Dietary Equol and Bone Metabolism in Postmenopausal Japanese Women and Osteoporotic Mice1,2

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Abstract

Equol binds to the estrogen receptor with greater affinity than its precursor, daidzein, an isoflavone found in soybeans. Inter-individual differences in ability to produce equol may lead to differential effects of isoflavone intervention on human health. Here, we review previously published work from our laboratory on equol producer status and bone health in human and in a mouse model of osteoporosis. We performed a 1-y, double-blind, randomized trial to compare the effects of isoflavone (75 mg of isoflavone conjugates/d; equivalent to 47 mg/d of the aglycone form) with those of placebo on bone mineral density (BMD), fat mass, and serum isoflavone concentrations in 54 early postmenopausal Japanese women classified by their equol-producer phenotype. Isoflavone intervention increased the serum equol concentration in equol producers but not in nonproducers (P < 0.04). The annualized changes in BMD in the total hip and intertrochanteric regions in the isoflavone-treated equol producers (–0.46 and –0.04%, respectively) were less than in the nonproducers (–2.28 and –2.61%, respectively). The annualized change in fat mass was lower in the equol producers compared with the nonproducers in the isoflavone group. The annualized changes in BMD and fat mass did not differ between the equol producers and nonproducers in the placebo group. Equol also inhibited bone loss and fat accumulation in estrogen-deficient osteoporotic mice. Our data suggest that prevention of bone loss and fat accumulation in early postmenopausal women by isoflavones may depend on an individual’s equol-producing capacity. J. Nutr. doi: 10.3945/jn.110.124842.

Effects of the intestinal metabolites of daidzein, equol, on bone metabolism

Epidemiological studies indicate that women who have high soy intake have less risk for osteoporosis than those consuming a typical Western diet (1). Additionally, several recently published studies report that isoflavone supplementation decreases risk for osteoporosis (2–4). In general, these studies administered isoflavones (40–200 mg/d) for 1–2 y and measured bone mineral density (BMD).4 The results from human studies are inconsistent (5). A recent meta-analysis of 10 randomized, controlled trials indicated that isoflavone intervention significantly attenuated bone loss in postmenopausal women (6,7). However, another meta-analysis concluded that isoflavones do not affect bone loss (8). One potential reason for these inconsistencies is individual differences in isoflavone metabolism. Recent studies suggest that the clinical effectiveness of isoflavones on bone metabolism might be due to individual differences in the ability to produce the daidzein metabolite, equol, in the intestine (9).

Equol is an isoflavone and a nonsteroidal estrogen (9). It binds to both estrogen receptors and induces transcription more strongly than other isoflavones, especially estrogen receptor-α (10). Moreover, equol is a chiral molecule, which exists as enantiomers R (+)-equol and S (–)-equol. In humans, the intestinal bacterial metabolism of daidzein to equol results in a strong metabolic chiral resolution that exhibits inter-individual variability in equol production. Several animals, including rats (12), mice (13), and chimpanzees (14), excrete equol. O-desmethylangolensin (O-DMA), another daidzein metabolite, is found in ~80–90% of the human population.1

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4 Abbreviations used: BMD, bone mineral density; Lc., Lactococcus; O-DMA, O-desmethylangolensin; OVX, ovariectomized.

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population (15,16) and equol is found in ~30–50% (9,17–20). The variability in mammalian equol production is considered to be dependent on individual differences in the intestinal microbiota responsible for equol production (9,21). Intestinal bacteria play an important role in isoflavone metabolism; young infants with undeveloped gut microflora do not produce equol (22–24) and germ-free animals also do not produce equol or O-DMA (25–28). Because some reports suggested a lower disease risk for equol producers than for nonproducers (9), there is a growing interest in certain bacterial strains that can produce equol. A number of strains involved in daidzein metabolism have been identified (29–31). However, the identification of equol-producing bacteria is complicated (9,11,21,25,26).

Lactococcus (Lc.), 20–92 homologous to Lc. Garvieae, is the only lactic acid bacterium to date known to produce equol directly from daidzein without producing O-DMA (32). Interestingly, the strain, Lc. 20–92, can also cleave glycosidic bonds of daidzein. Here we review our previously published work on equol producer status and bone health in humans and a mouse model of osteoporosis.

Effects of equol on bone metabolism in animals

Estrogen deficiency is associated with increased bone turnover and acceleration of bone loss, which leads to an increased susceptibility to bone fracture. A number of studies have reported that the soybean isoflavones, genistein and daidzein, dosed dependently inhibit bone loss in both female and male osteoporotic animal models without causing notable effects on reproductive organs (33). Similarly, equol may inhibit bone loss due to ovariectomy. We have reported that administration of equol (0.5 mg/d subcutaneously) inhibited bone loss of the whole body and femur in ovariectomized mice without uterine hypertrophy (34). Although 17β-estradiol administration (0.03 μg/d subcutaneously) prevented ovariectomized-induced bone loss from all regions, uterine hypertrophy occurred in mice (34). These results suggest that similar to selective estrogen receptor modulators, isoflavones, including equol, inhibit bone loss without estrogenic activity in the reproductive organs of estrogen-deficient animals. It is now recognized that one of the mechanisms by which estrogen deficiency causes bone loss is via stimulation of osteoclast formation, a process enhanced by several inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β. Furthermore, Nakamura et al. (35) recently reported apoptosis and Fas ligand expression upregulation in trabecular osteoclasts from wild type but not estrogen receptor knockout (ERα−/−;ERα−/−) mice after estrogen treatment. These results support a model in which estrogen regulates the life span of mature osteoclasts via the induction of the Fas/Fas ligand system and help explain the osteoprotective function of estrogen and selective estrogen receptor modulators, including isoflavones.

The role of equol status in the effects of isoflavones on bone health in humans

Equol production depends on an individual’s intestinal flora. Research has shown that ~30–50% of individuals in the population studied are capable of producing equol from daidzein (36). Lydeking-Olsen et al. (36) conducted a 2-y, randomized, placebo-controlled trial to investigate the effectiveness of isoflavone-supplemented soymilk (76 mg/d aglycone isoflavones content) for prevention of bone loss in postmenopausal women aged 41–75 y (mean age 58 y). The lumbar spine BMD for women who consumed soymilk with low levels of isoflavones (n = 22) decreased (4.2%; P = 0.01) by the end of the study; however, the lumbar spine BMD of women who consumed soymilk with isoflavones (n = 23) did not change compared with baseline. Equol producers (n = 10), defined by a cutoff level of 10 ng/mL (40 mmol/L) plasma equol, had a 2.4% increase in lumbar spine BMD compared with the 0.6% increase for equol nonproducers (n = 12) after the 2-y intervention with isoflavone-enriched soymilk.

We assessed the effects of equol-producing activity on BMD in postmenopausal Japanese women (37). Study participants were 68 healthy women aged 45–60 y who had undergone natural menopause within the previous 5 y, where menopause was defined as at least 12 mo beyond the last menstrual cycle. Fifty-four women (29/34 in the placebo, 25/34 in the isoflavone group) completed the 1-y intervention and their data were used for equol analysis. Study participants in the isoflavone group received a daily dose of 75 mg of isoflavone conjugates (38.3 mg daidzin, 0.2 mg malonyl-daidzin, 2.1 mg acetyldaidzin, 0.6 mg daidzein, 8.6 mg genistin, 0.6 mg acetylgenistin, 0.2 mg genisten, and 24.4 mg glycitin with glycitein) in capsule form (Fujilavone P40, Fujicco; 47 mg aglycon form) with dextrin and those in the placebo group received capsules containing only

FIGURE 1 Percent changes in BMD of the whole body (A), hip (B), femoral neck (C), and the intertrochanter region (D) in postmenopausal Japanese women who were equol (EQ) producers or nonproducers after 1 y of isoflavone (75 mg/d) or placebo treatment. Values are means ± SD, n = 10–15. *Different from EQ producers, P < 0.05. Adapted from (37) with permission by Wolters Kluwer/Lippincott, Williams & Wilkins.

*2S of 4S Supplement
dextrin. Fifty-six percent of study participants were equol producers, as assessed by equol production in fecal suspension incubated with daidzein under anaerobic conditions. In the placebo group, there were 15 and 14 equol producers and nonproducers, respectively; and in the isoflavone group, there were 15 and 10 equol producers and nonproducers, respectively. Serum daidzein concentrations, as determined by reverse-phase HPLC, increased to 3-fold the baseline value in equol producers and nonproducers who consumed isoflavone for 1 y \((P = 0.002)\). However, there was no difference in the serum daidzein concentration between equol producers and nonproducers. On the other hand, serum equol increased only in equol producers in the isoflavone group after 1 y \((P = 0.04)\). Serum equol did not change in equol producers in the placebo group. Because equol is a metabolite of daidzein, it has a slower plasma clearance rate than daidzein \((10)\). Therefore, whereas the serum levels of daidzein did not significantly different between the equol producers and nonproducers, the equol levels were significantly higher in the equol producers. Equol producers tended to have lower serum concentrations than nonproducers \((P = 0.15)\), but the longer half-life of equol in the bloodstream could explain why there were no significant differences in daidzein levels. After 1 y, serum genistein did not differ between equol producers and nonproducers in the isoflavone group.

In the isoflavone intervention group, the percent change in bone loss in the total hip \((-0.46\%)\) and the hip intertrochanteric region \((-0.04\%)\) of equol producers was lower \((P < 0.05)\) than that of equol nonproducers \((-2.28\% \text{ and } -2.61\%, \text{ respectively})\) \((\text{Student’s } t \text{ test}; \text{ Fig. 1}) (38)\).

A 2-factor analysis of covariance revealed no significant main effect on the percent change in BMD in any region after 1 y. The percent change in bone loss in the total hip and the hip intertrochanteric region did not differ between equol producers and nonproducers in the placebo group. Equol producers had a significantly lower annualized change in fat mass than nonproducers in the isoflavone group, but this was not the case for the placebo group. Based on these results, the effects of isoflavone on bone and fat mass might depend on equol-producing activity in postmenopausal Japanese women \((37)\). Isoflavone supplementation \((47 \text{ mg/d aglycone equivalent})\) with a normal diet for 1 y did not affect serum levels of estrogen, follicle-stimulating hormone, luteinizing hormone, progesterone, and androgen. However, there was no difference in the serum daidzein concentration between isoflavone producers and nonproducers, the equol levels were significantly higher in the equol producers. Equol producers had a significantly lower annualized change in fat mass than nonproducers in the isoflavone group, but this was not the case for the placebo group. Based on these results, the effects of isoflavone on bone and fat mass might depend on equol-producing activity in postmenopausal Japanese women \((37)\).

**Isolation of equol-producing bacteria from human feces**

Uchiyama et al. \((32)\) detected 3 equol-producing bacterial strains from human feces \((32,39)\). They selected the **Lc. Garvieae** strain as the 20–92 strain as the most appropriate bacteria for food usage, because it is homologous to **Lc. Garvieae**, which is widely used in Italian cheese.

Using real-time-PCR and the particular primer for **Lc. Garvieae**, we found that **Lc. 20–92** exists in the feces of postmenopausal Japanese women \((32)\). **Lc. Garvieae** was found in 35.3% of the postmenopausal Japanese women \((39)\). Interestingly, women with **Lc. Garvieae** were not always equol producers, suggesting that other bacteria may play a role in human equol production. Several other factors such as hydrogen gas and short chain fatty acids, which can affect the environmental conditions in the colon, may also be necessary for equol production.

**Conclusions**

Several factors such as race, age, diet, timing of exposure, and individual variations including genetic differences and variation in intestinal microflora influence the effects of isoflavones on bone health. The preventive effects of daidzein on bone loss in postmenopausal women might depend on an individual’s equol-producing capacity. In research from our laboratory, supplementation of the normal diet for 1 y with an additional 47 mg/d of isoflavone aglycon equivalent did not have adverse effects in postmenopausal women. Further studies are required to address the numerous questions on the potential benefits, mechanisms of action, and safety of isoflavones for postmenopausal women.

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**Literature Cited**


