

Review article

Phyto-oestrogens: where are we now?

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(Received 1 August 1997 – Revised 1 December 1997 – Accepted 18 December 1997)

Phyto-oestrogens have emerged from their esoteric role in animal husbandry following the hypothesis that the human Western diet is relatively deficient in these substances compared with societies where large amounts of plant foods and legumes are eaten. Evidence is beginning to accrue that they may begin to offer protection against a wide range of human conditions, including breast, bowel, prostate and other cancers, cardiovascular disease, brain function, alcohol abuse, osteoporosis and menopausal symptoms. Of the two main classes of these weak oestrogens, the isoflavones are under intensive investigation due to their high levels in soyabean. Like the 'anti-oestrogen' Tamoxifen, these seem to have oestrogenic effects in human subjects in the cardiovascular system and bone. Although previously only available from food, isoflavones are now being marketed in health-food supplements or drinks, and tablets may soon be available over the counter as 'natural' hormone-replacement therapy. In cancer, anti-oestrogenic effects are thought to be important, although genistein especially has been shown to induce wide-ranging anti-cancer effects in cell lines independent of any hormone-related influence. There are few indications of harmful effects at present, although possible proliferative effects have been reported. In infants, the effects of high levels in soya milk formulas are uncertain. The second group, lignans, have been less investigated despite their known anti-oestrogenic effects and more widespread occurrence in foods. Investigation of the possible benefits of phyto-oestrogens is hampered by lack of analytical standards and, hence, inadequate methods for the measurement of low levels in most foods. This problem may prove to be a major dilemma for regulatory authorities, clinicians and others wishing to advise the general public on whether these compounds really do have the health benefits attributed to them.

Phyto-oestrogens: Isoflavones: Lignans: Human disease risk

At least 12 200 natural substances, produced for structural, hormonal, attractant and chemo-protective purposes, have been identified in plant foods. They include the vitamins such as antioxidants vitamins C, E and folate, NSP (fibre), and the carotenoids, glucosinolates and flavonoids (National Research Council, 1996). These phytochemicals are of great interest because they could explain why diets containing large amounts of plant foods are associated with lower mortality and morbidity in adult life. Vegetarians, for example, have strikingly lower overall mortality rates than omnivores (Thorogood *et al.* 1994).

Within the flavonoid group are the isoflavones, phenols that are structurally similar to mammalian oestradiol (Setchell & Adlercreutz, 1988). These 'phyto-oestrogens' include daidzein and genistein which have been shown to bind to oestrogen receptors, albeit at comparatively low

levels (Shutt & Cox, 1972; Miksicek, 1995). Sheep grazing on Australian pastures containing a particular type of clover (*Trifolium* sp) rich in formononetin, which is converted to daidzein in the rumen during fermentation, developed a widespread infertility in the 1940s. This problem was traced to these non-steroidal weak oestrogens (Shutt, 1976). Soyabean is rich in daidzein and genistein and use of soyabean in captive cheetah (*Acinonyx jubatus*) in Cincinnati zoo was also shown to be responsible for an infertility syndrome, reversed by its removal from the feed (Setchell *et al.* 1987).

In common with many other weak oestrogens, in model systems the isoflavones have been shown to be anti-oestrogens, competing for oestradiol at the receptor complex, yet failing to stimulate a full oestrogenic response after binding to the nucleus (Tang & Adams, 1980). This

Abbreviations: SHBG, sex hormone-binding globulin.

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raises the possibility that they may be protective in hormone-related diseases, such as breast cancer (Setchell *et al.* 1984). The lignans enterolactone and enterodiol form another class of phenolic compounds. They are derived from colonic microbial fermentation of secoisolariciresinol and matairesinol in foods, and excreted in urine. Lignans have also been shown to bind to oestrogen receptors and to suppress oestrogen-stimulated responses (Waters & Knowler, 1982).

The significance of the structural similarity of the lignans and isoflavones to mammalian oestrogens and possible effects on cancer prevention were first promulgated in the early 1980s in publications of Setchell and of Adlercreutz (see Setchell & Adlercreutz, 1988). Since that time the literature on the possible health benefits of the isoflavones found predominantly in soyabeans has expanded exponentially, mainly in response to funding initiatives by the US government and soyabean industries, and more lately by European and UK Ministries of Food. Currently, there are several research fronts covering most of the major public health problems of Western societies. In addition to hormone-related and bowel cancers, phyto-oestrogens are under active investigation for their protective effects in other hormone-related conditions, such as menopausal symptoms, osteoporosis and heart disease.

The physiological effects of phyto-oestrogens have also created a marketing opportunity that has been utilized by industry, particularly in soyabean-producing countries such as the USA and Australia. Many 'health' supplements and drinks are now marketed as containing genistein, and tablets of extracts of isoflavones are also shortly to be marketed as 'natural' hormone-replacement therapies, available over the counter. These products will raise much public interest, requiring answers from the nutritional community.

The present status of knowledge in each of these areas will be discussed. Perhaps as a result of the economic importance of soyabean, and availability of research funds, most of the evidence relates to the isoflavones and soyabean. Despite their more widespread occurrence in foods, and greater consumption in Western populations, the lignans have received comparatively little attention.

Chemistry, analysis, food levels and metabolism

A major limitation to the study of metabolism of phyto-oestrogens in human subjects is the complexity of these substances, with at least fifteen different chemical forms of the isoflavones alone occurring in foods. In foods, the isoflavones are conjugated with a glucose (to form glycosides) and in soyabean, the major glycosides are daidzin, genistin and glycitin. These conjugated forms are not active oestrogenically (Miksicsek, 1995). Bacteria in the rumen of cows and sheep are known to deconjugate the glycosides to aglycones daidzein, genistein and glycitein, and some acid-hydrolysis occurs in the stomach (Kelly *et al.* 1993). The structure of these compounds is shown in Fig. 1.

The structure of two precursors of isoflavones that are important in clovers used as fodder crops are also shown in Fig. 1. In the sheep rumen, biochanin A is metabolized to

genistein, and formononetin is metabolized to daidzein. These two compounds do not occur in significant amounts in soyabean but they are the main isoflavones in extracts of clovers grown in certain soils, such as in Australia. Genistein is a known stimulant of N-fixing bacteria and high levels occur in these clovers probably because they are grown under N-limited conditions (Krishnan *et al.* 1995). N-fixation would also account for the importance of soyabean and other legumes as sources of isoflavones.

A further substance, coumestrol is found in lucerne (*Medicago sativa*), clovers, and at low levels in beans and peas. The structure of coumestrol is also shown in Fig. 1. Another substance with oestrogenic effects is zealalenone, produced by moulds contaminating cereal crops (Verdeal & Ryan, 1979).

The type of isoflavone differs according to how the soyabean is treated, and its origin. Japanese soyabean varieties contain different proportions of isoflavones to those grown in the USA, and fermented products, such as Miso, contain more unconjugated isoflavones rather than daidzin and genistin (Coward *et al.* 1993). Table 1 shows typical levels in soyabean flour, tofu and soyabean sauce (Reinli & Block, 1996). Low levels of some isoflavones are also found in other legumes, for example formononetin in mung beans, although absolute levels are uncertain due to analytical problems (see p. 395). Table 1 shows that levels in other foods are much lower than those in soyabeans.

Lignans are more widespread in plants where they form the building blocks of lignin in plant cell walls. Table 1 shows some estimated levels of precursors in foods (Thompson *et al.* 1991). Linseed (flax-seed) is a rich source.

No studies of the absorption of conjugated *v.* unconjugated isoflavones have been conducted in human subjects. Whilst it is generally thought that the glycoside cannot be absorbed, the structurally-related flavonoids (e.g. quercetin) are apparently better absorbed in the conjugated form, rather than unconjugated form (Hollman *et al.* 1995; Panganga & Rice Evans, 1997). After absorption in the small and probably large intestine, the isoflavones are re-conjugated (with sulfates and glucuronide) then excreted in urine and probably bile. Urinary and blood levels of isoflavones therefore increase with supplements of soyabean (see Table 2). However, only 7–30% of the ingested amount may be recovered in urine (Kelly *et al.* 1993; Cassidy *et al.* 1994; Xu *et al.* 1995). Blood levels increase within 30 min of consumption of a soyabean supplement, and begin to decline after 5 h ingestion, although elevated levels remain at 24 h (Morton *et al.* 1997).

The flora of the large bowel have another important role in the metabolism of the isoflavones, although in human subjects this is poorly documented. In the rumen of sheep, daidzein is converted to equol (see Fig. 1). Equol was shown to be the oestrogenic agent responsible for Clover disease in sheep, whereas genistein is metabolized to an inactive compound *p*-ethylphenol (Cox & Davies, 1988). However, in human subjects, genistein is found in human urine and may be the more metabolically-active compound (see p. 399). A number of other metabolites, including desmethylangolensin, are also excreted in human urine (Kelly *et al.* 1993). There is a wide individual variation in

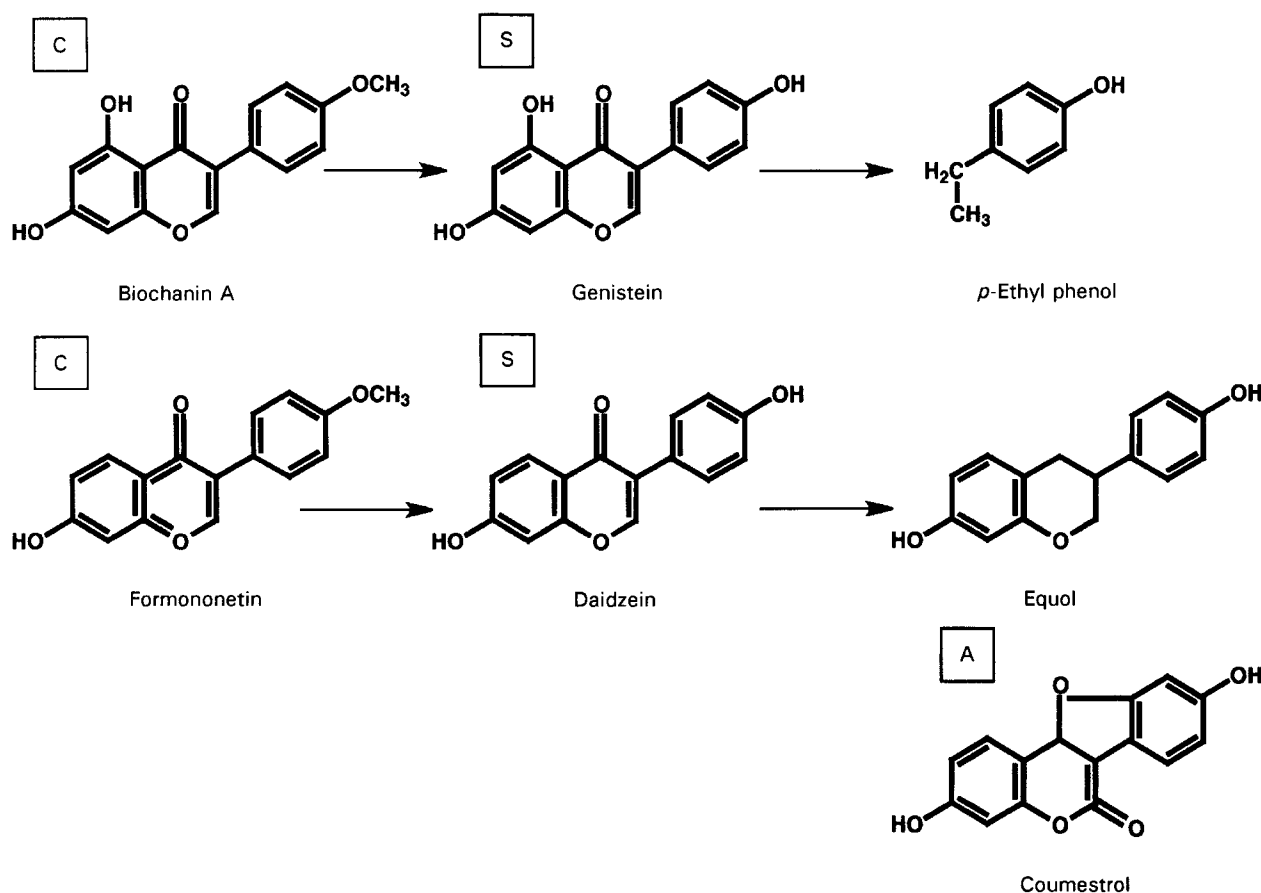


Fig. 1. Isoflavones occurring in foods and some of their metabolites. C, clover (*Trifolium* spp.); S, soyabean; A, lucerne (*Medicago sativum*).

Table 1. Approximate levels of isoflavones and lignans in foods

Food	Isoflavones* (mg/kg fresh wt)	Lignans† (mg/kg fresh wt)
Linseeds	0‡	600–3700‡
Soyabeans	1800	9
Tofu	2400	
Soyabean flour	1300–1650‡	1‡
Soya milk	450	
Soya sauce	150	
Peanuts		2
Brown rice		3
Whole wheat		5
Rye bread		12‡
Dried lentils		18
Dried beans	10–70	3
Fresh vegetables and fruit	1	1

* From Reinli & Block (1996), except where indicated.

† From Thompson *et al.* (1991), except where indicated.

‡ From Mazur *et al.* (1996)

the proportion of metabolites excreted, which may be a consequence of differential absorption of different isoflavones and probably individual differences in colonic flora species capable of metabolizing these compounds (Kelly *et al.* 1993). Some individuals do not produce equol, and limited studies suggest that high levels of complex carbohydrates, stimulating fermentation in the large bowel,

may result in greater breakdown of daidzein to equol (Cassidy, 1992).

Lignans also occur in plants, as secoisolariciresinol and matairesinol (Fig. 2), with glucose residues in the diglucosides attached to the phenolic or side-chain OH groups. During fermentation, the colonic bacterial flora remove the glucose groups and methyl groups to form the diphenols enterodiol and enterolactone which are structurally similar also to oestradiol (Fig. 2). After absorption, these also are excreted in urine. There are also comparatively limited studies of the metabolism of the lignans in human subjects. However, urinary levels of lignans increase substantially when supplements of linseed are fed to human subjects (see Table 2; Setchell *et al.* 1981; Lampe *et al.* 1994). There is also a moderate increase in urinary excretion following vegetable supplementation (Hutchins *et al.* 1995).

Apart from linseed and soyabean, where levels allow HPLC techniques for food analysis, few analyses of lignans and isoflavones in foods exist, although it is likely that they are not confined to plants. Soyabean is widely used in the food industry, and due to their content in milk, there are measurable levels of isoflavones in plasma from infants fed on cows' and human milk (Setchell *et al.* 1997).

GC-MS is accepted as the technique sufficiently sensitive for low levels (Mazur *et al.* 1996). However, before this, the compounds have to be hydrolysed and extracted from foods, when large losses can occur,

Table 2. Daily urine excretion (mg) of total isoflavones and lignans before and after supplementation with foods (Mean values and standard deviations)

Dietary regimen	Isoflavones		Lignans		Reference
	Mean	SD	Mean	SD	
Before soyabean*	0.01				Cassidy <i>et al.</i> (1994)
After 45 mg isoflavones as soyabean*	3.0				
Before soyabean*	2.5		2.8		Kelly <i>et al.</i> (1993)
After 72 mg isoflavones as soyabean*	17.4		2.3		
Before soyabean†	0.3	0.4	0.2	0.04	Karr <i>et al.</i> (1997)
After 36 mg as soyabean†	6.4	0.4	0.2	0.04	
Before linseed	0.6	0.33	0.9	1.25	Lampe <i>et al.</i> (1994)
After 10 g linseed	0.5	0.34	12.0	1.26	
Before fruit and vegetables	0.25	0.12	0.35	0.22	Hutchins <i>et al.</i> (1995)
After 600 g fruit, 310 g potatoes 165 g carrots	0.23	0.12	0.54	0.22	

* Group estimates of deviation not given.

† Mean of equal excreters and non-excreters given.

depending on the technique used and the isoflavone or lignan investigated. Several different solvents are in use, including ethanol, methanol, diethyl ether and acetone for extraction, and acid or enzymic methods for hydrolysis of glycosides. Not all methods incorporate internal standards, and deuterated standards, particularly of genistein, are insufficiently robust for the whole procedure, so that the losses encountered during extraction before GC-MS are unknown. As a result there is very little information available on isoflavones and the lignan precursors in foods, and most investigators have relied on urine and blood levels as markers of intake.

Table 3 shows some published information on urinary levels in different population groups (Adlercreutz *et al.*

1982, 1991). The pattern of excretion follows that of food intake, so that isoflavone excretion is only significant in Far Eastern populations eating soyabean. To achieve these urinary levels in Western populations, a macrobiotic diet has to be consumed. Urinary levels of lignans have been shown to be much greater than isoflavones in individuals consuming Western diets (Adlercreutz *et al.* 1991; Cassidy *et al.* 1991). This is a reflection of the fact that lignans are more widespread in foods than isoflavones, but there have been no formal studies of intake levels due to lack of information of levels of these compounds in foods. Messina (1995) has estimated Far Eastern intakes of isoflavones to be approximately 30 mg/d in Japanese populations. Studies to examine the hormonal effects of isoflavones in human

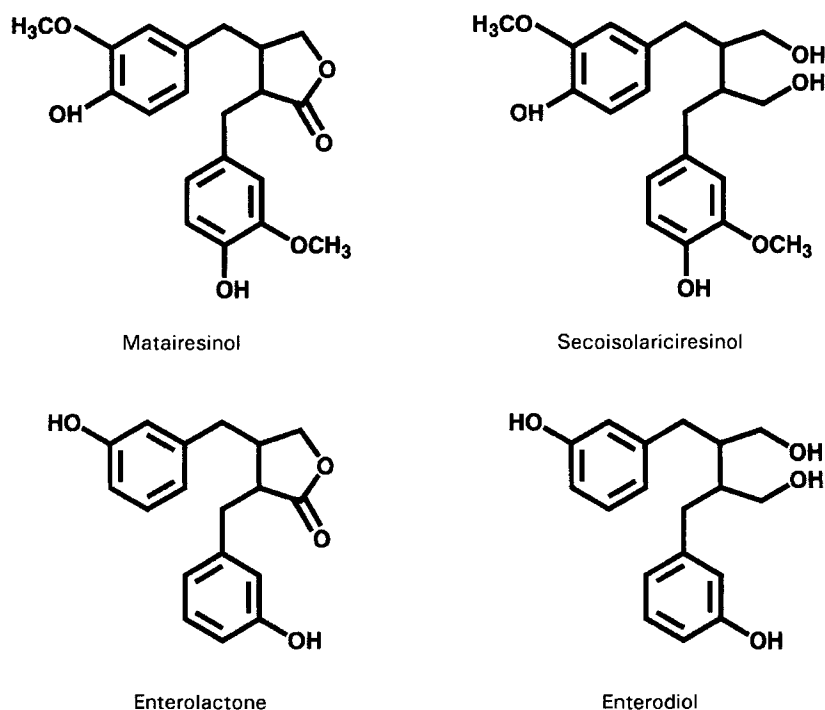
**Fig. 2.** Lignans occurring in foods and their metabolites in urine.

Table 3. Urine excretion (mg) of total isoflavone and lignans in different female populations (Mean values and standard deviations)

Group	Isoflavones		Lignans		Reference
	Mean	SD	Mean	SD	
Boston, USA; omnivores	0.11*		0.58		Adlercreutz <i>et al.</i> (1986)
Boston, USA; breast cancers	0.03*		0.31*		Adlercreutz <i>et al.</i> (1982)
Boston, USA; lactovegetarians	0.37*		1.23*		Adlercreutz <i>et al.</i> (1986)
Boston, USA; macrobiotic dieters	1.18*		5.99*		
Japanese	1.73	1.70	0.53	0.66	Adlercreutz <i>et al.</i> (1991)
Cambridge, UK; omnivores	0.05	0.07	0.22	0.26	Cassidy <i>et al.</i> (1991)

* No estimates of standard deviation given.

subjects have generally used amounts of soyabean which yield approximately 50 mg isoflavones.

Plant oestrogens and hormone-related conditions

Heart disease, osteoporosis, breast cancer, prostate cancer, and menopausal symptoms share a common epidemiology in that they are rare in Far Eastern populations eating traditional diets containing soyabean products compared with Western populations. However, with Westernization and loss of traditional eating patterns, the pattern of disease incidence is changing also in these countries.

Cross-sectional studies have shown higher phyto-oestrogen levels in urine (Table 3) and plasma of populations at lower risk of these diseases (Adlercreutz *et al.* 1982, 1991). However, there have been few detailed epidemiological studies relating intakes of phyto-oestrogens to individual risk of these diseases. For the time being, evidence that increased intakes would be beneficial is derived from experimental and physiological findings.

Breast cancer: physiological effects of diet

Breast cancer is the commonest cancer of women living in Western populations, and the incidence is rising in the UK (Coleman *et al.* 1993). The risk of breast cancer increases markedly with age, but its development is highly dependent on the hormones associated with ovarian function. (Bernstein & Ross, 1993). Although the rate of increase begins to slow after the menopause, events that occur premenopausally, and perhaps during adolescence, set the scene for later post-menopausal breast cancer. Established hormone-related risk factors include early menarche, late age at menopause, delayed age at first pregnancy, and, in post-menopausal women, elevated plasma free oestradiol concentrations. Family history is also important, although established genetic factors account for only about 4% of breast cancers in Western populations (Key & Pike, 1988; Toniolo *et al.* 1995; Berrino *et al.* 1996). The anti-oestrogen Tamoxifen is known to substantially reduce the risk of mortality from breast cancer by about 20% over 2 years and to reduce the incidence of cancer in the contralateral breast (Early Breast Cancer Trialists' Collaborative Group, 1988).

Hypotheses relating the international differences in breast cancer rates to diet have largely concentrated on fat, following the observation of Armstrong & Doll (1975) of very strong positive correlations between breast cancer and fat intake. A pooled analysis of twelve case-control studies found a significant elevation of relative risk with fat in post-menopausal breast cancer but not premenopausal breast cancer (Howe *et al.* 1990). Prospective studies, however, have not confirmed an effect of fat in adult life; a pooled analysis of seven prospective studies including data from 335 000 women found no evidence of a positive association between fat consumption and risk of breast cancer (Hunter *et al.* 1996). Intervention trials with low-fat diets have shown some effects on total or free oestradiol in post-menopausal women, although it is unclear whether these effects were attributable to changes in fat consumption or loss of body weight, which reduces sex hormone-binding globulin (SHBG) levels (Boyar *et al.* 1988; Prentice *et al.* 1990). There are no consistent effects of a low-fat diet in premenopausal women (Rose *et al.* 1987; Williams *et al.* 1989; Goldin *et al.* 1994).

More consistent effects in reducing oestrogen levels have been found when combined low-fat-high-fibre interventions were used (Woods *et al.* 1989; Rose *et al.* 1991; Crighton *et al.* 1992; Goldin *et al.* 1994). These effects are generally attributed to interference with the enterohepatic circulation of oestrogens and bile acids as a result of reduced β -glucuronidase (EC 3.2.1.31) production by microbial flora in the large intestine consequent upon increased levels of NSP (dietary fibre) entering the colon, together with increased binding of unconjugated steroids (Rose, 1991). It has also been proposed that NSP have independent effects on hormonal status on the basis of animal studies and a strong positive association between fibre intake and age of menarche in an ecological study (Hughes & Jones 1985; Rose, 1991; Arts *et al.* 1992). In case-control and prospective studies, adolescent cereal fibre intake has been related to age of menarche, plasma gonadotrophin and oestradiol levels and risk of breast cancer (Pryor *et al.* 1989; Ridder *et al.* 1991).

It has been suggested that these effects of fibre could have been brought about by phyto-oestrogens, since most fibre-containing foods are sources of lignans (Rose, 1991). However, there are no significant effects on serum oestradiol levels with lignans in premenopausal women (Phipps *et al.* 1993) and no consistent effects have been

found on pre- or post-menopausal serum oestrogens when supplements have been given in controlled interventions. One study with textured vegetable protein found an elevation of oestradiol in the follicular phase (Cassidy *et al.* 1994), another with soyabean milk found a depression of oestradiol throughout the cycle (Lu *et al.* 1996a) and another study with a soya drink caused an 'erratic' elevation throughout the cycle (Petrakis *et al.* 1996). No effects with soyabean were found in post-menopausal women (Baird *et al.* 1995; Petrakis *et al.* 1996).

It has also been suggested that the phyto-oestrogens would exert their effects via the stimulation of SHBG, reducing the proportion of free oestrogens circulating in plasma. This observation is based on cross-sectional comparisons and some studies with cell lines (Adlercreutz, 1995). However, SHBG is affected by, for example, changes in body weight, making cross-sectional comparisons difficult. In controlled intervention studies, there appear to be no effects of soyabean on SHBG levels in premenopausal women (Cassidy *et al.* 1994, 1995; Petrakis *et al.* 1996), nor in post-menopausal women (Baird *et al.* 1995; Petrakis *et al.* 1996). There is also no effect in men or premenopausal women receiving lignans (Shultz *et al.* 1991; Phipps *et al.* 1993). Hence, existing evidence to date suggests that a protective effect is unlikely to be brought about by lowering the levels of free oestrogens in plasma, since they do not seem to have a direct effect on serum oestradiol or SHBG levels.

Nevertheless, there is evidence for suppression of gonadotrophin output following consumption of phyto-oestrogens, with a lengthening of the human menstrual cycle. In sheep, coumestrol and genistein bind to receptors isolated from the pituitary and hypothalamus, although direct inhibition of gonadotrophins has not been shown (Mathieson & Kitts, 1980). In the first human study, soyabean supplements (as textured vegetable protein) containing 45 mg isoflavones/d were shown to suppress gonadotrophin output, increasing the length of the follicular phase of the menstrual cycle by 2–3 d (Cassidy *et al.* 1994). No effects were shown with supplements from which isoflavones had been removed (Cassidy *et al.* 1995). One early study showed no effects of soyabean substitution for meat in vegetarian women (Hill *et al.* 1986) but two later studies in non-vegetarian women have confirmed the effect of phyto-oestrogen containing foods on cycle length. Soya milk supplements induced a 3 d increase (Lu *et al.* 1996b), and linseed has also been found to increase the length of the luteal phase of the menstrual cycle, and to have no effects

on SHBG and serum oestrogen levels (Phipps *et al.* 1993). There is also evidence of effects on gonadotrophins in post-menopausal women (see p. 401).

Longer menstrual cycles would induce a lower lifetime exposure to oestradiol levels in the luteal phase which has been related to lower risk of both pre- and post-menopausal breast cancer (Henderson *et al.* 1985). These effects of soyabean in women are coupled with the fact that breast cancer rates used to be low and menstrual cycle lengths longer in Far Eastern populations where the traditional diet is rich in soyabean (Treloar *et al.* 1967). However, irregular rather than long menstrual cycles are generally related to lower breast cancer risk (Whelan *et al.* 1994; Henderson *et al.* 1985; den-Tonkelaar & de Waard 1996).

Two early prospective investigations reported significant protective effects of soyabean on breast cancer risk in Japanese women (Nomura *et al.* 1978; Hirayama, 1981). In a later case-control study in Singapore, a significant protective effect was shown in relation to pre-menopausal breast cancer risk (Lee *et al.* 1991). No relationship was shown in a case-control study in China, although post- and pre-menopausal risk were not studied separately (Yuan *et al.* 1995). The findings of Messina *et al.* (1994) in reviewing existing epidemiological studies are summarized in Table 4. In general, there is a trend towards a protective effect of soyabean in breast cancer, but these studies have generally used rather crude methods and did not distinguish between pre- and post-menopausal cancer.

Breast cancer: experimental studies

In vitro studies have established that phyto-oestrogens are weakly oestrogenic, since they have the ability to bind to mammalian oestrogen receptors to a low degree. Their affinity to receptors (from rabbit, sheep and rat uterine receptors, and a human cancer cell line) has been compared with oestradiol. Coumestrol has the greatest affinity, only ten to twenty times lower than oestradiol, and genistein about 100 times less; daidzein and equol bind about 1000 times less. Similar findings are evident when their ability to increase uterine weight of mice is studied (Verdeal & Ryan, 1979).

However, although these weak oestrogens bind to the oestrogen-receptor complex, they fail to stimulate a full oestrogenic response of replenishment of oestrogen receptors and protein synthesis. They have thus been characterized as antagonizing oestradiol and acting as both oestrogens and anti-oestrogens, at least in rats (Tang &

Table 4. Summary of soyabean and cancers in epidemiological studies (from Messina *et al.* 1994)

Type of cancer	Location of study	No. of studies published relating risk of cancer according to soyabean consumption			
		Total	Increased risk	No significant risk	Decreased risk
Breast	Japan, China, Singapore	5	0	2	3
Prostate	Japan, Hawaii	3	0	3	0
Colo-rectal	Japan, China, USA	8	2	3	3
Lung	China, Hong Kong	4	0	1	3
Stomach	Japan, China, Korea, Hawaii	14	1	6	7

Adams, 1980). Since they are eaten in comparatively high amounts, they are able, therefore, to interfere with the feedback system of the release of gonadotrophins and thus account for fertility problems in animals (Molteni *et al.* 1995). Enterolactone has also been shown to have anti-oestrogenic effects, suppressing rat uterine RNA synthesis induced by oestradiol (Waters & Knowler, 1982).

In animal models, soyabean products have generally reduced tumours induced by chemical carcinogens. Seven of nine studies have shown lower numbers of tumours in rats whose diet was supplemented with soyabean compared with those fed on a standard diet, and no study showed an increase in the numbers of tumours. These effects were not seen when feeding soyabean products which had been treated to remove the isoflavones (Barnes, 1995). The chemo-preventive effects are generally attributed to the anti-oestrogenic characteristics of isoflavones. In a careful study in which a purified laboratory diet free of isoflavones was fed, administration of genistein to newborn rats also reduced the numbers of tumours later induced by administration of carcinogen injections to fully-grown animals, perhaps because the early administration of genistein resulted in a more mature gland with less-susceptible structures to later initiation by the chemical carcinogen (Lamartiniere *et al.* 1995).

However, there are several other anti-cancer effects of isoflavones which are not related to their anti-oestrogenic properties. Genistein is known to inhibit tyrosine kinases, which are responsible for phosphorylating proteins required for the regulation of cell functions, including cell division. Hence, it has been shown to inhibit growth in many cell lines. These lines include those which do not have oestrogen receptors, which suggests that these effects may be independent of any anti-oestrogen effects. Genistein has also been shown to inhibit the DNA repair enzyme topoisomerase, and to act as an antioxidant, thus potentially preventing oxidative DNA damage (Peterson, 1995). In one cell line, genistein has been shown to cause changes characteristic of apoptosis, or programmed cell death, a protective mechanism induced in cells that have been damaged in order to prevent the proliferation of harmful mutations and possibly cancer (Pagliacci *et al.* 1994). It has also been shown to inhibit ras gene expression in a rat pheochromocytoma cell line (Nakafuku *et al.* 1992). In addition, genistein has been shown to inhibit angiogenesis, the formation of new blood vessels, an abnormal event which occurs as part of the growth and expansion of malignant tumours (Fotsis *et al.* 1993). It has been pointed out that many of these effects have been shown with very high concentrations, and not in cells treated with the levels likely to be achieved in plasma of human subjects eating foods containing phyto-oestrogens (Barnes, 1995); 100 µmol were needed for a significant suppression of angiogenesis for example, although proliferation was inhibited at 5 µmol levels and below (Fotsis *et al.* 1993). This compares with plasma levels of about 0.4 µmol/l in Japanese men and women fed on dietary supplements of isoflavones (Adlercreutz *et al.* 1993a,b; Morton *et al.* 1994), and peak levels of about 4 µmol/l in women receiving 126 mg isoflavones as soya milk, rising to 12 µmol/l when receiving 480 mg isoflavones/d (Xu *et al.* 1994, 1995).

Potential adverse hormonal effects of isoflavone supplementation

Despite the hypothesized beneficial effects of phyto-oestrogens in human cancer, two reports suggest that caution is necessary at this stage. In one study twenty-nine women took 60 g soyabean (containing 45 mg isoflavones) for 14 d and breast cell proliferation was assessed by [³H]thymidine labelling and the antigen Ki67 in biopsies before and after supplementation. Preliminary reports showed a significant increase in the proliferation rate of breast lobular epithelium (McMichael-Phillips *et al.* 1996). In another report, twenty-four women were studied for 6 months whilst taking a soya drink containing 38 mg genistein. Fluid was aspirated from the nipple of the breast for analysis of the cytology of epithelial cells, and to determine whether the volume and colour would be reduced, towards levels previously found in low-risk Japanese and Chinese women. Contrary to expectations, however, the risk factors increased, with an increased frequency of hyperplastic cells after soyabean supplementation (Petraakis *et al.* 1996).

Endometrial cancer risk is increased in women treated with the anti-oestrogen Tamoxifen, raising the possibility of a similar effect with phyto-oestrogens. However, there are no reports of increased risk associated with phyto-oestrogens. Incidence rates in Japan are somewhat lower than those in the UK (Parkin *et al.* 1992), and one report in Asian migrants to Hawaii found that soyabean consumption is associated with a reduced risk (Goodman *et al.* 1996).

There are, however, concerns about the long-term effects of phyto-oestrogens given to infants and young children. Coumestrol, found mainly in lucerne but also in clovers and at low levels in peas and beans, may behave differently to the isoflavones found mainly in soyabean. Coumestrol binds more strongly to oestrogen receptors, and may have more oestrogenic than anti-oestrogenic properties. When incorporated into the diets of lactating rats at low levels (100 µg/g; 50 mg human equivalent, i.e. amount which would have been consumed in an average human daily consumption of food of approximately 500 g), permanent oestrus was induced in female offspring and sexual activity was reduced in male offspring feeding on the supplemented milk (Whitten *et al.* 1995). No effects on the reproductive system of male and female prepubertal monkeys fed on high levels (approximately 600 mg human equivalents) of soyabean have been found over 3 months (Anthony *et al.* 1996).

In human subjects, breast milk contains comparatively low levels of soyabean phyto-oestrogens (equivalent to those in plasma; 20 ng/ml) but soya milk formulas contain at least ten times this amount (Bluck & Bingham, 1997). The possible effects of these relatively high levels in infancy are unknown, and currently breast milk or cows' milk formula are recommended for infant feeding, unless there is a clear indication that soya milk formula is required on medical grounds, for example in infants with cows' milk intolerance (Essex, 1996). This caution is endorsed by a recent report showing that infants fed on soya milk formulas have plasma isoflavone levels that are orders of magnitude greater than those of infants fed on human or cows' milk (Setchell *et al.* 1997).

Coronary heart disease

CHD accounts for 23 % of deaths in women in the the UK, and 30 % of deaths in men, although rates have been falling since the late 1970s in the UK. Rates are low in Far East countries, such as Japan, and also declining (Department of Health, 1994; Kesteloot *et al.* 1995).

CHD is a multi-factorial disease, for which the main established risk factors are raised serum cholesterol, raised blood pressure and smoking. In the UK, the proportion of both men and women who are hypertensive steadily increases with age. Compared with men, serum cholesterol levels are lower in women up to the age of 50 years. After the menopause, levels of serum cholesterol in women exceed those of men. In women therefore, the relative importance of CHD as a cause of death steadily increases with age, whereas in men, its importance declines after age 55–64 years (Department of Health, 1994).

The importance of lowering serum cholesterol in reducing the risk of CHD, and total mortality, is now well established, and a 1 % lowering of plasma cholesterol translates on a population basis to a 2–3 % reduction in risk of CHD (Department of Health, 1994; Scandinavian Simvastatin Survival Study Group, 1994). Cholesterol reduction can be achieved by a reduction in the saturated fatty acid content of the diet and by drugs. Both oestrogens in hormone-replacement therapy and the anti-oestrogen Tamoxifen lower LDL-cholesterol, although the decrease in LDL is complemented by an increase in HDL levels with hormone-replacement therapy (Prentice, 1990; Wolfe & Huff, 1995).

It is also well established that serum cholesterol levels can be reduced if animal protein is replaced with soyabean protein in controlled feeding trials. On average, a 9 % reduction in total cholesterol and a 13 % reduction in LDL-cholesterol have been reported in trials in which an average of 47 g soyabean protein was fed, but changes were greater in individuals with high initial cholesterol levels (Anderson *et al.* 1995). The NSP present in soyabean may account for some of the cholesterol-lowering effects found (Bakhit *et al.* 1994).

Following a suggestion of Setchell (1985), much of the effect of soyabean in lowering serum cholesterol has been attributed to the phyto-oestrogens (Anderson *et al.* 1995). Serum total and LDL-cholesterol levels have been found to be significantly lower in rhesus monkeys (*Macaca mulatta*) fed on soyabean protein containing isoflavones compared with those fed on isoflavone-free soyabean (Anthony *et al.* 1996). However, the mechanism is uncertain, since genistein is reported to both up- and down-grade LDL receptors, and some of the products used in studies reporting cholesterol-lowering effects contained unexpectedly low levels of isoflavones (Sitori *et al.* 1997).

As CHD is a multi-factorial disease, many other dietary factors are involved in affecting risk. LDL-lipoproteins are normally taken up by the liver, thus maintaining levels of serum cholesterol, but if they are oxidatively damaged, they are taken up by macrophages to form foam cells in the lining of arteries, initiating the first stages of atherosclerosis (Steinberg *et al.* 1989). The flavonoids, which are polyphenols, are antioxidants and may prevent oxidative damage. In *in vitro* systems, equol and *o*-desmethylo-

lensin, another metabolic product of daidzein, were shown to inhibit oxidation of LDL at low levels (0.1 μM) likely to be achieved when moderate levels of soyabean foods are consumed. Genistein and daidzein also inhibited oxidation at higher concentrations (1 μM ; Hodgson *et al.* 1996).

In addition, because of its effects on tyrosine kinases, genistein may have a role in suppression of the cellular processes which lead on to atherosclerosis, from foam cell to fatty streak to proliferation and invasion with fibrous plaques, rupture and ulceration. In cell lines, genistein has been found to inhibit the proliferation brought about by platelet-derived growth factor in the artery wall, and to interfere with release of inflammatory cytokines from macrophages. It also inhibits platelet aggregation and acts as a thromboxane-receptor agonist. Some of these effects are brought about at low levels. Genistein is also able to suppress the release of endothelial relaxing factor (NO) via its effect on inducible NO synthase (Raines & Ross, 1995; Wilcox & Blumenthal, 1995). No significant effects on platelet aggregation with genistein supplements (as soyabean) have been found so far in human subjects, although only one study has been reported (Gooderham *et al.* 1996).

Osteoporosis

Osteoporosis is defined as a condition in which the amount of bone per unit volume is decreased, but the composition remains unchanged. The bone becomes porous due to an imbalance in forming and resorbing bone cells, causing structural failure and predisposition to fracture. Whether or not sufficient bone is lost to cause fracture depends on the maximum bone mass achieved in young adulthood, the age at which bone loss begins, and the rate of loss, all of which are affected by hormonal, mechanical, genetic and nutritional factors. Hip fractures are particularly important since they lead to immediate disability and considerable morbidity. Hip and other osteoporotic fractures are set to increase rapidly, as the proportion of the UK population over 65 years increases (Department of Health, 1992). Hip fracture rates are low in Asian and African populations compared with those in North American and European populations (World Health Organization, 1994).

Osteoporosis in women is particularly associated with the menopause, since the loss of oestrogen accelerates bone loss. Hormone-replacement therapy prevents this loss, at least up to age 75 years, if taken for several years early on in the post-menopausal period (Felson *et al.* 1993). Dietary factors have also been investigated for their effect in achieving peak bone mass, and preventing bone loss in later life. Ca has been the subject of vigorous debate but a number of other dietary factors, including animal protein, have been investigated (Smith, 1993).

The hormonal effects of phyto-oestrogens, coupled with the comparative rarity of the disease in populations consuming soyabean, has also prompted investigation of their effects in osteoporosis. A synthetic analogue, ipriflavone, is known to be effective in inhibiting bone resorption in post-menopausal women, although its action is not thought to involve direct action with oestrogen receptors (Petilli *et al.* 1995). In addition, Tamoxifen is

known to prevent bone loss (Fentiman & Fogelman, 1993). So far, studies with phyto-oestrogens have mainly been confined to animal models, and in rats, soyabean protein has been shown to prevent bone loss by increasing formation which then exceeded resorption (Arjmandi *et al.* 1996). One preliminary report of a study in human subjects also showed that soyabean protein increases bone density over the short term (Erdman *et al.* 1996).

Hot flushes and vaginitis

One of the most disruptive and classic aspects of the menopause is the hot flush. In Western societies it is the most common symptom of the menopause, although the prevalence is much lower in Japan (Kronenberg, 1994; Knight & Eden, 1995). Hormone-replacement therapy generally alleviates the condition, as well as the vaginitis occurring at the menopause due to atrophy. This and the rarity of the problem in soyabean-consuming populations has prompted some investigations to determine whether phyto-oestrogens have a similar effect. There are as yet few reports, although further trials are reported to be in progress (Eden *et al.* 1996; Woods *et al.* 1996).

Hot flushes are related to the fall in circulating oestrogen, rather than absolute levels, and are associated with surges of gonadotrophins from the pituitary, particularly luteinizing hormone. These are not the cause of the flush, although they are under control of gonadotrophin-releasing-hormone neurones in the hypothalamus, which are in close proximity with thermoregulatory centres (Tataryn *et al.* 1979).

Two studies have shown that phyto-oestrogens, either as soyabean or lignans, lower plasma gonadotrophins after the menopause, although follicle-stimulating hormone rather than luteinizing hormone is lowered (Wilcox *et al.* 1990; Cassidy *et al.* 1997). However, two other studies showed no effects (Baird *et al.* 1995; Murkies *et al.* 1995). Only one study has examined hot flushes directly, and shown an improvement with 45 g raw soyabean flour/d, but an improvement occurred also with white wheat flour, which contains little phyto-oestrogens (Murkies *et al.* 1995).

Two studies have investigated the effects of phyto-oestrogen supplements on vaginal cytology, and found an increase in cell proliferation, which is an indication of oestrogenic activity with reversal of menopausal atrophy. One study used soyabean foods as the source of phyto-oestrogens, and the changes failed to reach significance. In the other study, a mixture of soyabean, clover and linseed was used, and significant effects found (Wilcox *et al.* 1990; Baird *et al.* 1995). No effects were found, however, in another intervention in which post-menopausal women were asked to add 45 g raw soyabean flour/d to their diet (Murkies *et al.* 1995).

Prostate cancer

Prostate cancer is the most common hormone-related cancer in men, and incidence has been rising rapidly, by about 3–4% per year, in the UK (Coleman *et al.* 1993). High-fat and -meat, low-NSP diets are currently linked to

increased risk of the disease, and, like breast cancer, it is comparatively rare in Far Eastern populations consuming soyabean. Isoflavone levels in prostatic fluid are much higher in Hong Kong and Chinese men consuming soyabean than in Portuguese and British men (Morton *et al.* 1997). Of the human epidemiological studies, only three (in Japan and in Japanese migrants to Hawaii) have been conducted, and results were not significant (see Table 4). In animal models, all three studies investigating the effects of soyabean showed reduced tumorigenesis. (Messina *et al.* 1994; Barnes, 1995).

There have been few studies of the effects of phyto-oestrogens on hormone levels. In small trials, both soyabean and linseed supplements had no effect on testosterone levels (Shultz *et al.* 1991; Lu *et al.* 1996; Cassidy *et al.* 1997). In one trial the gonadotrophin follicle-stimulating hormone was suppressed, but no effects were shown with linseed in another trial (Shultz *et al.* 1991; Cassidy *et al.* 1997). Clinical trials of soyabean in men with abnormal prostate specific antigen are reported to be in progress (Barnes *et al.* 1996).

There has been little investigation of the effect of the lignans, although they too have been proposed to be protective in prostate cancer (Adlercreutz, 1990). Like isoflavones, they also inhibit 5 α -reductase (Evans *et al.* 1995). Of the population groups studied, Portuguese men have markedly higher levels of lignans in prostatic fluid compared with British, Hong Kong and Chinese men (Morton *et al.* 1997). The incidence of prostate cancer in Portugal is higher than that in Hong Kong and China, but about half that of Britain (Parkin *et al.* 1992).

Other health-related effects of plant oestrogens

Bowel cancer

Bowel cancer is the second most common cancer in the UK, after breast cancer in women and lung cancer in men. Up to the 1960s it was rare in Japan and Far East countries, but incidence has increased rapidly, so that age-specific large-bowel cancer rates in males are now greater in Japan than in the UK (Bingham, 1996). These changes are attributed to Westernization of the Japanese diet, although there has been no formal examination of soyabean consumption in relation to trends in bowel cancer rates.

There have been eight case-control studies examining a role for soyabean in protection against colo-rectal cancer, in China, Japan, and in Japanese migrants to the USA. There is not a clear relationship, because studies have yielded either non-significant results, or protective or causative associations in about equal numbers (see Table 4; Messina *et al.* 1994). The initial hypotheses suggested the lignans to be the active chemo-preventive agent, but there has been little investigation of lignan intake or excretion in relation to bowel cancer (Setchell *et al.* 1981; Adlercreutz, 1984).

Colon cancer does not have a strong association with hormone status, but there are a number of other possible mechanisms whereby lignans or isoflavones could be involved in the aetiology of bowel cancer (see p. 399); for example, via suppression by genistein of inducible NO

synthase there may be a role for isoflavones to inhibit endogenous N-nitrosation that occurs when meat is consumed (Bingham *et al.* 1996). In animals, aberrant crypts are accepted early markers of colon cancer which can be induced by standard chemical carcinogens. One study has shown that linseed is able to reduce the numbers of aberrant crypts formed in such an animal model, and another study has shown that genistein has the same effect (Steele *et al.* 1995; Jenab & Thompson, 1996).

Other cancers, dementia and alcohol metabolism

Messina *et al.* (1994) have also collated results from case-control investigations of soyabean in relation to cancers at other sites, mainly conducted in Far Eastern populations. Results tend to show that increased soyabean consumption is also protective in lung and stomach cancer (Table 4). In addition, in a variety of different animal models of liver cancer, various soyabean products have protective effects and, as discussed previously, there are a number of mechanisms to account for a general reduction in tumours induced by such means (Barnes, 1995; Peterson, 1995). High levels of raw soyabean flour are known to cause pancreatic carcinomas in rats, probably due to the presence of a trypsin inhibitor. Levels are reduced on cooking or processing, however, and there is no epidemiological evidence that human populations consuming soyabean are at greater risk of pancreatic cancer (Bingham *et al.* 1994; Liener, 1995).

Hormone-replacement therapy is thought to improve cognitive function and perhaps reduce the onset of dementia (Erkkola, 1996). As a consequence of the probable similarities between hormone-replacement therapy and isoflavones, a possible role for isoflavones may emerge. The isoflavones also inhibit the human aldehyde and alcohol dehydrogenase isoenzymes which are responsible for the metabolism of alcohol and detoxification of acetaldehyde, raising the possibility that they could be used as a remedy for the treatment of alcohol abuse (Keung, 1993; Keung & Vallee, 1993). In hamsters, alcohol consumption can be suppressed by daidzein, although in rats the reduction in alcohol intoxication did not appear to be mediated by changes in liver dehydrogenase activity. (Xie *et al.* 1994; Keung *et al.* 1997).

Conclusions

Potentially the phyto-oestrogens would seem to have wide-ranging effects on hormonal-related conditions such as breast and prostate cancer, osteoporosis and conditions associated with the menopause, cardiovascular disease and possibly alcohol metabolism and dementia. Similar to the 'anti-oestrogen' Tamoxifen, the indications are that both oestrogenic and anti-oestrogenic effects, characteristic of weak oestrogens, may occur with supplementation, but a consensus awaits the findings of trials which are in progress in patients with these conditions and also with precursor cancer lesions such as polyps. Results from cell line studies have shown that genistein seems to have fundamental effects in controlling cell signalling, growth and gene

expression, which is probably also particularly important in cancer prevention.

There is, however, little evidence to support a role for these compounds in existing epidemiological studies, which have used crude methods, and have been conducted mainly in soyabean-consuming populations, with no distinction between pre- and post-menopausal state. No account of interaction with other dietary and constitutional factors has been taken; for example, hormonal status, *Helicobacter* infection, and genetic polymorphisms make a substantial contribution to most common cancers, osteoporosis and heart disease when combined with dietary factors.

The lignans have hardly been investigated both epidemiologically and in animal models, despite their known anti-oestrogenic effects. This is partly a result of lack of industrial funding, but also problems with analytical techniques, especially lack of internal standards, so that little information on a food level is available in order to investigate them epidemiologically. Prospective studies in which biological specimens have been collected are required in order to assess hormone and gene-nutrient interactions, and biomarkers of phyto-oestrogen intake in blood and urine. No lignan isolates are presently available for intervention trials.

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