05$ for both the regions). Significant differences were observed between equol producers and non-producers in the isoflavone group with regard to annualized changes in fat mass. No significant differences were observed in annualized changes in the BMD, and fat mass beteeen equol producers and non-producers in the placebo group. These data suggest that the preventive effects of isoflavones on bone loss and fat accumulation in early postmenopausal women depend on the equol-producing capacity of an individual.[96] The same authors measured serum concentration of E_2, follicle-stimulating hormone
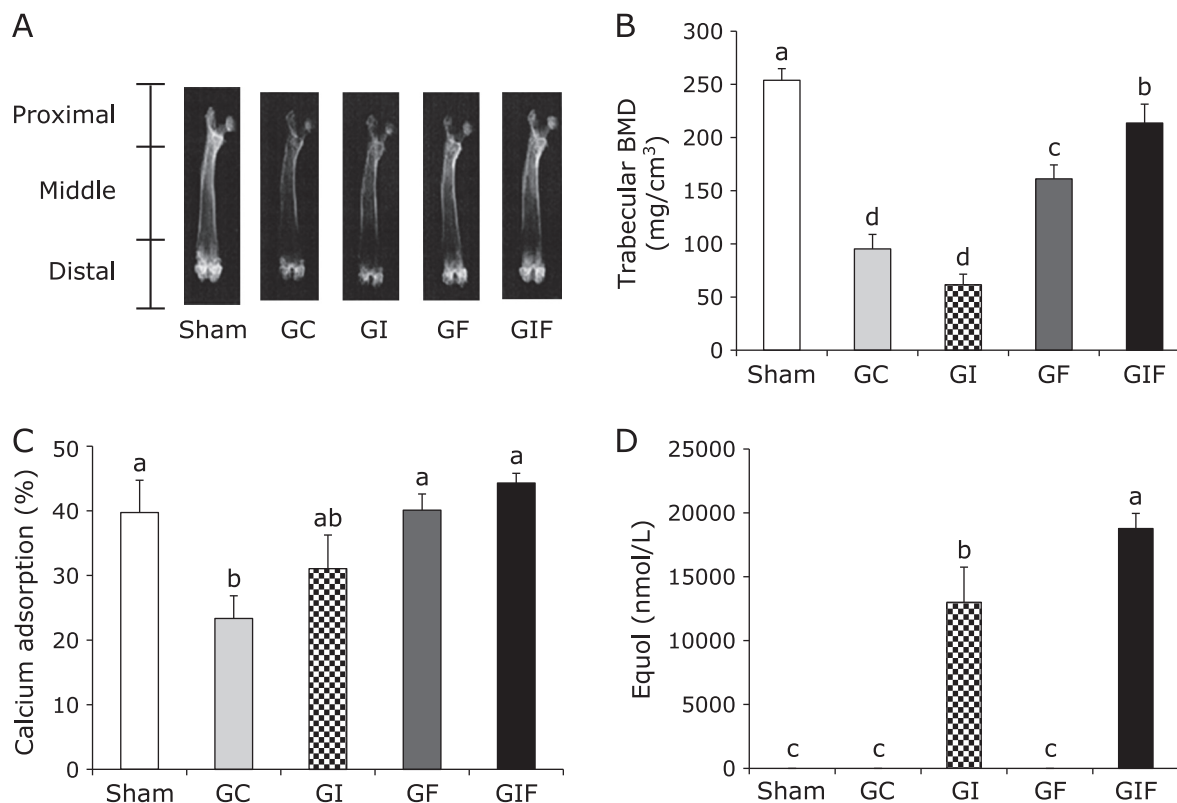
Fig. 3. Radiography of femora (A), distal metaphyseal trabecular bone mineral density (BMD) in feora using pQCT (B), apperent calcium absorption (%) (C), and serum equol concentration (D) collected from sham-operated (sham), gastrectomized (GX) rats fed a control diet (GC), 0.2% isoflavone glycoside diet (GI), 7.5% fructooligosaccharides (FOS) diet (GF), or a combination of 0.2% isoflavone glycoside, and 7.5% FOS diet (GIF) for 6 weeks. Values are expressed as means ± SE for each group. Bars not sharing a letter differ, $p<0.05$ (reference (82) with permission and modification).

(FSH), luteinizing hormone (LH), progesterone and thyroid hormone in their subjects to investigate the effects of isoflavone intake on hormone levels in postmenopausal women. No significant differences were observed in estrogenic hormone and thyroid hormone levels between the placebo and the isoflavone treatment groups. These results suggest that isoflavone intake for additional consumption with normal diet (total intake was 75 mg of aglycone equivalent/day) for a year did not affect serum hormone levels in postmenopausal Japanese women. Tosen *et al.*[97] also reported that natural *S*-equol supplements (10 mg/day) decreased bone resorption in postmenopausal equol non-producing Japanese women without adverse effects (hormonal changes).

In addtion, few studies have been conducted on bone metabolism with regard to *O*-DMA. Shoefer, Hur and their coworkers[30,32] showed that since the C-ring of daidzein is cleaved to *O*-DMA under anoxic conditions by *Clostridium sp.* or *Eubacterium ramulus*, the estrogenic activity of *O*-DMA is less than that of equol. In our *in vivo* and *in vitro* studies, the effects of *O*-DMA on bone and lipid metabolism in OVX mice and osteoclast cell cultures were weaker than those of equol.[104] According to an epidemiological study, Frankenfeld *et al.*[106] demonstrated that *O*-DMA producers had greater whole-body, leg and head BMD than *O*-DMA non-producers. Whole-body BMD among the *O*-DMA producers (geometric mean = 1.04 g/cm²) was 6% greater than whole-body BMD among the *O*-DMA non-producers (geometric mean = 0.98 g/cm²). Whole body and site-specific BMD did not differ between equol producers and non-producers.[99] Atkinson *et al.*[107] evaluated the relationship between daidzein-metabolizing phenotypes (equol and *O*-DMA production) and bone density and body consumption in pre-menopausal women (40–45 y, $n = 203$) in the United States in the absence of a soy

intervention. In this population of low soy consuming pre-menopausal women, there were no associations between daidzein-metabolizing phenotypes and hip, spine, femoral neck, or head BMD. They suggested that further studies in high soy consuming pre-menopausal women should assess whether interactions exist between phenotypes and soy consumption in relation to bone density and body consumption.[107]

Safety Evaluation of Isoflavones

After the shapes of the tablets and capsules were finalized and brought into effect by the health-promoting food system in April 2001, assessment of safety was specifically emphasized. As an initiative, Food Safety Commission of the Cabinet conducted a safety evaluation of soy isoflavones. In 2001, soybean isoflavone was approved as the principle ingredient in Foods for Specified Health Uses (FOSHU) aimed at individuals concerned about bone health. There are tea, soymilk and soft drink products that contain 40 mg of isoflavone conjugate, which is equivalent to 25 mg of the aglycone form. In 2004, applications for a tablet containing soy isoflavone aglycones as its principal ingredient, and a fermented food containing isoflavone aglycone in amounts exceeding the usual amounts in FOSHU were filed for approval. Foods with fortified or condensed isoflavones have not been consumed before, and there is a possibility that the tablets and capsules would be excessively consumed. Therefore, the Food Safety Commission issued a Notice, "Basic approaches to evaluating the safety of FOSHU containing soy isoflavones" in 2006.[108] According to this evaluation, (1) the upper limit of isoflavone aglycone intake from FOSHU was set at 30 mg/day for additional consumption with a normal diet; (2) The maximum

Table 2. Clinical trials (1998–2012) in postmenopausal women evaluating the effects of soy isoflavones and (S)-equol intakes (term: over 6 months)

Study cited	Location	Subject characteristics (mean age)	Main souse of isoflavones / Isoflavone dose (mg/day as aglycone)	Duration	Outcome reported
Am J Clin Nutr 1998; **68**: 1375S.	USA	Postmenopausal women (61 y)	Soy protein (54 mg/day)	6 months	Lamber BMC and BMD ↑
Am J Clin Nutr 2000; **74**: 844.	USA	Postmenopausal women (51 y)	Soy protein (80 mg/day)	6 months	Loss of lumbar spine BMC and BMD ↓
J Bone Miner Res 2002; **17**: 1904.	Italy	Postmenopausal women (52 y)	Isoflavone aglycone (45 mg/day)	1 year	Femoral and lumbar spine BMD ↑
J Clin Endclinol Metab 2003; **55**: 4740.	Hong Kong	Postmenopausal women (54 y)	Isoflavone aglycone (80 mg/day)	1 year	Loss of the total hip and trochanter BMC ↓, BMD →
Am J Clin Nutr 2004; **79**: 844.	United Kingdom	Postmenopausal women (55 y)	Isoflavone aglycone (43.5 mg/day)	1 year	Loss of lumbar spine BMC and BMD ↓
JAMA 2004; **292**: 65.	Netherlands	Postmenopausal women (67 y)	Soy protein (99 mg/day)	1 year	No change
Metabolism 2006; **55**: 423.	Japan	Postmenopausal women (54 y) classified based on their equol-producer phenotype	Isoflavone glycosides (47 mg/day)	6 months	Loss of sub-whole body and the total hip BMD in equol producers ↓ (No change in BMD between base line and post-intervention in any of the isoflavone treatment groups without the classification of equol-producer)
J Bone Miner Res 21: 780, 2006.	Japan	Postmenopausal women (54 y)	Isoflavone glycosides (47 mg/day)	1 year	Loss of femoral War's traiangle BMD ↓
Menopause 2007; **14**: 866.	Japan	Postmenopausal women (54 y) classified based on their equol-producer phenotype	Isoflavone glycosides (47 mg/day)	1 year	Loss of the total hip and intertrochantric BMD in equol producers ↓
Am J Clin Nutr 2008; **87**: 761.	Netherlands and France	Postmenopausal women (53 y) classified based on their equol-producer phenotype	Isoflavobe enriched foods (110 mg/day)	1 year	No change
Menopause 2009; **16**: 320.	USA	Postmenopausal women (54 y)	Soy protein (90 mg/day)	2 years	No change
Am J Clin Nutr 2009; **90**: 234.	USA	Postmenopausal women (73 y)	Soy protein (105 mg/day)	1 year	No change
Am J Clin Nutr 2010; **91**: 218.	USA	Postmenopausal women (55 y)	Isoflavone aglycone (80 and 120 mg/day)	3 years	A modest effect at the femoral neck
Menopause 2011; **18**: 563.	Japan	Postmenopausal equol non-producing women (55 y)	S-equol (10 mg/day)	1 year	Urinary deoxipiridinoline (bone resorption marker) ↓
J Clin Densitom 2011; **14**: 47.	USA	Postmenopausal women (54 y)	Isoflavone aglycone (80 mg/day)	3 years	Modestly beneficial for midshaft femur volimic BMD as time since last menstrual period ↑ and for mid-shaft femur strength-strain index as bone turnover ↑
Arch Intern Med 2011; **171**: 1363.	USA	Postmenopausal women (52 y)	Isoflavone aglycone (200 mg/day)	2 years	No change
Osteoporos Int 2012; **23**: 1571.	Taiwan	Postmenopausal women (55 y)	Isoflavone aglycone (300 mg/day)	2 years	No change

recommended level for safe isoflavone aglycone intake in the daily diet was set at 70–75 mg/day; (3) The intake of soy isoflavones from FOSHU was not recommended for pregnant women, infants and children.[85] Actually, it might be easy to exceed 75 mg of isoflavones in a standard Japanese daily diet. In USA, the Food and Drug Administration (FDA) indicated that, "25 grams of soy protein daily in a diet low in saturated fatty acid and cholesterol may reduce the risk of heart disease." This amount of soy protein (25 g) daily is very likely to equate to more than 75 mg of isoflavone. Which should we then choose, reducing the risk of heart disease or consuming a safe level of isoflavone? It appears that maximum recommended safe levels of safe isoflavone intake are not easy to evaluate and 75 mg of isoflavones may be low when considering daily diets in Japan.

Risk-Benefit Analysis of Isoflavones

We conducted an animal experiment to examine the dose-response relationship between isoflavone supplementation and bone and uterine weights in OVX mice.[109] The results indicated that administration of 0.2% isoflavone glycosides in the diet prevented bone loss and inhibited uterine hypertrophy, a risk factor for uterine cancer, in the OVX mice.

Among post-menopausal Japanese women as indicate above, individuals able to produce equol experience beneficial effects on bone loss.[96] To examine the effects of isoflavone intake on hormone levels in post-menopausal women in the same intervention study, we measured serum concentrations of E_2, FSH, LH, progesterone, and thyroid hormones.[110] The results indicated that,

in equol producers, around 75 mg of daily isoflavone intake showed beneficial effects on BMD after 1 year without any side effect (risk) on the level of sex and thyroid hormones.[109]

During the past few years, only the validity, that is the benefits, of FOSHU have been evaluated. More recently, risk evaluation has been promoted in Japan. Risks and benefits posed by functional foods including soy isoflavones should be collaterally evaluated in the near future, and risk-benefit analyses should be performed to investigate the correlation between the risks and benefits.

Conclusions

To increase equol production in the enteric environment of each individual, the development of probiotics using bacteria that have the ability to produce equol from daidzein is ongoing. Therefore, some bacterial strains that are capable of transforming daidzein to DHD or equol have been isolated. Recently, identifications of several enzymes related to daidzein metabolism and equol production were also developed in enteric bacteria. On the other hand, human race, animal species, sex, age, genetic background and several food factors such as non-digestible sugar and starch might affect isoflavone metabolism. Equol-producing phenotype could be a contributing factor for preventing bone loss. The equol-producing gene cluster has not been characterized yet. Detailed mechanisms of equol biosynthesis are expected to be clarified in the near future. Further, risk-benefit analyses of isoflavones and their metabolites should be performed.

Acknowledgments

Abbreviations

BMD	bone mineral density
Ca	calcium
DDRC	DHD rasemase
DHD	dihydrodaidzein
DHDR	DHD reductase
DXA	dual-energy X-ray absorptiometry
DZNR	NADH(H)-dependent daidzein reductase
ER	estrogen receptor
FDA	Food and Drug Administration
FOS	fructooligosaccharides
FOSHU	Food for Specified Health Uses
FSH	follicle-stimulaing hormone
GX	gastrectomized
HMG-CoA	3-hydroxy-3-methyl-glutaryl coenzyme A
HPLC	high performance liquid chromatography
IL1-β	interleukin-1β
Lc.	*Lactococcus*
LH	luteinizing hormone
O-DMA	*O*-desmethylangolensin
OVX	ovariectomized
PUFA	poly unsaturated fatty acid
pQCT	peripheral quantitative computed tomography
SERM	selective estrogen receptor modulator
THD	tetrahydrodaidzein
THDR	THD reductase
TNF-α	tumor necrosis factor-α

Conflict of Interest

No potential conflicts of interest were disclosed

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