Review Article

Health potential of soy isoflavones for menopausal women

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Abstract

Objective: To review the current literature on the effects of soy isoflavones, one class of phyto-oestrogens, on cardiovascular diseases, osteoporosis, cancer and climacteric symptoms.

Design: Many study designs were employed in the reports reviewed here, including prospective human trials, observational human studies, animal experiments and *in vitro* cell studies that explored the protective or preventive effects of soy isoflavones (genistein, daidzein and glycitein alone or mixed).

Setting: Diverse settings were employed, depending on study design.

Subjects: Human subjects, mostly menopausal or postmenopausal, were included, as were animal models and specific cell types.

Results: The findings were: (i) isoflavones plus soy protein together were needed to obtain the highly significant beneficial results on blood lipids and arterial dimensions; (ii) isoflavone treatments alone at high doses (relative to above) consistently improved bone parameters in rodent ovariectomized models, but not in humans or primates; (iii) isoflavones were not consistent in exerting positive effects regarding the prevention or treatment of cancers of the mammary glands, uterus and colon; and (iv) the effects of isoflavones on climacteric symptoms were not clear-cut.

Conclusions: The promise of soy isoflavones reducing chronic disease risk seems to be non-uniform, with the most conclusive benefits occurring in the prevention of cardiovascular diseases, but other organ systems, such as skeletal and reproductive tissues, may also benefit from the consumption of soy and soy-derived products.

Keywords
Phyto-oestrogens
Isoflavones
Genistein
Cardiovascular diseases
Blood lipids
Osteoporosis
Cancer
Climacteric symptoms
Menopause
SERMs

Phyto-oestrogenic molecules have received a great deal of attention over the last few years because of their potentially preventive roles against a few of today's most prevalent chronic diseases, namely, cardiovascular disease, osteoporosis and hormone-related cancers[1,2]. The particular plant molecules of interest are isoflavones, which are found in abundance in soybeans and their derivative foods, such as tofu, miso and others. Of the several isoflavones that are made by soybeans, genistein, in particular, has been experimentally shown to be the most efficacious in human subjects and animal models.

For the last several years, a concerted effort has been mounted to identify alternatives to traditional hormone replacement therapy (HRT). Pharmaceutical companies have been developing selective oestrogen receptor modulators (SERMs), which ideally will provide the beneficial effects of oestrogen replacement for bones, cardiovascular system and cognitive function, without adverse effects on breast and endometrial cancer. As a result, SERMs should not require co-administration with a progestin, which can eliminate the side effects that some women experience with that hormone. The first molecules of this new class of drugs to become available are tamoxifen and raloxifene, which exhibit many, but not all, of the qualities of an ideal HRT. Many consumers would also prefer to avoid synthetic molecules in favour of natural products. Soy phyto-oestrogens, namely isoflavones, are now being intensively investigated for their potential action as a SERM, i.e. an alternative to HRT.

This review covers the effects of isoflavones, predominantly genistein, on three common chronic diseases plus the role of isoflavones in ameliorating the hypo-oestrogenic symptoms of the menopause and the early postmenopausal period. In addition, the dual

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Table 1 Isoflavone content of soybeans and soy products[4]

Soy food	Daidzein (mg 100 g^{-1})*	Genistein (mg 100 g^{-1})*
Soybeans	84	111
Soybeans (roasted)	56	87
Soy flour	23	81
Tempeh	27	32
Tofu	15	16

*On a per dry weight basis.

cellular roles of isoflavones as both oestrogen agonists and antagonists are discussed.

Isoflavones: plant molecules with oestrogen-like properties

In the USA and in most western nations, the consumption of soy products is quite low, probably less than 5 g day^{-1} person^{-1}. In contrast the mean intakes in many Asian nations, for example by Japanese men and women, may be approximately 10-fold greater than in the USA. The intakes of the Japanese result in the highest levels of phyto-oestrogens measured in human subjects[3].

The isoflavone content of soybeans and their products illustrate the typical losses resulting from processing of the original bean; losses of isoflavones increase with each step[4]. Table 1 lists the isoflavone contents of various soy products.

Isoflavones are present in a variety of food legumes. The amounts present in a representative group of leguminous seeds and sprouts are listed in Table 2, but significantly higher amounts are found in soy products (Table 1). Soy and soy products take on added nutritional importance because they are the primary foods, if not the only foods, that supply isoflavones in the diets of human populations. Several reports include the content of phyto-oestrogens in diverse beans[5–8].

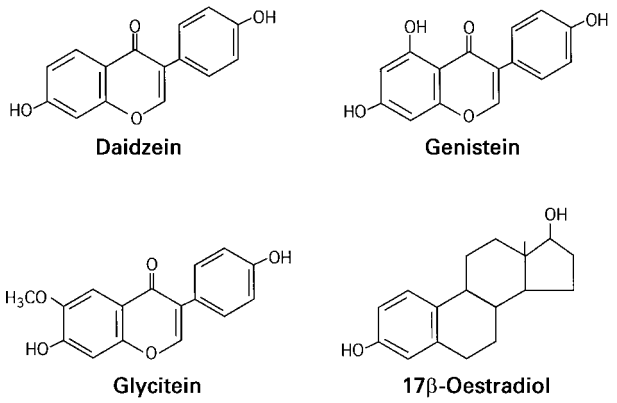

Fig. 1 Chemical structures of isoflavones and oestradiol-17β. Only a small difference exists in the structures of isoflavones, such as between genistein and daidzein (daidzein differs from genistein only by the absence of a hydroxyl group on the A ring), but this structural difference may greatly diminish the activity of an isoflavone, as in the case of daidzein

With the increasing availability of isoflavone-enriched supplements and new soy-containing food items in the marketplace, the total intake of isoflavones in the US population, and possibly in other western nations, will most likely rise substantially over the next few decades. Females are the more likely consumers of these soy-containing foods because of their belief in the potential prevention of osteoporosis, cardiovascular disease, hormone-dependent cancers and the reduction of symptoms associated with the menopause.

The chemical structures of the isoflavones in soy have similarities to that of oestradiol-17β, the most potent mammalian oestrogen (Fig. 1). Isoflavones in soy, namely, genistein, daidzein and glycitein, have several features in common with oestradiol-17β, including an aromatic A ring with a hydroxyl group substitution and a second hydroxyl group in the same plane (Fig. 1) at a distance similar to that in oestradiol[9].

Table 2 Isoflavone content of leguminous seeds and sprouts. Legumes were analysed for daidzein (D), genistein (G), coumestrol (C), formonetin (F) and biochanin A (B). Legumes that were found to have no detectable phyto-oestrogens include green peas, fava beans and lentils

Legume	Phyto-oestrogens (mg 100 g^{-1})	Phyto-oestrogen molecules
Seeds		
Green beans: fresh, raw	0.2	F
Lima beans: dry, raw	1.5	C
Garbanzo beans	1.5	B
Kidney beans: cooked	0.4	B
Pinto beans: dry	4.2	C, B
Great northern beans: dry	0.6	B
Black eyed beans: dry	1.7	B
Green split peas: dry	7.3	D
Mung beans: dry	0.6	F
Sprouts		
Clover sprouts	31.1	G, C, F, B

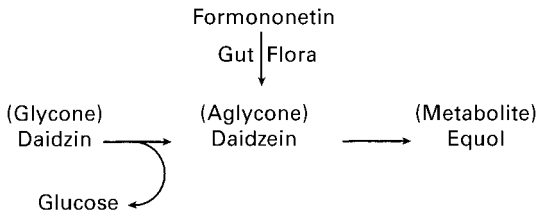

Fig. 2 Conversion of the isoflavone glycone, daidzin, to its aglycone, daidzein, in the gut lumen

In 1996, a new form of the oestrogen receptor (ER), ERβ, was described in rodent tissues. Since that time, a number of reports have documented the presence of ERβ in a wide variety of human and animal tissues, including the breast. Cells have been shown to vary in their distribution of ERα, the classic oestrogen receptor and ERβ molecules, depending on tissue. Reproductive cells, especially those of the breast and uterus, are rich in ERα, whereas other cells, such as in bone, have greater amounts of ERβ than ERα. This differential distribution of the two types of oestrogen receptors and the greater affinity of the isoflavones for ERβ relative to ERα suggest that the isoflavones have different effects in different tissues[10]. Isoflavones occupy the oestrogen receptors in cells, but occupancy time or affinity of isoflavones for the ERα receptor is much lower than that of oestradiol[11]. Of particular interest is the relative affinity of the ERβ receptor for the phyto-oestrogen genistein, which is approximately seven times that of ERα[10]. By binding to ERβ, phyto-oestrogens may have differential effects that may vary by site and individual. It is therefore important to consider ERβ in any future experimental assessments of the effects of phyto-oestrogens.

Most of the isoflavones in soy exist primarily as glucose conjugates or glycones. For example, genistein is an aglycone that results from the enzymatic cleavage of genistin. Similarly, daidzein is an aglycone that results from the removal of glucose from daidzin. These enzymatic changes are thought to result from the actions of gut bacteria. Figure 2 schematically illustrates the conversion of the glycone, daidzin, to its aglycone, daidzein, and its further metabolism to equol. Isoflavones may also be generated from precursor molecules, such as formononetin, through the action of gut flora (Fig. 2). Although most research has centred on the aglycone forms of genistein and daidzein, metabolites, such as equol, may contribute significantly to the biological effects of the isoflavone molecules. Equol and the other phyto-oestrogen metabolites are excreted in significant amounts in the urine of people who consume phyto-oestrogen-containing foods[12].

Extensive gastrointestinal processing of the natural products in food, including the removal of glycones from the soy protein fraction and the conversion of glycones to aglycones, must occur prior to intestinal absorption and distribution to body tissues. The isoflavones are fat-soluble molecules that are delivered from the gut to the tissues via chylomicrons. In addition, some of the metabolic products of isoflavones, in particular equol, may be transported in blood weakly associated with serum proteins. Equol, and possibly other isoflavones, are secreted by liver into bile and they therefore can cycle as part of the enterohepatic circulation[13,14]. This cycling may occur several times over one or more days and allows a steady-state concentration of these molecules to develop in blood as long as the intake of soy foods continues on a daily basis. Therefore, the enterohepatic circulation of these molecules enhances their long-term effects on tissues with oestrogen receptors. The caveat, though, is that soy foods must be eaten on a daily basis to maintain significant circulating concentrations.

Cells containing large numbers of oestrogen receptors, specifically breast and uterine cells, were studied initially in an attempt to understand better the specific actions of isoflavones. The critical observation that initiated experimental investigations of the isoflavones was that female sheep became reproductively inefficient following the ingestion of fresh coumestan-rich clover (subterranean), which led Australian scientists to conclude that excesses of subterranean clover-rich diet exerted adverse effects on uterine and possibly ovarian function[15].

Once absorbed and distributed to tissues of the body, aglycone isoflavones or their metabolites have two potential mechanisms of action at the cellular level. One fate is that the isoflavones are taken up by passive diffusion across the cell membrane and, via combination of the isoflavones with oestrogen receptors, they act on nuclear DNA as weak oestrogen agonists or antagonists. The classic action of oestrogenic isoflavones, using genistein as the model molecule, consists of several steps: (i) combination with the oestrogen receptor; (ii) dimerization of the genistein–oestrogen receptor complex with one of several possible molecules; (iii) activation of the oestrogen response element in DNA; (iv) stimulation of DNA-directed mRNA synthesis; and (v) the production of new protein molecules specific to the tissue cell type (Fig. 3).

A second action of phyto-oestrogens on cells, i.e. membrane protein (receptor) interactions, has recently been proposed and remains controversial. The isoflavones may interact with membrane proteins (receptors) and exert effects that are expressed through second messengers. The latter mechanism does not involve the genome directly, and it is typically short lived and more modulatory of existing cellular activities than the more potent genomic effects. Nevertheless, other steroid molecules, particularly 1,25-dihydroxycholecalciferol (calcitriol), also have similar membrane

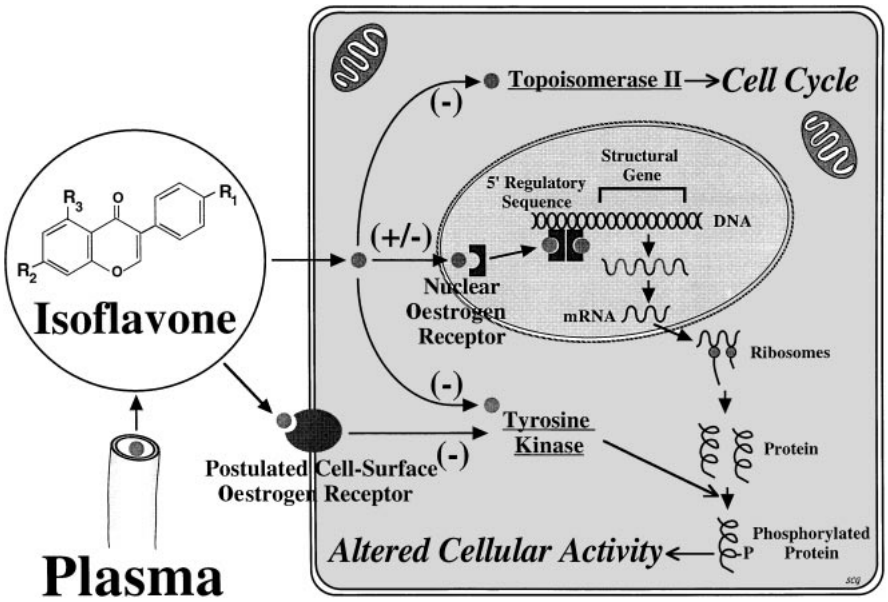

Fig. 3 Genomic and non-genomic actions of isoflavones on cells. Isoflavones, such as genistein, may interact with nuclear oestrogen receptors by either activating (+) or inhibiting (–) transcription of cell-specific genes. Non-genomic effects of these phyto-oestrogen molecules may include inhibitory effects on other molecules such as topoisomerase II and tyrosine kinase. (From Adlercreutz[1], with permission)

protein interactions[16,17]. In this mechanism, several possible second messengers, including kinase enzymes, are activated and they in turn stimulate or inhibit downstream pathways of metabolism and protein products (Fig. 3). The duration of this effect via a membrane protein interaction of isoflavones is considered to be short lived and of limited efficacy, unless the stimulating molecules are continuously available in blood and tissue fluids bathing cells.

Cardiovascular disease

Coronary heart disease (CHD) is the leading cause of death for postmenopausal women in westernized societies. For more than 25 years, data have accumulated suggesting that use of oestrogen replacement therapy is associated with about a 40–50% lower risk of CHD morbidity and mortality[18]. However, in spite of this evidence for a beneficial effect in preventing chronic diseases, only about 20% of postmenopausal women in the USA take HRT for 5 years or more[19]. The reasons for this poor use rate include fear of cancer and adverse side effects resulting from the progestin that is co-administered with oestrogen to prevent increased risk of endometrial cancer.

Because of the poor use rate of traditional HRT, considerable interest has arisen in finding alternatives that women will be willing to take. Recently, the results of the Heart and Estrogen/progestin Replacement Study (HERS) were published in which women who already had CHD were treated with either combined HRT (0.625 mg Premarin + 2.5 mg medroxyprogesterone acetate given continuously) or a placebo[20]. The combined HRT resulted in no advantage compared to the placebo for risk of a second coronary event through 5 years of treatment (relative hazard, RH = 0.99; 95% confidence interval, CI = 0.80–1.22). This study indicated that women who already had developed CHD did not benefit from this form of HRT (combined Premarin and Provera given continuously), supporting the idea that other forms of HRT (including different oestrogen or progestin molecules in combination) or alternatives to HRT should be explored. In addition, the negative results of the HERS trial underscore the importance of primary prevention. Soy phyto-oestrogens have been a focus of research recently as a potential therapy for postmenopausal women. Studies that have focused on soy phyto-oestrogens and cardiovascular disease in postmenopausal subjects are reviewed below.

Evidence that soy protein with phyto-oestrogens is beneficial

Human studies

Anderson *et al.*[21] published a meta-analysis of 38 clinical studies that compared the effects of soy protein treatment and a placebo on plasma lipids and lipoproteins, risk factors for cardiovascular disease. For studies to be included in this analysis, they had to have measured plasma lipid concentrations at baseline and used texturized soy protein or soy protein isolate as the active treatment. These authors found a

9.3% reduction in total cholesterol concentrations ($P < 0.05$) with soy treatment compared with the placebo after adjustment for baseline measurements. They also reported a significant reduction in low-density lipoprotein (LDL) cholesterol (12.9%) and plasma triglycerides (10.5%) with soy treatment. High-density lipoprotein (HDL) cholesterol concentrations were not significantly affected (2.4% increase). While these studies included both men and women, the results were not stratified by gender. Of interest, when the results were stratified into quartiles by baseline total cholesterol, those with the highest baseline measure had the greatest reduction in LDL cholesterol (24.0%) and the quartile with the lowest baseline total cholesterol had the smallest reduction (7.7%). Thus, overall, a moderate benefit of soy protein on plasma lipids and lipoproteins was found, and among those with the highest plasma cholesterol concentrations, a substantial improvement was shown with soy consumption. However, because the phyto-oestrogen content of the soy used in most of these studies is not known, it is impossible to determine what concentration is required to contribute to the improvement in plasma lipid concentrations. One conclusion from this analysis is that soy foods containing phyto-oestrogens have beneficial effects on plasma lipid concentrations.

In a recent study by Crouse *et al.*[22] the contribution of the phyto-oestrogens to the cholesterol-lowering properties of soy protein were investigated. This study included 156 moderately hypercholesterolaemic men and women (baseline LDL cholesterol >140 mg dl^{-1}) who were instructed to consume a low-fat, low-cholesterol diet (National Cholesterol Education Program Step 1 diet) for 4 weeks prior to the baseline measurements. The subjects were then randomized into one of five treatment groups, each of which received a protein supplement containing 25 g of protein. The treatment groups were: (i) casein protein; (ii) isolated soy protein (ISP) with 3 mg phyto-oestrogens; (iii) ISP with 27 mg phyto-oestrogens; (iv) ISP with 37 mg phyto-oestrogens; and (v) ISP with 62 mg phyto-oestrogens. The participants continued these treatments for 9 weeks. For the entire group, a trend toward lower LDL cholesterol concentrations was observed with increasing phyto-oestrogen content of the soy protein supplements, but only the supplement containing 62 mg of phyto-oestrogen resulted in significantly lower LDL cholesterol concentrations compared with the casein group (Fig. 4a). A greater benefit in LDL cholesterol reduction was found among the participants who had LDL cholesterol concentrations above the median at baseline. In this subset of participants, both the supplements containing 37 and 62 mg of phyto-oestrogen resulted in significantly lower LDL cholesterol concentration compared with the casein group. A significant dose–response trend

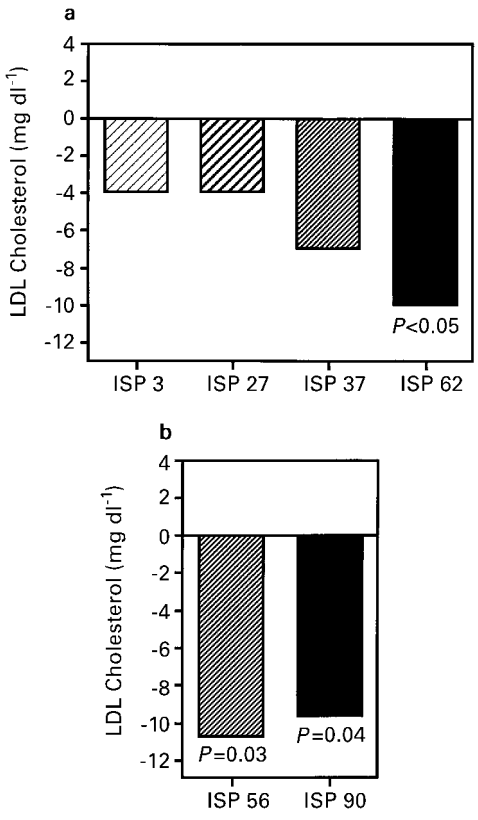

Fig. 4 Effects of isolated soy protein (ISP) with varying concentrations of isoflavones on plasma LDL cholesterol (mg dl^{-1}). Data are expressed as the difference from control group (casein or casein/non-fat dry milk) adjusted for baseline values. (a) Study used 25 g of protein per day in each of the groups (Crouse *et al.*[22]) (ISP3 = 3 mg isoflavones day^{-1}; ISP27 = 27 mg day^{-1}; ISP37 = 37 mg day^{-1}; ISP62 = 62 mg day^{-1}). (b) Study used 40 g of protein per day (Baum *et al.*[23]) (ISP56 = 56 mg isoflavones day^{-1}; ISP90 = 90 mg day^{-1})

was found, with increasing phyto-oestrogen intake resulting in increasingly lower LDL cholesterol concentrations. No significant effect of phyto-oestrogens was demonstrated on HDL cholesterol concentrations in this study. These data suggest that phyto-oestrogens in a supplement containing 25 g of soy protein can significantly lower LDL cholesterol concentrations and that the higher the phyto-oestrogen content (up to 62 mg day^{-1}), the better the response.

Baum *et al.*[23] reported the results of a randomized clinical trial that compared the effects of eating 40 g of soy protein daily, containing either 56 or 90 mg of phyto-oestrogens, with a casein/non-fat dry milk placebo which had no phyto-oestrogens. This study included 81 postmenopausal women who were moderately hypercholesterolaemic at baseline (baseline serum total cholesterol, 240–300 mg dl^{-1}). Women were instructed to follow the National Cholesterol Education Program Step 1 diet for 2 weeks prior to the baseline measurements and to continue this diet for the duration of the treatment period (6 months). Both soy

protein treatments resulted in significantly lower LDL and VLDL cholesterol and higher HDL cholesterol concentrations compared with the placebo, but no difference in lipid response was found between the two soy treatment groups (Fig. 4b).

Comparison of the data from the reports by Crouse *et al.*[22] and Baum *et al.*[23] might suggest that no further benefit on blood lipids can be attained with soy protein containing phyto-oestrogen levels higher than approximately 60 mg day^{-1}. It is also possible that the 40 g of soy protein consumed by the participants in the study by Baum *et al.*[23] overwhelmed any additional benefit that might have been seen with the higher phyto-oestrogen content. However, both studies suggest that phyto-oestrogen-containing soy protein can improve plasma lipid and lipoprotein concentrations. The study by Crouse *et al.*[22] further suggests that soy protein supplements containing low levels of phyto-oestrogens are not as beneficial as those containing higher amounts of these molecules.

Non-human primate studies

Additional information about the effects of soy protein and phyto-oestrogens can be derived from two studies in non-human primates. In the first, 85 male and 75 female cynomolgus monkeys were randomized into one of three treatment groups being fed: (i) casein and lactalbumin as the source of protein (control); (ii) soy protein isolate that was alcohol-washed to remove the phyto-oestrogens; or (iii) soy protein with the isoflavones intact[24,25]. The diets were identical in composition for macronutrients. The amount of isoflavones in the intact soy protein diet was approximately equivalent to a human dose of 140 mg day^{-1}. The isoflavone-containing soy diet significantly lowered LDL and VLDL cholesterol compared with the control group (about 35%) and increased HDL cholesterol (about 50%). The isoflavone-devoid soy diet resulted in only a slight reduction (about 8%) that was not significantly different from the control group, but significantly increased HDL cholesterol concentrations (about 20%). Coronary artery atherosclerosis measurements were done in 11 animals from each diet group and the cross-sectional area of the arterial lesions were largest in the control group (0.13 mm^2), smallest in the soy with isoflavones group (0.02 mm^2) and intermediate in the isoflavone-devoid soy group (0.06 mm^2). These data suggest that the soy isoflavones contribute greatly to the regulation of plasma lipid concentrations and atherosclerosis.

In the second study surgically postmenopausal monkeys were fed diets containing either: (i) alcohol-washed, phyto-oestrogen-devoid soy protein isolate (n=56); (ii) phyto-oestrogen-intact soy protein isolate (n=59) with a phyto-oestrogen amount equivalent to a dose for women of 120 mg day^{-1}; or (iii) isoflavone-devoid soy to which Premarin (conjugated equine oestrogens) (n=63) was added to approximate a woman's dose of 0.625 mg day^{-1} [26–28]. In Fig. 5 the effects of the soy phyto-oestrogens and traditional oestrogen therapy on plasma lipid concentrations are compared[26]. While both treatments lowered LDL and VLDL cholesterol, soy phyto-oestrogen administration resulted in significantly higher HDL cholesterol and significantly lower plasma triglycerides compared to traditional oestrogen therapy.

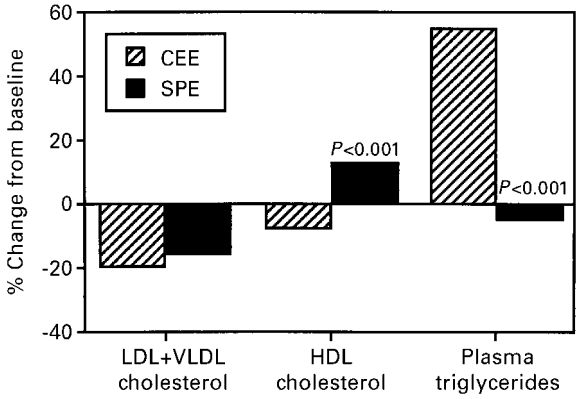

Fig. 5 Effects of treatment with soy phyto-oestrogens (SPE) or conjugated oestrogens (CEE) on cardiovascular risk factors and cardiovascular disease. Data are expressed as per cent difference from soy-fed control group (Anthony *et al.*[107])

In addition, the effects of these two treatments on atherosclerosis have been reported[27,28], and the soy isoflavones (approximately equivalent to a dose of 120 mg day^{-1} for women) appear to be equal to conjugated equine oestrogens (equivalent to a dose of 0.625 mg day^{-1} for women) in preventing atherosclerotic lesion development at some arterial sites (e.g. the internal carotid arteries) and less efficient at other sites (e.g. the common iliac artery). No explanation currently exists for the site-specific differences in atheroprotection of soy protein containing isoflavones. Differences in ERα and ERβ concentrations in various tissues and differential susceptibility of these sites to plasma lipid risk factors or direct effects of isoflavones on the arterial wall may be other explanations.

The above study in non-human primates[27,28] was the first to compare directly the effects of traditional HRT and soy phyto-oestrogens. The conclusion that can be made from this study is that soy phyto-oestrogens compare favourably with traditional oestrogen therapy in their effects on cardiovascular disease and its risk factors, but more studies are needed to confirm these findings.

Lack of effect of purified isoflavones

While abundant evidence exists that soy phyto-oestrogens, when fed in soy protein, have beneficial effects on the cardiovascular system and risk factors,

two recent studies in humans suggest that the purified isoflavones do not have the same effects. In a crossover study by Nestel *et al.*[29] peri- and postmenopausal women ($n=21$) were treated with 80 mg isoflavones in a tablet or a placebo pill for 5-week periods. They found the isoflavone pills had no effects on plasma lipid concentrations, although they did find a beneficial effect on systemic arterial compliance (a measure of elasticity of arteries). In a report by Hodgson *et al.*[30], dosing with a purified isoflavone tablet (55 mg day^{-1}) in a parallel arm, placebo-controlled study of 8 weeks' duration had no effect on LDL or HDL cholesterol concentrations in a group of men and women ($n=60$ per group).

Data from a study in non-human primates add further support to the lack of effect of purified isoflavones on plasma lipids. In a study by Greaves *et al.*[31], surgically postmenopausal monkeys ($n=20$ per group) were fed for 12 weeks with either: (i) a moderately atherogenic diet that had either casein and lactalbumin as the source of protein (C/L); (ii) the C/L diet with purified isoflavones added (C/L+Iso); or (iii) unextracted soy protein (Soy(+)). The isoflavone content of the C/L+Iso and Soy(+) diets were equivalent. The addition of purified isoflavones to the C/L-based diet (C/L+Iso) had no beneficial effects on plasma lipid/lipoprotein concentrations compared with the control group (C/L-based diet without isoflavones). In contrast, the isoflavone-containing soy protein-based diet (Soy(+)) reduced total cholesterol and LDL cholesterol, and increased HDL cholesterol.

These studies suggest that the isoflavones might require the presence of soy protein in order to beneficially affect plasma lipids. However, the finding of a beneficial effect of the purified isoflavone pills on systemic arterial compliance suggests that the purified isoflavone pills may have some biological effects[29].

Potential mechanisms

The evidence that soy with its phyto-oestrogens has a beneficial effect on cardiovascular disease seems convincing, and it appears that at least a portion of this benefit is modulated by effects on plasma lipids and lipoprotein concentrations. However, beneficial effects of soy with its phyto-oestrogens, and genistein in particular, on vascular reactivity, an indicator of vascular function, have also been reported[32]. Another potential benefit of the phyto-oestrogens, which has been shown *in vitro*, is the prevention of lipoprotein oxidation[33,34], which may blunt atherosclerosis since oxidized LDL particles are considered more atherogenic[35]. Phyto-oestrogens may also have direct effects on the artery wall and the cells involved in the promotion and progression of atherosclerosis. Genistein has been shown, *in vitro*, to inhibit the migration and proliferation of smooth muscle cells[36–38], to inhibit platelet activation[39,40] and aggregation[41,42], and to reduce platelet serotonin uptake[43]. Each of these processes has the capability to inhibit atherosclerosis initiation and progression.

Summary

Little doubt remains that soy with its phyto-oestrogens has beneficial effects on cardiovascular disease and its risk factors. A number of biological processes that may play a role in this protection include beneficial effects on plasma lipids and lipoproteins, improved vascular function, and possibly favourable effects on platelets and smooth muscle cells, cells that are important in atherosclerosis initiation and progression. An intervention incorporating soy with its phyto-oestrogens in a western-type diet that can favourably impact on cardiovascular disease morbidity and mortality (e.g. stroke, myocardial infarction) has not yet been undertaken.

Questions remain regarding dose and form of the phyto-oestrogens to maximize the benefits and minimize any potential risks. However, incorporation of soy with its phyto-oestrogens into a western diet may prove to be an important means of reducing cardiovascular disease and may have additional benefits for other chronic diseases.

Osteoporosis

The increase in skeletal fragility with ageing and the increase in bone fractures that result because of osteoporosis are public health concerns of enormous magnitude as the population ages, especially as more postmenopausal women are surviving to their 80s and 90s. A primary clinical preventive strategy has previously employed oestrogen replacement therapy or HRT, but the recently introduced SERMs may provide benefits to bone (and cardiovascular tissues) without adverse effects on reproductive tissues (i.e. cancer) as shown for oestrogens. The available experimental data on the effects of isoflavones (i.e. phyto-oestrogens) on bone suggest that these molecules, especially soy-derived genistein, may actually be naturally occurring SERMs[44].

Rodent studies

The effects of isoflavones on the skeletal tissue of experimental animals and humans have been found to be mixed, although the rodent studies have been highly consistent. The published reports are briefly reviewed in this section. Several rodent models have been examined, but little information is available for other species, such as the primate[45]. Most of the rodent experiments have employed ovariectomized (OVX) animals to demonstrate the effects of soy isoflavones, especially preparations that contain mixtures of

genistein, daidzein and glycitein. Effects of isoflavones on bone have been compared with those resulting from similar treatment with oestrogens. Experiments using the OVX adult female rat model have suggested increased bone mineral density at one or more sites was the primary outcome.

Studies from three different laboratories using OVX rats have shown that treatment with the isoflavones present in soy, either unpurified[46], partially purified[47] or purified[48] (as genistein) exerted significant improvement in bone mass and approximately the same per cent retention, which may approach the gain from treatment with replacement doses of oestradiol (Fig. 6). The age and body weights of the rats in these three different studies were similar, and the duration of treatment ranged from 14 days[47] to 30 days[46,48]. All three of the studies incorporated the phyto-oestrogen-containing preparation into the diet; however, the doses used are difficult to compare. The phyto-oestrogen content was not determined in the soy protein isolate in the Arjmandi *et al.*[46] study, and the phyto-oestrogen preparation used by Anderson *et al.*[47] was only partially purified.

Although the skeletal benefits of the isoflavones were comparable to those of oestrogens, the dose of isoflavones was approximately 100–1000-fold greater than that of oestrogens (oestradiol-17β or conjugated oestrogens). An additional benefit of the isoflavone treatment compared with oestrogens was the lack of any major effect of isoflavones on uterine weight. In these experiments, the isoflavone preparation appeared to act like a SERM because of the positive effects on bone without any obvious effects on the uterus, but further research is needed to establish that isoflavones are really SERMs.

Although the results from Blair *et al.*[48] suggest that co-administration with soy protein is not required to show bone-conserving effects of soy isoflavones in the OVX rodent model, the effects of a range of doses have not been determined. Blair *et al.*[48] used purified genistein at a single high dose. Only the study by Anderson *et al.*[47] used more than one dose, and they reported that the lowest dose was the most effective, with less effect at the two higher doses, i.e. a biphasic response.

Human studies

Only a few reports on the skeletal effects of genistein or of a genistein-rich isoflavone preparation on human subjects have been published, and in these studies bone mineral density at several skeletal sites or the whole body was the primary outcome. Additional publications on effects of isoflavones on bone mineral density are anticipated in the next few years. One study using a soy protein isolate containing 56 or 90 mg phyto-oestrogens showed that older postmenopausal

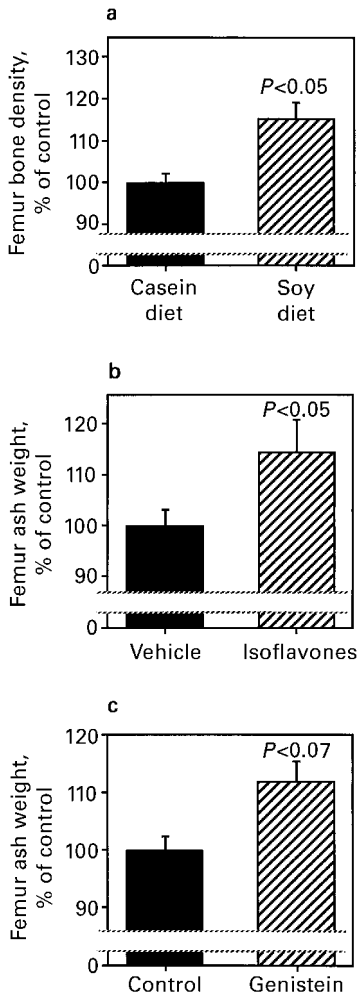

Fig. 6 Effects of (a) soy protein, (b) purified soy extract and (c) genistein on bone mass in ovariectomized (OVX) rats. (Data from Arjmandi *et al.*[46], Anderson *et al.*[47] and Blair *et al.*[48])

women responded to the higher, but not the lower, dosage with significantly improved lumbar vertebral bone mineral density (Fig. 7)[49].

In vitro *studies*

In studies of isolated osteoblast-like cells in culture in which the media are changed frequently, the continuous dosing of cells with an isoflavone is easy to undertake, whereas in animal or human models it is less certain that sufficiently high blood concentrations can be maintained over long enough periods (e.g. weeks to months) to have a significant impact on bone cells. For this reason, isolated cell studies provide an advantage because the cells are continuously bathed with an isoflavone, much like the maintenance of a uniform concentration of isoflavones in the blood because of the enterohepatic circulation. The critical element is to provide isoflavones in the culture media at all times at a dose or doses sufficient to exert a threshold response. Our studies with isolated

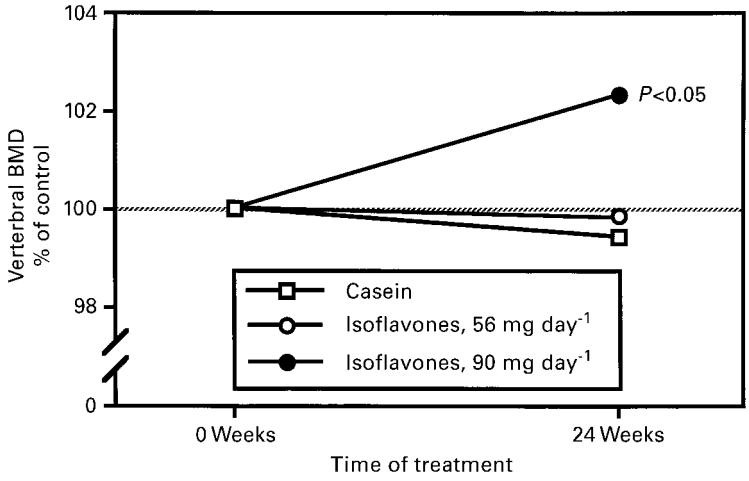

Fig. 7 Effects of purified soy extract on lumbar bone mineral density (BMD) in elderly women after almost 6 months of treatment. (Adapted from Potter *et al.*[49])

osteoblast-like MC3T3-E1 cells, treated with genistein between days 2 and 4 in culture, then incubated for a total of 12 days, suggest that genistein inhibits the synthesis and secretion of IL-6, an interleukin known to stimulate the activity of osteoclasts in bone (X. Chen *et al.*, unpublished data, 1999). This inhibitory effect of genistein on IL-6 is similar to the inhibition of IL-6 following treatment of these cells with oestradiol (Fig. 8). The significance of these data is that genistein acts very much like oestrogens in inhibiting the resorptive activities of osteoclasts.

An alternative mechanism for decreased osteoclastic bone resorption by genistein is a direct effect via inhibition of tyrosine kinase activity in osteoclasts. Blair *et al.*[48] reported that genistein, at doses achievable in the circulation, can significantly decrease the amount of bone degraded by isolated chicken osteoclasts *in vitro*. Either a direct or indirect effect of genistein or other phyto-oestrogens to decrease osteoclastic bone resorption in the absence of normal levels of circulating oestrogens would be a potential benefit.

The potential for phyto-oestrogens to prevent loss of bone mass has been demonstrated in experimental rodent models, but convincing data have been more difficult to obtain from primate and human studies. It is possible that the few primate and human investigations published thus far may either have used doses that were too low or the duration of treatment may have been too brief. The results of other studies are being awaited.

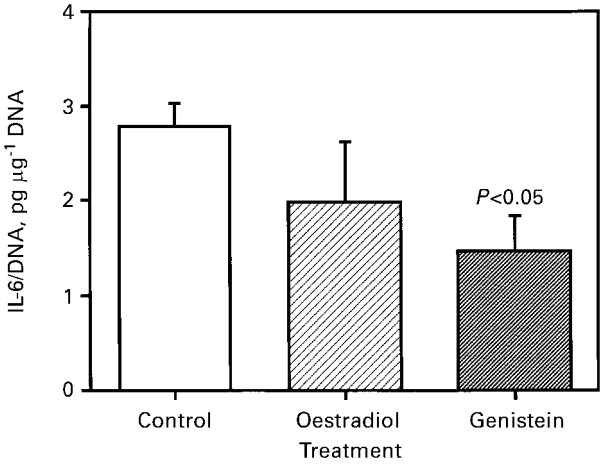

Fig. 8 *In vitro* cell studies of osteoblasts (MC3T3-E1) showing the inhibition of IL-6 production by the cells in a similar manner to that found for oestradiol. IL-6 is a known stimulator of osteoclastic resorption, and these data demonstrate that genistein acts on these cells in a very similar way to oestradiol (X Chen *et al.*, unpublished data)

Cancer

Demographic and epidemiological evidence for anticancer effects

Asian women have a relatively lower risk of breast, endometrial and colonic cancer, compared with western women[50–52]. The risk of breast cancer increases in Asian immigrants to the USA[53] and in urban subpopulations within Japan[54], suggesting that environmental factors are responsible for the difference. Asian diets typically contain less fat and a higher proportion of vegetables, including soy products, when compared with western diets. Among the seven case–control studies of soy intake and breast cancer risk published to date, there is more evidence for breast cancer protection by soy phyto-oestrogens in premenopausal women than in postmenopausal women (Fig. 9)[55–61]. Therefore, there may be context-dependent effects of soy phyto-oestrogens on cancer risk, depending on life stage and/or hormonal status.

Only one epidemiological study of endometrial

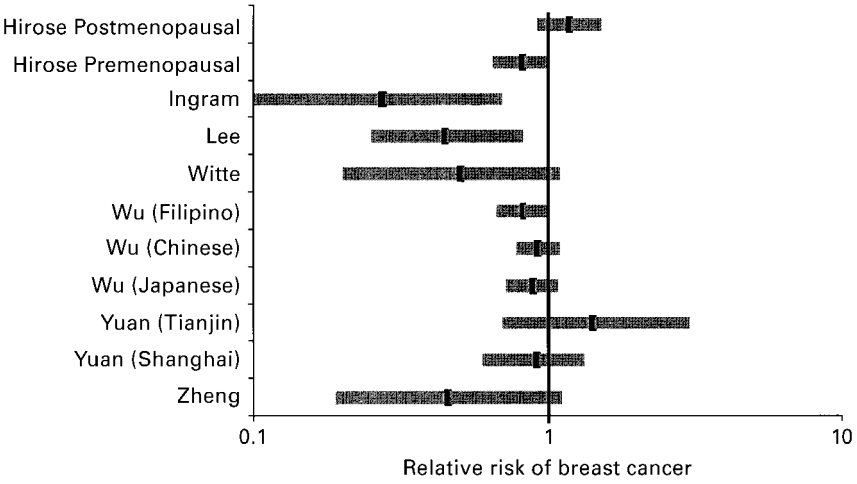

Fig. 9 Case–control studies of soy intake or soy isoflavone excretion and breast cancer risk[56,61]. The distribution of risk estimates in studies including premenopausal women suggests a relatively greater protection of soy phyto-oestrogens against premenopausal women than postmenopausal women[55–59,61]

cancer and soy phyto-oestrogen intake has been conducted to date. In this case–control study, women in the highest versus the lowest quartile of soy intake had a relative risk of 0.46 (95% CI 0.26–0.83) for the development of endometrial cancer[62], i.e. about a 50% lower risk of endometrial cancer among the quartile with the highest intake of soy.

Epidemiological studies of colonic cancer consist of demographic observations similar to those made for breast and endometrial carcinoma[50] and relatively few case–control studies. Greater intakes of vegetables, including soybeans, are associated with lower risk of colon cancer[63,64], but the contribution of phyto-oestrogens *per se* to this effect has not been clearly isolated. The mechanisms by which soy phyto-oestrogens might diminish colon cancer risk is unclear since consumption of soy milk decreased circulating oestradiol in women in one study[65], while in another the use of HRT was inversely correlated with colon cancer[66].

Animal studies

Approximately two-thirds of the more than two dozen rodent studies have shown a protective effect of soy or soy phyto-oestrogens against chemically-induced mammary neoplasms[67]. Neonatal exposure to the soy phyto-oestrogen, genistein, has produced a mammary cancer-protective effect later in life[68]; a similar finding has been made for exposure at puberty[69]. However, it is worthwhile to note that soy phyto-oestrogens promote the growth of human MCF-7 breast cancer cells *in vitro* and that the growth of these cancerous cells injected into nude mice has been promoted by administration of dietary genistein[70]. Because of the paucity of endometrial cancer models, animal studies have been limited to uterotrophic assays or intermediate marker assays.

Soy phyto-oestrogens clearly are not mammotrophic or uterotrophic in macaques, when given at doses similar to those attained in the Asian diet. Recent work has shown that the oestrogenic or anti-oestrogenic effect of soy phyto-oestrogens is dependent on hormonal context. In monkeys given a hormone-replacement dose of oestrogen, dietary soy supplementation decreased oestrogen-induced proliferation in both mammary and uterine tissues and, therefore, could potentially decrease the cancer-promoting effect of oestrogen[71]. Other studies in rats have indicated that the effects of dietary oestrogens and oestrogen-replacement therapy are interactive and dose-dependent, such that soy phyto-oestrogens may increase breast cell proliferation at low doses of oestrogens, but may decrease breast cell proliferation when given in combination with high doses of oestrogens (JM Cline, unpublished data).

Animal studies of colorectal cancer and soy have produced conflicting results. Induction of atypical colonic crypts by azoxymethane has been inhibited by soy intake in rodent models[72,73]; however, the development of colonic tumours induced by the same carcinogen has been promoted by soy intake[74]. The development of colonic neoplasms in the transgenic ApcMin mouse (bearing the murine homologue of the familial polyposis gene) was not inhibited by soy isoflavones given in a 'western-type' diet[75]. Treatment with other specific components of soy, for example the Bowman–Birk inhibitor[76] and soybean saponins[73], has resulted in diminished tumorigenesis.

Anticancer mechanisms

There are a number of mechanistic reasons why soy phyto-oestrogens may be cancer-protective, including a variety of antiproliferative effects, modulation of steroid hormone-metabolizing enzymes and binding

proteins, induction of apoptosis (programmed cell death) and possible antiangiogenic effects.

The most extensively studied isoflavone is genistein. Genistein binds with approximately 0.4% of the affinity of oestradiol to the oestrogen receptor[11]. Several investigators have shown that in the absence of oestrogen, isoflavones have weakly oestrogenic effects, whereas in the presence of oestrogen, they may exert an antagonistic effect[77,78]. This suggests a competitive, oestrogen receptor-mediated mechanism. However, the generality of this phenomenon has not been shown for all tissues, and particularly not for the mammary gland. It may be that the weak oestrogenicity of genistein has little to do with its breast cancer-protective effects[79]. At higher doses ($>10\,\mu mol\,l^{-1}$), genistein has antiproliferative effects in MCF-7 breast cancer cells without respect to their oestrogen receptor positivity[80]. This effect is, therefore, presumably not dependent on the receptor, and may be mediated by genistein's potent inhibition of tyrosine kinase activity (see Fig. 3)[81]. Genistein is also an inhibitor of DNA topoisomerase[82], which may be an integral part of its differentiation-inducing properties. It is also an antioxidant[83], and induces apoptosis in breast cancer cell lines *in vitro*[84]. Furthermore, genistein also has antiproliferative and antiangiogenic effects on vascular endothelial cells *in vitro*[85], leading to the hypothesis that its antiangiogenic properties may be responsible for the relatively indolent nature of breast carcinomas in Asian women. Genistein also induces expression of the inhibitory growth factor, transforming growth factor (TGF) β, which diminishes epithelial cell proliferation in the breast[86].

When considering the cancer-protective effects of soy, it is important not to have a preconceived notion of the compounds responsible for the benefits. Also present in soy products are a number of compounds other than genistein and other isoflavones that may exert anticancer effects, such as saponins[87], phytic acid[88] and protease inhibitors[89]. The degree to which they might contribute to cancer prevention, either alone or synergistically with phyto-oestrogens, should not be ignored.

Recent developments in oestrogen receptor biology

In addition to ERβ, splicing variants of the oestrogen receptor are variably expressed in both tumours and normal tissues[90]. These variants may be constitutively active even in the absence of oestrogen, or may have dominant negative activity (i.e. they may bind oestrogen but fail to activate the oestrogen response element). In the normal mammary gland, such variants may account for interindividual differences in breast cancer susceptibility, and may contribute to unexpected responses to chemopreventive agents and hormonal treatments. In tumours, such oestrogen receptor variants may account for the failure of some immunohistochemically oestrogen receptor-positive tumours to respond to hormonal therapy. For example, the presence of the exon-3 deleted variant is associated with tumours that are oestrogen-receptor positive but progesterone-receptor negative[91].

Potential cancer risk associated with phyto-oestrogens

Some recent *in vitro* and *in vivo* studies suggest that the growth of normal and neoplastic breast epithelium is promoted by phyto-oestrogens[70,92,93]. The widely used MCF-7 breast cancer cell line responds with proliferation to doses of genistein in the low micromolar range both *in vitro* and *in vivo*[70]. These data may indicate risk of tumour promotion in women with inapparent or recurrent breast cancer; however, it is worthwhile to note that MCF-7 cells are cancerous and transformed. Animal inoculation studies with these cells, therefore, address promotion but not the initiation step in carcinogenesis. Important differences exist between MCF-7 and normal breast epithelial cells. For example, MCF-7 cells excrete less of the inhibitory factor TGF-β than non-epithelial cells and respond with proliferation to genistein, while normal human mammary epithelial cells respond by growing less[86]. The basis for this difference between normal and neoplastic cells may lie in the paracrine regulation of the breast. For example, in normal breast epithelial cells, sex steroid receptor expression and proliferation hardly ever occur in the same cells, implying paracrine signalling or temporal separation; in contrast, in breast tumours, the receptors and proliferation occur together[94]. With respect to non-neoplastic breast tissue, some concern is raised by the work of McMichael-Phillips *et al.*[93], who found that short-term (2 weeks) treatment with dietary soy protein isolate ($60\,mg\,day^{-1}$) may increase proliferation of breast epithelial cells in women. This finding, too, could represent a cancer-promoting effect. Thus, while the weight of the data from epidemiological and anticarcinogenic studies suggests a cancer-protective effect, data also exist that support the opposite conclusion.

Summary

The rate differentials, migratory data and time trends of cancer risk in human populations indicate that an unknown epigenetic factor in the western lifestyle predisposes women to cancers of the breast, endometrium and colon. The contribution of vegetable intake to lower cancer risk is clear; however, the degree to which one can attribute the anticancer effect to soy phyto-oestrogens is still uncertain. Part of this problem is the result of recent developments in our knowledge of oestrogen receptor function; 'oestrogenicity' can no

Table 3 Summary of published trials on the effect of isoflavone supplementation on hot flushes

Study	Protein (g day^{-1})	Isoflavone (mg day^{-1})	Treatment duration (weeks)	Hot flush severity (% change)	Hot flush frequency (% change)
Murkies *et al.*[103]	45	74	12	NR	NE
Brzezinski *et al.*[104]	NR	NR	12	NR	NE
Albertazzi *et al.*[105]	40	76	12	NR	–15
Washburn *et al.*[106]	20 (10 BID)	34 (17 BID)	12	–22	NE

BID, twice daily; NE, no effect; NR, not reported.

longer be easily defined. Not all women are likely to be affected equally by consumption of soy phyto-oestrogens, because of interindividual differences in phyto-oestrogen metabolism[95] and potentially because of differences in oestrogen receptor types between women. The benefit of soy phyto-oestrogens is clearer for dietary exposures early in life and for premenopausal women. However, because the *in vitro* data suggest that normal breast cells and cancerous breast cells respond differently to phyto-oestrogens, the benefit for older women and breast cancer survivors remains to be determined. Understanding the effects of soy phyto-oestrogens will require (i) careful consideration of the hormonal status and phyto-oestrogen-metabolizing status of women participating in soy prevention trials; (ii) further exploration of the relevant paracrine mediators in the tissues of interest; and (iii) quantification of the effects of different oestrogen receptors, such as ERβ, for which soy phyto-oestrogens may have particular affinity.

Climacteric symptoms

Women in the Pacific Rim report fewer symptoms related to hypo-oestrogenism during the climacteric than western women[96,97]. Soy-containing foods are much more prevalent in East Asian diets than in western diets[98]. Soy is also a rich source of several oestrogenic isoflavanoid compounds (e.g. genistein and daidzein)[98]. These observations suggest that the isoflavones in soy may have oestrogen agonist properties. Genistein suppresses gonadotropin-releasing hormone (GnRH)-induced leutinizing hormone (LH) secretion in OVX rats, and also prevents progesterone-induced suppression of LH in OVX rats[99–101]. A diet enriched with 60 g of soy protein (45 mg isoflavones) per day prolonged the follicular phase of the menstrual cycle and suppressed the mid-cycle GnRH-induced LH and follicle-stimulating hormone surges in premenopausal women[102]. These experimental observations suggest that the isoflavones in soy may have oestrogen antagonist properties. Since more than 70% of western women experience hot flushes in the climacteric, and since soy may represent a dietary alternative to more traditional postmenopausal HRT, it is important to consider the impact of soy, as an isoflavone-rich food, on hot flushes as a measure of their effect on climacteric symptoms in general. Table 3 summarizes the published trials that have examined the effect of isoflavone supplementation on hot flushes.

The four studies, all randomized and blinded, offer interesting contrasts. The Murkies *et al.* study utilized a phyto-oestrogen-poor control treatment, wheat flour, and the subjects were instructed to finish the prescribed treatment over the course of the entire day[103]. The Brzezinski *et al.* trial utilized an unsupplemented, isoflavone-poor diet as its control, and instructed the subjects randomized to a 'phyto-oestrogen rich' diet to consume an appropriate amount of isoflavone-rich foods during the day[104]. The Albertazzi *et al.* trial utilized a casein (isoflavone-free) supplement as the control[105], and the Washburn *et al.* trial utilized a carbohydrate supplement (isoflavone free) as the control[106]. Except for the Washburn trial, the soy supplements were administered at least twice daily. Washburn *et al.*[106] found no effect on either hot flush frequency or severity in his once daily treatment regimen (34 mg isoflavones), but did find reduced hot flush severity when the dose was administered at two time points instead of one (17 mg BID isoflavones). The Murkies *et al.*[103] trial reported improvement in both hot flush and menopausal symptom scores in both treatment groups compared to baseline, but the improvements were not different between treatments. Brzezinski *et al.*[104] did not compare the effects of treatment between groups, although it appears that improvements in total menopausal symptoms score and hot flushes were similar in both the isoflavone-supplemented and control diet groups. However, the vaginal dryness score appeared to be more improved in the isoflavone-supplemented group compared with the control diet group[104]. The trial by Albertazzi *et al.*[105] found significantly greater reductions in hot flush frequency and severity among isoflavone users.

Several inferences may be taken from these trials. The 'bioavailability' of isoflavones in the isolated soy protein preparations may be better than that found in whole foods. Increasing the frequency of dosing may improve symptom relief. While a dose as low as 10 g of isolated soy protein twice daily decreased the frequency of hot flushes in a single study[106], it appears that a dose of at least 20 g twice daily is necessary to reduce hot flush severity. All treatments included were a combination of both the isoflavones and either the

whole foods they are found in or isolated soy protein. No published reports were found regarding the efficacy, if any, of preparations containing only the isoflavones themselves (exclusive of soy protein). Finally, while the effect of isoflavones on menopausal symptoms is both real and clinically relevant for both hot flush severity and frequency, the effect does not appear to be as great as that of traditional pharmacological oestrogen replacement therapy.

Public health implications

In terms of both health promotion and chronic disease prevention, the potential public health impact of soy consumption on a daily basis could be important. Research findings suggest that reductions in cardiovascular disease, osteoporosis and perhaps reproductive and other cancers may be possible with routine consumption of soy products containing isoflavones. The challenge will be getting consumers, especially those in the western nations of the world, to modify their eating habits by including generous amounts of soy and its derivative foods in their diets on a daily basis. Nutrition education will be important in developing behavioural changes in the selection and use of these soy-containing foods.

Growing evidence from several sources indicates that the potential exists for selective activity of dietary phytochemicals, depending on the oestrogen receptor subtype, which may vary between different individuals and in different tissues. Therefore, these molecules may be naturally occurring SERMs. Although evidence continues to emerge that phytochemicals decrease the incidence of breast cancer, many observations are only phenomenological. Much work needs to be done to explore basic mechanisms. Regardless of whether the aggregate effect of phyto-oestrogens diminishes, or even raises, breast cancer risk, widespread consumption of phytochemicals in the human diet (and via supplements) makes it imperative that we understand their effects, particularly as they relate to differing developmental and functional stages of breast epithelia.

To suggest that soy-derived isoflavones can replace oestrogen replacement therapy or HRT in the treatment of postmenopausal women seems premature, but for those women who refuse HRT therapy because of a family history of breast cancer or for other medical or personal reasons, soy with isoflavones may offer an alternative treatment with some benefits for chronic disease risk.

Conclusions

A few major conclusions can be drawn from the established effects of isoflavones from soy and soy products on tissue structures and functions.

1. The isoflavone effects appear to be tissue specific, i.e. in some tissues these soy-derived molecules act like oestrogens (agonists), whereas in others they may act as oestrogen antagonists. In their actions as agonists or antagonists in different tissues, isoflavones appear to behave as SERMs, a class of drugs with similar agonistic and antagonistic properties.
2. Dosages of isoflavones need to be quite high, in the 60–100 mg day^{-1} range, in order to exert their beneficial effects on cells of different types present in tissues throughout the body. A threshold for efficacy on cardiovascular tissue and bone seems to be greater than 60 mg day^{-1} while effects on climacteric symptoms may be seen at lower doses, i.e. approximately 20 mg day^{-1}.
3. Administration of isolated soy isoflavones, i.e. pure genistein, has not proved to be as effective in promoting beneficial effects on cardiovascular tissues as the same dose of isoflavones administered together with soy protein. This result suggests that a diet containing soy and its phyto-oestrogens may be more likely to promote these health benefits than isoflavone supplements alone. Bone studies in rodent models, however, yielded similar results whether proteins accompanied the isoflavone or not.
4. Isoflavone concentrations in blood appear to be optimized and stabilized by the continuous recycling of these molecules via the enterohepatic circulation. These mechanisms ensure that concentrations of phyto-oestrogens are maintained at sufficiently high levels throughout fairly long periods, much like the elevation of oestrogens during parts of the menstrual cycle, during pregnancy and with oestrogen replacement therapy.
5. Specific benefits of soy-containing isoflavones have been documented for reducing blood lipids and relaxing arterial walls, but beneficial effects have been more difficult to establish for bone health during the menopause despite some studies with favourable results. The effects of isoflavones on the prevention of specific cancers remains highly uncertain. The minimum dosages of isoflavones at which benefits are obtained may vary from tissue to tissue, and the role of isoflavone consumption as part of soy-derived foods or together with isolated soy protein remains to be determined.
6. Little concern exists about excessive doses of isoflavones from consumption of soy foods. However, the introduction of purified isoflavone supplements to consumers increases the possibility that toxic or adverse health effects could occur with high dosages.

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