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Risks and Benefits of Soy Phytoestrogens in Cardiovascular Diseases, Cancer, Climacteric Symptoms and Osteoporosis

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Contents

Abstract

Phytoestrogens, plant chemicals classified as isoflavones, coumestans and lignans, display estrogen-like activity because of their structural similarity to human estrogens and exhibit high affinity binding for the estrogen receptor β. They are common components of food items such as grains, beans, fruits and nuts. Isoflavones are primarily found in soybeans and foods made from soy. In particular, significant therapeutic properties have been generally attributed to soy isoflavones, but most of the claims have been poorly, or not at all, confirmed by well designed clinical trials. Such is the case of the purported role of soy isoflavones in reducing plasma cholesterol levels. This link is now not supported by many authors or by appropriately designed clinical studies. The role of isoflavones in cancer prevention, particularly of tumours under endocrine control (breast, prostate and others) is again only supported by weak to nonexisting clinical evidence. A similar case is that of the prevention/treatment of postmenopausal symptoms and osteoporosis. Disturbing data have been reported on potential negative effects of soy isoflavones on cognitive function in the aged, particularly

relating to tofu intake. Recent studies have finally indicated a potential role for soy isoflavones in inducing chromosomal changes in cells exposed *in vitro* and potentiating chemical carcinogens. These findings may not, however, be extrapolated to clinical conditions. Available data do not appear to unequivocally support beneficial effects of soy isoflavones, and warn against their wide use, in the absence of satisfactory clinical findings.

The presence of relatively high concentrations of isoflavones in soybeans has been recognised since 1931;[1] genistein glycoside was first isolated from soybeans 10 years later.[2] The hormonal properties of isoflavones were first suspected after the observation in the mid-1940s of an infertility syndrome in sheep, ingesting clover containing high levels of the isoflavones formononetin and biochanin A.[3] These are metabolised by intestinal bacteria to equol, a unique isoflavone with a high affinity for the estrogen receptors. Equol was later found in human urine following soy consumption and levels of isoflavones in the blood and urine far exceeded those of endogenous estrogens,[4,5] leading to the hypothesis that isoflavones could be biologically active.

The abundance of certain isoflavone phytoestrogens (from now on only referred to as 'isoflavones') in Asian diets and the lower rates of 'Western' diseases such as coronary heart disease, as well as breast, prostatic, and colon cancers in such populations have suggested a protective role for these mostly soy-derived substances.[6,7] The possibility that isoflavone intake may have antiatherogenic and anticancer effects has received support from plausible underlying mechanisms of protection, including plasma lipid modifications, antioxidant effects, vascular reactivity changes as well as hormonal actions, leading to reduced cancer risk and incidence.[8,9] Based on these postulated mechanisms, the use of soy isoflavones has been encouraged in the management of hormone-related symptoms, such as menopausal flushes,[10] and diseases such as osteoporosis.[11]

However, most, if not all, of these therapeutic claims are only supported by weak or nonexisting clinical evidence. In addition, serious concerns have been raised about the potential toxicities of isoflavones from soy.[12] It is, therefore, mandatory to carefully evaluate the pros and cons of soy isoflavone treatment in a variety of clinical conditions.

1. Chemical Characteristics and Food Sources

The plant chemicals displaying estrogen-like activities and high affinity binding to the estrogen receptor (ER)[13] may be classified into isoflavones, coumestans and lignans. The major isoflavones, genistein and daidzein, commonly exist as glycosides, and they may also be derived from their precursors, biochanin A and formononetin. Coumestrol and 4′-methoxycoumestrol are the most important food derived coumestans with estrogenic activity. The estrogenic lignans enterodiol and enterolactone are derived from the compounds secoisolariciresinol and matairesinol.[7]

After consumption of isoflavones or lignans by humans, heterocyclic phenols with a structure similar to estrogens are formed by a complex enzymatic metabolic conversion in the gastrointestinal tract.[5,14] The chemical structures of the 2 major phytoestrogens, genistein and daidzein, and of the major lignan enterolactone, compared with the natural estrogen estradiol and the antiestrogen tamoxifen are shown in figure 1. Daidzein is eventually metabolised to both equol and *O*-desmethylangolensin (*O*-DMA). Genistein is metabolised to 6′-hydroxy-*O*-DMA.[14] In this review only the biological properties of soy isoflavones will be discussed.

Isoflavone absorption and utilisation require a series of de-conjugation and conjugation steps. Conjugated isoflavones are excreted into the urine as well as into bile. After excretion into bile, deconjugation by gut bacteria and re-absorption may

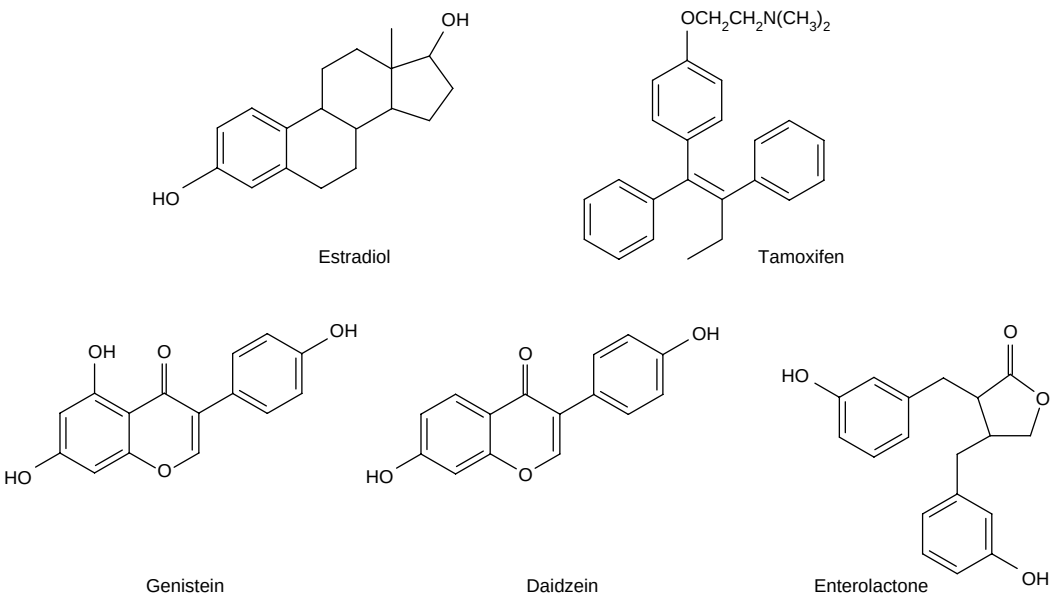

Fig. 1. Chemical structures of the natural estrogen estradiol, the estrogen antagonist tamoxifen, the 2 soy isoflavones genistein and daidzein and the lignan enterolactone.

take place, resulting in further metabolism and degradation in the intestine.[15] Levels of isoflavone metabolites vary widely between individuals, though their excretion is generally highly correlated with dietary intake[16].

Isoflavones are primarily found in soybeans and food derived from soybeans. Soy bean contains approximately 0.2 to 1.6mg of isoflavones/g dry weight.[17] Chick peas and other legumes, such as mung beans, and mushrooms, as well as clover, are other isoflavone sources. Urinary phytoestrogen excretion studies clearly indicate that soybean consumption is only significant in populations in the Far East, the mean daily isoflavone intake in Asian populations having been estimated as approximately 30 mg/day.[18] In Western populations, beans and peas (45%), tea and coffee (25%), nuts (10%), and grains, rice and cereals (5%) are main dietary sources of isoflavones.[6,7]

Large scale epidemiological studies as well as observational studies have been hampered by the lack of easily applicable measurements of isoflavone exposure. Excretion in overnight or 24-hour urine samples has been reported to vary with diet type.[18-20] Furthermore, individuals show high variability in the metabolic response to, in particular, the soy isoflavones daidzein and genistein, suggesting a highly variable metabolic capacity or differentially active metabolic pathways. White North American women excrete relatively high levels of coumestrol and lignans, whereas Latino women excrete more genistein.[20] In Asian populations, isoflavone excretion is particularly high because of a high consumption of soy-based foods.

2. Isoflavone Intake and Cardiovascular Protection

There is a general consensus that populations consuming predominantly vegetable-derived products, have a lower incidence of cardiovascular diseases. A variety of mechanisms have been postulated in support for this observation. Numerous comparative studies dating way back from Ignatowski[21] have suggested that soy protein, versus

animal protein, may exert a cardiovascular protective activity.

2.1 Effects on Plasma Lipid Levels

A large number of more and less recent clinical studies, predominantly from our group, have clearly pointed out a cholesterol lowering effect of soy proteins in patients with hypercholesterolaemia including patients with concomitant diabetes mellitus.[22-25] In 1995 Anderson et al.,[26] in a widely quoted meta-analysis, convincingly concluded that soy-protein rich diets are responsible for a significant reduction in cholesterol levels, particularly in patients with elevated baseline cholesterol levels. Reduction in low density lipoprotein-cholesterol (LDL-C) levels ranged from 7.1 mg/dl (3.3%) for patients with baseline total cholesterol levels of <200 mg/dl to 68.1 mg/dl (24%) for patients with baseline total cholesterol levels of >335 mg/dl (fig. 2). The meta-analysis suggested that soy isoflavones may account for up to 60 to 70% of the hypocholesterolaemic effect.[26] Contrary to this suggestion there was, instead, clear evidence that essentially all studies in patients with severe hypercholesterolaemia had been conducted using isoflavone free products.[27] Earlier[28] and more recent[29-32] *in vivo* and *in vitro* studies with soy protein or well defined fractions thereof have furthermore convincingly confirmed that the cholesterol lowering mechanism is most likely ascribable to LDL receptor (LDL-R) activation, induced by these protein components. Such an effect is very evident, following soy protein intake, in mononuclear cells isolated both from patients with familial hypercholesterolaemia[33] and also from postmenopausal women with moderate cholesterol elevations.[34]

The issue of the isoflavone composition of soy products has gained interest in the last 2 years, particularly after the US Food and Drug Administration permitted promotion of soy protein as a dietary tool to reduce cardiovascular risk.[35] The issue as to whether soy phytoestrogens are really responsible for the cardiovascular benefits, and particularly for the cholesterol lowering activity, was essentially based on the results of animal studies that indicated that ethanol extraction of intact soy protein resulted in a product unable to reduce plasma cholesterol levels in primates.[36,37] Ethanol extraction removes, among other compounds, soy isoflavones.

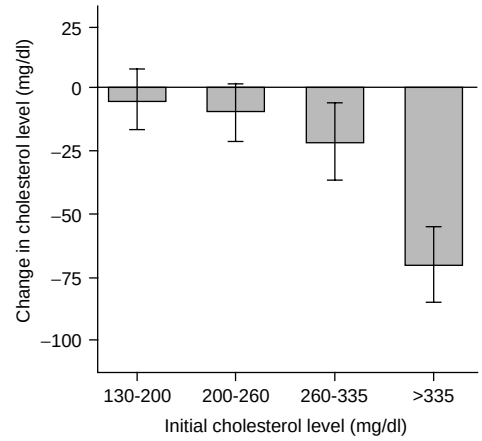

Fig. 2. Reductions in plasma cholesterol level observed in clinical trials with soy protein as they relate to the baseline cholesterol level.[26] It is apparent that only patients with significant baseline cholesterol level elevations benefit from the intake of soy protein.

Clinical studies have led, however, to divergent and mostly negative findings. Two clinical studies, one in men with moderate hypercholesterolaemia[38] and the other in premenopausal women with normal cholesterol levels,[39] comparing whole soy flour derived products and similar ethanol washed products both showed a better hypocholesterolaemic activity with the former. However, such studies should be viewed with caution, after carefully conducted animal studies have given a clear indication that the 'ethanol wash' has no hypocholesterolaemic activity *per se*.[40,41]

In fact, data from 3 clinical studies where purified soy isoflavone supplementation were given to menopausal and perimenopausal women[42,43] and individuals of both genders,[44] as well as data from studies on pre- and postmenopausal women receiving isoflavones from red clover,[45,46] all showed no effect on plasma lipid levels, in spite of inconsis-

tent positive findings on other end-points, such as arterial vasomotility. Daily doses of isoflavones (mainly genistein/daidzein) ranged between 55 and 90mg. All of these studies involved individuals with normal lipid levels. It is therefore of particular interest that a very recent study, in postmenopausal women and including patients with severe hypercholesterolaemia also produced negative findings.[47]

A recent consensus paper,[48] indicates, however, that both soy protein and isoflavones may be needed for maximal cholesterol-lowering effect of soy, also recommending soy protein foods in a diet low in saturated fat and cholesterol, to promote heart health. It is of course, difficult at present to single out possible mechanisms whereby isoflavones may exert any additional effects to those exerted by vegetable protein rich foods. In a recent study evaluating a possible cooperative interaction, obese and lean Zucker rats received either a casein or a soy protein enriched diet, the latter either high or low in isoflavones.[49] While the soy protein diets were markedly hypocholesterolaemic versus the casein one, the soy protein diet with the high isoflavone content proved slightly more hypocholesterolaemic and with a significantly higher effect on liver cholesterol and triglyceride levels, thus potentially suggesting a lipolytic and cholesteryl ester hydrolytic effect of isoflavones.[49] Cooperative interaction may result from an accumulation of isoflavones in the enterohepatic circulation, thus exerting liver specific effects.[50] In a study on LDL-R knock-out mice it appeared that LDL-R deficient animals do not respond with a plasma cholesterol reduction to isoflavones, thus again suggesting a potential effect on LDL-R.[51] A choleretic effects in rats is supported by a number of studies,[52,53] but this effect may not be clinically significant in the humans, where bile flow regulation is considerably different from rats.

At this point, it is clear that proteins are responsible for the major beneficial effects on cholesterol regulation. The effect of isoflavones may be, at best, cooperative, also potentially inducing additional stimulatory effects (e.g. on apolipoprotein AI production).[54]

2.2 Antioxidant Activity

Soy protein, compared with casein consumption, may lead to a significant decrease in arterial lipid peroxidation. The antioxidant effects of isoflavones *in vivo* and *in vitro*, studied on a variety of cell systems, have been reviewed by Kurzer and Xu[55] who concluded that isoflavones act as antioxidants directly or indirectly by enhancement of the antioxidant activities of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase.

In a study by Tikkanen et al.,[56] 6 healthy volunteers consumed soy protein containing 60mg of isoflavones per day and the effects on LDL oxidation were examined by measuring copper-induced oxidation. Two weeks of soy consumption significantly prolonged LDL oxidation lag time by approximately 20 minutes. In a study on 46 surgically postmenopausal nonhuman primates, arterial lipid peroxidation levels were about 17% lower in the group fed soy protein isolate with the isoflavones, compared with the group fed casein and lactalbumin as the protein source.[57]

These findings confirm *in vitro* data indicative of reduced LDL oxidation in the presence, particularly, of genistein.[58,59]

Very recently Wiseman et al.[60] evaluated the resistance of isoprostane F_2 and of LDL to the *in vitro* formation of diene conjugates induced by copper in 24 individuals (19 postmenopausal women and 5 men) who consumed a texturised soy containing either high (556 mg/day) or low (1.9 mg/day) levels of isoflavones for 17 days. The isoflavone rich diet induced significantly lower 8-epi-prostaglandin F_2 levels (–19.5%; $p < 0.03$) versus the comparison product, suggesting, therefore, a reduced LDL oxidability. A similar reduction of LDL conjugated dienes was reported in patients with hyperlipidaemia receiving a soy diet providing 86mg isoflavone/2000 Kcal/day,[61] but in the only study using isoflavone pills (86 mg/day), already reported,[45] there was no evidence of a re-

duced LDL oxidability. A similar finding was reported in patients with hypertension given 55mg daily of an isoflavone supplement previously shown to reduce LDL oxidability *in vitro*.[62]

2.3 Vascular Reactivity and Cellular Proliferation

Data of potential therapeutic interest have been provided on the activity of isoflavones on vessel wall motility. Soy protein isolate with isoflavones has also been shown to improve vascular function in both nonhuman and human primates. Studies in monkeys fed for 6 months with soy-based diets indicate that ethanol extraction (low-isoflavone diet) leads to arterial constriction (–6.2%) in response to acetylcholine, whereas the unextracted isoflavone rich diet dilates monkey arteries (+6.4%).[63] While there are reservations on this conclusion, based on the previously presented data, the intravenous administration of genistein (30 minutes before testing) caused dilatation (+3.3%) in the previously constricting arteries of female macaques, fed a low isoflavone diet.[63]

Thus, like mammalian estrogens, dietary soy isoflavones may enhance the dilator response to acetylcholine of atherosclerotic arteries in female monkeys. Reportedly, in 18 postmenopausal women,[64] a previously abnormal endothelium-dependent, flow-mediated dilatation in the brachial artery, improved by +5.3% after the women took a daily beverage of 40g soy protein containing 80mg isoflavones for 1 month. In a study,[42] mentioned previously in section 2.1, involving 21 peri- and postmenopausal women, treatment for 5 weeks with 80 mg/day of purified soy isoflavones, improved systemic arterial compliance by 26%, with no effects on LDL and high density lipoprotein-cholesterol levels. However, a more recent study in a similar series of women, given purified isoflavones, failed to confirm these findings and again did not report any plasma lipid changes.[43]

Very recently the effects of an acute intravenous administration of genistein or daidzein was evaluated in healthy humans of both genders.[65] Genistein was infused at concentrations of 10 to 300 nmol/min, with each dose administered for 6 minutes. At the 2 highest doses significant increases in forearm arterial flow in both men and women were recorded. Similar effects were exerted by equimolar concentrations of 17β-estradiol, infused in the same way. Both genistein and 17β-estradiol effects were antagonised by the nitric oxide (NO) synthase antagonist *N*-monomethyl-l-arginine. Genistein also potentiated the forearm vasodilatation induced by acetylcholine, but no had effects on the NO independent vasodilator nitroprusside. These findings are certainly of interest and indicate that genistein may be the only active isoflavone in vasomotolity. However, caution should be exercised, since genistein may be erratically absorbed after oral administration [15] and achieved venous plasma concentrations were about 8- to 10-fold higher than those observed after oral intake of high isoflavone soy proteins.[66]

Effects of phytoestrogens on haemostasis and fibrinolysis have not been studied so far, with the exception of a small study in nonhuman primates fed soybean protein with and without isoflavones (ethanol washing) reporting a small protection against flow reduction after the activation of platelets by collagen infusion.[67] A possible relation with smaller platelet volumes in whole soy protein fed animals was suggested.[68]

Studies *in vitro* have suggested that the isoflavones also affect smooth muscle cells that are involved in atherosclerosis progression. Genistein is known to bind rather weakly to the classical ER but with much higher affinity to ERβ (see section 3)[69] and may, therefore, display more potent effects in tissues expressing ERβ. Mäkelä et al.[70] recently measured the expression of ER and ERβ in the arteries of rats after endothelial denudation, a technique that promotes atherogenesis. Seven days after injury, expression of both the ER and ERβ was increased, but ERβ was overexpressed to a much greater extent (approximately 30 times more). Treatment with various subcutaneous doses (0 to 2.5 mg/kg) of either 17β-estradiol or genistein in rats resulted in protection against neointima formation. Equivalent doses of 17β-estradiol and

genistein were equally effective. Furthermore, a number of authors have reported that genistein inhibits the migration and proliferation of smooth muscle cells.[71,72]

No hard data are available on the effects of isoflavones on clinical atherosclerosis, as assessed by carotid intima-media thickness, or coronary angiography. A very recent report in monkeys with and without diabetes mellitus suggest markedly reduced delivery of lipoproteins into monkey arteries after isoflavone rich soy diets.[73] In our laboratory, we have recently shown reduction in volume, after intravascular ultrasound monitoring of focal arterial lesions, in the rabbit carotid after isoflavone free soy protein intake (Sirtori et al., unpublished data).

3. Hormone-Dependent Tumours

Rates of hormone-related cancers (breast, ovary, endometrium and prostate) are highest in populations with Western diets, which are relatively rich in fat and meat and poor in fibre, and lowest in Asian populations who eat plant-based diets.[74,75] Isoflavones have been hypothesised as a key factor in these differences. The ground for this hypothesis has been based on a number of studies suggestive of specific hormonal effects of isoflavones.

In the standard model of the ovariectomised rat, isoflavones have antiosteoporotic[11,76] but only weakly uterotrophic effects.[77] The discovery of a second ER, ERβ,[78] for which genistein and the natural estrogen 17β-estradiol display close affinities, has provided a new perspective on the pleiotropic nature of these effects. ERs are intranuclear binding proteins, of which the ERα is linked to the classical hormonal effects, including endometrial proliferation and mammary enlargement, whereas ERβ seems instead to be responsible for the growth promoting effects of estrogens on nongonadal tissues and also for the vascular effects of the hormones. ERβ is expressed in differing amounts and by different cells[79] versus the classical ERα. Soy isoflavones are approximately one-third as potent as estradiol on ERβ and only 1/1000 as potent on ERα.[69] Thus, soy isoflavones can be viewed as a type of selective estrogen receptor modulator (SERM). Whether they may be of clinical help because of this selectivity is, however, difficult to conclude.

Several other biological aspects of the interaction of isoflavones with target endocrine tissues underline the complexity of their mechanism. After early reports that genistein had inhibitory effects on the epidermal growth factor receptor tyrosine kinase activity,[80] many investigators attributed most of the effects of the isoflavones to the inhibition of other kinases, although there were no consistent demonstrations of such an inhibition. Genistein may exert its antiproliferative effects through transcriptional processes rather than directly on tyrosine kinase. If so, then the variable effects of genistein and other isoflavones in estrogen-sensitive tissues may depend on the production of paracrine and autocrine growth factors that cause proliferation also of cells that do not express ERα or ERβ. Not all such factors may stimulate cell division, however. Genistein dose-dependently increases both expression[81] and production[82] of transforming growth factor β , an inhibitor of epithelial cell growth in both normal and transformed breast epithelial cells. Again acting by ERβ-dependent mechanisms, genistein inhibits both metastases[83-85] and angiogenesis,[86,87] 2 important processes that may lead to death from cancer.

The final effects of isoflavones may also be mediated by different metabolic handling. Human breast cancer cells convert isoflavones to phase I and phase II metabolites;[88,89] under cell culture conditions simulating oxidative bursts, isoflavones are converted to halogenated and nitrated derivatives.[90] Modifications of the isoflavone molecules may thus influence their binding to ERα or ERβ or to other protein targets.

SERMs are currently considered to be able to stimulate only the AF-1 constitutive activation domain of the ER, and not the ligand-dependent activation domain AF-2, thereby acting as a partial ER agonists and partial antagonists.[91,92] Soy

isoflavones may possibly act in a similar way as SERMs.

3.1 Breast Cancer

In humans, limited evidence points in the direction of a protective effect of isoflavone intake on breast cancer,[93-95] although some reports paradoxically suggest that there may be higher breast cell proliferation after soybean supplementation.[96,97] Some positive studies based on urine measurement of isoflavones are hampered by the fact that urine samples were taken after a diagnosis of breast cancer[98] and women may thus have changed their usual food intake. Epidemiologists suggest that, possibly, the lower breast cancer incidence in Asian women may be more likely because of increased parity than to soy isoflavones intake.[99] A possible reduction of endometrial cancer[100] has also been suggested in isoflavone consumers.

Animal studies are inconclusive about a potentially increased cancer risk attributable to isoflavones,[36,101-103] as might be expected analogous to the effects of estradiol. Soybean estrogens given to nonhuman primates in the diet at doses scaled from those given to women, did not modify vaginal cytologic maturation index[100] or organ weights.[36] The same observation was made in surgically postmenopausal rats.[102] Furthermore, no proliferative effects were observed in breast and endometrial tissue of nonhuman primates.[101] Very recently, no effects of intact or isoflavone-depleted soy protein on *N*-nitroso-*N*-methylurea-induced rat mammary tumourigenesis were reported.[104]

Soy consumption has been observed to both increase[105] and decrease[106,107] plasma estrogen levels; 1 study reported increased nipple aspirate volume,[105] suggesting estrogenic effects on the breast. Other observations include no effects on endometrial biopsies[108] or plasma sex hormone–binding globulin levels.[8,105,107-109] An increased menstrual cycle length has been reported by some authors.[106,109]

Available data for soy isoflavones on breast cancer risk thus suggest either a neutral or a mildly beneficial effect.[110] Mechanistic grounds for suggesting an increased isoflavone intake in order to reduce breast cancer risk appear rather tenuous. In a recent editorial it was questioned whether genistein should be rated as anti- or pro-breast cancer.[111]

3.2 Prostate Cancer

Prostate cancer incidence seems to decrease with increasing isoflavone intake.[66,112] In epidemiological studies fat and meat show a positive and cereal a negative association with prostate cancer mortality.[75] A decreased prostate cancer risk has been found in men who were Seventh-Day Adventists,[113] having a high consumption of beans, lentils or fruits (rich sources of flavonoids) and in men of Japanese ancestry living in Hawaii,[114] consumers of rice and tofu, both rich in isoflavonoids.

Japanese men consume approximately 6mg of daidzein and 10mg of genistein per day, leading to concentrations possibly sufficient to inhibit cell proliferation,[115] to interfere with growth factor tyrosine kinases[82] or topoisomerase II[115,116] and to inhibit angiogenesis.[86] Reduced luteinising hormone secretion following isoflavones, as also seen in women[8] could lead to decreased testosterone synthesis and lower prostate cancer risk.

Despite the high fat intake, the incidence of prostate cancer in Finland is, however, lower than that in the US, but much higher than in Japan. Assays of lignans and isoflavonones in urine by gas chromatography-mass spectrometry have revealed very high total excretion in the Japanese, intermediate levels in Finland, and low levels in the US.[117] Japanese individuals have 5 to 100 times higher levels of isoflavones (genistein, daidzein, and their metabolites) than individuals living in most other countries. In Finland, the urinary excretion of lignans dominates, being higher than in both Japanese and American individuals. These findings thus suggest a contribution to the lower prostate cancer incidence by both isoflavones and lignans.

Very recently Urban et al.[118] evaluated the effects of 40 g/day of soy protein with and without isoflavones (hot ethanol extracts as previously discussed) in elderly men with elevated prostate specific antigen (PSA). The isoflavone rich product

significantly increased plasma levels of genistein, daidzein and metabolites, and moderately reduced cholesterol levels. It failed, however, to affect either plasma levels of PSA or of the soluble p105 component of the p185erbB-2 proto-oncogene. In a previous study, 4 × 40mg daily doses of isolated clover isoflavones, for 1 week, led to extensive apoptosis in prostate biopsies at prostatectomy.[119] This was, however, not the result of a controlled investigation and, at present, evidence in favour of a positive effect of isoflavones on prostate cancer appears weakly supported.

In human prostate cancer cell studies, genistein and its precursor biochanin A inhibit cell growth at relatively high concentrations.[120] Studies on human prostate cancer cells, extended to different lines (LNCaP, DU-145 and PC-3) and to a number of other isoflavones, lignans and flavones also measuring PSA both intra- and extracellularly, have indicated that some metabolites may be more active than the original isoflavones.[121]

While the association of androgens with prostate cancer has long been known, the role of the estrogens remains controversial. Neonatal estrogenisation of male mice with diethylstilbestrol results in the development of dysplastic changes in the prostate, including lesions resembling human intraperitoneal neoplasia. At the age of 9 months, 8 of 10 mice fed a soy-free diet showed severe dysplastic changes, but in a group receiving a soy-containing diet, only 3 of 10 animals had severe dysplasia, possibly consequent to an antiestrogenic effect.[122] Treatment of prostate cancer with estrogens results in inhibition of growth, but estrogens have also been shown to be associated with both benign prostatic hyperplasia and prostate cancer.[121] On the other hand, Japanese men have lower prostate weights than do Western men at similar ages.[6]

Theoretically, dietary estrogenic compounds could therefore be both beneficial and deleterious with regard to prostate disease. The beneficial effects may also be mediated via mechanisms not involving the estrogen receptor, as above indicated for breast cancer. These may involve inhibition of tyrosine and other protein kinases, 3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase, 5α-reductase and aromatase, all mechanisms displayed by phytoestrogens.[112,115,123]

As for the case of breast cancer, demonstrations of a clear cut activity of soy isoflavones on prostate cancer risk are not convincing. Although it appears unlikely that isoflavones may lead to an enhanced risk of prostate disease, current evidence[118] does not suggest that isoflavone intake, as currently available, may affect major biological markers of prostate cancer.

4. Other Hormone-Dependent Effects of Isoflavones

4.1 Osteoporosis

Evidence for an estrogenic bone-preserving effect of isoflavones has been provided in a number of studies. Recently, Potter et al.[124] investigated whether supplementation with 2 different doses of soy isoflavones for 6 months had effects on bone mineral density and content. Significant increases ($p < 0.05$) occurred in both the bone mineral content and density in the lumbar spine but not elsewhere in the soy isolate group (90 mg/day of phytoestrogens), versus the control group. However, in a more recent controlled study, an evaluation of the effects of soy protein isolates, containing different amount of isoflavones (from 8 to 130 mg/day), on pre- and postmenopausal women, failed to detect significant changes in the markers of bone turnover, with a possible reduction of osteocalcin, insulin-like growth factor (IGF)-1 and IGFB-3 following high dose isoflavone in the postmenopausal group, thus not supporting any useful effect on bone turnover.[125] Generally larger bone sparing effects are noted in rat models of ovarian deficiency,[126] but such findings have not been reproduced in a similar primate model.[127]

In a very recent Japanese study, evaluating daily intakes of isoflavones in 478 postmenopausal women, there was evidence of significantly different bone mineral densities, adjusted to years since menopause, in the highest intake compared with

the lowest intake category ($p < 0.01$), within the early and late postmenopausal groups.[128] However, data not supportive of beneficial effects on bone in humans have come from a 10-year follow-up study of postmenopausal women in The Netherlands. In this study, no association was found between bone changes and the excretion of genistein/daidzein, whereas higher urinary equol and enterolactone excretion was associated with increased rates of bone loss, although the study was not powerful enough to detect statistically significant associations.[129] In women with osteoporosis and osteoporotic fractures, in view of the current evidence indicating clear beneficial effects of hormone replacement therapy (HRT) and mild effects of soy, they should not be encouraged to consider soy isoflavones as a useful alternative for treatment.[130]

4.2 Menopausal Complaints

Observational studies and clinical trials of HRT in postmenopausal women have generally shown a reduction in the number and severity of vasomotor symptoms (e.g. hot flushes or night sweats) and a potentially beneficial effect on cognitive decline and dementia in older adults (see section 4.3). Population-based studies, indicate, however, that only 12 to 21% of US postmenopausal women currently use HRT, suggesting that there is a large group of women who either will not or cannot use HRT.[131,132] Efforts are therefore underway to identify agents that may have a better risk/benefit profile.

It is generally stated that Japanese women have delayed menopause and reduced postmenopausal symptoms. Observational studies in Japanese women indicate that vasomotor symptoms may be nearly 10-fold lower than in the US or other Western women.[133] Japanese women show approximately a 100-fold higher excretion of isoflavonoids in the urine compared with American or Finnish women. Postmenopausal women consuming 34 to 165 mg/day of isoflavones in isolated soy proteins, soymilk or texturised vegetable protein products experience increased levels of sex-hormone binding globulin,[134] decreased levels of urinary estrogens and no effect on endometrial biopsy results.[135,136]

Clinical studies on the effects of soy isoflavones on the vasomotor symptoms of menopause have, however, provided mixed results. A modest decrease in the frequency and severity of hot flushes was reported in a number of studies. In an open study with soy versus wheat flour, both reduced hot flushes (by 40 and 25%, respectively) and menopausal symptom score versus previous scores on an unrestricted diet.[137] A few studies using higher doses of isoflavones (50 to 80 mg/day), enrolling women with more vasomotor symptoms at baseline (4 to 7 symptoms/day) and with larger sample sizes, have shown mildly beneficial effects on self-reported frequency and severity of vasomotor symptoms.[10,134,138] In the largest of these, a double-blind, parallel, multicentre, randomised placebo-controlled trial, 51 postmenopausal women (age range 48 to 61 years) took 60g of isolated soy protein and 53 (age range 45 to 62 years) took 60g of placebo (casein) daily for 12 weeks. By the end of the twelfth week, patients taking soy had a 45% reduction in their daily hot flushes versus a 30% reduction obtained with placebo ($p < 0.01$).[138] Very recently in a study involving 177 pre- and postmenopausal women with hot flushes and a history of mammary carcinoma, a crossover double blind trial of soy capsules rich in isoflavones (600mg 3 times a day) for 4 weeks did not result in any significant subjective improvement versus a placebo.[139] There appeared to be a slight preference for the placebo in treated women.

At the doses tested for all the soy phytoestrogen preparations, the effects on vasomotor symptoms were much smaller than those observed with traditional HRT. Soy isoflavones generally increase endometrial proliferation modestly[140] or not at all.[141] No evidence of bleeding, breast tenderness or gastrointestinal symptoms was otherwise reported in postmenopausal women.

As a general note, it should be underlined that the placebo effect in essentially all studies was around 30%[99] and the differential effect exerted by soy products was generally modest. In a recent

review, it was suggested that current data are insufficient to draw definitive conclusions about the use of isoflavones as an alternative to HRT.[133] Furthermore, a consensus opinion of the Northern American Menopause Society only recommended that menopausal women consume whole foods that contain isoflavones, especially for the cardiovascular benefits of these. It also suggested a level of caution to be observed in making these recommendations.[142]

4.3 Cognitive Function

Observational studies and clinical studies of HRT have reported, among other things, potential beneficial effects on cognitive decline and dementia in older women.[143,144] Theoretically, phytoestrogens could be thus expected to improve cognitive function, particularly verbal functioning, as suggested for conventional HRT.[145]

For cognitive decline and soy isoflavone intake, scientific data are mixed. Uncontrolled observational studies suggested that increased tofu in the diet was associated with declines in cognition in a study of Japanese American men in Hawaii.[146] On the other hand, animal data indicate potentially beneficial effects of soy isoflavones on neuroanatomy and simple memory tasks.[147] This issue remains unclear, because no human trial data are available documenting the short or long term effects.

Recently, the hypothesis that estrogen intake may reduce cognitive decline in older age has lost credibility, after a controlled study involving patients with Alzheimer's disease treated with conjugated estrogens showed, if anything, a slight worsening on the Dementia Rating Scale,[148] Furthermore, a longitudinal study established in 1965 in Hawaii recently reported that increased tofu intake in midlife may be associated with increased cognitive dysfunction in later life.[149] These data need more adequate support from prospective studies, but certainly advise caution in promoting isoflavone intake as a potential preventive therapy for age-related symptoms.

5. Potential Genotoxicity and Selective Endocrine System Toxicity of Isoflavones

Possible direct adverse cellular effects of isoflavones were relatively underrated until Kulling and Metzler[150] reported the induction of micronuclei in cultured Chinese hamster V70 cells exposed to genistein and coumestrol (found in alfalfa and clover). Induction of micronuclei, i.e. acentric chromosomal fragments, is indicative of clastogenic activity. Daidzein did not appear to induce such an effect. In a follow up study, human cultured lymphocytes were exposed to 50 to 75 mol/L coumestrol or 25 mol/L genistein for 6 hours *in vitro*. A clear induction of structural chromosomal aberrations was observed by cytogenetic analysis[151] (fig. 3). Again, daidzein did not induce chromosomal aberrations even at 100 mol/L.

It appears therefore that some, but not all isoflavones, carry a risk of genetic toxicity. Genistein is a potent inhibitor of tyrosine kinase[80] and this is generally rated as an anticarcinogenic effect, because several growth factor receptors and oncogenes are regulated by tyrosine phosphorylation.[81] Tyrosine phosphorylation, on the other hand, also plays an important role in the response of cell cycle check points to DNA damage. Progression from G2 into the M-phase is controlled by cyclin-dependent kinases regulated by tyrosine phosphorylation and inhibition of kinases may inactivate the checkpoint and hence damaged cells may enter mitosis without delay.[152] If such damaged cells survive, permanent genetic alterations may result. A similar perturbation on a cyclical process by phytoestrogens has been recently described in the oviduct cells.[153] In these, genistein, daidzein and other phytoestrogens, at concentrations similar to those of estradiol, induce the synthesis of leukaemia inhibitory factor (LIF), a glycoprotein essential for embryo implantation; influencing LIF synthesis in a noncyclic fashion may lead to tubal infertility.

Concentrations of 25 mol/L of the isoflavone genistein, reported as potentially genotoxic, are somewhat higher than those found in plasma after

Fig. 3. Metaphase spreads of human peripheral blood lymphocytes stained with Giemsa exposed to 25 mol/L genistein for 6 hours. Arrows indicate chromosomal damage (courtesy of Professor Sabine Kulling).

the intake of soy based formulas, which contain isoflavone levels ranging from 17 to 30 mg/L, equivalent to 63 to 110 mol/L.[12] Concentrations of isoflavones in the plasma of 4-month-old infants, fed exclusively soy-based infant formulas, is approximately 4 mol/L, of which 60% is genistein; lower levels (0.2 to 1 mol/L) are found in adults consuming soy-based diets.[12] Thus, the active concentration of genistein, found to induce structural alterations in human chromosomes is only about 10-fold higher than that observed in humans on soy diets. Based on these observations recently it has been hypothesised that genistein may play a role in the development of childhood leukaemia.[154]

The apparent paradoxical genotoxic activity of, particularly, genistein, in the face of the rather large consensus that this phytoestrogen may be an anticarcinogen, is supported by recent data on Chinese hamster lung cells, ovary cells and human lymphocytes exposed to ethanol extracts of a commercial soybean processing by-product.[155] Whereas in fact fractions containing daidzein and genistein de-

press 2-acetoxyacetylaminofluorene (2AAAF) induced DNA damage, the genotoxic impact of 2AAAF is enhanced by purified genistein at 100 mg/L (369 mol/L).

At present, all data indicating a possibly enhanced genotoxic risk after isoflavone intake should be viewed with caution, considering that, up to now, no clinical data indicative of an enhanced cancer risk, have been provided. However, it also should not be underrated that similar *in vitro* findings might be sufficient for halting the clinical development of synthetic drugs.

Among other cellular effects of genistein, a direct apoptotic activity should be mentioned. This has been repeatedly linked to its potential antiproliferative and antidifferentiating effects in many neoplastic cells. Apoptosis can be induced in cells such as thymocytes,[156] leukaemia cells[157] and testis cells.[158] However, recent worry has been raised, by the observation that genistein, in a similar manner to sodium azide and dexamethasone, can induce of cell death in cultures of the testicular cell lines TM3, TM4 and GC-1.[159] The mechanism, for both genistein and dexamethasone is mainly apoptotic and the 2 agents demonstrate significant synergism. Genistein-induced apoptosis can be observed at concentrations as low as 10 mg/L (36.9 mol/L).[158,159] While an apoptotic activity of genistein on cancer cells might appear as a desirable phenomenon, particularly in view of the synergism with dexamethasone, this activity, when exerted on normal cells, may be a matter of concern.

A final endocrine tissue sensitive to genistein is the thyroid. Earlier studies had indicated the potential of soy-containing formulas to induce goitre and hypothyroidism in infants, occasionally with the development of autoimmune thyroid disease.[160] More recently, the responsible component has been identified in soybean isoflavones. In the presence of the iodide ion, genistein and daidzein can block thyroid peroxidase, catalysing tyrosine iodination by acting as a substrate, and thus yielding mono-, di- and triiodoisoflavones.[161] Genistein can thus inhibit thyroxine synthesis. The inhibitory concentration $(IC)_{50}$ values for inhibition of thyroid peroxidase catalysed reactions by genistein and daidzein are about 1 to 10 mol/L, i.e. concentrations in the range of levels (about 1 mol/L) measured the plasma of humans consuming soy products.[12]

6. Conclusions

An overview of the potential therapeutic effects of isoflavones shows that data on the effects of regular dietary intake mainly stem from studies in which isoflavones were present in the habitual diet. Attempts to quantify intake by information on the daily diet combined with concentrations measured in food, would be more desirable since isoflavone concentrations in urine or blood reflect no more than the prior 24-hour intake.[7]

Observational studies or epidemiological surveys of dietary intake of soy-based foods point to a higher likelihood of beneficial effects on various end-points versus controlled investigations us-

Table I. Potential beneficial effects of natural estrogens, soy isoflavones and soy protein on different end-points[99]

End-point	Estrogens	Soy isoflavones	Soy protein
Lipoproteins	+	?	++
Vascular function	+	+?	+
Atherosclerosis	+	?	+
Brain	?	–?	?
Bone	+	±	?
Cancer	±	±	?
Menopause	+	?	?
Significant adverse effects	+	?	?

+ = beneficial; ++ = very beneficial; ± = contrasting data; ? = undefined; -?= uncertain negative findings; +? = uncertain positive findings.

ing purified isoflavones. While, for example, there was an almost general consensus that isoflavones might be responsible to a large extent for the cardiovascular risk reduction induced by soy,[26] there is now conclusive evidence that this is in fact not the case.[48] Similar situations, that is the non-reproducibility of observational studies in well controlled clinical investigations, have been seen with antioxidants [162] and HRT[163] in the prevention of coronary disease. In both, apparently positive results from epidemiological/observational studies were not confirmed by placebo controlled investigations.

In an attempt to compare the effects of soy isoflavones, soy proteins and traditional HRT, current scientific data are summarised in table I. Beneficial effects on the cardiovascular system were observed with soy isoflavones and traditional HRT; relief of menopausal symptom and control of osteoporosis are significantly better with HRT, while no adverse effects on breast or endometrial cancer occur with isoflavones. Few data exist on the effects of isoflavones on cognition (retrospective data are a matter of concern), whereas uncontrolled studies have observed beneficial effects with HRT. Taken in aggregate, soy isoflavones should not be viewed as a viable alternative to HRT, but a large number of randomised clinical trials assessing both dietary soy supplements and isoflavone pills are currently underway. When these are completed, we may be better able to re-evaluate this issue.

The large availability of isoflavone formulations, of different content and composition appears, at present, not to be justified by clinical evidence and potentially major adverse effects may occur in the face of absent or very dubious clinical benefits.[164,165] European authorities have taken the position of not allowing isoflavone health claims to be promoted.[166] At present, the positive effects of soy protein intake, mainly in the cardiovascular area, also achieved with natural products containing isoflavones, should allow this natural mixture to be taken within indications well supported by clinical studies. Evaluation of alternative vegetable protein sources, free or relatively poor in isoflavones, should also be encouraged.

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