**Cancelo Hidalgo MJ**
Hospital Universitario de Guadalajara - Universidad de Alcalá - Guadalajara (España)

# Isoflavones and bone health

Correspondence: M.J. Cancelo Hidalgo - Servicio de Ginecología y Obstetricia - Hospital Universitario de Guadalajara - C/ Donante de Sangre, s/n - 19002 Guadalajara (Spain)
e-mail: mcanceloh@sego.es

## Summary
Phytoestrogens are a family of plant-derived components that present a steroid structure and can act in the estrogen receptor. They contain both estrogenic and antiestrogenic properties, depending on the tissue in which they act.
The potential mechanisms by which phytoestrogens can affect cell activities have been divided into genomic and non-genomic effects. The former act through estrogen receptors, and the latter are mediated by cellular proteins. The active mechanism of soy isoflavones in bone may be beneficial, as they act by stimulating the activity of the osteoblasts. On the other hand, through the RANK-L/OPG system they bring about a decrease in osteoclast survival and activity. This article reviews *in vitro* studies, in animals and humans, that involve isoflavones and bone health to ascertain how these substances affect those postmenopausal women who use them in treatment or prevention of the climacteric syndrome.
In general, the global assessment of human studies shows variability in the design, in the variety of isoflavone sources, in the time of the analysis and in the dose. In addition, the variability in the bioavailability and metabolism of isoflavones between the subjects must be considered. All this makes it difficult to obtain consistent conclusions.
To sum up, some positive results justify the need for further research. From a clinical point of view, isoflavones are used in women with climacteric symptoms who cannot or do not wish to undergo hormone therapy. They would not be indicated for treating osteoporosis, but those women who use them at the right doses and time can expect a benefit in maintaining bone mass.

**Key words:** *bone health, soy, isoflavones.*

## Introduction

The estrogenic deficit derived from decreased ovarian function leads to increased bone remodeling, with a negative balance that contributes to a loss of bone mass. The result is the increased risk of developing osteopenia, osteoporosis and, as a consequence, increased risk of fracture.

Hormone therapy is considered a very effective treatment for the relief of climacteric symptoms. It has been shown to have a beneficial effect on bone with reduction of vertebral and hip fracture even in non-osteoporotic postmenopausal women[1], but information about the increased risk of some chronic diseases have markedly increased the interest of clinicians and women for alternatives to this treatment. Some of the most popular are based on food or phytoestrogen supplements.

Of all the natural alternatives currently under study, phytoestrogens and their components, isoflavones, seem to offer the greatest potential for bone loss prevention.

## Isoflavones and bone metabolism

Phytoestrogens are a group of plant-derived compounds that have been shown to have both estrogen agonist and antagonist properties, depending on the tissue where they act.

Based on their chemical structure, phytoestrogens are divided into four main classes:

1.- Isoflavones
2.- Stilbenes
3.- Coumestans
4.- Lignans

Isoflavones are the best known, with their main representatives genistein and daidzein. They are found in significant quantities in soybeans. The chemical structure is similar to 17β estradiol and can bind to estrogen receptors (ER). The binding of a phytoestrogen to ER may result in partial activation of the same (agonist effect) or displacement of an estrogen molecule, which reduces receptor activation (antagonistic effect).

They have an affinity for ER that is lower than that of estradiol. The affinity and the time of occupation of the isoflavones by the β receptor is about 30 times higher than by the α receptor. In tissues, there is a different distribution of these receptors, suggesting that they exert selective tissue effects depending on the tissue in which they act. In the reproductive tissue, especially the uterus and breast, the type predominates, while the bone tissue has a greater amount of receptors β[2]. In addition, isoflavones present other actions independent of ER, such as enzymatic inhibition or antioxidant activity.

The exact mechanisms of the effect of isoflavones and other components of soy on bone are still not fully understood. It has been postulated that the main effect would be genomic through ERs, but other non-genomic effects have also been verified[3].

The presence of ER in osteoblastic cells and genistein binding to ER have been demonstrated. The result appears to be increased bone formation by activating osteoblasts through the genomic mechanism involving the activation of the nuclear estrogen receptor. A variety of non-genomic mechanisms have also been described, including the inhibition of tyrosine kinase and topoisomerase II[4]. On the other hand, it has been shown that daidzein induces apoptosis of osteoclasts[5].

Another mechanism of action proposed more recently is through increased osteoprotegerin synthesis (OPG) by the osteoblast. In a cohort of osteopenic postmenopausal women, genistein administration compared with placebo showed that the level of RANK-L was lower ($p<0.001$ vs. placebo) and that of OPG higher ($p<0.001$ vs. placebo) in the follow-up over one and two years[6].

Another possible mechanism of action is the different behavior of soy protein compared to animal proteins versus intestinal calcium absorption[7]. The consumption of soy protein produces a lower urinary calcium excretion than the intake of animal protein[8]. This could have clinical importance regarding recommendations on health habits for postmenopausal women, suggesting the substitution of animal protein for soy protein.

In summary, from the physiological point of view of bone remodeling, these findings support the hypothesis of a stimulating effect of osteoblasts and a possible inhibiting effect of osteoclast recruitment (via RANK-L), as well as a shortening of their half-life to promote its apoptosis. The result would be an antiresorptive effect with positive balance towards the formation mediated by the OPG, and with action in the ER, but also through the action in certain enzymes[9].

### *In vitro* studies

Many basic research studies indicate the positive effect of isoflavones on the variables related to bone metabolism, osteoporosis, fracture and bone quality. The MC3T3-E1 osteoblastic cells have been cultured in medium containing various concentrations of daidzein, showing a significant increase in alkaline phosphatase activity and protein content. This effect is completely counteracted by adding an antiestrogen such as tamoxifen, which indicates the stimulatory effect on proliferating and differentiating the osteoblastic cells MC3T3-E1 mediated through the ER. The effect of genistein on osteoblastic cells seems to be the same as that of daidzein[10].

On the other hand, genistein inhibits osteoclast activity directly through tyrosine kinase inhibition, which in turn inhibits bone resorption[11].

## Studies in animals

Most studies of phytoestrogens action on bone, in animal experiments, have been carried out in ovariectomized rat (OVX) models and some in primates. They vary considerably depending on whether the administration route has been subcutaneous, continuous parenteral injection or oral feeding. In general, the analyzed product is isoflavones, either pure compounds (mainly genistein) or soy proteins, with or without their isoflavones, but with a wide variety of doses used.

Controls have been made with casein or semipurified diets. In several studies, the effect of phytoestrogens has been compared with conjugated equine estrogen or estradiol.

The main objectives have been the variation of the trabecular and/or cortical bone mass, bone mineral density (BMD) and the mechanical resistance in some studies. Secondary objectives included variation in markers of bone turnover and effects on uterine weight.

In general, the effects of isoflavones in the skeletal tissue of experimental animals have been consistent in the sense of showing a favorable effect of isoflavones on bone.

The first studies examined the effects of soybean milk[12] and soybean protein[7] compared to casein in the animal model of OVX rats. The rats fed soy diet showed significantly higher bone density in the femur and lumbar spine than the rats in the control group. The question of whether the effect was due to the protein itself or to the presence of isoflavones in soy is not clarified in these initial studies. To clarify this question, the OVX rats were fed a diet containing 44 µmol/day of genistein. The control rats were fed an identical diet in which the isoflavones were eliminated. The results showed that genistein was effective in reducing bone loss in OVX rats, supporting the hypothesis that it would act as an osteoclast inhibitor[4].

In another study, soy protein isolate proved to be as effective as estradiol in controlling bone loss after ovariectomy was carried out in rats[13].

However, in another study, which showed a significant IGF-1 mRNA increase in the groups treated with isoflavone, and in a dose-dependent way, no significant effect on bone density was found[14].

Using techniques such as DXA, the trabecular bone volume of the distal femoral metaphysis was reported to be markedly reduced in OVX mice, showing the genistein capacity to restore this loss[15].

In a randomized trial that studied the ability to reverse bone loss already established by daily intake of soy isoflavones in the long term and in different doses (20, 40 or 80 mg/kg/day for 84 days), and carried out in rats from which ovaries were removed and rats that underwent simulated surgery conserving the ovaries[16], the BMD was significantly lower in the OVX rats than in those that underwent sham surgery. Feeding with isoflavones did not affect BMD in this population. Neither induced changes in uterine weight, indicating the absence of uterotrophic effect. The anti-osteoclastic activity induced by isoflavones occurred in a dose-dependent manner. However, although isoflavone administration reduced bone turnover, it did not reverse the already established bone loss. These results support the idea that consumption of soy isoflavones may have a more preventive than curative role in bone health.

The importance of neonatal exposure to isoflavones has been reported. The analysis of BMD and bone resistance in mice in adulthood is higher when they have had an intrauterine exposure to genistein and/or daidzein[17].

In bone quality analysis, genistein retained the biomechanical quality of trabecular bone regardless of microstructure parameters, such as density or length of microfractures, mineral apposition rate or BMD[18].

However, studies in primates do not concur with results in OVX rats. In premenopausal *cynomolgus* monkeys (*Macaca fascicularis*), a high-isoflavon content soybean diet did not significantly affect bone characteristics, BMD, or bone biomarker measurements[19]. In ovariectomized monkeys, no effect of soy phytoestrogens in the diet was observed for any bone mass measurement[20], and soy protein alone did not prevent the increase in bone turnover[21].

In summary, the effect of isoflavones in basic research (*in vitro* studies and animal models) points to:

- Reduction of markers of bone resorption
- Increase in markers of bone formation
- Preservation of bone structure and quality
- Preservation of bone resistance to fracture

## Human studies

*Observational studies*

Observational studies of dietary intervention have shown similar findings to the *in vitro* effects of phytoestrogens in bone cell cultures and markers of bone turnover, which are indirectly consistent with the reduction of bone remodeling.

Most observational studies on bone markers have been conducted in women living in countries where the population has a relatively high intake of phytoestrogens. These have found a significant inverse correlation between isoflavone intake and urinary excretion of bone resorption markers pyridinoline and deoxypyridinoline in postmenopausal women of Asian countries[22].

Among Asian populations, several observational studies show that postmenopausal women who consume soy foods, and therefore isoflavones, present the highest BMD of the lumbar and/or hip spine[23], as in American populations of Japanese origin[24,25]. A greater peak of bone mass and the maintenance of this bone mass in young women has been described[26], and a lower loss in perimenopausal women[27] and postmenopausal women[28]. This effect has not been shown in breast cancer survivors[29].

In adults who live in Western countries, the data are limited, so it is difficult to draw conclusions about the relationship between phytoestrogen intake and BMD or the rate of fractures, since their consumption is generally insignificant in these countries[30]. A study in American white women found a decrease of 18% in resorption markers in those with high intake of genistein in the diet[28].

*Clinical studies*

The biggest problem with clinical trials in humans that analyze the effect of isoflavones on bone is the great variability in terms of design, source of the products analyzed, dosage and, especially, the relatively short duration in order to accurately

detect significant changes in the BMD. In addition, a confounding factor in isoflavone treatment studies is the variability in the bioavailability and metabolism of isoflavones among subjects.

Subjects vary from low to moderate and high metabolizers. Therefore, even if the same dose of isoflavone is administered, a variability in response can be expected. Daidzein is metabolized to equol by the gut microbiota in approximately 30% of people. This metabolite is biologically more active than its precursor[31].

A one-year randomized, double-blind, placebo-controlled trial administering equol supplements (10 mg/day) to 93 non-equol menopausal Japanese menopausal women showed that the intervention increased the concentrations of this metabolite in serum and urine in a dose-dependent manner. Urinary deoxypyridinoline decreased significantly, with a -23.94% change in the group receiving equol supplement compared to a change of -2.87% in the placebo group (p=0.020). In addition, BMD was maintained in the treated group, which decreased in the placebo group[32].

There are few reports of studies in premenopausal women, and it is not possible to draw conclusions about the impact of phytoestrogens on bone in them[33,34]. The administration of soy rich in isoflavones had no effect on BMD in healthy young adult women with normal menstruation[35].

In another 24-week study conducted in 69 perimenopausal women, the effect on bone loss of administering soy protein rich in isoflavones (80.4 mg/day), soy protein poor in isoflavones or casein (4.4 mg/day) was analyzed. The control group had a significant loss of bone, while the treatment rich in isoflavones attenuated lumbar spine bone loss[27]. A study of similar design in postmenopausal hypercholesterolemic women obtained similar results[36].

According to this information, it is possible that the inclusion of soy products containing isoflavones in diets of perimenopausal women can attenuate bone loss and decrease the risk of osteoporosis. However, in another study conducted in apparently healthy early postmenopausal white women (51-56 years), consumption of foods containing soybean isoflavone aglycone at 110 mg/day for one year did not prevent postmenopausal bone loss nor did it affect bone turnover[37]. Similar results are shown in another study in late postmenopausal women, with one year of follow-up, in which, although changes were identified in the markers, it did not occur in BMD, even when the results were analyzed by producers and non-producers of equol[38].

The cooperative effects of isoflavones and exercise on bone and lipid metabolism were analyzed in 128 postmenopausal women during 24 weeks randomly assigned to 4 groups: placebo; placebo combined with walking (3 times a week); Isoflavone intake (75 mg of isoflavone conjugates per day); and isoflavone combined with walking. The combination of isoflavones and exercise showed favorable effects on serum lipids and body composition of postmenopausal women. The findings of this study suggest that the preventive effects of isoflavones on bone loss depend on the individual's intestinal microbiota for the production of equol[39], although other studies do not find differences depending on the producer or non-producer phenotype of equol[37].

A meta-analysis of ten randomized, placebo-controlled trials, related to the effects of soy isoflavone intake on BMD of the lumbar spine, included 608 women who were administered soy isoflavones in doses of 44-160 mg/day with a treatment time of 4-24 months. In conclusion, the intervention with isoflavones significantly attenuated the bone loss of the spine in menopausal women. These favorable effects are more marked when more than 90 mg/day of isoflavones are administered. The beneficial effect would be evident after consumption for 6 months[40].

However, another meta-analysis that included ten randomized, placebo-controlled trials of at least one year and that analyzed 896 women indicated that supplementation with soy isoflavones is unlikely to have a significant favorable effect on BMD. in the lumbar spine and the hip. Similar results were obtained in subgroup analyses by sources of isoflavones (soy protein vs. isoflavone extract) and ethnic differences (Asian vs. western). Only the analysis according to the dose equal to or greater than 80 mg/day compared to lower doses tended to have a weak beneficial effect on the BMD of the lumbar spine[41].

On the effects of isoflavone intake on markers of bone remodeling, the results of nine randomized trials in which 432 subjects were included in a meta-analysis. It was concluded that the intervention with isoflavones significantly inhibits resorption and stimulates bone formation, according to the response of bone turnover markers. These favorable effects occur even if <90 mg/day of isoflavones are consumed or if the intervention lasts less than 12 weeks[42].

The effects of isoflavones on bone strength in humans are unknown. It has been indicated that the treatment with soy isoflavones over 3 years was modestly beneficial in the measurement of the volumetric bone mineral density of the medial femur, as well as in the force-deformation index[43].

A recent meta-analysis[44] analyzed the effect of isoflavones on BMD. We included 21 studies with 2,652 postmenopausal women. The results indicated that in the lumbar spine, treatment with isoflavones is associated with a significant increase in BMD compared to the control. In the femoral neck, the number of studies that provide this information is 18 (n=1,604), also finding a significant change. The studies that used isoflavones aglycone found better results compared to the control, being higher the effects to the studies that used the glycosylated forms.

Genistein reduced the urinary excretion of pyridoline and deoxypyridoline by increasing the levels of alkaline phosphatase and insulin-like growth factor-1 (IGF-1), without showing changes

in the ultrasound measurement of the endometrial thickness. The authors concluded that treatment with isoflavones exerts a moderate beneficial effect against bone loss related to estrogen deprivation in post-menopause. The effect seems to be related to the aglycone form of the isoflavones.

## Effect on fracture risk

The only information on the effect of isoflavones on fracture risk is derived from some population studies. There are no clinical trial data on fracture.

A prospective study of a large Asian cohort[45] of 24,403 postmenopausal women with no history of fracture or cancer, followed for a mean of 4.5 years, and after adjusting for age, socioeconomic status, osteoporosis risk factors, and other dietary factors, found a relationship of fracture risk with the consumption of soy protein or isoflavones, with an inverse relationship that was more pronounced in women in early menopause. The authors concluded that soy consumption can reduce the risk of fracture in postmenopausal women, especially among those approaching menopause.

## Conclusions

Evidence from epidemiological and prospective cohort studies indicates a positive effect of isoflavone intake on the risk of osteoporosis and fragility fracture.

There are several mechanisms of action that explain the actions of isoflavones on bone and, although the exact mechanisms involved are not fully understood, it seems that the consumption of soy isoflavones attenuates bone loss induced by menopause by decreasing resorption and training stimulation.

As shown consistently in both *in vitro* and animal studies, isoflavones appear to stimulate bone formation through action on osteoblasts, being able to inhibit bone resorption by acting on osteoclasts and thus establishing a positive balance

Human studies show variability in the results due, at least in part, to the different methodology used, the variety of isoflavone sources, the doses used and the time of the analysis; to which we must add the variability of the bioavailability and the metabolism of the isoflavones between the subjects, being sometimes difficult to separate the results of a possible genetic and environmental influence.

The studies reviewed show evidence of a beneficial effect of soy isoflavones on bone health in perimenopausal and postmenopausal women when soy protein with high isoflavone content is incorporated into the diet. This could be an adequate strategy to improve the bone health of postmenopausal women.

The evidence is insufficient to recommend the consumption of isoflavones for the prevention or treatment of osteoporosis, but in those women taking adequate doses of isoflavones, lower BMD loss related to estrogenic deprivation can be expected.

The results of the studies show some positive results, which justifies the need to carry out additional clinical trials in which it would be desirable to have a larger sample population and a longer duration than allowed, in addition to demonstrating the effect of isoflavones on the biochemical markers of bone remodeling, bone density and bone quality, investigate the effect on the prevention of fractures.

**Conflict of interests:** The author declares no conflict of interest.

## Bibliography

1. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA. 2002; 288(3):321-33.
2. Kuiper GG, Carlsson B, Grandien K, Enmark E, Häggblad J, Nilsson S, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology. 1997;138:863-70.
3. Anderson JJ, Anthony M, Messina M, Garne SC. Effects of phyto-oestrogens on tissues. Nutr Res Rev. 1999; 12(1):75-116.
4. Blair HC, Jordan SE, Peterson TG, Barnes S. Variable effects of tyrosine kinase inhibitors on avian osteoclastic activity and reduction of bone loss in ovariectomized rats. J Cell Biochem. 1996;61(4):629-37.
5. Rassi CM, Lieberherr M, Chaumaz G, Pointillart A, Cournot G. Down-regulation of osteoclast differentiation by daidzein via caspase 3. J Bone Miner Res. 2002; 17(4):630-8.
6. Marini H, Minutoli L, Polito F, Bitto A, Altavilla D, Atteritano M, et al. OPG and sRANKL serum concentrations in osteopenic, postmenopausal women after 2-year genistein administration. J Bone Miner Res. 2008; 23(5):715-20.
7. Arjmandi BH, Alekel L, Hollis BW, Amin D, Stacewicz-Sapuntzakis M, Guo P, et al. Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. J Nutr. 1996;126:161-7.
8. Breslau NA, Brinkley L, Hill KD, Pak CY. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. J Clin Endocrinol Metab. 1988;66(1):140-6.
9. Zheng X, Lee SK, Chun OK. Soy isoflavones and osteoporotic bone loss: a review with an emphasis on modulation of bone remodeling. J Med Food. 2016;19(1):1-14.
10. Sugimoto E, Yamaguchi M. Stimulatory effect of daidzein in osteoblastic MC3T3-E1 cells. Biochem Pharmacol. 2000;59(5):471-5.
11. Williams JP, Jordan SE, Barnes S, Blair HC. Tyrosine kinase inhibitor effects on avian osteoclastic acid transport. Am J Clin Nutr. 1998;68:1369S-74S.
12. Omi N, Aoi S, Murata K, Ezawa I. Evaluation of the effect of soybean milk and soybean milk peptides on bone metabolism in the rat model with ovariectomized osteoporosis. J Nutr Sci Vitaminol. 1994;40:201-11.
13. Arjmandi BH, Birnbaum R, Goyal NV, Getlinger MJ, Juma S, Alekel L, et al. Bone-sparing effect of soy protein in ovarían hormone-deficient rats is related to its isoflavone content. Am J Clin Nutr. 1998;68:1364S-8S.
14. Arjmandi BH, Getlinger MJ, Goyal NV, Alekel L, Hasler CM, Juma S, et al. Role of soy protein with normal or reduced isoflavone content in reversing bone loss induced by ovarian hormone deficiency in rats. Am J Clin Nutr. 1998;68:1358S-63S.
15. Ishimi Y, Miyaura C, Ohmura M, Onoe Y, Sato T, Uchiyama Y, et al. Sective effects of genistein, a soybean isoflavone, on B-lymphopoiesis and bone loss caused by

estrogen deficiency. Endocrinology. 1999;140:1893-900.
16. Picherit C, Bennetau-Pelissero C, Chanteranne B, Lebecque P, Davicco MJ, Barlet JP, et al. Soybean isoflavones dose-dependently reduce bone turnover but do not reverse established osteopenia in adult ovariectomized rats. J Nutr. 2001;131(3):723-8.
17. Kaludjerovic J, Ward WE. Neonatal exposure to daidzein, genistein, or the combination modulates bone development in female CD-1 mice. J Nutr. 2009;139(3):467-73.
18. Dai R, Ma Y, Sheng Z, Jin Y, Zhang Y, Fang L, et al. Effects of genistein on vertebral trabecular bone microstructure, bone mineral density, microcracks, osteocyte density, and bone strength in ovariectomized rats. J Bone Miner Metab. 2008;26(4):342-9.
19. Lees CJ, Kaplan JR, Chen H, Jerome CP, Register TC, Franke AA. Bone mass and soy isoflavones in socially housed, premenopausal macaques. Am J Clin Nutr. 2007;86(1):245-50.
20. Register TC, Jayo MJ, Anthony MS. Soy phytoestrogens do not prevent bone loss in postmenopausal monkeys. J Clin Endocrinol Metab. 2003;88(9):4362-70.
21. Lees CJ, Ginn TA. Soy protein isolate diet does not prevent increased cortical bone turnover in ovariectomized macaques. Calcif Tissue Int. 1998;62(6):557-8.
22. Horiuchi T, Onouchi T, Takahashi M, Ito H, Orimo H. Effect of soy protein on bone metabolism in postmenopausal women. Osteoporosis. 2000;11:721-4.
23. Somekawa Y, Chiguchi M, Ishibashi T, Aso T. Soy intake related to menopausal symptoms, serum lipids, and bone mineral density in postmenopausal Japanese women. Obstet Gynecol. 2001;97:109-15.
24. Fukui Y, Miura A, Nara Y, Uesugi T, Yamori H, Yamori Y. Relationship between urinary isoflavones and bone metabolism in postmenopausal Japanese women. J Nutr. 2000;130:686S.
25 Rice MM, LaCroix AZ, Lampe JW, van Belle G, Kestin M, Sumitani M, et al. Dietary soy isoflavone intake in older Japanese American women. Public Health Nutr. 2001;4(5):943-52.
26. Ho SC, Chan SG, Yi Q, Wong E, Leung PC. Soy intake and the maintenance of peak bone mass in Hong Kong Chinese women. J Bone Miner Res. 2001;16(7):1363-9.
27. Alekel DL, Germain AS, Peterson CT, Hanson KB, Stewart JW, Toda T. Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. Am J Clin Nutr. 2000;72(3):844-52.
28. Mei J, Yeung SS, Kung AW. High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women. J Clin Endocrinol Metab. 2001;86(11):5217-21.
29. Baglia ML, Gu K, Zhang X, Zheng Y, Peng P, Cai H, et al. Soy isoflavone intake and bone mineral density in breast cancer survivors. Cancer Causes Control. 2015;26(4):571-80.
30. Guthrie JR, Ball M, Murkies A, Dennerstein L. Dietary phytoestrogen intake in mid-life Australian-born women. Climacteric. 2000;3:254-61.
31. Wu J, Oka J, Ezaki J, Ohtomo T, Ueno T, Uchiyama S, et al. Possible role of equol status in the effects of isoflavone on bone and fat mass in postmenopausal Japanese women: a double-blind, randomized, controlled trial. Menopause. 2007;14(5):866-74.
32. Tousen Y, Ezaki J, Fujii Y, Ueno T, Nishimuta M, Ishimi Y. Natural S-equol decreases bone resorption in postmenopausal, non-equol-producing Japanese women: a pilot randomized, placebo-controlled trial. Menopause. 2011;18(5):563-74.
33. Song YS, Paik HY. Soybean intake and bone mineral density change in Korean college women. Epidemiological observations. J Nutr. 2002;132:616S.
34. Di Leo C, Tarolo GL, Bestetti A, Tagliabue L, Del Sole A, Aliberti G, et al. Osteoporosis and phytoestrogens: an assessment of bone mineral density via quantitative peripheral computed tomography in milk-egg-vegetarian women in the premenopause. Radiol Med. 2000;99:250-7.
35. Anderson JJ, Chen X, Boass A, Symons M, Kohlmeier M, Renner JB, et al. Soy isoflavones: no effects on bone mineral content and bone mineral density in healthy, menstruating young adult women after one year. J Am Coll Nutr. 2002;21(5):388-93.
36. Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman JW. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. Am J Clin Nutr. 1998;68:1375S-9S.
37. Brink E, Coxam V, Robins S, Wahala K, Cassidy A, Branca F; PHYTOS Investigators. Long-term consumption of isoflavone-enriched foods does not affect bone mineral density, bone metabolism, or hormonal status in early postmenopausal women: a randomized, double-blind, placebo controlled study. Am J Clin Nutr. 2008;87(3):761-70.
38. Kenny AM, Mangano KM, Abourizk RH, Bruno RS, Anamani DE, Kleppinger A, et al. Soy proteins and isoflavones affect bone mineral density in older women: a randomized controlled trial. Am J Clin Nutr. 2009; 90(1):234-42.
39. Wu J, Oka J, Higuchi M, Tabata I, Toda T, Fujioka M, et al. Cooperative effects of isoflavones and exercise on bone and lipid metabolism in postmenopausal Japanese women: a randomized placebo-controlled trial. Metabolism. 2006;55(4):423-33.
40. Ma DF, Qin LQ, Wang PY, Katoh R. Soy isoflavone intake increases bone mineral density in the spine of menopausal women: meta-analysis of randomized controlled trials. Clin Nutr. 2008;27(1):57-64.
41. Liu J, Ho SC, Su YX, Chen WQ, Zhang CX, Chen YM. Effect of long-term intervention of soy isoflavones on bone mineral density in women: a meta-analysis of randomized controlled trials. Bone. 2009;44(5):948-53.
42. Ma DF, Qin LQ, Wang PY, Katoh R. Soy isoflavone intake inhibits bone resorption and stimulates bone formation in menopausal women: meta-analysis of randomized controlled trials. Eur J Clin Nutr. 2008;62(2):155-61.
43. Shedd-Wise KM, Alekel DL, Hofmann H, Hanson KB, Schiferl DJ, Hanson LN, et al. The soy isoflavones for reducing bone loss study: 3-yr effects on pQCT bone mineral density and strength measures in postmenopausal women. J Clin Densitom. 2011;14(1):47-57.
44. Lambert MNT, Hu LM, Jeppesen PB. A systematic review and meta-analysis of the effects of isoflavone formulations against estrogen-deficient bone resorption in peri- and postmenopausal women. Am J Clin Nutr. 2017;106(3):801-11.
45. Zhang X, Shu XO, Li H, Yang G, Li Q, Gao YT, et al. Prospective cohort study of soy food consumption and risk of bone fracture among postmenopausal women. Arch Intern Med. 2005;165(16):1890-5.