Comprehensive evaluation of the role of soy and isoflavone supplementation in humans and animals over the past two decades

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Soy and soy-based foods are considered healthy, particularly in many Asia-Pacific countries, where soy products have long been consumed. Soy and soy-related products have been found to help prevent the occurrence of cardiovascular diseases and certain types of cancer, such as breast and prostate cancer. These products can also have antioxidative effects that alleviate hot flashes during menopause and bone loss. These biological and therapeutic functions are primarily due to the isoflavones derived from soy, whose structure is similar to the structure of 17-β-oestradiol. Despite the many health benefits for humans and animals, the application of isoflavones remains controversial because of their anti-oestrogenic properties. We focused on general information regarding isoflavones, as well as their structure, function, and application. We summarized evidence showing that dietary or supplemental isoflavones exert protective effects on the health of humans and animals. Based on the literature, we conclude that soy foods and isoflavones may be effective and safe; however, more high-quality trials are needed to fully substantiate their potential use.

KEYWORDS
antioxidant, bone loss, hot flashes, Isoflavones, review

1 | INTRODUCTION

Soybeans were a staple crop in East Asia long before written records were made. Soybeans are a source of many nutrients, such as proteins, lipids, and carbohydrates, as well as functional compounds, such as α-tocopherol (Ishii & Tanizawa, 2006). Consumer focus on isoflavones in soybean-based products has increased significantly in recent years (Messina, Rogero, Fisberg, & Waitzberg, 2017). A new report published by the United States Department of Agriculture indicates that sales of food-grade soybeans, which were processed into tofu, bean curd snacks, and soy milk, increased by 63% between 2010 and 2015 in China (ATO Beijing Staff, C B, 2017). The possible benefits for humans have led to increased soy product consumption. Over the past 2 decades, reports have investigated the potential health benefits of soy. Most of these studies have attributed the beneficial effects to biologically active components, commonly referred to as phytochemicals. For example, Karinat and FenuSMART™ can alleviate climacteric syndrome and cardiovascular disease in perimenopausal women (Kirchenko, Myasoedova, et al., 2017; Shamshad Begum, Jayalakshmi, et al., 2016). However, many studies have focused on isoflavones.

Isoflavones, including genistein, daidzein, and glycitein, are similar in structure to 17-β-oestradiol (Figure 1) and can bind to oestrogen receptors (ERs). Isoflavones are natural molecules present in edible plants, particularly in soybeans, red clover, and kudzu root (Messina, 2016; Rietjens, Louisse, & Beekmann, 2017). They were first reported in connection with negative effects because breeding problems were observed in Australian female sheep grazing on isoflavone-rich red clover, which led to an investigation of isoflavones in the 1940s (O’Leary, 1964). In 1987, Setchell proposed that the fertility problems observed in cheetahs were related to soy isoflavones in the standard animal diet (Setchell, Gosselin, et al., 1987). These adverse health effects may be due to the anti-oestrogenic properties of isoflavones, which may act as endocrine disruptors (EDs). In contrast, isoflavones also have various beneficial health effects, including the relief of menopausal symptoms such as hot flashes and osteoporosis in older women. Asian populations in China and Japan consume more soybeans and soy products than populations in North America. Additionally, certain types of cancer, such as breast, prostate, and colon cancer, have a lower incidence of occurrence in Asia than in the West (Adlercreutz, Mousavi, et al., 1992), possibly because isoflavones have oestrogenic
Isoflavones (daidzein, glycitein) were identified as soy isoflavones. Until 1973, three aglycones (genistein, daidzein, and glycitein) were considered sources of isoflavones. Soy isoflavones include two types of compounds, aglycones and glycosides. Certain aglycone forms of isoflavones are absorbed, and others are the formation of the active compounds daidzein and genistein. The safety issues associated with isoflavones are also discussed.

**FIGURE 1** Chemical structure of isoflavones

activity under certain conditions. Therefore, many postmenopausal women perceive phytoestrogens, such as isoflavones, in food supplements to be natural alternatives to conventional hormone replacement therapy (Poluzzi et al., 2014). In addition to their oestrogenic activity, isoflavones exhibit antioxidative effects both in vivo and in vitro. For example, isoflavones strongly scavenge free radicals, particularly in patients with diabetes (Umeno, Horie, Murotomi, Nakajima, & Yoshida, 2016). Genistein exerts anticancer, anti-inflammatory, and cardioprotective effects; in addition, genistein reduces free-radical-related tissue injury via antioxidant activity (Han, Tian, et al., 2009). Based on these findings, isoflavones must be treated with consideration in terms of both their benefits and associated hazards.

Concerns regarding the safety of isoflavones are based largely on their oestrogen-like properties; these properties are also considered responsible for the potential health benefits of isoflavones. Many countries are paying increased attention to the safety of soy foods and products, such as soy infant formulas, which are widely used. Furthermore, the European Food Safety Authority (EFSA) conducted an evaluation of the risk to peri- and postmenopausal women who consume foods supplemented with isoflavones (Mitchell, Arteaga, et al., 2015).

This review was established on the basis of the information from 1996 to 2017 available from PubMed, which was found using the terms “soy isoflavones,” “daidzein,” and “genistein” cross-referenced with the terms “hot flashes,” “hot flushes,” “osteoporosis,” “bone mineral density,” “antioxidant,” and “oxidative stress”. Articles selected for analysis were published in English and involved in vivo or in vitro studies of humans or animals.

We discuss the structure, characteristics, and mode of action of isoflavones and summarize the main effects of isoflavones on organisms. The safety issues associated with isoflavones are also discussed.

**2 | GENERAL CHARACTERISTICS OF ISOFLAVONES**

Isoflavones are phytoestrogens found in plants such as red clover, linseed, and soybeans. Among foods, soybeans are one of the richest sources of isoflavones. Soy isoflavones include two types of compounds, aglycones and glycosides. Until 1973, three aglycones (genistein, daidzein, and glycitein) were identified as soy isoflavones. The remaining 97–98% of isoflavones are glycosides, including genistin, daidzin, glycitin, and their variants. The concentration of isoflavones in soy depends on the soybean type, climate, and region of cultivation. Eleven soybean types from America and Japan have been analysed. The isoflavone content of dry soybeans ranges from 1.2 to 4.2 mg/g (Pilsakova, Riecansky, & Jagla, 2010; Wang & Murphy, 1993). The isoflavone content of soy products is associated with the methods used for extraction and processing. Although soymilk and tofu contain 2 mg of isoflavones per gram of protein, soy flour contains 5 mg because baking does not alter isoflavone content (Brynin, 2002).

The glucose conjugates of isoflavones are inactive in plants. Upon consumption by humans, they are metabolized by the gastrointestinal system, and the glucose residue is removed, resulting in the formation of the active compounds daidzein and genistein. Certain aglycone forms of isoflavones are absorbed, and others are further metabolized into equol in the intestine. Equol is produced by intestinal bacteria in humans that are equol producers, and because it has a greater affinity than daidzein to ERs, it is one of the most bioactive isoflavones.

**3 | MECHANISM OF ACTION OF ISOFLAVONES**

Isoflavones can bind to ERs because isoflavones are similar in structure to 17-β-oestradiol. ERs have two forms, ERα and ERβ, which are distributed among different tissues.

ERs vary in their binding affinity for isoflavones (Morito, Hirose, et al., 2001). Genistein has a higher affinity for ERβ than for ERα (20–30 times), but the affinity of all isoflavones for ERs is lower than that of oestradiol. Thus, isoflavones can exhibit oestrogenic and anti-oestrogenic behaviour, according to the level of endogenous oestradiol and the number and type of ERs (Cassidy, Bingham, & Setchell, 1994). Isoflavones exert agonist effects at low endogenous oestrogen concentrations; however, isoflavones also exhibit anti-oestrogenic properties (Collins, McLachlan, & Arnold, 1997).

Sex-steroid activity is altered by exposure to isoflavones in humans and animals. In one study, 20 postmenopausal women were randomly assigned to receive a soymilk diet or a placebo for 10 weeks. The results showed that isoflavones may significantly increase levels of sex-hormone-binding globulin (Pino et al., 2000). In contrast, another study showed that sex-hormone-binding globulin levels did not change after 4 weeks of soy supplementation (Baird, Umbach, et al., 1995). The differences between these results are likely due to sex, age, and isoflavone exposure duration, among other factors. A schematic of the proposed role of soy isoflavones in the management of human and animal health is shown in Figure 2.

**4 | EFFECTS OF ISOFLAVONES**

Soy and isoflavones are reported to alleviate climacteric symptoms and reduce the risk of cancer. Therefore, we summarize the most recent systematic review on soy isoflavones (see Table 1).
4.1 Isoflavones reduce the frequency of hot flashes

Hot flashes are the most common symptom driving postmenopausal women to seek treatment. The incidence of hot flashes among menopausal women varies. For example, the incidence of hot flashes in Asian women is 18–25% (in China or Japan) but is 53% among African-American women and 70–85% in Caucasian women (Boulet, Oddens, Lehert, Vemer, & Visser, 1994). In 1992, Adlercreutz et al. proposed that isoflavones alleviate hot flashes because Japanese women were observed to have a low prevalence of hot flashes. This finding could potentially be attributed to racial, cultural, and dietary differences, particularly because Asian diets commonly include foods with high levels of phytoestrogens, such as soybeans, tofu, and miso.

In a low-oestrogen environment, soy isoflavones exhibit potent oestrogenic activity because there is little competition. Therefore, in postmenopausal women, soy isoflavones may display more oestrogenic properties. Many studies have examined the effects of isoflavone-rich foods since the first clinical trial was performed in 1995 (Murkies et al., 1995), but the results of these studies are mixed.

Some reviews have summarized the effect of isoflavones on hot flashes in menopausal women. A systematic review of randomized controlled trials conducted between 2004 and 2011 concluded that isoflavones significantly reduce hot flashes during the menopausal transition and after menopause. In five trials of soy isoflavone preparations, two preparations (6 g of soy germ extract and 25 g of soy protein in soy nuts) significantly decreased the frequency of hot flashes (Thomas, Ismail, et al., 2014). A meta-analysis and systematic review, examining the efficacy of phytoestrogens in relieving menopausal symptoms, concluded that phytoestrogens may reduce the frequency of hot flashes without serious side effects in menopausal women (Chen, Lin, & Liu, 2015). A comprehensive review, assessing 39 nonhormonal drug studies (including 16 isoflavone trials), showed that soy isoflavones were more effective than sertraline, venlafaxine, paroxetine, gabapentin, and clonidine (Taku, Melby, Kronenberg, Kurzer, & Messina, 2012). Recently, a review of 62 studies, including 6,653 women, concluded that individual phytoestrogen interventions, such as dietary and supplemental soy isoflavones, were associated with improvements in the frequency of daily hot flashes (pooled mean difference, −0.79 [−1.35 to −0.23]) (Franco, Chowdhury, et al., 2016). Although different results have been published, all of the studies concluded that isoflavones affect hot flashes.

As an isoflavone, genistein may exert dose- and time-dependent effects on the frequency of hot flashes. A subgroup analysis showed that products containing higher levels (18.8 mg/day) of genistein were approximately 50–200% more potent than products containing lower levels (15 mg/day). A meta-regression analysis revealed that the decrease in hot flash frequency in longer-duration studies (>weeks) was approximately three-fold greater than that in shorter-duration trials (Taku, Melby, Kronenberg, Kurzer, & Messina, 2012).

As previously mentioned, some women can convert daidzein to equol (i.e., are equol producers), and studies have been performed to compare outcomes between equol producers and non-producers (Zaheer & Humayoun, 2017). In Japan, equol supplements have shown promise for the management of hot flashes in women, during menopause (Aso, 2010).

Clinical trials on the subject have also been performed. One randomized placebo-controlled trial included 30 postmenopausal

![FIGURE 2 Schematic of the proposed role of soy isoflavones in the management of human and animal health. ERs = oestrogen receptors↑ Increase, ↓ Decrease [Colour figure can be viewed at wileyonlinelibrary.com]](image)
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<td>Kou, Wang, et al. (2017)</td>
<td>Effect of soybean protein on blood pressure in postmenopausal women: A meta-analysis of randomized controlled trials</td>
<td>The consumption of soy protein (≥25 g/day) is associated with significant decreases in blood pressure in postmenopausal women, and decreases in blood pressure are strongly related to the isoflavone dose in the soy protein.</td>
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<td>Chaivon-Demersay, Azzout-Marniche, et al. (2017)</td>
<td>A systematic review of the effects of plant compared with animal protein sources on features of metabolic syndrome</td>
<td>Soy protein associated with isoflavones may prevent the onset of risk factors associated with cardiovascular disease, that is, hypercholesterolemia and hypertension.</td>
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<td>Zhong et al. (2016)</td>
<td>Association between dietary Isoflavones in soy and legumes and endometrial cancer: A systematic review and meta-analysis</td>
<td>There is a weak inverse association between higher consumption of dietary isoflavones from soy products and legumes and endometrial cancer risk.</td>
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<td>Zheng, Lee, and Chun (2016)</td>
<td>Soy isoflavones and osteoporotic bone loss: A review with an emphasis on modulation of bone remodeling</td>
<td>Soy isoflavones have the potential to decrease bone resorption and enhance bone formation. Further study is warranted.</td>
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<td>Tse and Eslick (2016)</td>
<td>Soy and isoflavone consumption and risk of gastrointestinal cancer: A systematic review and meta-analysis</td>
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<td>Song, Zeng, Ni, and Liu (2016)</td>
<td>The effect of soy or isoflavones on homocysteine levels: A meta-analysis of randomised controlled trials</td>
<td>Soy and isoflavones are not associated with a reduction in homocysteine levels.</td>
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<td>Jiang, Botma, Rudolph, Husing, and Chang-Claude (2016)</td>
<td>Phyto-oestrogens and colorectal cancer risk: A systematic review and dose–response meta-analysis of observational studies</td>
<td>High exposure to isoflavones is not associated with the risk for colorectal cancer.</td>
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<td>Ghazanfarpour, Sadeghi, and Roudsari (2016)</td>
<td>The application of soy isoflavones for subjective symptoms and objective signs of vaginal atrophy in menopause: A systematic review of randomised controlled trials</td>
<td>Soy isoflavones may decrease vaginal symptoms in postmenopausal women.</td>
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<td>Fang et al. (2016)</td>
<td>Soy isoflavones and glucose metabolism in menopausal women: A systematic review and meta-analysis of randomized controlled trials</td>
<td>Genistein plays an important role in improving glucose metabolism because of its low heterogeneity.</td>
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<td>Abdi, Alimoradi, Haqi, and Mahdizad (2016)</td>
<td>Effects of phytoestrogens on bone mineral density during the menopause transition: A systematic review of randomized, controlled trials</td>
<td>In menopausal women, isoflavones likely exert beneficial effects on bone health, but there have been controversial reports about changes in BMD.</td>
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<td>Franco et al. (2016)</td>
<td>Use of plant-based therapies and menopausal symptoms: A systematic review and meta-analysis</td>
<td>Composite and specific phytoestrogen supplementation is associated with modest reductions in the frequency of hot flashes and vaginal dryness but no significant reduction in night sweats.</td>
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<td>Yu, Jing, Li, Zhao, and Wang (2016)</td>
<td>Soy isoflavone consumption and colorectal cancer risk: A systematic review and meta-analysis</td>
<td>Soy isoflavone consumption in Asian populations and in case–control studies is significantly associated with a reduced colorectal cancer risk, particularly with soy foods/products.</td>
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<tr>
<td>Amiot, Riva, and Vinet (2016)</td>
<td>Effects of dietary polyphenols on metabolic syndrome features in humans: A systematic review</td>
<td>Diets rich in polyphenols, such as the Mediterranean diet, which promotes the consumption of diverse polyphenol-rich products, could be an effective nutritional strategy for improving the health of patients with metabolic syndrome.</td>
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<td>van Die et al. (2016)</td>
<td>Phytotherapeutic interventions in the management of biochemically recurrent prostate cancer: A systematic review of randomised trials</td>
<td>Soy isoflavones are safe and well tolerated. No recommendation can be made for the use of these agents in managing prostate cancer morbidity or mortality.</td>
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<td>Lehert, Villaseca, Hogervorst, Maki, and Henderson (2015)</td>
<td>Individually modifiable risk factors to ameliorate cognitive aging: A systematic review and meta-analysis</td>
<td>Individual choices can and do affect cognitive ageing. However, the study does not make special recommendations in the absence of stronger evidence indicating meaningful effectiveness.</td>
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<td>Xu, Du, and Xu (2015)</td>
<td>A systematic, comparative study on the beneficial health components and antioxidant activities of commercially fermented soy products marketed in China</td>
<td>Fermented soybean products may contain higher levels of antioxidant and nutritious substances.</td>
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<td>Cheng, Chen, et al. (2015)</td>
<td>Do soy isoflavones improve cognitive function in postmenopausal women? A meta-analysis</td>
<td>Soy isoflavone supplementation appears to have a positive effect on improving summary cognitive function and visual memory in postmenopausal women.</td>
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<tr>
<td>Chen et al. (2015)</td>
<td>Efficacy of phytoestrogens for menopausal symptoms: A meta-analysis and systematic review</td>
<td>Phytoestrogens appear to reduce the frequency of hot flashes in menopausal women without serious side effects.</td>
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TABLE 1 (Continued)

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<td>Thomas et al. (2014)</td>
<td>Effects of Isoflavones and amino acid therapies for hot flashes and co-occurring symptoms during the menopausal transition and early post menopause: A systematic review</td>
<td>Isoflavones significantly reduce hot flashes and co-occurring symptoms during and after the menopausal transition.</td>
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<td>D’Adamo and Sahin (2014)</td>
<td>Soy foods and supplementation: A review of commonly perceived health benefits and risks</td>
<td>Moderate amounts of traditionally prepared and minimally processed soy foods may offer modest health benefits while minimizing the potential for adverse health effects.</td>
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<td>Mourouti and Panagiotakos (2013)</td>
<td>Soy food consumption and breast cancer</td>
<td>There is no definite conclusion in this paper.</td>
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<tr>
<td>Fritz, Seely, et al. (2013)</td>
<td>Soy, red clover, and Isoflavones and breast cancer: A systematic review</td>
<td>Soy consumption may be associated with a reduced risk of breast cancer incidence, recurrence, and mortality. More evidence should be gathered to confirm safety before high-dose isoflavones are used.</td>
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<td>Castelo-Branco and Soveral (2013)</td>
<td>Phytoestrogens and bone health at different reproductive stages</td>
<td>The increase in bone mineral density and decrease in bone resorption markers by isoflavones are mediated by equol production, reproductive status, supplement type, isoflavone dose and intervention duration.</td>
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<td>Wei, Liu, Chen, and Chen (2012)</td>
<td>Systematic review of soy isoflavone supplements on osteoporosis in women</td>
<td>Soy isoflavone supplements significantly increase bone mineral density and decrease the bone resorption marker urinary deoxyripyridinoline.</td>
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<td>Taku et al. (2012)</td>
<td>Extracted or synthesized soybean isoflavones reduce menopausal hot flash frequency and severity: Systematic review and meta-analysis of randomized controlled trials</td>
<td>Soy isoflavone supplements, derived by extraction or chemical synthesis, significantly reduce the frequency and severity of hot flashes.</td>
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<tr>
<td>Castelo-Branco and Cancelo Hidalgo (2011)</td>
<td>Isoflavones: Effects on bone health</td>
<td>There is a positive relationship between isoflavones and bone health. More well-designed trials are needed to clarify the effects of isoflavones.</td>
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<tr>
<td>Taku et al. (2010)</td>
<td>Effects of soy isoflavone supplements on bone turnover markers in menopausal women: Systematic review and meta-analysis of randomized controlled trials</td>
<td>Soy isoflavone supplements moderately decrease the bone resorption marker deoxyripyridinoline, but do not affect the bone formation markers bone alkaline phosphatase or osteocalcin in menopausal women.</td>
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<td>Ricci, Cipriani, Chiaffarino, Malvezzi, and Parazzini (2010)</td>
<td>Soy isoflavones and bone mineral density in perimenopausal and postmenopausal Western women: A systematic review and meta-analysis of randomized controlled trials</td>
<td>Soy isoflavones mixtures are not effective in decreasing bone loss in peri- and postmenopausal Western women.</td>
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<td>Huber, Imhof, and Schmidt (2010)</td>
<td>Effects of soy protein and isoflavones on circulating hormone concentrations in pre- and postmenopausal women: A systematic review and meta-analysis</td>
<td>Isoflavone-rich soy products decrease follicle-stimulating hormone and luteinizing hormone in premenopausal women and may increase oestradiol in postmenopausal women.</td>
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<td>Hooper, Madhavan, Tice, Leinster, and Cassidy (2010)</td>
<td>Effects of isoflavones on breast density in pre- and postmenopausal women: A systematic review and meta-analysis of randomized controlled trials</td>
<td>Isoflavones from different sources have no effect on breast density in postmenopausal women and have a small effect in premenopausal women.</td>
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<td>Bolanos, Del Castillo, and Francia (2010)</td>
<td>Soy isoflavones versus placebo in the treatment of climacteric vasomotor symptoms: Systematic review and meta-analysis</td>
<td>It is difficult to establish conclusive results because of the high heterogeneity among studies.</td>
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women who received a placebo, 30 who received 84 mg of soy germ isoflavones, and 30 who received 126 mg of soy germ isoflavones for 6 months. The study concluded that soy germ isoflavones may improve menopausal symptoms, as assessed by hot flashes and the modified Kupperman Index (Ye, Wang, et al., 2012). Another 6-month, parallel-group, double-blind, randomized, placebo-controlled trial included 270 Chinese postmenopausal women, who were randomly assigned to receive one of three treatments: 40 g of soy flour, 40 g of low-fat milk powder, and 63 mg of daidzein, or 40 g of low-fat milk powder. The results showed that neither whole soy nor purified daidzein significantly alleviated hot flashes (Liu, Ho, Woo, Chen, & Wong, 2014). A randomized, blinded study in postmenopausal women found that a daily dose of 54 mg of genistein improved vasomotor symptoms by diminishing vascular inflammation and, more specifically, visfatin production (Bitto, Arcoraci, et al., 2016). In another trial, 100 menopausal women were randomly divided into two groups and received either 900 mg of gabapentin or 60 mg of isoflavones for 3 months. At the end of the 12 weeks, both groups showed significant improvement in hot flashes (Singhal & Shullai, 2016). Most studies have found that isoflavones may reduce the frequency of hot flashes in postmenopausal women. However, other studies have found that soy protein containing isoflavones does not improve quality of life in postmenopausal women (Kok, Kreijkamp-Kaspers, Grobbee, Lampe, & van der Schouw, 2005).

### 4.2 Isoflavones prevent bone loss

With increasing life expectancy, bone loss is becoming a worldwide problem and is expected to increase in menopausal women. Although hormone replacement therapy is an effective treatment for bone loss, it has been reported to be associated with an increased risk of breast cancer and other diseases (Schilling, Ebert, Raaijmakers, Schutze, & Jakob, 2014). Soy isoflavones have attracted considerable attention in the context of bone loss in recent years. In 1993, a study found that hip-fracture rates were significantly lower in Hong Kong than in the United States (Ho, Bacon, Harris, Looker, & Maggi, 1993). This difference may be related to the fact that soy food products rich in isoflavones are staples for Asian women.

Numerous studies have focused on the effect of isoflavones on bone loss in menopausal women. Several double-blind studies in humans have shown that isoflavones help to reduce bone loss. In 2015, Pawlowski, Martin, et al. observed the effect of isoflavones on bone calcium levels in postmenopausal women; 24 postmenopausal women received one of five soy isoflavone preparations (52, 114, 105, 161, or 220 mg/day) for 50 days. The most effective soy intervention (105.23 mg of total isoflavones/day) increased bone calcium retention by 7.6%. Genistein, at 52.85 mg/day, increased bone calcium retention by 3.4%, but there were no additional benefits at higher amounts (113.52 mg/day). In another 6-month, double-blind study, 70 subjects received GBB, which consisted of genistein (30 mg/day), vitamin D3 (800 IU/day), vitamin K1 (150 μg/day), and polysaturated fatty acids. The subjects supplemented with (genivi™ bone blend) GBB maintained their femoral neck bone mineral density (BMD; Lappe, Kunz, et al., 2013). A similar design was used in ovariec-
tomized rats. The soy isoflavone extract plus vitamin D3 was more effective than any supplement alone in increasing ostein expression (Chang, Hu, et al., 2013). Data from one experiment on rats indicate that soy isoflavones improved femur bone quality by increasing histomorphometric parameters and the content of glycosaminoglycans and mature type-I collagen fibres (Santos, Fiorencio-Silva, et al., 2014).

The beneficial effects of isoflavones include not only slowing bone loss but also stimulating bone formation. In a 2-year, randomized, double-blind, placebo-controlled trial, 198 postmenopausal women received 54 mg of genistein per day, whereas 191 received a placebo. At the end of the experiment, the levels of bone formation promoters, such as bone-specific alkaline phosphatase and insulin-like growth factor I, were increased by supplementation with genistein (Marini, Minutoli, et al., 2007). In another similar study, 389 osteopenic, postmenopausal women were treated with either genistein (54 mg/day) or a placebo. All patients received calcium and vitamin D3 in therapeutic doses. After 3 years, genistein was found to have significantly reduced the levels of pyridinoline, serum carboxy-terminal cross-linking telopeptide, and the soluble receptor activator of nuclear factor κB ligand; in addition, genistein increased the levels of bone-specific alkaline phosphatase, insulin-like growth factor 1, and osteoprotegerin (Marini, Bitto, et al., 200)8. Positive effects were also demonstrated by a study in female rats; equol was found to increase BMD by stimulating bone formation more efficiently than daidzein (Tousen, Ishiwata, Ishimi, & Ikegami, 2015).

However, some studies have obtained different results, that is, no significant differences between isoflavones and a control (Levis et al., 2011) (Alekel, Van Loan, et al., 2010; Tai, Tsai, et al., 2012). These different findings may be associated with study design components such as dosage, duration and administration method.

The exact mechanism of action of isoflavones on bone is not yet fully understood. Isoflavones may act on two cell types, osteoclasts...
and osteoblasts, to mediate bone remodelling. A study in osteoblasts suggested that isoflavones such as genistein and daidzein might modulate bone remodelling through ERs by regulating target-gene (interleukin 8 and serum- and glucocorticoid-inducible kinase 1) expression through CAMP regulatory-element motifs (Tang, Zhu, Liu, Wang, & Ni, 2011), possibly because their structures allow them to bind ERs and exhibit oestrogenic activity (Atmaca, Kleerekoper, Bayraktar, & Kucuk, 2008). Another study in primary human trabecular osteoblasts showed that genistein increased osteoprotegerin mRNA levels and protein secretion two- to six-fold (Viereck et al., 2002). Some studies on osteo-
classt have shown that isoflavones can inhibit osteoclast formation (Lee, Kim, & Jang, 2014), particularly when combined with carotenoids (Tadaishi, Nishide, Tousen, Kruger, & Ishimi, 2014). Therefore, soy isoflavones may exert beneficial effects on bone loss in postmenopausal women by activating osteoblasts and inhibiting osteoclasts.

4.3 Isoflavones act as antioxidants

Oxidative stress is a metabolic disturbance caused by excessive reactive oxygen species (ROS) or weak antioxidant activity. ROS impair cellular structure and function by damaging proteins, lipids, and nucleic acids (Freeman & Crapo, 1982; Mantle & Preedy, 1999). Antioxidant/oxidant balance is preserved by both enzymatic and nonenzymatic systems. Malondialdehyde (MDA) is produced by polyunsaturated fatty acid peroxidation, and the activities of enzymes, including superoxide dismutase (SOD), catalase, glutathione peroxidase, and lactate dehydrogenase, reflect the degree of cell injury.

The composition and effects of soy have been studied extensively. Several clinical trials have suggested that soy diets decrease oxidative stress. A randomized, double-blind, controlled trial was conducted to determine the effect of 6 and 12 months of supplementation with soy isoflavones on MDA in 182 Indonesian postmenopausal women, aged 47–60 years. The results showed that supplementation with isoflavones could decrease the MDA concentration (Puspamin, Suyatna, Mansyur, & Hidajat, 2013). In vitro, genistein, daidzein, and equol are effective free-radical scavengers and demonstrate their high antioxidative capacity by scavenging free radicals and increasing antioxidant protein expression. First, isoflavones are reported to scavenge free radicals, and second, isoflavones may increase the expression of antioxidant enzymes, such as metallothionein (MT). A study conducted in Caco-2 cells showed that 100 μM genistein increased the expression of MT mRNA 15-fold (Kameoka, Leavitt, Chang, & Kuo, 1999). In 2006, a similar result was observed in HepG2 cells: MT antioxidant genes (MT1A, MT2A, MT1E, and MT1X) were significantly up-regulated by 100 μM genistein (Chung, Kang, et al., 2006). Overall, isoflavones are effective antioxidants in animals and may act by scavenging free radicals and increasing antioxidant protein expression.

Isoflavones also act as antioxidants in chickens. Broiler chicks were fed a basal diet or a basal diet supplemented with genistein (5 mg/kg), hesperidin (20 mg/kg) or a mixture of supplements. The results showed that at 0 and 15 days, the groups fed a supplemented diet showed significantly reduced MDA levels in breast meat (Kamboh & Zhu, 2013). At 42 days old, chickens given 20 mg/kg isoflavones showed slightly reduced MDA production in the plasma, and significantly reduced MDA production was observed in the breast muscles after supplementation with 20, 40 or 80 mg/kg (Jiang et al., 2007). In a model of oxidative damage caused by A1254 in chicken testicular cells, supplementation with 10 g/ml of daidzein restored SOD activity, reduced glutathione content and significantly increased lipid peroxidation (Mi, Zhang, Zeng, Liu, & Liu, 2007). Another study in chicken intestinal epithelial cells showed that equol can protect intestinal epithelial cells from oxidative damage by promoting antioxidant gene expression, increasing antioxidant enzyme activity, and enhancing antioxidant capacity (Lin, Jiang, Zheng, & Gou, 2016).

Although the results of several studies support the abovementioned findings, some authors argue that the evidence is ambiguous. A study in 42 subjects aged >50 years with high cholesterol (low-density lipoprotein cholesterol 3.36 mmol/L) showed that diets relatively high in soy protein or soy phytoestrogens had little effect on plasma antioxidant capacity and oxidative stress biomarkers (Vega-Lopez, Yeum, et al., 2005). These results are consistent with those of a study in ovariec-tomized women, which showed that 12 weeks of isoflavone administration did not affect catalase or SOD (Mittal et al., 2014).

The antioxidant activity of isoflavones operates through two mechanisms. First, isoflavones are reported to scavenge free radicals, and this activity may be associated with the structures of the isoflavones. Genistein and daidzein containing three and two OH substitutions showed stronger antiperoxyl radical activity than did Trolox, an α-tocopherol analogue (Cao, Sofic, & Prior, 1997). Both the number and the position (e.g., 4′) of the hydroxyl group are relevant. Therefore, genistein is the most potent inhibitor of H2O2 and O2− caused by 12-O-tetradecanoylphorbol-13-acetate and xanthine/xanthine oxidase in HL-60 cells, whereas daidzein is second in terms of effectiveness (Wei, Bowen, Cai, Barnes, & Wang, 1995). One study investigated the reactivity rate constant and found that genistein was a powerful radical scavenger at physiological pH (Zielonka, Gebicki, & Grynkiewicz, 2003). Second, isoflavones may increase the expression of antioxidant proteins, such as metallothionein (MT). A study conducted in Caco-2 cells showed that 100 μM genistein increased the expression of MT mRNA 15-fold (Kameoka, Leavitt, Chang, & Kuo, 1999). In 2006, a similar result was observed in HepG2 cells: MT antioxidant genes (MT1A, MT2A, MT1E, and MT1X) were significantly up-regulated by 100 μM genistein (Chung, Kang, et al., 2006). Overall, isoflavones are effective antioxidants in animals and may act by scavenging free radicals and increasing antioxidant protein expression.

5 SAFETY OF ISOFLAVONES

Herbal medicinal products, such as nutraceuticals, are expected to be safe, effective, and of appropriate quality (Pferschy-Wenzig & Bauer,
CONFLICTS OF INTEREST
The authors have declared that there are no conflicts of interest.

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