

Soy — relieving the symptoms of menopause and fighting osteoporosis

Nima Kotecha and Brian Lockwood explore the growing evidence supporting the therapeutic use of soy in hormone-related conditions

The menopause is signalled by a woman's last menstrual period and is defined as the permanent cessation of menstruation resulting from loss of ovarian follicular activity.¹ At menopause, many women experience a decrease in their quality of life. The major symptoms associated include:

- Vasomotor symptoms
- Localised atrophy of genitalia
- Osteoporosis
- Coronary heart disease
- Psychological problems

The most common vasomotor symptoms displayed by women include hot flushes, night sweats and palpitations. Psychological problems experienced are sleep deprivation, forgetfulness, difficulty concentrating and depression. Many women also suffer from vaginal dryness, consequently leading to loss of libido. These symptoms are all linked to the declining and erratic production of oestrogen by the ovaries.²

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Osteoporosis is defined as a reduction in bone density¹ and is another common symptom experienced by menopausal women. The lack of oestrogen results in an increase in osteoclastic bone resorption and consequently there is an overall increase in bone turnover. More bone is resorbed than replaced and there is an associated increase in the rate of bone loss which may continue for five to 10 years.

Hormone replacement therapy

Hormone replacement therapy (HRT) is most commonly prescribed for the relief of menopausal symptoms and for protection against bone loss and ischaemic heart disease. However, reports in the media on the long-term effects of HRT have deterred its use, due to the associated risks of breast cancer, myocardial infarctions and stroke. As a result, increasing numbers of women have turned to complementary and alternative medicines for relief from menopausal symptoms. This article focuses on the potential use of soy isoflavones for treating menopausal symptoms.

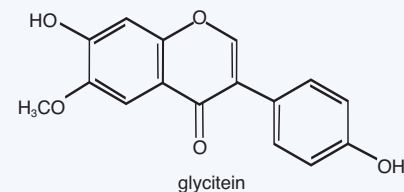
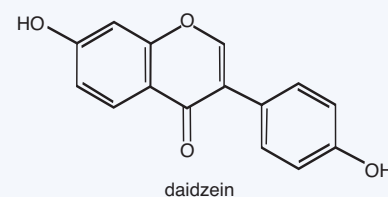
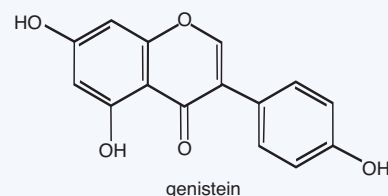
Isoflavones

Soy isoflavones are also referred to as phytoestrogens because they have properties similar to selective oestrogen modulators.³ Isoflavones are found naturally in a variety of

plants, including fruits and vegetables, and are most abundant in leguminous plants such as soy. Soybean contains a complex mixture of biologically active chemicals. One of the main constituents of the soybean beneficial to health is the protein, which contains isoflavones. Isoflavones are a subclass of a larger and more ubiquitous group of nutrients known as flavonoids.

Chemical structure

The main isoflavones in soybeans are genistein (4',5'-trihydroxyisoflavone) and daidzein (4',7'-dihydroxyisoflavone), and their respective β -glycosides, genistin and daidzin.⁴ The abundance of genistein appears to be higher than daidzein in soybeans and soy products. There are also small amounts of another isoflavone in soybeans, glycitein (4',7'-dihydroxy-6-methoxyisoflavone) and its glycoside, glycitin.⁴ The chemical structures of genistein and daidzein are shown below.



The main isoflavones of soy

The basic structure of isoflavones consists of two benzene rings, which are linked by a heterocyclic pyrane ring. One hydroxyl group (-OH) is found attached to each benzene ring. As can be seen from the chemical structures, genistein and daidzein differ by

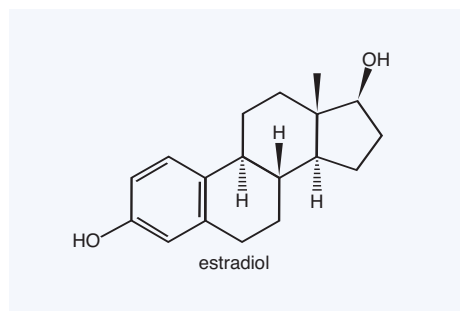


A diet rich in soy and soy products can provide certain therapeutic benefits

one hydroxyl group on the A ring of the isoflavone structure. In plants, isoflavones are inactive when present in the bound form as glycosides, but when the sugar residue is removed, these compounds become activated.⁵ Both the metabolite and parent compound are liable to absorption.

17 β -Estradiol is one of the most potent endogenous oestrogens in humans. The structures of isoflavones are similar to the structure of 17 β -estradiol in two ways:

- Both have an aromatic ring with a hydroxyl group attached to it
- A nearly identical distance exists between two hydroxyl groups in both⁶



The structure of estradiol

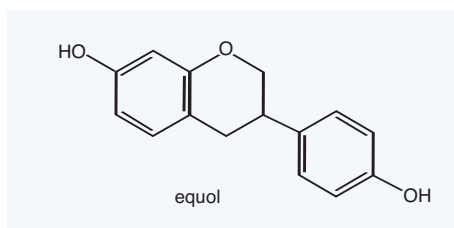
Since the chemical structure of isoflavones is similar to that of oestrogen it is not surprising that they bind and interact with the oestrogen receptor (ER), predominantly the ER β form of the receptor and thereby exert a weak oestrogenic effect. However, the isoflavones have much lower binding affinities to oestrogen receptors than estradiol. In addition they have been shown to have lower potential to stimulate alkaline phosphatase production in bone cells compared with estradiol.⁷

The structural differences between oestrogens and isoflavones may explain why isoflavones have more selective effects in different oestrogen-responsive tissues. Bone contains only ER β , but ER α and ER β are both located in the vascular system, the placenta, the uterus and in breast tissue.⁸ Isoflavones (particularly genistein) have greater affinity for ER β as opposed to ER α and this may explain why they have positive effects on the central nervous system, blood vessels and bone, although they have little or no effect on breast and endometrial tissue. The different affinities of isoflavones to ER α and ER β , and the different tissue distribution of these two oestrogen receptors, could possibly offer an explanation for the inconsistent effects of isoflavones that have been reported on menopausal symptoms and osteoporosis.

Clinical importance of equol

Equol is not found in soy but is a product of the intestinal bacterial metabolism of daidzein⁹, one of the main isoflavones in soyfoods. It possesses oestrogenic activity, having affinity for both oestrogen receptors ER α and ER β .

However, equol is not produced in adults



The structure of equol

who lack the intestinal bacteria that are required to metabolise daidzein in soy products. It has been proposed that the ability to make equol may hold the clue to the effectiveness of soy protein diets when employed in the treatment or prevention of hormone-dependent conditions.⁹ The failure to distinguish those subjects who are "equol-producers" from "non-equol producers" in previous clinical studies could possibly explain the variance in reported data on the health benefits associated with soy.⁹ Equol was formed in only 45 per cent of the postmenopausal women in one particular study⁹, but in those capable of making equol, lumbar spine bone mineral density (BMD) increased by 2.4 per cent (compared with the control group) while there was no significant change in BMD in the "non-equol producers".

Mechanism of action

Soy isoflavones have been shown to reduce menopausal symptoms, but the exact mechanism of action is yet to be determined. Several studies have hypothesised possible mechanisms.

Oestrogen receptors (ERs) bind to endogenous oestrogens and isoflavones, as well as to other environmental oestrogen-like molecules. When isoflavones reach the target tissue, they cross the cell membrane by passive diffusion, bind to ERs in the cytosol and form an isoflavone-ER complex. This complex then translocates into the nucleus for activation of the oestrogen response element (ERE), which is involved in the regulation of DNA-directed mRNA synthesis and the production of new proteins.¹⁰ Therefore, by this mechanism, isoflavones can bind to oestrogen receptors and so directly affect transcription of oestrogen-regulated gene products.

Another explanation is that isoflavones act through their antioxidant effects. The antioxidant properties of isoflavones are associated with the presence of hydroxyl groups at positions 4' and 5' on the aromatic ring.¹¹ Furthermore, there appears to be a positive synergy between phytoestrogens and other antioxidants.¹² This may be important in disease processes that involve oxidative stress, eg, in reducing low density lipoprotein (LDL) oxidation in atherosclerosis. Apart from protecting lipid-carrying proteins, phytoestrogens may also prevent the oxidation of critical enzymes in the signal transduction pathways through protection of cysteine groups. This property is not, however, governed by their oestrogen-like structures, but rather their anti-oxidant properties, suggesting that the overall effect of isoflavones may appear to be

like that of an oestrogen or an anti-oestrogen.¹⁰ It should be noted that the antioxidant properties of genistein may be responsible for its proposed anti-cancer properties.

Some isoflavones, such as genistein, can also bind with membrane receptors and function as tyrosine kinase inhibitors¹⁰, which are involved in protein phosphorylation during cell proliferation. In this way, isoflavones can influence the cell cycle and metabolism, through second messengers in the cytoplasm.¹⁰ In addition, genistein inhibits DNA topoisomerase II and ribosomal S6 kinase, both of which may lead to protein-linked DNA strand breaks in cancerous cells.⁵

Studies have shown an increase in the amount 17 β -estradiol in the presence of isoflavones, which has suggested that isoflavone supplementation increases oestrogen levels. They may have an indirect effect due to isoflavones acting on sex hormone-binding globulin. Isoflavones might compete with estrogens for this protein.¹³

Mechanism of action on bone cells

Researchers have hypothesised that a diet rich in isoflavones has a protective effect on bone. Krurzer and Xu¹³ reviewed several studies that include possible mechanisms of action to explain the beneficial effect of phytoestrogens on bone loss. These mechanisms include prevention of calcium loss, beneficial effects on osteoblasts, and influences on the secretion of calcitonin, which suppresses bone resorption. Both genistein and daidzein suppress osteoclast activity by a number of possible mechanisms. These include induction of apoptosis; activation of protein tyrosine phosphates; inhibition of cytokines; and changes in intracellular calcium and membrane depolarisation, which are all involved in bone turnover.¹⁴

Oestrogen receptors (only β) have been found in osteoblasts. The phytoestrogen-ER complex may bind to EREs and may thus cause alteration in the production of certain proteins as described earlier.

Bone remodelling is the function of the activity of both osteoblasts and osteoclasts. Osteoblasts respond to changes in the activity of osteoclasts, the bone resorbing cells.¹⁴ Osteoblasts are responsible for secreting bone formation-related proteins, such as alkaline phosphatase and osteocalcin. Besides this, osteoblasts are also capable of synthesising many other cytokines such as interleukin-6 and osteoprotegerin. These cytokines have been demonstrated to have critical roles in the regulation of osteoclast differentiation and activities.¹⁵ Soy isoflavones may, therefore, have indirect effects on osteoclasts by mediating cytokine production in osteoblasts.

Isoflavones for menopausal symptoms

Menopausal symptoms, especially hot flushes, have been reported to vary in incidence levels between women in different countries. In Asia (mainly South East Asia, Japan and China), only 14–25 per cent of women experience hot flushes, compared with 70–80 per

cent of women in western countries such as the UK and Denmark.¹⁶ The reason for these differences may possibly be due to the high proportion of soy isoflavones consumed in the traditional Asian diet that may have an influence on the body's response to the changing hormone levels at menopause. These findings have encouraged research into the potential benefits of soy isoflavones on menopausal symptoms.

Critical analysis of many trials has led to the suggestion that there is some evidence for the use of soy to alleviate menopausal symptoms. In addition there appears to be no safety concerns with soy products in short-term use.¹⁶⁻¹⁸ However, other studies have failed to demonstrate a reduction of menopausal symptoms.¹⁹⁻²¹

Another double-blind, placebo-controlled trial of four months' duration suggested that soy isoflavone treatment (100mg per day) may be a safe and effective alternative therapy for many menopausal symptoms.²² In that particular study it was noted that genistein and daidzein plasma concentrations peak 6-8 hours after ingestion, thus the choice of administering 33.3mg every eight hours was made.

The main adverse effects of soy products reported have been gastrointestinal disturbances such as nausea. However, allergy to soy has been noted¹⁶ and several subjects in randomised controlled trials (RCTs) have suffered from unpalatability of the treatment, especially in studies that have involved soy drinks.²³ It is of vital importance that tolerance be balanced with efficacy to achieve therapeutic effectiveness, because if patients are not able to tolerate soy they are less likely to be compliant. In one trial that used soy drinks²³, 10 out of 263 women volunteers dropped out of the study due to intolerance to the drink.

Risks of long-term effects of therapeutic soy use are largely unknown, although epidemiological data do not seem to indicate that serious problems exist.¹⁶

Even though many clinical trials have been conducted, the understanding of the potential health benefits of soy isoflavones is far from complete. The lack of clarity in determining any potential benefit of soy is most significantly due to methodological variation in trials. The most important factors include the menopausal status of the participating women, dosage of soy (ie, isoflavone content) and the outcome measures that have been used to test the efficacy.

Trials carried out have ranged from four weeks' duration¹⁹ to six months²⁰, making it difficult to compare the findings directly. The vast majority of the trials do not state specific compositions of their soy treatment, and therefore it is not possible to make conclusions on specific isoflavone quantities. Equally, the quality of the soy product used or the bioavailability of the isoflavone content may not have been assessed. Furthermore, as mentioned earlier, there is great variability in the ability to metabolise daidzein to its

metabolite equal⁹ between individuals. Isoflavone content of trial materials needs to be used at standardised levels to determine the most effective dose.

Plant sources that are rich in isoflavones also contain other compounds that may have the potential to interact with phytoestrogens, interfering with their activity and bioavailability. Consideration must therefore be given to these compounds as well. In addition, some phytoestrogens may act as oestrogen agonists or oestrogen antagonists depending on their structure and concentration. Isoflavones have been shown to have oestrogen agonist effects in low endogenous oestrogen concentrations and oestrogen antagonist effects in a more oestrogenic environment.²⁴ Thus one would expect isoflavones to have oestrogenic effects after the onset of menopause due to the low endogenous oestrogen concentration, and oestrogen antagonist effects before the onset of menopause when the endogenous oestrogen concentration is high. The oestrogen antagonist activity of phytoestrogens may be partially explained by their competition with endogenous 17 β -oestradiol for the oestrogen receptors.⁵

Use in breast cancer survivors

Soy isoflavones have also been tested for their efficacy in reducing menopausal symptoms in postmenopausal breast cancer survivors. The efficacy of the agents tested in breast cancer survivors has been variable, leaving vasomotor symptoms inadequately treated or ineffective in many women.²³ Previous studies have claimed positive effects of soy on hot flushes, but have had limitations in that they have been small, or not blinded, or have found only a minimal reduction or insignificant reduction in hot flushes.²³ Therefore, at present there is insufficient evidence to qualify soy or phytoestrogens as a suitable alternative to HRT in the treatment of hot flushes. Further study is required into safe and effective treatments for vasomotor symptoms, and the long-term safety of phytoestrogens in breast cancer survivors must be determined as well.

Osteoporosis

The continual loss of bone in the elderly is a natural process associated with aging. Women have a higher incidence of osteoporotic fractures than men due to their lower peak bone mass but, in addition, the abrupt decrease in oestrogen secretion in postmenopausal women accelerates bone loss.⁵ Soyfoods and isoflavones have received considerable attention for their potential role in preventing and treating osteoporosis. However, more long-term studies are needed on bone density and fracture rates, to determine safety, efficacy and appropriate dosage.

Drug therapy for osteoporosis

Drug therapy for osteoporosis can be divided functionally into two main categories:

■ **Antiresorptive agents** These are drugs that inhibit bone resorption, and therefore re-

duce bone turnover. Antiresorptive drugs such as oestrogens, calcitonin and bisphosphonates are most effective in the prevention of osteoporosis.

■ **Formation-stimulating agents** These stimulate bone formation, exerting an anabolic effect. Formation-stimulating regimens such as sodium fluoride or monofluorophosphate, parathyroid hormone fragments, and anabolic steroids are of potential value in the treatment of established osteoporosis, where bone mass is already low and benefit from antiresorptive drugs is likely to be small.²⁵

Ipriflavone is a synthetic derivative of naturally occurring isoflavones. It is a new non-hormonal agent that has become widely available in various countries for use in the treatment of osteoporosis, used at a dose of 200mg three times daily. Although its mechanism of action is still not fully understood, both *in vivo* and *in vitro* studies indicate that the drug inhibits osteoclast-mediated bone resorption, and stimulates bone formation in some systems.²⁵

The role of soy-containing phytoestrogens in bone health is an area of growing interest and is based on observational studies among Asian women showing that a higher consumption of phytoestrogens is associated with higher values of BMD and consequently a lower incidence of osteoporotic fractures. Three lines of evidence have been used to illustrate the effectiveness of isoflavones in bone: animal research; human studies; and *in vitro* cell investigations.¹⁰

Animal research

Ovariectomised (OVX) rodents (mainly rats) have mostly been used as models to examine the effect of isoflavones on bone loss caused by oestrogen deficiency. Current reports on animal studies have been highly consistent in demonstrating that soy isoflavones, either administered alone or with soy protein in the diet, increase bone mineral content (BMC) or BMD compared with control animals.²⁶

Human investigations

When compared with animal studies, data from human studies are limited, and have shown contradicting results. Clinical trials in involving postmenopausal and perimenopausal women, analysing BMC and BMD, have demonstrated that isoflavones can significantly increase bone mineral density at the lumbar spine.^{27,28} However, these studies had limitations in that they were of short duration with small sample sizes. The studies, which were ranging from four to 16 weeks' duration, are short with respect to bone metabolism because the bone remodelling cycle can be as long as 80 weeks.²⁹ It would therefore be premature to assume that soy with isoflavones has a significant long-term bone-sparing effect or that soy reduces bone fractures of the spine. The findings need to be confirmed by longer studies of two to three years' duration.

Bone turnover has mainly been assessed by the bone resorption markers pyridinoline and deoxypyridinoline, and the bone formation marker osteocalcin. Some studies are promising and suggest that isolated soy protein may have a protective role on bone maintenance³⁰ and that daily supplementation of soybean isoflavones (61.8mg) for four weeks is associated with a significant reduction in the excretion of bone resorption markers (pyridinoline and deoxy-pyridinoline).³¹ In postmenopausal women, diets rich in soyfoods have resulted in significant increases in serum osteocalcin concentrations.²⁸

However, other researchers have reported that soy supplementation over a three-month period did not produce any significant changes in the two pyridinoline markers of bone resorption³² and that soy protein isolate containing high concentrations of isoflavones was not protective against bone loss in early menopausal women.³³

Trials have shown a greater response to oestrogen treatments in the spine than in the hip due to the higher content of trabecular bone in the spine.³⁴ Trabecular bone is known to have a higher turnover rate than does cortical bone that is found in the hip. Thus, the lumbar spine, which is relatively high in trabecular bone, should be more sensitive to compounds that are thought to affect remodelling, such as oestrogens and phytoestrogens.³⁴

In a recent study, daily administration of 54mg genistein reduced postmenopausal bone mineral loss at the femoral neck and lumbar spine as effectively as hormone replacement therapy with 1mg per day oestrogen.³⁵ These findings add to the existing evidence that soy intake may be beneficial for bone conservation in postmenopausal women. However, further studies, ie, longer-term RCTs, are needed to elucidate the components of soy protein, to deduce optimal dosages and the time of life that soy is most effective in maintaining bone mass.

***In vitro* studies of osteoblast-like cells**

Numerous *in vitro* studies with human and animal osteoblasts and with osteoclasts have been carried out, yielding consistent observations of direct effects of phytoestrogens and isoflavones on both cell types. Daidzein and genistein have been found to have a stimulatory effect on protein synthesis and on alkaline phosphatase release by various types of osteoblast cells *in vitro*.¹⁴ Osteoprotegerin (OPG), a member of the tumour necrosis factor receptor superfamily, prevents bone resorption by a paracrine mechanism.¹⁴ It has now become apparent that osteoclast activity is modulated through osteoblasts via OPG. The cytokine receptor/activator of nuclear factor- κ (RANKL)¹⁴ stimulates osteoclast differentiation and function with higher levels of RANKL expression leading to increased bone resorption.

Additional components in soy

There are additional components in soy products that may also be responsible for the

prevention of bone loss or stimulating bone formation. The main ones include calcium and vitamin K2 (menatetrenone). Calcium is a component of many soy products and vitamin K2 is present in fermented soybeans (also known as tofu). Vitamin K2 is known to stimulate bone formation and prevent bone loss.³⁶ A significant correlation was found between BMD, the number of years since the onset of menopause, and consumption of fermented soybeans, suggesting that the effects of calcium, vitamin K2, and isoflavones might be synergistic.³⁷ Fermented soybeans have been shown to be consumed in higher quantities in eastern rather than in western Japan, correlating with epidemiologic data that show a lower incidence of osteoporotic bone fractures in women in eastern Japan.³⁷ This evidence might indicate that consumption of fermented soybeans is required to achieve the synergistic effects of calcium, vitamin K2, and isoflavone, rather than consuming isolated isoflavone extracts from soy.

Safety issues

The isoflavones, individually or combined, are considered to be safe up to quite high doses. Human toxicity studies of isoflavones suggest that doses ranging from 1–16mg/kg body weight are reasonably safe.¹⁰ The higher doses may prove to be necessary to prevent osteoporosis. At the higher doses being recommended for prevention of bone loss in postmenopausal women, little concern has been raised about adverse effects.

A few studies in rodents and isolated cells, however, suggest that isoflavones may not be totally safe. Genistein at high doses such as 600mg per day has been shown *in vitro* to inhibit cell growth and induce apoptosis.³⁸ In addition, some reproductive disturbances, such as uterotrophic effects, have been reported in animals fed a diet rich in isoflavones or other phytoestrogens.³⁹ Although the effect of soy and isoflavones on bone health constitutes to be an exciting area of research, no firm conclusions about safety can be reached at present.

Uncertainties

In order to achieve a significant reduction in total cholesterol, and the cardiovascular benefits reported for soy, one may require soy protein together with the isoflavones. However, for skeletal effects, pure genistein alone (or a combination of genistein with other isoflavones) is effective.¹⁰ It remains debatable whether a combination of isoflavones with soy proteins is necessary for optimal skeletal maintenance.

It is yet to be determined whether split doses of isoflavones over 24 hours would be more effective than a single dose of daily isoflavones or vice versa. The long-term safety of isoflavones, either mixed with soy protein or as purified supplements, remains to be examined in human subjects.¹⁰ The amounts of isoflavones consumed in standard food products such as tofu is generally re-

garded as safe but the higher quantities in supplements used over long periods is a concern to many researchers.

Soy intake recommendations

There is now a wide range of new foods containing soy readily available in supermarkets for those who wish to increase phytoestrogens in their diet. Specifically to alleviate menopausal symptoms and to prevent osteoporosis for which HRT is commonly used, phytoestrogens have been suggested as an alternative. In addition to this, isoflavones are now directly extracted from soya and red clover for use as an additive to non-soya foods.

All the studies reviewed on menopausal symptoms and osteoporosis have examined different quantities of soy or isoflavones, making it difficult to assign an exact value for recommendation. Generally, reports on the efficacy of soy isoflavones in reduction of menopausal symptoms suggest the targeted delivery is approximately 50mg of isoflavones per day. Since dose levels for other known phytoestrogens have not yet been deduced, it should be noted that this recommendation is focused on specified isoflavones (genistein and daidzein) rather than other phytoestrogens present in soy. Much of the justification regarding the selection of a dose of 50mg isoflavones/day (0.5–1.0 mg/kg body weight per day) is based on the presumed average intake of isoflavones in adults in China, Japan and Taiwan.

For the prevention of osteoporosis, however, soy isoflavones have to be consumed in much higher doses. A six-month trial in postmenopausal women reported that subjects consuming 90mg of isoflavones contained in soy protein have a two per cent increased bone mass compared with those on 56mg of intake contained in the same protein level.³⁰ Another study²⁷ revealed that soy protein isolate containing 80mg of isoflavones, attenuated bone loss in the lumbar region of perimenopausal women. Based on these findings and a number of human studies, for osteoporosis prevention a dose between 60 and 100mg of isoflavones per day may be needed. Toxicity studies of human subjects suggest that purified isoflavones are safe at doses at least twice as high as used in reported studies.¹⁰

Traditional soyfoods have an protein-to-isoflavone ratio of approximately 300:1; therefore, consuming 15g soy protein will result in consuming approximately 50mg isoflavones. These amounts of soy protein and isoflavones are provided by approximately two servings of traditional soyfoods and are likely to be efficacious for those diseases for which soy is proven to be beneficial.⁴

Conclusion

There is a growing body of literature on the subject of phytoestrogens that suggests that they may provide substantial health benefits, but there are still gaps in our understanding of them and their impact on human health and

safety. Phytoestrogens can be a significant contributor of oestrogens of dietary origin and may have health effects that are especially relevant to women's health, due to hormone-associated diseases in women. The plant isoflavones share structural similarities with endogenous oestrogens and *in vitro* studies have shown that the isoflavones can bind to oestrogen receptors. Functionally, it appears that the phytoestrogens may exert both oestrogenic and anti-oestrogenic effects, depending on circulating levels of endogenous sex hormones.

Although the purity, potency and effectiveness of the soy extracts are not well established, they are popularly believed to be safe and effective for the treatment of menopausal symptoms. It is clear, however, that much research is required to define clearly the pharmacological effect of dietary isoflavones and that future studies are of longer duration and are carried out using standardised quantities and structurally characterised mixtures of compounds, or with isolated phytoestrogens.

The benefit of isoflavones in the diet is to help maintain a healthy balance between the activities of osteoblasts and osteoclasts, resulting in better bone conservation and the potential prevention of osteoporosis, and associated fractures.¹⁰ However, as mentioned earlier, further research is needed into the long-term bone sparing effects of isoflavones so that their potential benefits in the prevention of osteoporosis can be confirmed.

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Note added in proof

Since this manuscript was submitted for publication, two relevant publications have appeared. An investigation into the enantiomeric form of equol has shown that the S-enantiomer is the exclusive product of human intestinal bacterial synthesis from soy isoflavones. The S-form has high affinity for ER β , whereas R-equol is relatively inactive.¹ A prospective cohort study of soy food consumption in 24,403 postmenopausal Chinese women over four and a half years has shown that soy consumption halved the number of osteoporotic fractures within a period of 10 years post-menopause.²

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