Effects of phyto-oestrogens on tissues

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Abstract

Recent investigations on the effects of phyto-oestrogens on various tissues have revealed that these diverse molecules may improve human health, particularly by protecting against certain chronic diseases. After a brief examination of the food sources, structures, and general cellular actions of the major phyto-oestrogens, current research findings on cardiovascular disease, skeletal tissues, and reproductive cancers are reviewed. Phyto-oestrogen concentrations in blood may be maintained at high levels in those consuming soyabean (*Glycine max*)-based food daily at several meals and exert their effects on target cells through either genomic effects via the classical oestrogen receptors or non-genomic effects mediated by membrane-bound oestrogen receptors or other cellular proteins. The expression of oestrogen receptor (OR) subtypes alpha (α) and beta (β) varies across tissues, and cells that preferentially express OR-β, which may include bone cells, are more likely to respond to phyto-oestrogens. Conversely, reproductive tissues contain relatively more OR-α and may, thus, be differently affected by phyto-oestrogens. Soyabean phyto-oestrogens appear to prevent the progression of atherosclerosis through multiple interactions, including lowering of plasma lipids and lipoproteins, increased vasodilatation and, possibly, decreased activation of blood platelets and vascular smooth muscle cells. However, a favourable impact on cardiovascular disease morbidity and mortality by a soyabean-enriched western-type diet remains to be shown, and unresolved questions remain regarding dose and form of the phyto-oestrogens in relation to risks and benefits. The isoflavones of soyabean have been shown consistently to have bone-retentive effects in animal studies by several investigators using rodent models, although intakes must be above a relatively high threshold level for a lengthy period of time, and little or no extra benefit is observed with intakes above this threshold level. The reports of modest or no effects on prevention of bone loss in human and non-human primate studies respectively, may be due to the limited doses tested so far. The relationship between soyabean-food intake and cancer risk has been more extensively investigated than for any other disease, but with less certainty

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about the benefits of long-term consumption of phyto-oestrogen-containing foods on prevention of cancer. The observations that breast and prostate cancer rates are lower in Asian countries, where soyabean foods are consumed at high levels, and the high isoflavone content of soyabeans have led to examination of the potential protective effects of phyto-oestrogens. Establishing diet–cancer relationships has proved difficult, in part because of the conflicting data from various studies of effects of soyabean-diets on cancer. Epidemiological evidence, though not impressive, does suggest that soyabean intake reduces breast cancer risk. The isoflavone genistein has a potent effect on breast cancer cells *in vitro*, and early exposure of animals to genistein has been effective in reducing later development of mammary cancer. Thus, continuous consumption of soyabean foods in early life and adulthood may help explain the low breast cancer mortality rates in Asian countries. Although the evidence for a protective effect against prostate cancer may be slightly more supportive, more research is needed before any firm conclusions can be made about the phyto-oestrogen–cancer linkages.

Phyto-oestrogens: Soyabean : Cancer: Bone: Blood lipids

Introduction

Practically all plant foods contain small amounts of the diverse phyto-oestrogen molecules that have the potential to improve health. Phyto-oestrogens, especially the soyabean-derived isoflavones, are receiving great scrutiny as food supplements for the purposes of both enhancing the health of tissues and preventing several common diseases, such as cardiovascular diseases, cancers of reproductive tissues, and osteoporosis. Food phyto-oestrogen molecules can be divided into two major chemical classes: isoflavones and coumestans. The isoflavones are found predominantly in soyabeans (*Glycine max*), whereas coumestans are produced primarily by clovers (genus *Trifolium*) and some legumes. These molecules function as antioxidants in plants, but in mammalian tissues these natural products act as agonists, or partial agonists, of oestrogens.

This review covers the following topics relating to phyto-oestrogens: food sources, general cellular actions, effects on cardiovascular and skeletal tissues, and cancer chemoprevention.

Food sources of phyto-oestrogens

Relatively few foods contribute significant amounts of phyto-oestrogens to the human diet, and the cultural variation in consumption of those foods, particularly soyabean products, results in a wide range of intakes of these molecules. The use of soyabean products in Asian nations contributes to the highest levels of phyto-oestrogens measured in human subjects, e.g. Japanese men and women have intakes that are ten-fold or greater than those of the USA population average (Adlercreutz *et al.* 1993; Messina, 1995).

Phyto-oestrogens, both isoflavones and coumestans, are present in a variety of legumes consumed by Americans. The highest concentration of the major isoflavones (genistein and daidzein) are found in soyabeans and soyabean products as shown in Fig. 1 for genistein and
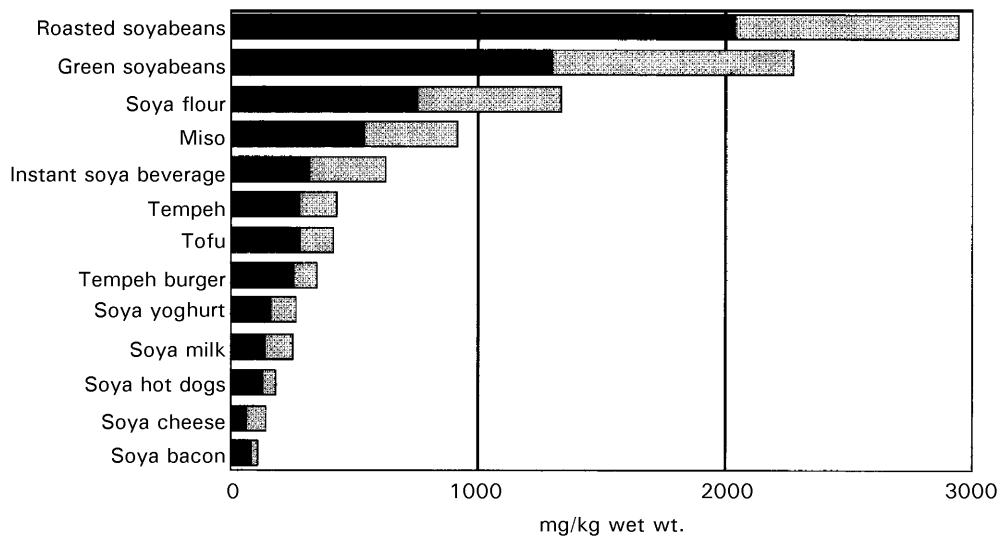
Fig. 1. Isoflavone contents of various soyabean products. Isoflavone contents of soyabean foods are expressed as mg/kg food (wet wt.) (■), Genistein; (▒), daidzein. Note that the genistein:daidzein ratio is approximately 3:2 to 2:1 for a variety of soyabean foods. Taken from Coward *et al.* (1993) and Wang & Murphy (1994).

daidzein. Clearly the concentrations of isoflavones in the derivative or second generation soyabean foods are lower than those of the soyabeans themselves or soyabean products made directly from soyabeans. The concentration of isoflavones decreases in the derivative soyabean foods because of dilution with other constituents and losses during processing. For instance, many of the second generation soyabean products are made from soyabean concentrate that has been extracted with ethanol to make the food more palatable. The aqueous–ethanol extraction method can remove > 90 % of the isoflavones, although less rigorous extraction methods are generally used. The isoflavones appear to be resistant, however, to degradation by commonly used food preparation methods, e.g. baking, frying, etc.

While coumestrol is an important phyto-oestrogen in animal diets, the coumestans are present in only a few foods commonly consumed by humans. Table 1 lists those foods that contain potentially significant amounts of coumestrol, the primary coumestan consumed by humans.

Food processors and manufacturers of dietary supplements have obviously become aware of the interest in the potential benefits of phyto-oestrogen consumption on prevention of cardiovascular disease, cancer, and osteoporosis. The available data consist of a mixture of epidemiological studies examining the relationship between consumption of natural foods

Table 1. Coumestrol content of foods*

Food item	Coumestrol (mg/kg)
Clover (*genus Trifolium*) sprouts	280·6
Round split peas (*Pisum sativum*), dry	81·1
Kala chana (*Cicer arietinum*)	61·3
Lucerne (*Medicago sativa*) sprouts	46·8
Pinto bean (*Phaselous vulgaris*) seeds, dry	36·1
Large lima bean (*Phaselous limensis*) seeds, dry, raw	14·8

*From Franke *et al.* (1994).

containing phyto-oestrogens and disease prevalence, a relatively few studies of human subjects in which purified or enriched phyto-oestrogen preparations are taken prospectively, and a burgeoning number of animal and *in vitro* studies. The increased availability of isoflavone-enriched supplements and new food items containing significant amounts of natural or supplemented phyto-oestrogens will probably lead to an increase in consumption of these molecules by the population. Because of the connection of the phyto-oestrogens with reduced risk of osteoporosis and cancer, particularly breast cancer, women are more likely to choose to increase their consumption of these dietary molecules.

Structures of major phyto-oestrogens

The phyto-oestrogens can interact with oestrogen receptors, and the similarity of their structures to the 17β-oestradiol molecule is believed to explain this mimicry. These molecules share several features in common with the oestradiol structure (Fig. 2), including a pair of hydroxyl groups separated at a similar distance; one hydroxyl group is a substituent of an aromatic A ring while the second hydroxyl lies at the opposite end of the molecule (Miksicek, 1993).

The oestrogen-like structures of the phyto-oestrogens help explain the ability of these molecules to occupy the oestrogen receptor; however, the interaction is not equivalent since both the occupancy time and affinity for the receptor are significantly reduced for phyto-oestrogens (Miksicek, 1994). In addition, small differences in the structures of individual isoflavones dramatically alter their oestrogenicity. Daidzein and genistein share identical structures except for an additional hydroxyl group on the A ring of genistein; however, genistein may have up to 5–6-fold greater oestrogenic activity in some assays (Markiewicz *et al.* 1993).

The isoflavones are present in foods in multiple chemical forms. The aglycone form is considered the active form of the molecules, but they may be only a small percentage of the total isoflavones. The glucoside (glycone) form and acetyl- and malonylglucosides are the
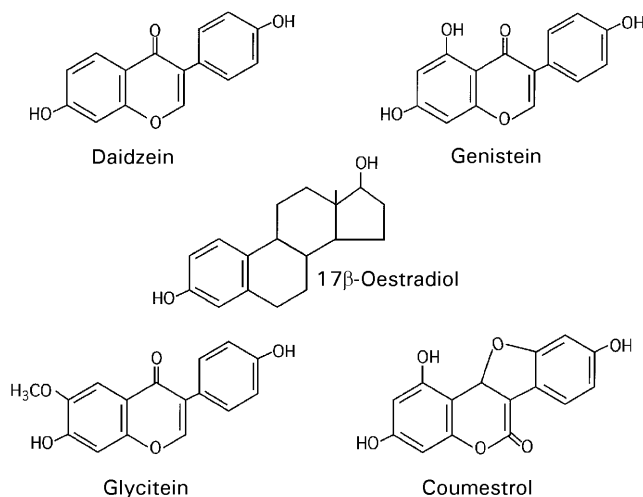

Fig. 2. Chemical structures of the major phyto-oestrogen molecules in comparison with 17β-oestradiol. The phyto-oestrogens are drawn with the B ring up to emphasize the similarity to the oestradiol molecule.

major forms found in soyabean and soyabean products. Some of the fermented soyabean products, such as fermented bean curd, have aglycones as the major isoflavone form (Wang & Murphy, 1994). The conversion of isoflavones from glucosides to aglycones is accomplished in the gut, and metabolism also includes generation of the primary isoflavone molecules, genistein and daidzein, from precursors with lower oestrogenicity (Fig. 3). Biochanin A and for-mononetin, found in clover sprouts and a few other legumes, are readily converted by gut flora to genistein and daidzein respectively. Genistein can be further metabolized in the gut to compounds such as 4-ethylphenol, and daidzein to compounds such as equol, dihydrodaidzein, and O-desmethylangolensin. Although genistein and daidzein have been used for most experimental studies of isoflavone action, data suggest that the daidzein metabolite equol may also exert significant biological effects (Hodgson et al. 1996). Equol, which has a greater antioxidant effect than other phyto-oestrogens, is often the isoflavone found in highest concentration in the blood and urine.

General cellular actions of phyto-oestrogens

Cells containing large numbers of oestrogen receptors (OR), specifically breast and uterine cells, were studied initially in an attempt to understand better the actions of specific phyto-oestrogens. The original observations that female sheep became reproductively inefficient following the ingestion of fresh subterranean clover (Trifolium subterraneum) led Australian scientists to conclude that excesses of coumestan-rich clover exerted adverse effects on uterine and possibly ovarian function (Bennetts, 1946).

Other investigators have demonstrated that the amounts of phyto-oestrogens required to produce classical oestrogenic effects on reproductive tissues similar to that of a physiological dose of 17β-oestradiol are approximately 1000-fold or greater in experimental animal models. In isolated cell lines of bone cells, however, the amounts of phyto-oestrogens required for equivalent effects of 17β-oestradiol may be about the same (JJB Anderson, T Ohue and SC Garner, unpublished results). This significant difference in the effects of oral dosages, approximately 10^{-3} or greater, could result both from inefficient absorption of the bioactive
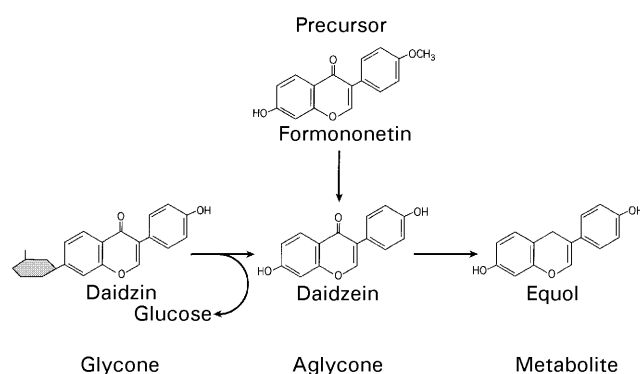

Fig. 3. Phyto-oestrogen metabolism. Daidzein, the aglycone form of this phyto-oestrogen, may be derived from intestinal metabolism of the aglycone or other precursor molecules. Further metabolism of the phyto-oestrogen may also yield metabolites with oestrogenic activity, e.g. equol.
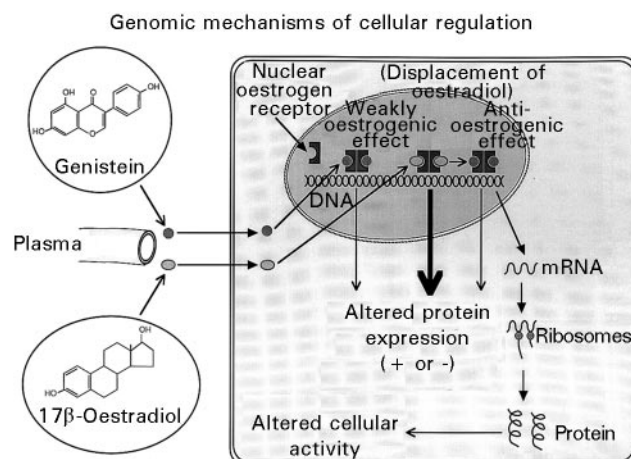
Fig. 4. Classical genomic mechanism through which oestrogens and phyto-oestrogens act. Binding of the oestrogen or phyto-oestrogen molecule, e.g. genistein, to the nuclear oestrogen receptor initiates dimerization of the receptor and interaction with oestrogen response elements on DNA. Activation or inhibition of gene transcription results in altered protein expression. Binding of a phyto-oestrogen may be interpreted to result in either a weakly oestrogenic effect or an anti-oestrogenic effect based on displacement of oestrogen molecules.

molecules (Xu *et al.* 1995) and differential effects of these compounds in various tissues (Anthony *et al.* 1996; Anderson *et al.* 1998).

Once the fat-soluble phyto-oestrogens are delivered from the gut to the tissues via chylomicrons, they have two potential fates at the cellular level: (1) they are taken up by passive diffusion across the cell membrane and act via the classical oestrogen mechanism as weak agonists; or (2) they interact with membrane proteins (receptors) and exert an effect that is expressed through second messengers in the cytoplasm (see below).

The classical action of phyto-oestrogens, using genistein as the model molecule, consists of several steps: combination with OR in the cytosol, movement of the genistein–OR complex into the nucleus for activation of the oestrogen response element after dimerization with one of several possible molecules, stimulation of DNA-directed mRNA synthesis, and the production of new protein molecules specific to the tissue cell-type (Fig. 4).

Oestrogen regulation of genes has also been shown to involve an alternative pathway in which the occupied oestrogen receptor combines with c-Fos and c-Jun oncoproteins and regulates gene transcription via AP-1 sites (Gaub *et al.* 1990; Weisz & Rosales, 1990; Umayahara *et al.* 1994).

Since the discovery of a second type of OR, OR-β (Kuiper *et al.* 1996), tissues have been found to vary in their expression of OR-α and OR-β. Some cells, especially breast and uterus, have been reported to be rich in OR-α, whereas others, such as bone, are rich in OR-β (Kuiper *et al.* 1997; Onoe *et al.* 1997), although this description may be an oversimplification. A differential distribution of OR, which have varying affinities for oestrogen agonists, may be part of the explanation for the dissimilar responses of various tissues to any single oestrogen agonist and the altered response of a single tissue to different oestrogenic molecules. For example, a selective oestrogen receptor modulator (SORM), like raloxifene, has little effect on repro-

Table 2. Relative binding affinities and relative potencies of major phyto-oestrogens compared with 17β-oestradiol

Molecule	Relative binding affinity*	Relative potency†
Oestradiol	1·0	1·0
Coumestrol	0·13	0·0020
Genistein	0·0125	0·0008
Daidzein	0·0010	0·00013
Biochanin A	0·00005	< 0·000006

* Adapted from Miksicek (1994). The relative binding affinities are calculated as the inverse of the concentration excess, relative to 17β-oestradiol, required to give 50 % inhibition of specific binding of 17β- [^{3}H]oestradiol to oestrogen receptors expressed in COS-7 cells, e.g. a 20 000-fold excess of biochanin A is required for 50 % inhibition.

† Adapted from Markiewicz et al. (1993). The relative potencies are based on the concentrations of phyto-oestrogens that exert one-half of their maximum effects (EC_{50}) on alkaline phosphatase (EC 3.1.3.1) in endometrial adenocarcinoma cells (Ishikawa-Var I) with the potency of oestradiol set at 1.0.

ductive tissues, but has bone-conserving properties. (These diverse properties of molecules with oestrogenic activity has led to the generation of the term SORM.) Phyto-oestrogens, especially genistein, are thought to act like SORM, although additional experimental work is needed to clarify the actions of specific phyto-oestrogens on OR.

Phyto-oestrogens have generally been reported to have lower binding affinity for oestrogen receptors (Miksicek, 1994) and lower potency in producing oestrogenic effects (Markiewicz et al. 1993) compared with 17β-oestradiol. As shown in Table 2, the relative binding affinities with the oestrogen receptor and relative potencies in inducing alkaline phosphatase (EC 3.1.3.1) activity in endometrial adenocarcinoma cells in culture have a similar rank order for major phyto-oestrogens. The values for relative binding affinities of phyto-oestrogens, however, tend to be several-fold higher compared with oestradiol than the corresponding potency in the endometrial cell line. Other cell types, including cells of the cardiovascular system, bone cells, and cancer cells of many different tissues may have different responses to the various phyto-oestrogens.

Steroid molecules, which include oestrogen and the other sex hormones, adrenal steroids, and 1,25-dihydroxyvitamin D, have recently been suggested to have effects on membrane-bound receptors that do not involve activation of the genomic machinery of the cell (Nemere, 1995, 1996; Machelon et al. 1996; Benten et al. 1997; Le Mellay et al. 1997). Several researchers have reported that addition of 17β-oestradiol to osteoblasts and other cells in culture stimulates increases in intracellular Ca^{2+} within seconds to minutes (Picotto et al. 1996; Le Mellay et al. 1997; Benten et al. 1998). The changes in this potential intracellular second messenger are much too rapid to involve changes in protein synthesis via the nuclear steroid receptors. Although it has not been demonstrated experimentally, phyto-oestrogens probably are also recognized by the putative cell-surface oestrogen receptors. In addition, other effects of phyto-oestrogens that involve activation of several possible second messengers, including kinase enzymes, may in turn stimulate or inhibit downstream pathways of metabolism and protein products. Many of these potential mechanisms of action of phyto-oestrogens are illustrated in Fig. 5.

The duration of the effects of phyto-oestrogens mediated via an interaction with membrane protein(s) is considered to be short-lived and with limited efficacy, unless the stimulating molecules are continuously available in solutions bathing cells. In studies of isolated cells in culture in which the media are frequently exchanged, the continuous dosing of cells with a phyto-oestrogen is easy to visualize. In animal models or humans, it is less certain that suffi-
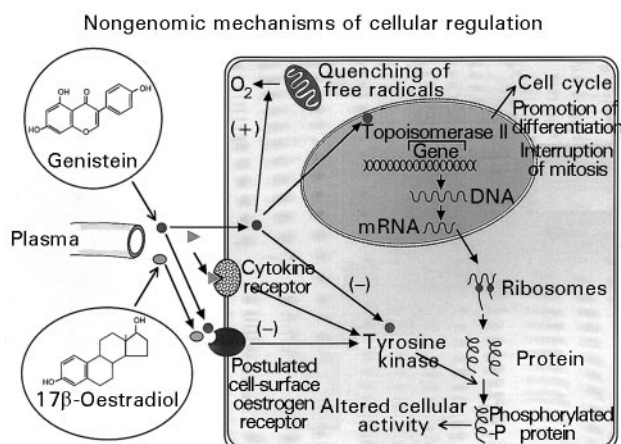
Fig. 5. Non-genomic mechanisms through which oestrogens and phyto-oestrogens may act. Oestrogens and phyto-oestrogens may bind to the postulated cell-surface oestrogen receptor to alter cellular activity. Phyto-oestrogens, particularly genistein, may have other effects via inhibition of tyrosine kinase (*EC* 2.7.1.112) activity, quenching of free radicals, or inhibition of topoisomerase II (*EC* 5.99.1.3), which alters the cell cycle.

ciently high blood concentrations can be maintained over long periods, i.e. weeks to months, to have a significant impact on cells. Studies of isolated immortal osteoblast-like cells (ROS and ROS.SMER) treated with genistein suggest that the membrane mechanism of action can modestly, but significantly, upregulate the cellular production of alkaline phosphatase approximately 20–30 % above basal unstimulated production (JJB Anderson, T Ohue and SC Garner, unpublished results). At the same time, genistein appears to have little or no effect on the classical OR mechanism in these cells, as shown by lack of any inhibitory effect of the pure oestrogen antagonist, ICI 182,780 (during the first 4–6 d in culture) (JJB Anderson, T Ohue and SC Garner, unpublished results).

In summary, both true oestrogens and phyto-oestrogens exert their effects on target cells containing viable OR by two mechanisms, the classical OR-induced series of steps, and the new membrane-protein interaction. Elevated concentrations of oestrogens can be maintained throughout fairly long periods, such as during parts of the menstrual cycle and during pregnancy. Similarly, phyto-oestrogen concentrations in blood may also be maintained at fairly high levels by ingestion of soyabean products at several meals each day, as is the case of many Japanese who consume 30–50 g soyabean-containing foods/d (Adlercreutz *et al.* 1993; Messina, 1995). Therefore, exposure of cells to oestrogens and/or phyto-oestrogens during these periods would be presumed to activate both the OR and the membrane proteins. Tissues with greater numbers of OR-α, e.g. breast and uterus, would probably be more affected by true oestrogens than by phyto-oestrogens, but tissues with higher expression of OR-β (e.g. bone) might be more responsive to phyto-oestrogens.

Effects of phyto-oestrogens on cardiovascular disease and its risk factors

Phyto-oestrogens have recently been shown to have several effects on cardiovascular tissues. These effects may reduce the risk of cardiovascular diseases.

Effects of soyabean protein on plasma lipids and atherosclerosis

The replacement of animal protein in the diet with soyabean protein has long been associated with hypocholesterolaemic and atherosclerosis-preventive effects (Meeker & Kesten, 1941). Anderson *et al.* (1995*b*) have reported on a meta-analysis assessing the effects of dietary soyabean protein on plasma lipid concentrations in which soyabean protein supplementation resulted in a reduction of approximately 13 % in plasma LDL-cholesterol concentrations, 11 % reduction in plasma triacylglycerol, and 2 % increase in HDL-cholesterol. If one stratifies the results by baseline total plasma cholesterol concentrations, the effects of dietary soyabean protein supplementation on total cholesterol and LDL-cholesterol concentrations are most robust among those with the highest plasma cholesterol at baseline. Recently, Nagata *et al.* (1998) reported a significant trend for lower plasma cholesterol concentrations with increasing soyabean intake after adjustment for potential confounders for both men (*n* 1242, *P* for trend = 0·0001) and women (*n* 3596, *P* for trend = 0·0001) in a prospective cohort study in Takayama City, Japan. These studies suggest that soyabean proteins may be useful for improving plasma lipoprotein concentrations and for prevention of cardiovascular disease.

Cardiovascular protective components of soyabean

While the cardioprotective effects of soyabean protein are well accepted, the component(s) responsible for these effects is currently the subject of much research. During the 1970s and early 1980s the amino acid composition was investigated for its effect on plasma lipid and lipoprotein metabolism and its role in atherosclerosis inhibition.

Huff *et al.* (1977) reported one such study that seems to reflect the overall findings of these studies. Groups of rabbits (*n* 6–10 per group) were fed on diets that contained either casein or soyabean protein or mixtures of amino acids that duplicated the amino acid composition of each protein. For casein or its amino acid mixture the plasma cholesterol concentrations of the two groups were found to be essentially the same. However, for soyabean protein the amino acid mixture was not as hypocholesterolaemic as the intact protein, providing evidence that there were components of the intact soyabean other than the amino acids that independently lowered plasma cholesterol concentrations or interacted with the protein moiety to affect lipoprotein metabolism favourably.

More recently, evidence has accumulated that the component of soyabean protein responsible for a large part of its cardioprotective effects is an alcohol-extractable molecule(s) (Sugano & Koba, 1993; Anthony *et al.* 1996, 1997; Balmir *et al.* 1996; Kirk *et al.* 1998; Tovar-Palacio *et al.* 1998). The alcohol-extractable components that may have the potential to lower plasma lipid concentrations are the saponins, phytosterols and isoflavones. The bulk of evidence regarding saponins is that they exert no hypocholesterolaemic effect in the presence of soyabean protein (Potter *et al.* 1993). In addition, most soyabean protein isolates have low amounts of phytosterols because of delipidation and extensive processing required to obtain the isolates (Anderson & Wolf, 1995). The isoflavones (non-steroidal phyto-oestrogens), which are largely removed from soyabean protein when it is washed with alcohol, have been reported to alter plasma lipid concentrations and are currently being actively investigated for their role in cardiovascular disease protection.

Effects of soyabean phyto-oestrogens on plasma lipids and lipoproteins

Several studies provide evidence that the phyto-oestrogens in soyabean modulate plasma lipid and lipoprotein concentrations, but the data are not entirely consistent. For the purpose of this review, only published reports for which the study design allows an assessment of the iso-

flavones (phyto-oestrogens) as the likely mediating factor on cardiovascular endpoints will be included.

In one report (Balmir *et al.* 1996), rats (*n* 6 per group) were fed for 28 d on diets containing soyabean protein extracted with ethanol to remove the phyto-oestrogens (soya(−)), unextracted soyabean protein with the phyto-oestrogens intact (soya(+)), casein (casein), or casein with 0.36 mg alcohol-extract of soyabean flour added per g protein (casein(+). The alcohol-extract was treated with acetone to precipitate sugars, saponins and proteins; thus it was principally a phyto-oestrogen-containing extract. All of the diets contained moderate amounts of fat, predominantly saturated fats, and were cholesterol-free. The groups fed on the casein(+), soya(−), and soya(+) diets had significantly lower LDL-cholesterol concentrations than the casein group, but the former three groups were not significantly different from each other. The HDL-cholesterol concentrations tended to be higher in the two groups fed on the diets containing isoflavones relative to the isoflavone-devoid groups. In a second study (Balmir *et al.* 1996) with hamsters (*n* 10 per group), the casein, casein(+), and soya(+) diets were the same as described earlier. Two additional groups were included in this study, a group fed on the unextracted soyabean protein with 0.36 mg isoflavone-containing extract added per g protein (soya(++)) and a group fed on casein with 0.72 mg extract added per g protein (casein(++)). The effects of all these diets on LDL- +VLDL-cholesterol concentrations were examined. The soya(+), soya(++), and casein(+) groups had significantly lower LDL- +VLDL-cholesterol concentrations than the casein group. The LDL- +VLDL-cholesterol concentration of the casein(++) group was also lower than for the casein group but not significantly so. Therefore, while both soyabean protein and the isoflavone-containing extract lowered LDL- +VLDL-cholesterol concentrations, no additional benefit was seen for the groups receiving the higher dose of isoflavone extract (casein(++) and soya(++)) in these two species.

Kirk *et al.* (1998) have reported on a study in which they fed diets containing unextracted soyabean protein isolate (soya(+)) or alcohol-washed soyabean protein (soya(−)) to C57BL/6 (wild-type) mice and LDL receptor-deficient (LDLr-null) mice. The wild-type (soya(−), *n* 16; soya(+), *n* 20) were treated for 10 weeks and the LDLr-null groups (soya(−), *n* = 9; soya(+), *n* 8) for 6 weeks. Among the wild-type mice the soya(+) group had significantly lower total and LDL- +VLDL- cholesterol concentrations than the soya(−) group. HDL-cholesterol concentrations were not different between groups. Among the LDLr-null mice, no significant difference was observed in total plasma cholesterol between diet groups. The authors interpreted these differences in response of the two strains of mice to suggest that soyabean isoflavones might lower plasma cholesterol concentrations by increasing LDL receptor activity, thus the lack of effect of the isoflavone-containing soyabean protein to lower plasma cholesterol in the LDL receptor-deficient mice.

Studies in non-human primates support the hypothesis that soyabean phyto-oestrogens play a primary role in modulating plasma lipid and lipoprotein concentrations (Anthony *et al.* 1996, 1997, 1998). In a cross-over study in young rhesus monkeys (*Macaca mulatta*) (*n* 25), the animals were fed on diets containing soyabean protein isolate from which the isoflavones had been extracted with ethanol (soya(−)) or with the isoflavones intact (soya(+)) (Anthony *et al.* 1996). Plasma LDL- +VLDL-cholesterol concentrations were significantly lower with the soya(+) diet in both males and females. Additionally, in the females the soya(+) diet consumption resulted in significantly improved HDL-cholesterol concentrations relative to when they were fed on the soya(−) diet.

In another study with non-human primates (Anthony *et al.* 1997, 1998), young cynomolgus monkeys (*Macaca fasicularis*) (males, *n* 77; females, *n* 70) were fed on diets containing either

casein and lactalbumin (casein), alcohol-extracted soyabean protein isolate (soya(−)), or unextracted soyabean-protein isolate (soya(+)) as the source of protein. The diets were identical for other components. During the 14-month treatment period, plasma lipid concentrations were measured. There was a robust LDL- + VLDL-cholesterol-lowering effect and HDL-cholesterol-raising effect ($P = 0.0001$) of the phyto-oestrogen-intact soyabean protein relative to the phyto-oestrogen-extracted soyabean observed in both males and females ($P = 0.0001$ for the combined data for each measure). A large part of the benefit of soyabean protein is lost when it is alcohol-washed. It seems likely that the loss of the beneficial effects of soyabean protein isolate on plasma lipids and lipoproteins is due to the extraction of the phyto-oestrogens.

Additional support for the hypothesis that the isoflavones are an important modulator of plasma lipoprotein concentrations comes from a recently completed study by Crouse *et al.* (1998). In this randomized clinical trial, mildly hypercholesterolaemic men (*n* 94) and women (*n* 62) were treated with protein supplements (25 g protein in each) that contained casein, alcohol-extracted soyabean protein isolate (3 mg isoflavones), or soyabean protein isolate that contained 27 mg, 37 mg, or 62 mg isoflavones/d. The treatment phase lasted 9 weeks. The 62 mg isoflavone group (soya(++)) had significantly lower LDL-cholesterol concentrations than the casein group ($P < 0.05$) while the alcohol-extracted soyabean protein (soya(−)) had no effect. The authors also reported a dose–response relationship with progressively lower total and LDL-cholesterol concentrations with increasing isoflavone dose.

Baum *et al.* (1998) recently completed a study comparing the effects of soyabean protein isolate containing two different concentrations of isoflavones with a control protein (casein and non-fat dry milk) in postmenopausal women (total *n* 66). All subjects received 40 g protein/d and the isoflavone doses for the two soyabean protein groups were 56 mg/d (soya(+)) or 90 mg/d (soya(++)). The soya(++) protein was similar in isoflavone content to that used in the study described earlier by Crouse *et al.* (1998). The difference in isoflavone dose with this product, 90 mg/d for Baum *et al.* (1998) and 62 mg/d for Crouse *et al.* (1998), results from the difference in the amount of protein given each day, 40 g *v.* 25 g, respectively. Both doses of isoflavones resulted in significantly lower LDL- + VLDL-cholesterol and higher HDL-cholesterol concentrations relative to the control group. No evidence was found of a dose–response effect on these endpoints.

The beneficial effects of soyabean phyto-oestrogens on plasma lipids and lipoproteins appear convincing. The LDL cholesterol-lowering effects of soyabean isoflavones have been shown in various animal models, including mice, rats, hamsters, cynomolgus and rhesus monkeys, and human subjects. The LDL-cholesterol-lowering effects have been seen when isoflavone-containing extracts have been added to casein-based diets, a significant attenuation of LDL-cholesterol lowering has been demonstrated when the isoflavones are removed from soyabean protein by alcohol extraction, and a dose–response effect on LDL-cholesterol lowering has been shown with increasing isoflavone concentration in the dose range of 3–62 mg isoflavones/d. Studies with transgenic mice suggest that the benefits of isoflavones on cholesterol lowering may be mediated through upregulation of LDL-receptor activity.

Effects of soyabean phyto-oestrogens on vascular reactivity

Vascular reactivity in response to acetylcholine is a measure of vascular function and can be evaluated by repeated quantitative angiography. Changes in the diameter of a coronary artery

are measured after intracoronary infusion of acetylcholine, which induces endothelium-mediated vasodilation in normal arteries and constriction in atherosclerotic arteries of oestrogen-deficient subjects. Honoré *et al.* (1997) compared the effects of unextracted soyabean protein isolate (soya(+), *n* 5) and phyto-oestrogen-extracted soyabean protein (soya(−), *n* 6) in young pre- and peripubertal rhesus monkeys and reported that the soya(+) diet significantly improved vascular reactivity. They also repeated this test in the soya(−) group and administered genistein (the principal phyto-oestrogen in the soyabean protein isolate) intravenously 20 min before angiography and found that genistein had similar oestrogen-like effects on vascular reactivity (vasodilatation) relative to soya(−) treatment. These data further support the hypothesis that the phyto-oestrogens, and genistein in particular, are the components in soyabean responsible for cardioprotection.

Effects of soyabean phyto-oestrogens on atherosclerosis

A much smaller body of experimental evidence supports a protective effect of soyabean phyto-oestrogens on atherosclerosis. One piece of evidence is from a study of young cynomolgus monkeys in which the animals were fed on diets containing either casein and lact-albumin, alcohol-extracted soyabean-protein isolate (soya(−)), or unextracted soyabean protein isolate (soya(+)) as the source of protein (Anthony *et al.* 1997). Coronary artery atherosclerosis extent was quantified on a subset of monkeys from each treatment group (soya(−), *n* 18; soya(+), *n* 15). Average lesion size in the soya(+) group was approximately 70 % smaller (*P* = 0·05) than in the soya(−) group (MS Anthony, TB Clarkson and TB Bullock, unpublished results). These data would suggest that the alcohol-extractable material, probably the phyto-oestrogens, inhibits atherogenesis.

In another study in surgically postmenopausal cynomolgus monkeys, the effects of soyabean with its phyto-oestrogens were compared with those of conjugated equine oestrogens (Premarin® (Wyeth-Ayerst Laboratories, Philadelphia, PA, USA), the most commonly used oestrogen-replacement therapy in the USA) on atherosclerosis in the iliac artery (Anthony & Clarkson, 1998). Animals were randomized into one of three groups: (1) a group fed on a diet containing isoflavone-extracted soyabean protein (soya(−), *n* 56), (2) a group fed on soyabean protein with the phyto-oestrogens intact (soya(+), *n* 59), or (3) a group fed on the isoflavone-
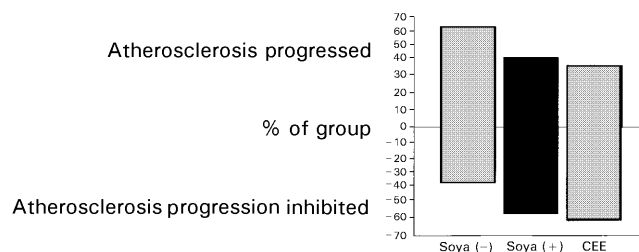

Fig. 6. Effects of phyto-oestrogen-extracted soyabean protein (soya(−), *n* 56), phyto-oestrogen-intact soyabean protein (soya(+), *n* 59) or conjugated equine oestrogens (CEE, Premarin® (Wyeth-Ayerst Laboratories, Philadelphia, PA, USA), *n* 63) on atherosclerosis progression in the common iliac artery of surgically postmenopausal cynomolgus monkeys (*Macaca fasicularis*). Data are expressed as the percentage of each group in which atherosclerosis progressed or was inhibited. Soya(+) *v.* soya(−), *P* = 0·03; CEE *v.* soya(−), *P* = 0·007; soya(+) *v.* CEE, *P* = 0·62. Adapted from Anthony & Clarkson (1998).

extracted soyabean protein with Premarin® (*n* 63) added to approximate the usual dose for women. Atherosclerosis progression in the common iliac artery was evaluated after 3 years of treatment. Fig. 6 shows the percentage of each group that demonstrated atherosclerosis progression or inhibition of atherosclerosis progression. These data suggest that both soyabean phyto-oestrogens and conjugated equine oestrogen inhibit atherosclerotic plaque progression relative to the phyto-oestrogen-extracted soyabean protein-treated control group, and the effects of the two treatments are similar.

Kirk *et al.* (1998) recently evaluated the effects of diets containing either intact or phyto-oestrogen-extracted soyabean on atherosclerosis in two strains of mice, LDL receptor-deficient (LDLr-null) and wild-type (C57BL/6) mice. Among the wild-type mice, lesion area was significantly smaller in those fed on intact or unextracted soyabean protein compared with phyto-oestrogen-extracted soyabean protein, but no difference in the extent of atherosclerosis was seen in the LDL receptor-deficient mice. The effects on atherosclerosis are similar to those for plasma and LDL-cholesterol (described earlier), suggesting that isoflavones modulate plasma cholesterol concentrations by increasing LDL-receptor activity and thereby inhibiting atherogenesis.

While the data are just now accumulating, soyabean protein containing phyto-oestrogens appears to protect against atherosclerosis progression. The magnitude of the effect appears to be comparable to that of traditional postmenopausal oestrogen therapy (Premarin®). The LDL receptor appears to play a role in modulating the effects of the isoflavones. These data on the benefits of soyabean protein containing isoflavones, which reduce the extent of atherosclerosis, are encouraging for the prospect of reducing the risk of CHD.

Lack of an effect of purified isoflavones on plasma lipid concentrations

Two recent reports have evaluated the effects of purified phyto-oestrogen pills on cardiovascular disease risk factors. The first was a randomized, placebo-controlled cross-over trial, in which menopausal and perimenopausal women (*n* 21) were treated with either purified isoflavone pills containing about 80 mg isoflavones (45 mg genistein, 34 mg daidzein, 3 mg glycitin) or a placebo for 5-week periods (Nestel *et al.* 1997). These investigators found improved systemic arterial compliance with isoflavone pill treatment, but no effect on plasma lipids. The second half of the cross-over was extended to a 10-week period to see if additional treatment time was required for plasma lipid effects, but there continued to be no effect of purified isoflavones on the plasma lipid concentrations. In a second randomized, placebo-controlled, double-blind trial, forty-six men and thirteen postmenopausal women were treated with purified phyto-oestrogen pills containing 55 mg isoflavones (16 mg biochanin A, 30 mg genistein, 8 mg formononetin, 1 mg daidzein) isolated from subterranean clover, for 8 weeks (Hodgson *et al.* 1998). No effect of the purified phyto-oestrogen pills was found on plasma lipid and lipoprotein concentrations.

In considering these studies together with the two previously cited human studies (Baum *et al.* 1998; Crouse *et al.* 1998), one of the differences is that where no cholesterol-lowering effect of the phyto-oestrogens was shown, the subjects were not selected to be hypercholesterolaemic. Thus Crouse *et al.* (1998) reported no significant effect on total cholesterol or LDL-cholesterol with the soyabean protein supplements in the half of the subjects with LDL-cholesterol concentrations below the median at baseline. Other possible explanations for a lack of effect of purified isoflavones include: (1) the phyto-oestrogens are not the active agent: it is some other unidentified component that is removed with alcohol extraction and is present in soyabean in

concentrations parallel to the phyto-oestrogens; (2) the phyto-oestrogens are inactivated when they are isolated and purified and (3) an enabling factor in soyabean protein allows the phyto-oestrogens to have the beneficial effects on plasma lipids and lipoproteins.

Discussion

Considerable evidence exists that soyabean with its phyto-oestrogens has a beneficial effect on cardiovascular disease, preventing the progression of atherosclerosis by interaction with multiple biological processes. A large part of this benefit appears to be mediated by effects on plasma lipid and lipoprotein concentrations. Soyabean phyto-oestrogens, and genistein in particular, also impact beneficially on vascular reactivity. Another potential benefit of soyabean phyto-oestrogens, which has been shown *in vitro*, is prevention of lipoprotein oxidation (Hodgson

et al. 1996; Ruiz-Larrea *et al.* 1997). The resulting decrease in oxidized LDL particles, which are considered more atherogenic (Witztum & Steinberg, 1991), may decrease the risk of atherosclerosis. The phyto-oestrogens in soyabean protein may also have some direct effects on the artery wall and the cells involved in promotion and progression of atherosclerosis. Genistein has been shown, *in vitro*, to inhibit the migration and proliferation of smooth muscle cells (Fujio *et al.* 1993; Shimokado *et al.* 1994, 1995), to inhibit platelet activation (Kuruvilla *et al.* 1993; Murphy *et al.* 1993) and aggregation (Asahi *et al.* 1992; McNicol, 1993), and to reduce platelet serotonin uptake (Helmeste & Tang, 1995). Each of these effects of genistein may delay the development of atherogenesis.

It remains to be shown that an intervention incorporating soyabean with its phyto-oestrogens into a western-type diet can favorably impact cardiovascular disease morbidity and mortality (e.g. stroke, myocardial infarction). Questions remain regarding dose and form of the phyto-oestrogens to maximize the benefits and minimize any potential risks. Incorporation of soyabean with its phyto-oestrogens into a western diet, however, may prove to be an important means for prevention of cardiovascular disease and may have additional benefits for other chronic diseases.

Areas for further investigation

Several gaps in our knowledge of phyto-oestrogens remains to be investigated if these molecules are to be considered as a preventive therapy for cardiovascular disease:

(1) The appropriate dose of phyto-oestrogens required for cardiovascular benefit is uncertain.
(2) The relative potency of each of the two principal phyto-oestrogens in soyabean, genistein and daidzein, of the much less abundant glycitin, and of the metabolites (e.g. equol, *O*-desmethylangolensin, dihydrodaidzein, 4-ethylphenol) needs to be determined and compared with parent compounds. Whether some optimum ratio of the phyto-oestrogens exists should also be determined.
(3) The question remains why purified isoflavone preparations appear to have no effect on plasma lipid and lipoprotein concentrations, although step-wise reductions in LDL-cholesterol levels occur with increasing doses of isoflavones administered in soyabean protein.
(4) For soyabean phyto-oestrogens to be considered for primary prevention of cardiovascular disease, their safety in other organ systems must be established.

Effects of phyto-oestrogens on skeletal tissues

The published data supporting a beneficial effect of phyto-oestrogens on bone mass are limited, but reports have been rather consistent in support of positive bone effects of isoflavones and other phyto-oestrogens in human subjects, most experimental animal models, and bone explants or bone cells *in vitro*. An increase in bone mass could result from either increased bone formation by osteoblasts or decreased bone resorption by osteoclasts, and would thus help to prevent the development of osteoporosis. Although the cellular mechanism of action of phyto-oestrogens on bone may involve either osteoblasts or osteoclasts, OR are present in relatively low numbers in osteoblasts while mammalian osteoclasts are not believed to express these receptors. Thus, a genomic effect of phyto-oestrogens, consistent with their ability to interact with the ligand-binding portion of the OR *in vitro*, may underlie a direct effect of these molecules on osteoblasts, while other responses of osteoblasts or osteoclasts may result from either indirect mechanisms or non-genomic actions (see later).

Investigations of phyto-oestrogen effects on bone in human subjects

In human subjects, phyto-oestrogen molecules may benefit bone mass by direct effects via bone cells; however, the consumption of soyabeans or soyabean protein, as an extract or hydrolysate, apparently has a conservatory effect on Ca. In contrast to the animal protein lactalbumin (a milk protein), isolated soyabean protein, which contains isoflavones, exerts a modest decrease in urinary Ca excretion during both the postprandial and 24 h periods (Anderson *et al.* 1987). In addition, the consumption of soyabean protein stimulates glucagon during the postprandial period at a much greater rate than lactalbumin. Based on the results of this study, however, it may have been the isoflavones that exerted the favorable effect on Ca retention following soyabean consumption. Whatever the acute mechanism of soyabean protein (containing iso-flavones) on Ca conservation is, it is highly significant, and it may help to offset lower Ca intakes among vegetarians and counter other potentially adverse dietary factors in the diets of vegans (Anderson & Miller, 1998).

Post-menopausal women in general may benefit from dietary supplementation with iso-flavone-enriched soyabean products to maintain bone mass. A report (Potter *et al.* 1998) of soyabean supplementation of sixty-six postmenopausal women compared bone measurements at baseline and 6 months after feeding a soyabean-protein supplement enriched in genistein and daidzein (doses of approximately 56 and 90 mg of isoflavones/d), compared with treatment with a casein/non-fat dry milk supplement (no isoflavones). The higher dose resulted in significant improvements ($P < 0.05$) in both bone mineral density and content of lumbar vertebrae relative to the control group, with no significant benefit in subjects receiving the lower dose. These findings, which illustrate the positive responses of vertebral bone mineral density of the postmenopausal women in the study, suggest that a threshold dose exists for any benefit of isoflavones on bone mineral density. Longer prospective studies examining the effects of phyto-oestrogens on bone parameters of postmenopausal women are needed to clarify effective doses and whether other skeletal sites will also benefit from isoflavones over a longer time frame of treatment.

Some evidence is also available that consumption of soyabean proteins can reduce urinary markers of bone resorption. Pansini *et al.* (1997) reported that non-osteoporotic, post-

menopausal women who consumed 60 g isolated soyabean proteins daily for 3 months had significant reductions in both deoxypyridinoline and N-telopeptide excretion.

In addition to the limited data on effectiveness of naturally-occurring isoflavones in preventing bone loss in postmenopausal women, the bone-conserving effects of chronic treatment with ipriflavone, a synthetic isoflavone, in women with low bone mass have been reported. Gennari *et al.* (1997) studied 453 postmenopausal women in a double-blind, placebo-controlled, 2-year study with random assignment of women to either ipriflavone (200 mg three times per day) or placebo; all women received 1 g Ca/d orally. The final outcome showed a significant ($P < 0.05$) difference between the placebo groups, which lost 1·6 % at one study centre and 3·5 % of bone mass at the second centre, and the ipriflavone group, which maintained bone mass (approximately 0 % loss).

Data on the effectiveness of phyto-oestrogens in promoting bone accretion and bone retention in young adult women are also needed in addition to longer trials with these dietary molecules in older women. An increase in peak bone mineral density of young women may possibly delay or prevent development of osteoporosis in later life. Crucial data are required from longer human trials with isoflavones establishing the long-term benefits of these nonsteroidal molecules.

Animal model experiments of phyto-oestrogen effects on bone

Some of the limited number of published reports on the skeletal effects of phyto-oestrogens on animals are highlighted here.

Rodent studies. The most commonly used animal model for examining the effect of molecules with the potential for anti-osteoporotic actions has been the ovariectomized (OVX) rat. Three different studies have been reported in which OVX rats have been treated with either soyabean with its naturally-occurring isoflavones, a genistein-rich soyabean isoflavone preparation, or purified genistein. Feeding an isoflavone-containing soyabean protein-based diet to OVX rats resulted in retention of significantly more bone than feeding a casein-based diet (Arjmandi *et al.* 1996). Similarly, OVX lactating rats treated with a genistein-rich soyabean isoflavone preparation for 14 d retained significantly more bone mass than vehicle-treated controls (Anderson *et al.* 1995*a*; JJB Anderson, WW Ambrose and SC Garner, unpublished results). Purified genistein also promoted a similar improvement in femur bone mass of OVX rats (Blair *et al.* 1996). The common factor among these three studies was the presence of isoflavones, including genistein. The studies by Anderson *et al.* (1995*a*; JJB Anderson, WW Ambrose and SC Garner, unpublished results) found that soyabean isoflavones had an apparent biphasic effect on bone retention. A similar biphasic response in which lower doses had significant effects on bone while higher doses produced less benefit has been reported for bone-derived cells in culture as discussed later. Further studies of the effects of phyto-oestrogen molecules over a broad dose range are needed to determine whether a potentially narrow range exists for beneficial effects of phyto-oestrogens as suggested by these findings of a biphasic relationship.

The research reported above involved the isoflavones found in soyabean products. These molecules have been studied most frequently because soyabean is the most common source of phyto-oestrogens in the human diet. However, the human diet also includes many legumes, such as lima beans (*Phaseolus limensis*), pinto beans (*Phaseolus vulgaris*), and sprouts of clover (genus *Trifolium*) or lucerne seeds (*Medicago sativa*) (Franke *et al.* 1994), that contain

significant amounts of another phyto-oestrogen, coumestrol (Table 1). Dodge *et al.* (1996) found significant effects of coumestrol but not of genistein, on prevention of bone loss in rats after ovariectomy. Coumestrol administered orally appeared to be equally effective compared with ethinyl oestradiol in preventing bone loss, but doses at least 100-times higher were required. In another study, injection of coumestrol to OVX rats twice per week has been reported to cause a similar significant improvement in femur bone mass to that shown with oestradiol (Draper *et al.* 1997). Interestingly, the same report contained results of an experiment in which dietary supplementation with an isoflavone-containing extract from subterranean clover did not significantly improve the bone mass of OVX rats. A similar finding was obtained by JJB Anderson, WW Ambrose and SC Garner (unpublished results) for young, growing OVX rats, which had modest, but not significant, gains in femur bone mass after 4 weeks of treatment with a genistein-rich soyabean isoflavone extract.

Significant bone retentive effects of the synthetic isoflavone, ipriflavone, have also been reported. Cecchini *et al.* (1997) found that either 400 mg/kg body weight of ipriflavone mixed in the food of OVX rats or subcutaneous injection of 27·2 g/kg of 17β-oestradiol per day could decrease bone resorption to values of sham-operated animals. Although ipriflavone produced similar bone effects to that of oestradiol, no significant uterotrophic effect was observed.

Non-human primate studies. Although clearly valuable, relatively few primate studies of the effects of phyto-oestrogens on bone mass have been published and positive effects of isoflavones on retention of bone after ovariectomy in non-human primates have been difficult to demonstrate. One prospective study in which primates were treated with a single dose of isoflavones (28 mg per animal) for 2 years has been published (Jayo *et al.* 1996). No positive effects of the isoflavones on bone measurements were reported. The investigators suggested that the lack of significant improvement in bone mass in this experiment could have resulted from the use of a single dose of isoflavones, which may have been too small to produce a positive effect on bone mass or that the Ca level in the diet (equivalent to a human intake of about 800 mg/d) was too low. Additional research has been reported by the same group (Register *et al.* 1997) in which they fed OVX macaques (genus *Macaca*) for 6 months on either casein/lactalbumin or soyabean protein diets with and without oestrogen. Serum Ca was significantly increased in the soyabean protein-fed groups compared with the casein/lactalbumin groups, which is consistent with the finding of Anderson *et al.* (1987) that soyabean protein could conserve Ca in humans.

An additional study in OVX cynomolgus monkeys examined the rate of bone formation in cortical bone (Lees & Ginn, 1998). All animals were OVX, and bone was collected 7 months after feeding on either casein or soyabean-based diets with and without treatment with oestradiol. While oestradiol significantly reduced bone formation rates at periosteal, osteonal, and endocortical surfaces, the only statistically significant effect of the soyabean diet was an increase in bone formation at the endocortical surface. The authors conclude that phyto-oestrogens in the dietary soyabean isolate are effective in improving cardiovascular risk factors but not bone under the same conditions.

The difficulty of demonstrating positive effects of isoflavones on retention of bone after ovariectomy may be consistent with the concept that the dose of isoflavones necessary to demonstrate gains in bone tissue is relatively high, about 90 mg/d. This requirement is congruent with studies using rodent models. Also the animal experimental results suggest that a threshold dose of isoflavones needs to be consumed for a lengthy period of time, i.e. months to years, before any measurable effect on bone can be observed. Doses higher than the threshold appear to be no more beneficial than the minimal effective dose; very high doses are less effective, and if too high, they may even be toxic.

In vitro *bone cell studies of phyto-oestrogens*

The use of bone tissue or osteoblast-like and osteoclast-like cells in culture have provided important information about the effects of phyto-oestrogen molecules on bone activity.

Bone tissue in organ culture. A single published study has examined the effects of phyto-oestrogens on bone tissues in organ culture. Tsutsumi (1995) reported that coumestrol inhibited bone resorption and stimulated bone mineralization in 9-d-old chick embryonic femur explants. In addition, the increased bone resorption resulting from treatment with parathyroid hormone, the hormonal form of vitamin D, or prostaglandin E_2 could be inhibited by coumestrol or 17β-oestradiol. The author reported that the optimal effects were obtained with approximately 10^{-5} M-coumestrol, which is about 100–1000-fold higher than the optimal concentration of oestradiol (Miksicek, 1994).

Osteoblast-like cells. Studies of osteoblast-like cells in culture have shown that they respond to phyto-oestrogens. Different cell lines with osteoblast-like properties have been examined *in vitro* to confirm and extend the research on effects of phyto-oestrogens on bone *in vivo*. An important finding from at least two laboratories has been the biphasic response of osteoblast-like cells to a wide range of concentrations of phyto-oestrogens (Benvenuti *et al.* 1991; Anderson & Garner, 1997).

Benvenuti *et al.* (1991) tested the effects of ipriflavone, a synthetic flavone molecule with a structure closely related to the isoflavones, on bone cells *in vitro*. They found the alkaline phosphatase (*EC* 3.1.3.1) activity of UMR-106 osteoblast-like cells responded to increasing doses of ipriflavone with a biphasic response (Fig. 7). The peak activity of alkaline phosphatase of approximately three times that of the baseline for control cells was found at 10^{-10} M-ipriflavone.

The effects of genistein and 17β-oestradiol on alkaline phosphatase activity have also been examined in the ROS-SMER-14 osteoblast-like cell line (Fig. 8) (T Ohue, SC Garner and JJB Anderson, unpublished results). It was found that both the ROS-SMER-14 and its parent ROS 17/2·8 cell lines responded with modest increases in alkaline phosphatase activity. Although the ROS.SMER-14 cells express approximately eight times as many oestrogen receptors as the ROS 17/2·8 cells (Migliaccio *et al.* 1992), the responses of alkaline phosphatase to addition of
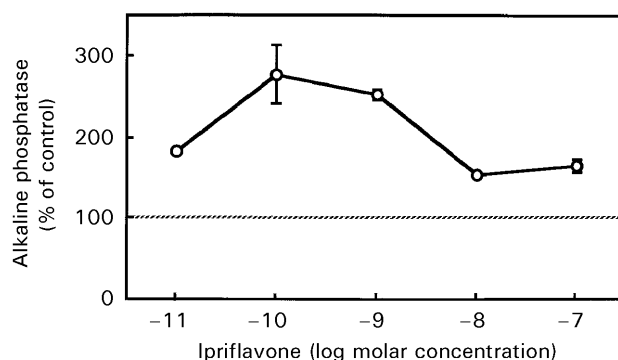
Fig. 7. Ipriflavone, a synthetic isoflavone, exerts a biphasic effect on alkaline phosphatase (*EC* 3.1.3.1) activity in UMR-106 osteoblast-like cell line (adapted from Benvenuti *et al.* 1991). Vertical lines represent SE.

genistein (10^{-10}–10^{-8} M) were similar in both cell lines, suggesting that other mechanisms of action of phyto-oestrogens may be operating in these cells. At higher (10^{-6}–10^{-4} M) concentrations of genistein and 17β-oestradiol both ROS.SMER-14 and ROS 17/2·8 cells assumed a more rounded appearance with reduced contacts with other cells and the culture plate. The presence of DNA ladders after gel electrophoresis suggests that this an apoptotic rather than a toxic effect (JJB Anderson, T Ohue and SC Garner, unpublished results). The differential effects of genistein and 17β-oestradiol on alkaline phosphatase and cell morphology suggest that some effects of these molecules may be through similar mechanisms of action, while some bone-related effects may involve non-oestrogenic actions of genistein or other phyto-oestrogens. A closely related phyto-oestrogen, daidzein, did not produce a biphasic response in the same osteoblast-like cells (T Ohue, SC Garner and JJB Anderson, unpublished results).

Studies of osteoblast-like cells in culture demonstrate that these cells respond to phyto-oestrogens and related molecules. Comparison of the effects of isoflavones and chemically similar molecules, however, demonstrates that various cell lines may respond differently to phyto-oestrogens. Further, the responses of bone cells *in vivo* may be different from those observed *in vitro*, and it is likely that our knowledge of the mechanisms of action of phyto-oestrogens on osteoblasts will continue to unfold in the next few years.

Osteoclast-like cells. Inhibition of osteoclastic cell activity *in vivo* by oestrogens, and possibly phyto-oestrogens, is believed to be mediated via osteoblasts since mammalian osteoclasts are generally considered not to express oestrogen receptors. Despite this postulated relationship *in vivo*, however, direct inhibitory effects of phyto-oestrogens have been demonstrated on avian osteoclasts *in vitro* by Blair *et al.* (1996). They found that genistein, but not the structurally similar molecule daidzein, could exert this effect. Both Blair *et al.* (1996) and Williams *et al.* (1998) proposed that isoflavones inhibited activities of osteoclast-like cells by interfering with specific enzymatic systems, such as the tyrosine kinase (*EC* 2.7.1.112) activity of epidermal growth factor receptor protein, i.e. a non-genomic mechanism. Tobe *et al.* (1997) assayed parathyroid hormone-stimulated bone resorption by mixed cultures of mouse bone cells on dentine slices *in vitro*. The number of resorption pits was inhibited by calcitonin and significantly stimulated by daidzein but not genistein or ipriflavone. Further research will be required to resolve these reports of divergent actions of phyto-oestrogens on osteoclasts *in vitro*.
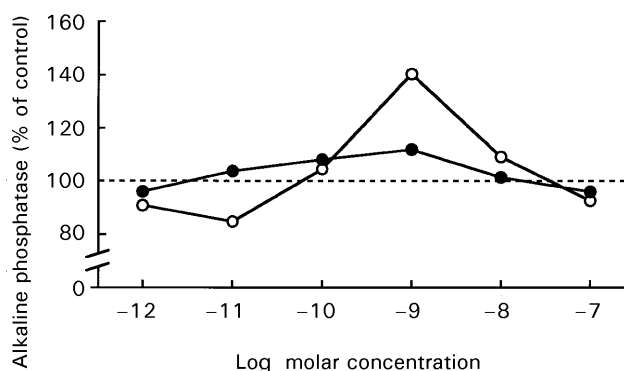

Fig. 8. Alkaline phosphatase (*EC* 3.1.3.1) activity is significantly increased by both genistein (○) and 17α-oestradiol (●) in ROS.SMER-14 osteoblastic cells (T Ohue, SC Garner and JJB Anderson, unpublished results).

Summary.   Taken together these studies suggest that isoflavones, and possibly other phyto-oestrogens, may operate by both oestrogen-receptor-mediated pathways and by pathways that involve so-called non-genomic mechanisms that could involve both the putative cell-surface oestrogen receptor and enzymatic pathways such as tyrosine kinase (see later). Isolated bone cells and cell lines will continue to provide valuable models for future studies of the effects of the many non-steroidal oestrogen-like molecules on the health benefits of soyabean-derived isoflavones and other phyto-oestrogens.

Mechanisms of action of phyto-oestrogens on osteoblasts and osteoclasts

Phyto-oestrogens, like oestrogens, are thought to interact at the cellular level by two major mechanisms: genomic and non-genomic.

Interactions of phyto-oestrogens with the oestrogen receptor.   The oestrogen receptor is believed to act primarily through altering the production of specific proteins within the cell by binding to regulatory sites on DNA molecules (oestrogen response elements (ORE)) and increasing or decreasing expression of specific genes. Depending on the affinity of the phyto-oestrogen molecule for the OR, binding of a phyto-oestrogen to the receptor may result in partial activation of the receptor (weak agonistic effect) or displacement of an oestrogen molecule, which may reduce receptor activation (antagonistic or anti-oestrogenic effect).

Phyto-oestrogens combine with the OR but at lower affinities than 17β-oestradiol. The affinities of selected phyto-oestrogens for the oestrogen receptor in comparison to 17β-oestradiol are listed in Table 2. Miksicek (1993) has demonstrated that diverse naturally occurring and synthetic molecules can activate human nuclear oestrogen receptors in an *in vitro* reporter assay. Although the non-steroidal oestrogens are less potent than 17β-oestradiol, they do generate the same maximal response when the dose tested is high enough. These findings suggest that the cumulative effect of the many molecules of plant origin with weak agonism may be a significant factor in human health, including the maintenance of bone mass.

Nearly all cell types are believed to be affected by oestrogens in some way because OR are present in almost all mammalian cells that have nuclei, with the possible exception of osteo-clasts. The response of different cells may depend in large part, therefore, on the number of OR, which may be approximately $100-1000$ times higher in reproductive tissues than in most other cells of the body, including bone cells. Migliaccio *et al.* (1992) reported that oestrogens could activate oestrogen receptors present in both the wild type ROS 17/2.8 cells and in the ROS.SMER-14 cells stably transfected with mouse uterine OR.

The genomic effects of oestrogens are believed to be mediated via an intracellular oes-trogen receptor that activates gene transcription by interacting with ORE. A second intra-cellular OR molecule has recently been discovered (Kuiper *et al.* 1996). The classical OR is now designated OR-α and the more recently discovered, structurally similar, protein has been named OR-β. Initial reports suggest that the phyto-oestrogens, genistein and coumestrol, have a significantly higher affinity for OR-β than for OR-α (Kuiper *et al.* 1997).

Both OR-α and OR-β are expressed in bone cells (Arts *et al.* 1997; Onoe *et al.* 1997). Recently published reports indicate that the expression of OR-α and/or OR-β may vary during differentiation of osteoblasts *in vitro*. The pattern of expression, however, may be dependent on the particular cells being studied. While Onoe *et al.* (1997) found that primary cultures of rat osteoblasts expressed OR-β at a relatively constant level during 28 d in culture, the expression of OR-α increased by $7-8$-fold in parallel with osteoblastic differentiation. Bodine *et al.* (1998)

also examined OR-α expression in primary rat osteoblasts. They found a 70-fold increase in OR-α mRNA levels by days 22–25 of culture. In contrast, Arts *et al.* (1997) found that a human osteoblastic cell line (SV-HFO) had approximately 10-fold higher levels of OR-*β* mRNA expression in mineralizing (day 21) cells *v.* proliferating (day 6) cells, but OR-α mRNA increased only approximately 2-fold.

OR-α and OR-*β* can form heterodimers (Cowley *et al.* 1997; Pettersson *et al.* 1997) and, thus, the responsiveness of cells may differ depending on the relative expression of the two receptor subtypes. Paech *et al.* (1997) examined the differential ligand activation of OR-α and OR-*β* with an ORE reporter construct and with an AP-1 reporter construct. Although oestradiol and a panel of ligands, including tamoxifen and raloxifene, had almost the same activation profile with the ORE reporter construct with both OR, the responses of the AP-1 reporter construct were distinctly different. Oestrogen activated the AP-1 reporter construct in the presence of OR-α but not OR-*β*, while tamoxifen was a potent activator of both OR-α and OR-*β*. The next few years promise to be an exciting period of research into the role of these two oestrogen receptor subtypes in explaining some of the differential effects of oestrogenic compounds, including phyto-oestrogens, on bone cells compared with reproductive tissues.

Non-genomic mechanisms. Phyto-oestrogens may interact with other cellular proteins in addition to the classical oestrogen receptor. The existence of membrane-bound receptor for oestrogenic molecules has been proposed (Watson *et al.* 1995). In addition, genistein has been demonstrated to inhibit tyrosine kinase (Akiyama *et al.* 1987) and topoisomerase II (*EC* 5.99.1.3) (Okura *et al.* 1988). An important function of the phyto-oestrogens in plant cells is as antioxidants, and it is likely that they could perform the same function in animal cells. Proposed actions of phyto-oestrogens through non-genomic mechanisms are supported by the direct action of phyto-oestrogens on osteoclasts (Williams *et al.* 1998), which appear to lack oestrogen receptors. The major effect of phyto-oestrogens on osteoclasts may still be mediated via osteoblasts however, since secretion of cytokines by osteoblastic cells is believed to be the major mechanism for regulation of osteoclastic activity in response to calcaemic hormones and oestrogen.

The potential mechanisms by which phyto-oestrogens may affect cellular activities are illustrated in Figs. 4 and 5. The mechanisms of action are divided into the genomic and non-genomic effects of phyto-oestrogens. The genomic effect involves OR and activation of the ORE, whereas non-genomic actions involve membrane receptors and cytosolic enzymes.

Discussion

These more recent investigations of the oestrogenic activities of the isoflavones, either as partial oestrogen agonists or anti-oestrogens (inhibitors of natural oestrogen activity), are clearly relevant to bone health and disease. Soyabean isoflavones, mainly genistein and daidzein, have now been shown by at least four different laboratories to conserve bone in OVX-rodent models. They probably have similar bone conservatory effects in higher mammalian species. The phyto-oestrogen coumestrol, which is not commonly consumed by humans, may be more potent than the soyabean isoflavones in preserving bone mass. Clearly the application of phyto-oestrogens for the prevention and treatment of osteoporosis is approaching.

These published reports support the concept that a threshold dose of isoflavones needs to be consumed for a sufficiently long period of time, i.e. months to years depending on the species, before any measurable effects on bone mass and density can be observed. A further

complexity appears to be that consumption of higher doses does not convey further benefit of isoflavones, with higher doses possibly being less effective. Although it is possible that extremely high doses may even have adverse effects, such events are not likely from food sources alone. However, the increasing availability of isoflavone-enriched food supplements and purified phyto-oestrogens in capsule form should be viewed with caution because of our current lack of knowledge of the potential toxicity of these molecules. Greater information from human studies currently in progress, however, is needed before the efficacy of these preparations in human subjects is known. The use of soyabeans and their products make this approach, as opposed to drug formulations that preserve bone, more attractive to many post-menopausal women.

The consumption of practically any soyabean product means that isoflavones will also be consumed from these foods in amounts that depend on the degree of processing (see Fig. 1). The doses of phyto-oestrogens that are effective in *in vitro* studies are approximately 10^{-8} M, but these concentrations are difficult to translate to either animal models or human studies. Nevertheless, the only positive effects of phyto-oestrogens on bone observed so far in post-menopausal women have been small and limited to the lumbar vertebrae. Although data have not yet been published from several human studies in progress, additional evidence for skeletal benefits of soyabean isoflavones is anticipated in the next few years. It may also be necessary to accumulate data from studies lasting decades to assess the effects of lifelong intakes of soyabean products at levels similar to those of high-consuming Asian populations.

Soyabean intake and cancer risk

Within the past few years soyabean foods and certain soyabean components have attracted considerable research attention for their potential role in cancer prevention and treatment. A critical early development in this field was the conclusions of a workshop by the National Cancer Institute, USA, in 1990 which led to the Institute allocating significant funds toward investigating the anticancer potential of soyabean components (Messina & Barnes, 1991). Understandably, initial interest in the anticancer effects of soyabean focused primarily on breast cancer. Early enthusiasm for this relationship was based largely on three primary observations: (1) the low breast cancer mortality rates in Asian, soyabean food-consuming countries; (2) animal work showing that soyabean inhibits chemically induced mammary carcinogenesis (Barnes *et al.* 1990); and (3) the potential anti-oestrogenic effects of soyabean isoflavones.

A comprehensive review by Messina *et al.* (1994) noted that the evidence supporting the potential anticancer effects of soyabean, although far from conclusive, was by no means limited to breast cancer. Published data suggest that soyabean may reduce the risk of a variety of cancers and, in particular, cancer of the prostate. Consequently, this section on cancer focuses primarily on breast and prostate cancer, and in particular the potential anticarcinogenic effects of the soyabean isoflavones. Although several different putative anticarcinogens have been identified in soyabeans, including phenolic acids (Ramakrishna *et al.* 1989), phytosterols (Rao & Janezic, 1992), protease inhibitors (Bowman Birk Inhibitor and Kunitz inhibitor; Kennedy & Manzone, 1995), inositol hexaphosphate (Graf & Eaton, 1993), saponins (Koratkar & Rao, 1997), and melanoidins (miso) (Kamei *et al.* 1997), the evidence most strongly supports a role for the isoflavones in the hypothesized anticancer effects of soyabean.

Isoflavones as anticarcinogens

As noted previously, three primary aglycone isoflavones are present in soyabeans: genistein, daidzein and glycitein. Glycitein is present in relatively small amounts and little is known about its biological activity, although it has been shown to be a weak oestrogen (Sonn *et al.* 1997). The isoflavone daidzein has exhibited anticancer effects both *in vitro* (Hirano *et al.* 1989; Higashi & Ogawara, 1994) and in animal models (Jing *et al.* 1993); however, the greatest focus has been on genistein. Recently, the Chemopreventive Branch of the Division of Cancer Prevention and Control, National Cancer Institute, National Institute of Health in the United States published its clinical development plan for genistein (Chemopreventive Branch and Agent Development Committee, 1996).

Literally hundreds of *in vitro* studies show that genistein can inhibit the growth of a wide range of both hormone-dependent and hormone-independent cancer cells with an IC_{50} of between approximately 5×10^{-6} and 10^{-4} M (approximately $2-25\ \mu g/ml$). Genistein has been shown to be effective in inhibiting growth of breast cancer cell types and many others (Table 3). In addition, genistein has been shown to inhibit the metastatic activity *in vitro* of both breast (Scholar & Toewa, 1994) and prostate cancer cells (Santibáñez, 1997), independent of effects on cell growth.

In a variety of cell lines, including cells derived from human gastric cancer (Yanagihara, 1993), oestrogen-receptor positive and negative human breast carcinoma cell lines (Shao *et al.* 1998), leukaemic cells (Spinozzi, 1994), melanoma cells (Rauth *et al.* 1997), and colon cancer cells (Kuo, 1996), genistein arrested cell cycle progression at G_2-M. These studies also showed that the addition of genistein to cancer cells induces differentiation and apoptosis.

The precise mechanism(s) by which genistein inhibits the growth of cancer cells has not been identified, but several have been proposed. Although the antioxidant properties of geni-stein (Wei *et al.* 1993) may contribute to the anticancer effects observed *in vitro*, it is more likely that these effects result from the ability of this isoflavone to modulate multiple processes associated with neoplastic transformation (Table 4). In addition to these effects, Peterson *et al.* (1996) reported that genistein increased the *in vitro* concentration of transforming growth factor beta.

Soyabean intake and breast cancer

As noted earlier, in addition to the low breast cancer mortality rates in Asian countries, much of the enthusiasm for the anticancer effects of soyabean foods was because weak oestrogens,

Table 3. Selected cancer cell types inhibited by genistein *in vitro*

Cell type	Reference
Breast cancer	Peterson & Barnes, 1991, 1996
	Pagliacci *et al.* 1994
Prostate cancer	Peterson & Barnes, 1993
	Naik *et al.* 1994
	Kyle *et al.* 1997
Melanoma	Rauth *et al.* 1997
Leukaemia	Makishima *et al.* 1993
Gastric cancer	Matsukawa *et al.* 1993
Colon cancer	Kuo, 1996

Table 4. Multiple processes associated with neoplastic transformation that may be modulated by genistein

Protein target of genistein	Reference
Tyrosine-specific protein kinase	Akiyama *et al.* 1987
Mitogen-activated protein (MAP) kinases	Thorburn & Thorburn, 1994
DNA topoisomerase II (*EC* 5.99.1.3)	Constantinou *et al.* 1990
Epidermal growth factor-induced phosphatidylinositol turnover	Imoto *et al.* 1988
S6 kinase activation	Linassier *et al.* 1990

including isoflavones, have been posulated to function as anti-oestrogens *in vivo* (Geynet *et al.* 1972; Martin *et al.* 1978). On a molar basis, relative to physiological oestrogens, isoflavones are quite weak, possessing between 1×10^{-4} and 1×10^{-3} the activity of 17β-oestradiol (Folman & Pope, 1966; Mayr *et al.* 1992; Markiewicz *et al.* 1993). Although data are conflicting on this point, limited support exists for the notion that isoflavones are anti-oestrogenic. In fact, more than 30 years ago Folman & Pope (1966) demonstrated in female mice that subcutaneous injection of genistein inhibited oestrone-stimulated increases in uterine weight. More recently, genistein (Wang *et al.* 1996) and equol (Sathyamoorthy & Wang, 1997) were shown to inhibit oestradiol-stimulated pS2 mRNA expression in MCF-7 cells. In addition, genistein, daidzein and equol were found to down-regulate oestrogen receptor mRNA expression.

The prevailing hypothesis has been that isoflavones exert anti-oestrogenic effects when placed in a high oestrogen environment, such as exists in premenopausal women, and oestrogenic effects in a low oestrogen environment, such as in postmenopausal women. This hypothesis has some support; for example, Mäkelä *et al.* (1995) found that soyabean consumption led to an increase in uterine weight in OVX mice not given the synthetic oestrogen diethylstilbestrol, but to a decrease in uterine weight in mice given diethylstilbestrol (Fig. 9). Also, Foth & Cline (1998) found that soyabean-feeding partially antagonized the stimulatory effect of oestrogen on endometrial and breast cancer tissue in OVX macaques, although soyabean had slight stimulatory effects on proliferation in the breast tissue of animals not given oestrogen.

Another potentially important observation related to the anti-oestrogenic hypothesis was reported by Cassidy *et al.* (1994, 1995), who found that premenopausal women consuming 60 g textured soyabean product (containing 45 mg isoflavones) experienced a 2.5 d increase in the length of their follicular phase, whereas no change was noted in women fed on a similar amount of soyabean from which the isoflavones had been chemically removed (Cassidy *et al.* 1995). Particularly noteworthy is the finding that serum follicle stimulating hormone and luteinizing hormone levels decreased significantly in response to the consumption of soyabean isoflavones.

Findings that soyabean increases follicular phase length may be quite relevant to the soyabean–breast cancer hypothesis because some data indicate that longer cycles, which are typical of Asian women, are associated with a reduced risk of breast cancer (Olsson *et al.* 1983; Yuan *et al.* 1988). However, Wu & Pike (1995) have pointed out that in the studies by Cassidy *et al.* (1994, 1995), serum oestrogen levels were not decreased, whereas serum oestrogen levels are typically lower in Asian compared with western women. In contrast to Cassidy *et al.* (1994, 1995) however, Lu *et al.* (1996*a*) observed an increase in menstrual cycle length in response to soyabean consumption and a decrease in serum oestrogen levels. The difference between the studies is perhaps a result of the much higher isoflavone intake in the latter study. To complicate this issue further, however, two recent studies failed to find an effect of soyabean on menstrual cycle length or oestrogen levels (Duncan *et al.* 1998; Haggins *et al.* 1998).
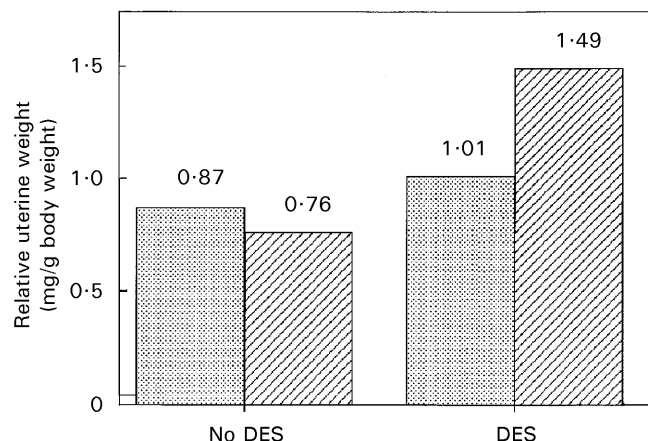
Fig. 9. Relative uterine weight of mice fed on a soyabean-containing or soyabean-free diet with or without diethylstilbestrol (DES). Outbred Han-NMRI mice were fed on either an Ewos R3 diet that contained roasted soyabean meal (70 g/kg diet) (▦) or the equivalent soyabean-free non-purified diet (Ewos R403) in which casein was substituted for soyabean meal (▨). Diets were with (6 μg/kg) or without DES. The uterine weight of animals fed on soyabean without DES (n 32) was significantly higher than the uterine weight (n 33) of animals not fed on soyabean. The uterine weight of the animals (n 18) fed on soyabean and DES was significantly lower than the uterine weight of animals (n 22) fed on DES but not on soyabean. Statistical significance (two-way ANOVA) of effect of dietary soyabean, $P < 0.05$; effect of DES, $P < 0.001$; interaction between dietary soyabean and DES, $P < 0.001$. Adapted from Mäkelä *et al.* (1995).

Although some evidence suggests that isoflavones are anti-oestrogenic under some conditions, other research supports a primarily oestrogenic effect of isoflavones (Mäkelä *et al.* 1994; Dees *et al.* 1997; Santell *et al.* 1997; Zava & Duwe, 1997). Importantly, genistein has a biphasic effect on oestrogen receptor-positive breast cancer cell growth *in vitro*; at micromolar concentrations growth inhibition occurs, whereas modest growth stimulation occurs at lower concentrations. The lower concentrations, however, may more accurately reflect those likely to occur *in vivo* (Zava & Duwe, 1997). Consistent with these findings is work by Santell *et al.* (1997), who found that genistein increased uterine weight in OVX Sprague-Dawley rats without inhibiting the increase in uterine weight caused by oestradiol administration.

Finally, two studies involving premenopausal women concluded that soyabean exerts oestrogenic effects on breast tissue. In one, nipple aspirates of secreted breast fluids, which have been shown to be an indicator of breast cancer risk by Wrensch *et al.* (1991), increased in response to soyabean consumption over a 4-month period, as did the number of atypical cells in the breast fluid (Petrakis *et al.* 1996). In the other study, McMichael-Phillips *et al.* (1996) found that the rate of DNA synthesis by breast cells taken from biopsies of normal breast tissue from women with benign or malignant breast disease was enhanced by 2 weeks of soyabean feeding. Although increased cell proliferation has traditionally been regarded as a factor increasing cancer risk, recent work with the non-steroidal anti-inflammatory drug, sulindac, calls this notion into question. Sulindac increases colon cell proliferation in mice but decreases chemically-induced colon tumours (Moorghen *et al.* 1998). In the case of soyabean, perhaps the increase in cell proliferation is a compensatory response to an increase in apoptosis, as high doses of genistein have been shown to induce apoptosis *in vitro* (Shao *et al.* 1998).

Although the clinical implications of these studies in premenopausal women are under debate, when the *in vitro*, animal, and human data are considered, it is difficult to conclude that soyabean and/or isoflavones are necessarily anti-oestrogenic in premenopausal women. As noted previously, however, genistein inhibits the growth of both oestrogen receptor-positive and-negative breast cancer cells (Peterson & Barnes, 1991). Thus, even without exerting anti-oestrogenic effects, soyabean/genistein may decrease breast cancer risk through non-hormonal mechanisms (see earlier and Fig. 5).

Epidemiology. Although several epidemiological studies have considered the relationship between soyabean intake and breast cancer risk, most were conducted before the increased interest in the potential anticancer effects of soyabean foods. Consequently, especially in the earlier studies, some concern exists about the completeness of the dietary soyabean data. Not surprisingly, nearly all of the epidemiological studies were conducted in Asia.

The first published study, conducted in Japan, was a case–control study involving 212 cases and equal numbers of hospitalized and neighbourhood controls, that was actually designed to examine the effects of dietary fat on breast cancer risk (Hirohata *et al.* 1985). No association was noted between fat intake from soyabean products and cancer risk, although specific information on soyabean intake was not provided. In a prospective study in Hawaii involving nearly 7000 subjects, Nomura *et al.* (1978) used the soyabean intakes of the men in this cohort as a surrogate for their wives' intakes to examine the effect of soyabean on breast cancer risk. Dietary uptake was recorded during two different time periods, 1965–8 and 1971–5. No association during the first time period was found, but during the second period, an inverse association was noted between intake of miso soup and cancer risk and a trend for a decreased risk for tofu intake. In agreement with these findings, in a prospective study, Hirayama (1986) found a significant graded inverse association between the intake of soyabean paste soup and subsequent risk of breast cancer among 142 857 women in Japan followed for 17 years.

More recently, a case–control study by Lee *et al.* (1991) in Singapore (*n* 200 cases, 420 controls) found that the regular consumption of soyabean foods was associated with a marked decreased risk of breast cancer in premenopausal women (upper fifth *v.* lowest fifth of the intake of total soyabean products; odds ratio 0·39, 95 % CI 0·19, 0·77; $P < 0.02$), but not in postmenopausal women. Similarly, a Japanese case–control study (*n* 1186 cases, 23 163 controls) also found that soyabean intake (tofu intake $\geqq 3$ times/week *v.* < 3 times/week) was associated with a decreased risk of breast cancer in premenopausal women (odds ratio 0·81, 95 % CI 0·65, 0·99; $P < 0.05$), but not postmenopausal women (Hirose *et al.* 1995). In contrast to these studies, a case–control study involving two different locations in China, Shanghai (*n* 534 cases, 534 controls) and Tianjin (*n* 300 cases, 300 controls), failed to find an association between soyabean food intake and breast cancer risk in either pre- or postmenopausal women (Yuan *et al.* 1995).

The only case–control study (*n* 596 cases, 958 controls) thus far conducted in the USA to examine the relationship between soyabean intake and breast cancer risk found that tofu consumption was protective in both pre- (adjusted odds ratio 0·67) and postmenopausal Asian women (adjusted odds ratio 0·60) (Wu *et al.* 1996). However, the overall intake of tofu among the subjects in this study was relatively low; the highest quartile of intake included women who consumed tofu as little as fifty-five times/year. Also, tofu was protective primarily in Asian women born in Asia who migrated to the West and not in Asians born in the USA (Wu *et al.* 1998). Finally, in the prospective Iowa Women's Study involving over 34 000 women, after 8 years of follow-up, tofu intake was associated with a modest decrease (adjusted relative risk 0·76; no consumption *v.* any consumption) in postmenopausal breast cancer risk. This differ-

ence was not statistically significant ($P = 0.22$) (Greenstein *et al.* 1996). Not unexpectedly, however, only 2.9 % of the cohort reported eating any tofu.

In addition to the limited number of epidemiological studies that quantified soyabean intake, Ingram *et al.* (1997), in a study involving 144 pairs of cases and population controls matched for age and area of residence, found an inverse relationship between the risk of both pre- and postmenopausal breast cancer and the urinary excretion of isoflavonoids (three 24 h collections; for cases, urine samples were collected before surgery). After controlling for age at menarche, alcohol consumption and fat intake, the adjusted odds ratio (lowest *v.* highest quartile) for equol, an isoflavan, was 0.27 ($P = 0.009$). However, urinary excretion of genistein was not measured and the interassay CV for the control urine pool sample for the individual phyto-oestrogens ranged from 10.9 % to 42.6 %. Also, no markers were included to assess the completeness of urine collections. Perhaps more importantly, based on the urinary collection of daidzein and equol, soyabean consumption even in the highest quartile of intake was quite modest.

Animal studies. Several animal studies have examined the effect of diets containing soyabean isoflavones on experimental mammary cancer (Table 5); four studies used diets that included soyabean-protein isolate (> 90 % soyabean protein), four non-fermented, whole soyabean products, two miso, and two isoflavones. Overall, these studies provide modest support for a protective effect of soyabean/isoflavones.

Of the four studies using soyabean protein isolate, two found a protective effect (Barnes *et al.* 1990; Hawrylewicz *et al.* 1991), whereas two did not (Carroll, 1975; Hsueh & Park, 1990). However, neither study in which protective effects were observed reported a reduction in tumour incidence. Also, in one of those studies (Hawrylewicz *et al.* 1991) the addition of methionine to the soyabean diet eliminated much of the difference in tumour number, although tumour volume was still significantly reduced.

Each of the four studies that used non-fermented, whole soyabean products reported protective effects (Troll *et al.* 1980; Gridley *et al.* 1983; Barnes *et al.* 1990; Gotoh *et al.* 1998*b*). In the study by Troll *et al.* (1980), however, raw soyabeans were used and the body weight of soyabean-fed rats was 10 % lower than that of control animals. Also, no effects on tumour incidence were found in any of the other three studies. In the study by Barnes *et al.* (1990), although tumour number was reduced by approximately 50 %, no dose response was observed among the three different diets containing 50, 100 and 200 g soyabean/kg diet). Furthermore, the reduction of tumour number in the study by Gotoh *et al.* (1998*b*) appeared to be a function primarily of a delay in tumour appearance. Finally, although Gridley *et al.* (1983) found that textured soyabean protein did slightly increase the latency period for spontaneous tumours, final cancer incidence was not affected.

Two studies incorporated the fermented soyabean product, miso, into their laboratory diets. In one study, Baggott *et al.* (1990) found that the number of dimethylbenz(a)anthracene-induced mammary tumours was decreased by approximately 30 % in rats fed on a diet containing 200 g miso/kg diet. In the other study, by Gotoh *et al.* (1998*b*), a diet containing 100 g miso/kg diet reduced N-nitroso-N-methylurea (MNU)-induced mammary tumour number by about 50 %. As pointed out earlier, however, tumour inhibition appeared to be related to a delay in tumour appearance in the rats consuming whole soyabeans. In a follow-up study (Gotoh *et al.* 1998*a*), a diet containing 100 g miso/kg diet was again shown to be effective in reducing tumour number by about 50 %. In addition, tamoxifen also significantly reduced tumour number, and the combination of tamoxifen and miso acted synergistically to inhibit tumour development. This combination significantly reduced both tumour incidence and number. The authors speculated that the increase in the number of oestrogen receptors in the tumour

Table 5. Effects of soyabeans, soyabean products and isoflavones on mammary cancer in laboratory rodents*

Reference	Induction method	Treatment groups	Results and comments
Troll *et al.* 1980	X-irradiation	Control Raw soyabeans (500 g/kg diet)	Tumour incidence: control 70 %, soyabean 44 %; $P < 0.01$. Soyabean-fed animals weighed less than controls (265 v. 331 g).
Gridley *et al.* 1983	Spontaneous	Milk protein (110 or 330 g/kg diet) Beef protein (110 or 330 g/kg diet) Fish protein (110 or 330 g/kg diet) Soyabean TVP (110 or 330 g/kg diet)	Tumour latency ranged from 48 to 84 d, no significant differences.
Barnes *et al.* 1990	MNU	Control Powdered soyabean chips (50, 100 or 200 g/kg diet)	Tumours per rodent: control 7·9, 50 g soyabean/kg diet 3·5, 100 g soyabean/kg diet 5·3, 200 g soyabean/kg diet 4·2. Values estimated from the figures presented. No statistics presented. Latency was increased in soyabean-fed groups. Tumour incidence not affected.
Gotoh *et al.* 1998*b*	MNU	Control Soyabeans (20 or 100 g/kg diet)	Tumours per rat (100 g soyabean/kg diet 1·1, control 2·2; $P < 0.05$). No statistical differences in incidence.
Carroll, 1975	DMBA on day 50, 60, or 75	Control SPI	Tumours per group ranged from 16 to 34 with no significant differences for soyabean v. control. Day refers to time DMBA was administered.
Hsueh & Park, 1975	DMBA	Control SPI	No significant difference in tumour incidence (control 85 %, SPI 79 %)

Barnes et al. 1990	DMBA	Control SPI (100 or 200 g/kg diet)	Tumours per rat (100 g SPI/kg diet 2·3, 200 g SPI/kg diet 2·3, control 3·6. Values were estimated from the figures presented. No statistics presented. Latency was increased in soyabean fed groups. Incidence was not affected.
Hawrylewicz et al. 1991	MNU	Control SPI (190 g/kg diet)	Tumour incidence (control 80 %, SPI 42·3 %; $P < 0.01$). Latency (control 93 d, SPI 125 d; $P < 0.05$). Tumours per rat (control 1·6, SPI 0·8; not statistically different).
Baggott et al. 1990	DMBA	Control Miso (200 g/kg diet)	Tumour incidence (control 100 %, miso 80 %; $P = 0.05$). Latency (control 78 d, miso 106 d; $P = 0.04$). Tumours per rat (control 4·1, miso 2·95; not statistically different).
Gotoh et al. 1998a,b	MNU	Control Miso (100 g/kg diet)	Tumours per rat (control 2·2, miso 1·2; $P < 0.05$). Incidence (control 80 %, miso 60 %; not statistically different).
Constantinou et al. 1996	MNU	Control Genistein (4 mg/kg BW) Daidzein (4 mg/kg BW)	No significant differences in tumour incidence or tumours per rat.
Murillo et al. 1998	MNU	Control Genistein (20 mg/kg BW)	Data presented are for palpable tumours. No difference between groups.

TVP, textured vegetable protein; MNU, N-nitroso-N-methylurea; DMBA, 7,12-dimethylbenz(a)anthracene; SPI, soyabean protein isolate; BW, body weight.
*All studies presented above used Sprague-Dawley rats except for Gridley et al. (1983) which used C3H/HeJ mice.

increased the hormone dependency of the tumours, thereby potentiating the effects of the anti-oestrogen, tamoxifen. Furthermore, in a study designed to examine possible therapeutic effects, the combination of tamoxifen and miso significantly inhibited tumour size, when administered after tumours had already formed, in comparison with the control and tamoxifen groups. Interestingly, Baggott *et al.* (1990) attributed the protective effects of miso to the high Na content of this food.

An additional two studies examined the effects of isolated isoflavones on chemically-induced mammary cancer in mature animals. Constantinou *et al.* (1996) found that neither isolated genistein (0·8 mg/d) nor daidzein affected MNU-induced mammary tumour incidence. Although both isoflavones reduced tumour number by approximately 20 %, this reduction was not statistically significant. Similarly, Murillo *et al.* (1998) found that genistein had no effect on MNU-induced mammary carcinogenesis. When genistein was combined with the synthetic isoflavone, ipriflavone, which also was ineffective by itself, tumour number was reduced by approximately 50 %.

In contrast to the unimpressive effects of isoflavones in adult animals, several reports have shown that early exposure, during either the neonatal or prepubertal period of life, to genistein (subcutaneous or dietary administration) markedly inhibits the development of dimethylbenz(a)anthracene-induced mammary tumours in rodents and increases the latency period (Brown & Lamartiniere, 1995; Lamartiniere *et al.* 1995; Murrill *et al.* 1996). Genistein appears to reduce carcinogenesis by stimulating mammary development and making this tissue less sensitive to the carcinogenic effects of dimethylbenz(a)anthracene. Finally, genistein (0·75 g/kg diet) was found to stimulate the growth of MCF-7 (human breast cancer cell line) cells implanted into OVX athymic mice (Hsieh *et al.* 1996). The growth of oestrogen-independent MDA-MB-231 cells, a human breast cancer cell line, however, was inhibited by a high (3 g/kg diet), but not a low (0·75 g/kg diet), dose of genistein, although food intake (but not body weight) was reduced by 10 % at the higher level of genistein (Santell *et al.* 1998).

Overall strength of the evidence for breast cancer. Although an intriguing hypothesis, the scientific evidence is only modestly supportive that the adult consumption of soyabean reduces breast cancer. The limited epidemiological data are somewhat supportive of the hypothesis, but mostly for premenopausal breast cancer. In fact, one case–control study (Wu *et al.* 1996) found soyabean intake to be inversely related to risk of postmenopausal breast cancer whereas three studies that included four different populations did not (Lee *et al.* 1991; Hirose *et al.* 1995; Yuan *et al.* 1995). Furthermore, in the one study in which soyabean was protective against postmenopausal breast cancer, protection was limited primarily to Asian immigrants, not USA-born Asians. Although Ingram *et al.* (1997) found the urinary excretion of equol to be inversely related to breast cancer risk, it would appear that overall soyabean intake in that study was quite low. Also, unfortunately, in general in western populations it is difficult to control for all differences between soyabean food consumers and non-consumers that potentially affect breast cancer risk since significant amounts of soyabean foods are not commonly consumed. Perhaps soyabean intake contributes to the low breast cancer mortality rates in Asian countries, not simply because of the consumption of soyabean foods in adulthood, but because of the soyabean foods these women first consumed as young girls.

The most impressive evidence comes from the animal studies showing that exposure to genistein during the early stages of life reduces the later development of chemically-induced mammary cancer. However, findings from studies of adult animals in which isolated isoflavones were administered were not impressive. In general, those studies showing a reduction in carcinogenesis found only that tumour number, not tumour incidence, was affected. Also as discussed previously, there were concerns about many of the studies in which soyabean was shown to be protective.

Clearly, genistein inhibits both oestrogen-dependent and oestrogen-independent breast cancer cells *in vitro*. However, the genistein concentration required to inhibit cell growth may be higher than the concentration that can be achieved *in vivo* in humans consuming soyabean foods and is probably higher than the genistein concentration of free-living Japanese people. A plasma genistein concentration of 276 nmol/l was observed in free-living Japanese men (*n* 14) (Adlercreutz *et al.* 1993), but in humans fed on soyabean foods and isolated genistein, plasma genistein concentrations have reached the low micromolar level (Xu *et al.* 1994, 1995; King & Bursill, 1998). However, most of the genistein molecules in plasma are either bound to protein (Arnold *et al.* 1996; Martin *et al.* 1996) or conjugated with glucuronic acid (Adlercreutz *et al.* 1995) and are, therefore, likely to be biologically inactive. It is worth noting, however, that Peterson & Barnes (1996) found that much lower genistein concentrations are required to inhibit the growth of non-transformed human breast epithelial cells in comparison with breast cancer cells.

It is clear therefore that given the potent *in vitro* effects of genistein, and the protective effects shown by some animal and epidemiological studies, little question remains that the relationship between soyabean intake and breast cancer risk warrants further investigation.

Prostate cancer

Prostate cancer mortality rates vary markedly among countries. An interesting observation related to the occurrence of prostate cancer is that clinical prostate cancer rates vary much more than the rates of latent prostate cancer. For example, the incidence of clinical prostate cancer in the USA among white men is 10–15-fold higher than the Japanese rate, whereas the overall incidence of latent prostate cancer is only about 50 % higher (Yatani *et al.* 1989). Also, recent work suggests that the Japanese incidence of prostate cancer may actually be under-diagnosed by as much as 80 %, making the true ratio of incidence to mortality much higher in Japan than in the West (Shibata *et al.* 1997). These observations suggest that in some populations, such as the Japanese, the growth of prostate tumours is slower and/or that the onset of prostate tumours occurs later in life. Speculation abounds that the intake of soyabean foods may be one factor contributing to the low Japanese prostate cancer mortality rate, although the data in support of this hypothesis, while intriguing, are limited.

Epidemiological studies. Not surprisingly, limited human data exist upon which to evaluate the soyabean–prostate cancer hypothesis, as only a few epidemiological studies have been conducted in this area. In a Japanese cohort, no significant association between soyabean paste soup and prostate cancer risk (Hirayama, 1979) was noted nor was a consistent relationship for miso soup with prostate cancer evident when comparing cancer patients with patients with hyperplastic disease and controls (Oishi *et al.* 1988). In contrast, a prospective study by Severson *et al.* (1989) involving 7999 men in Hawaii who were followed for 18–21 years found that the consumption of tofu was associated with a markedly reduced risk of prostate cancer ([2]1/week *v.* [3]5/week; age- adjusted relative risk 0·35), although this difference did not quite reach statistical significance ($P = 0·054$), and the number of men with tumours in each of the tertiles was quite small.

Of potential relevance to the effects of isoflavones on prostate cancer risk is the finding that not only are isoflavone levels higher in prostatic fluid in men from soyabean food-consuming countries but that isoflavones appear to be concentrated by about twofold in the prostatic fluid relative to plasma (Morton *et al.* 1997). Also worthy of note is a recent report that significant apoptosis occurred in the prostatic specimen from a man with adenocarcinoma who had taken

isoflavones (160 mg/d) derived from red clover (*Trifolium pratense*) one week before surgery (Stephens, 1997). The red clover extract contained both genistein and daidzein as well as the methylated isoflavones, biochanin-A and formononetin, from which genistein and daidzein, respectively, are derived. Finally, in a short-term study by S Barnes (unpublished results), soyabean intake did not affect prostate-specific antigen levels in men with elevated prostate-specific antigen levels.

In vitro *effects of genistein.*   Genistein inhibits the growth of both androgen-dependent and androgen-independent prostate cancer cells *in vitro* (Peterson & Barnes, 1993; Naik *et al.* 1994; Kyle *et al.* 1997). Also, as noted previously, independent of cell growth, genistein has been shown to inhibit the metastatic potential of prostate cancer cells, an effect that is associated with a decrease in the tyrosine phosphorylation of an unidentified protein (Santibáñez *et al.* 1997).

In addition to the effects of genistein on signal transduction noted previously, other mechanisms by which genistein/isoflavones could reduce prostate cancer risk also are operating. For example, oestrogens have been used successfully as a form of hormone therapy for metastatic prostate cancer (Pienta & Esper, 1993); thus, theoretically, the oestrogenic effects of isoflavones may be protective. Also, some data indicate that in genital skin fibroblasts and benign hyperplastic prostate tissue, genistein inhibits the activity of 5α-reductase (*EC* 1.1.1.64), the enzyme that converts testosterone into dihydrotestosterone, which stimulates the growth of prostate tissue (Evans *et al.* 1995). Ross *et al.* (1992) have shown that biomarkers of 5α-reductase activity are higher in Caucasian and black men compared with Japanese subjects, a finding that may contribute to differences in prostate cancer rates between these populations. The *in vitro* data from Evans *et al.* (1995) are consistent with findings from Lu *et al.* (1996*b*), who reported that after one month of soyabean milk consumption (1·04 litres/d), serum levels of 3α,17β-androstanediol glucuronide, a metabolite of dihydrotestosterone, were significantly reduced.

Animal studies.   Only recently has the effect of soyabean on prostate cancer in laboratory animals been studied. One study relevant to this topic, but which did not involve prostate cancer *per se*, found that after 9 months of feeding a diet containing soyabean to Han-NMRI mice, the incidence of prostatic dysplasia, which may be viewed as a pre-neoplastic prostate lesion, was markedly reduced compared with the incidence in mice not fed on soyabean (30 % *v.* 80 %, $P < 0.04$) (Mäkelä *et al.* 1995). At 12 months, however, the difference between the two groups was reduced (64 % *v.* 86 %). These findings are consistent not only with the epidemiological data noted above, but also with the results of a study of MNU- induced prostate tumours in Lobund-Wistar rats (Pollard & Luckert, 1997). Rats fed on a diet containing soyabean, but with a very low level of isoflavones, had latency periods of 7·3 (pre-MNU group) and 9·3 months (post-MNU group), compared with 10·0 (pre-MNU group) and 10·6 months (post-MNU group) for animals fed on a diet containing soyabean with a high isoflavone content (Fig. 10).

Five studies were identified that examined the effect of soyabean/genistein on tumour development in rats implanted with prostate cancer cells (Naik *et al.* 1994; Wang *et al.* 1995; Schleicher *et al.* 1996; Zhang *et al.* 1997*a*; Zhou *et al.* 1998). Four of these studies, all of which have been published in abstract form only, reported protective effects. Zhang *et al.* (1997*a*) found that in rats fed on a diet containing soyabean flour (330 g/kg diet) and implanted with Dunning R3327 PAP (rat prostate adenocarcinoma) tumours, tumour growth was significantly retarded at 16 weeks compared with animals fed on the control diet. Similarly, Schleicher *et al.* (1996) found that genistein (50 mg/kg body weight), given under the skin in the dorsal scapular area every 12 h starting at the time of tumour cell transplantation, inhibited the development of
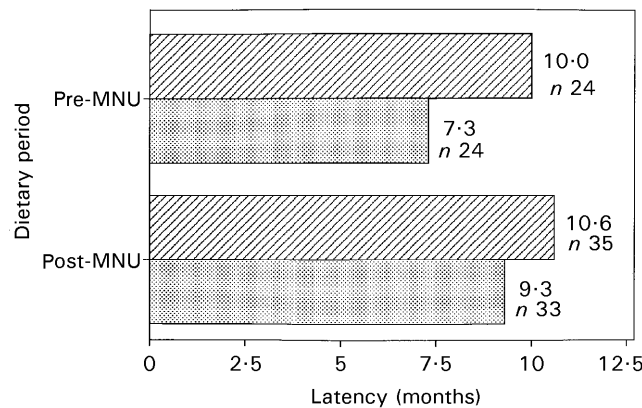
Fig. 10. Tumour latency period in rats fed on high and low isoflavone-containing diets. Rats were fed on a diet that included 200 g/kg soyabean protein isolate containing either 1·27 mg genistein and 0·42 mg daidzein per g protein (high isoflavone, (▨)) or 0·077 mg genistein and 0·025 mg daidzein per g protein (low isoflavone, (▦)) Tumours were initiated by inoculation with a single dose of N-nitroso-N-methylurea (MNU) followed by two subcutaneous implants of testosterone propionate. Diets were fed either before or following MNU administration. Trials were terminated 14 months after onset. Adapted from Pollard & Luckert (1997).

prostate tumours in rats implanted with prostate carcinoma cells. Rats given genistein developed fewer tumours and fewer invasive tumours and no genistein-treated animals developed lung metastases. In agreement with these studies, Zhou *et al.* (1998) found that the addition of an isoflavone rich concentrate to an AIN-76 diet (Anonymous, 1977) significantly reduced tumour volume in SCID (Severe, Combined ImmunoDeficiency) mice inoculated subcutaneously with 2×10^6 LNCaP (human prostate adenocarcinoma cell line) cells. Finally, Wang *et al.* (1995) found that dietary genistein (1 g/kg diet), when added to a high-fat (40·5 % of energy) diet, inhibited the growth of LNCaP cells implanted into nude mice.

Insight into a possible mechanism for the inhibitory effects of genistein against implanted prostate cancer cells comes from Dalu *et al.* (1996) who found that in Lobund-Wistar rats, dietary genistein (1 g genistein/kg diet) decreased the weight of the dorsolateral and ventral prostates and inhibited the expression of tyrosine-phosphorylated proteins. This study is the first to demonstrate that, *in vivo*, genistein inhibits a key cellular pathway. Related to this finding are those of Geller *et al.* (1998) who found that genistein inhibited the incorporation of [3H]thymidine (a measure of tissue growth) in a dose-dependent fashion (at concentration ranging from 1–15 µg/ml) by 44 % and 86 % in cultured benign prostate hypertrophy tissue.

In contrast to the favourable results discussed above, Naik *et al.* (1994) found that, although genistein inhibited prostate cancer cell growth *in vitro*, when Copenhagen rats were injected in the right flank with the metastatic MAT-Lylu prostate cancer cell line, oral doses of genistein of 0·07, 0·143 and 0·285 mg/kg body weight per d did not inhibit the development of prostate tumours. Higher doses of genistein (0·143, 0·285 and 0·428 mg/kg body weight per d) injected intraperitoneally also had little effect on tumour growth. The dietary levels used by Naik *et al.* (1994) were much lower than the levels used in the studies in which protective effects were observed, but they more closely approximate human dietary intake.

Overall strength of the evidence for prostate cancer.   The evidence that soyabean reduces prostate cancer risk is limited, but the available data tend to be more supportive of a benefit in terms of a reduction in prostate cancer risk than they are for breast cancer although this relationship is still quite speculative. Only a few epidemiological studies have examined this issue, and although one study did suggest that tofu intake was protective against prostate cancer, the association did not reach statistical significance (Severson *et al.* 1989). Several studies did find that soyabean/genistein inhibited the growth of prostate cancer cells implanted into rodents (Wang *et al.* 1995; Schleicher *et al.* 1996; Zhang *et al.* 1997*a*; Zhou *et al.* 1998), and one study found that genistein inhibited a key cellular pathway *in vivo* (Dalu *et al.* 1996). However, when a relatively low dose of genistein (in comparison with these studies) was used, Naik *et al.* (1994) did not observe growth inhibition *in vivo*. A very intriguing finding reported by Morton *et al.* (1997) is that, relative to plasma, isoflavones appear to be concentrated in the prostatic fluid. This difference in concentration suggests that the relatively high levels of genistein required to inhibit prostate cancer cell growth *in vitro* and *in vivo* may be applicable to humans consuming soyabean foods.

Discussion

The two primary observations of low breast and prostate cancer rates in Asian countries, where soyabean foods are consumed at high levels, and high isoflavone content of soyabeans raised initial interest in the relationship between soyabean food intake and cancer risk. However, only within the past 10 years has the diet–prostate cancer linkage been seriously investigated and establishing diet–cancer relationships has proved to be difficult, in part because many of the data relevant to the soyabean–cancer relationship are conflicting.

Overall, the evidence is more supportive for a protective effect against prostate cancer than breast cancer. In addition to the evidence already presented, several intriguing observations about the possible anticancer effects of soyabean/isoflavones related to breast, prostate, and other cancers make further research in this area compelling. For example, genistein has been shown to inhibit angiogenesis *in vitro* (Fotsis *et al.* 1993). Although the concentration of genistein initially reported to inhibit angiogenesis (IC_{50}, 150 μmol/l) is certainly much higher than the genistein concentration likely to be achieved *in vivo*, more recent work has shown that a much lower (IC_{50}, 8 μM) genistein concentration is effective in inhibiting angiogenesis (Adlercreutz & Mazur, 1997). A possible explanation for the higher genistein concentration required to inhibit angiogenesis in the earlier report may have resulted from incomplete solubilization of genistein in the media.

Results from several recent animal studies also suggest that soyabean/isoflavones may inhibit tumour metastases (Yan *et al.* 1997, 1998; Menon *et al.* 1998), including the metastases of human breast cancer cells (Connolly *et al.* 1997). In addition, daidzein has recently been shown to enhance immune function *in vitro* (Wang *et al.* 1997) and in animals (Zhang *et al.* 1997*b*), although Hendrich *et al.* (1998) did not find an effect of short-term (7 d) soya milk consumption on natural killer cell activity in young adults. Finally, results from a preliminary human study indicated that short-term soya milk consumption reduced lymphocyte DNA damage (Mitchell & Collins, 1998). Thus, a variety of potential mechanisms exists through which soyabean foods may inhibit the cancer process that are equally applicable to a variety of cancers (Table 3). Human studies in which the effects of soyabean consumption on biomarkers

of cancer risk are examined are sorely needed and more research in general is needed before anything approaching a firm conclusion can be made.

Overall conclusions

Investigations of phyto-oestrogens over the previous decade have demonstrated that these isoflavone molecules have diverse actions on mammalian cells, largely related to their oestrogen-mimetic properties.

Classical genomic actions of oestrogens have been used to explain the positive effects of these steroid molecules on both cardiovascular and bone tissues. Similarly, the potential for stimulation of growth of cancer cells in breast and uterine tumours is probably due to their effects on protein expression. Although the phyto-oestrogens were originally discovered because of their ability to mimic the actions of oestrogens on reproductive tissues in livestock, more recent research suggest that many potential effects of both oestrogens and phyto-oestrogens may be mediated by either cell-surface steroid receptors or molecules that belong to intracellular second-messenger systems. Understanding these alternative mechanisms of action will be critical for defining the role of phyto-oestrogens for both their beneficial and, potentially, adverse effects.

Human studies that are underway will probably demonstrate whether short-term consumption of phyto-oestrogens can produce health-promoting effects on tissue function and clarify effective doses for various organ systems. The epidemiological studies involving high soyabean-consuming Asian populations have been based on individuals with a lifetime pattern of daily intakes of phyto-oestrogen-containing foods from diverse soyabean products. The evidence that soyabean, with its phyto-oestrogens, is beneficial for cardiovascular diseases and its risk factors seems convincing. Likewise, the beneficial effects of soyabean phyto-oestrogens on bone are rather consistent, although longer term studies are required to be conclusive. Whether favourable effects of soyabean phyto-oestrogens on bone and cancer risk exist will require further information to develop a consensus.

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