Physiological effects of phyto-oestrogens in relation to cancer and other human health risks

BY AEDIN CASSIDY*

Nutritional Chemoprevention and Gut Metabolism Group, Medical Research Council, Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH

Effets physiologiques des phytoestrogènes en relation avec le cancer et autres risques sur la santé de l'homme

RÉSUMÉ

Les aliments d'origine végétale contiennent des phytoestrogènes dont la structure est semblable à l'oestrogène des mammifères, l'estradiol-17β. Ils ont une affinité pour les récepteurs de l'oestrogène entre 1/500 et 1/1000 comparée à celle de l'oestrogène-17β, et ils produisent des réponses oestrogéniques typiques et prévisibles lorsqu'ils sont administrés aux animaux. Dans les modèles animaux et dans les systèmes expérimentaux *in vitro*, les phytoestrogènes se montrent capables d'agir à la fois comme agonistes partiels et comme antagonistes partiels, avec des effets primaires favorisés par la compétition avec l'estradiol-17β pour le récepteur de l'oestrogène.

Les isoflavones, administrés comme produits de protéine de soja, ont modifié le statut hormonal et la régulation physiologique du cycle menstruel chez des femmes avant la ménopause. Aucun de ces changements n'a été observé lorsque le soja a été remplacé par de l'Arcon F, un produit de soja sans isoflavone. Ces résultats suggèrent donc que les oestrogènes végétaux présents dans le soja exercent potentiellement un effet oestrogénique faible sur l'axe hypothalamique–pituitaire–gonadique. Il est possible que l'état de conjugaison des isoflavones de l'alimentation influence les effets physiologiques observés, les isoflavones inconjugués étant potentiellement plus efficaces. Dans des essais en appartement métabolique sur des femmes ménopausées, on a observé que 60 g/jour de soja sur une période de quatre semaine supprimaient les niveaux de luteinizing hormone (LH), alors que 40 g/jour de graine de lin (une riche source de lignanes) sur une période de six semaines causaient une suppression significative de LH aussi bien que de follicle-stimulating hormone.

Il est possible que les modifications hormonales et du cycle menstruel induites par la protéine de soja et la graine de lin sur les femmes avant et après la ménopause soient potentiellement bénéfiques au regard des facteurs de risque du cancer du sein. Des preuves épidémiologiques, des résultats d'études sur des animaux, et certains travaux *in vitro* suggèrent également que les phytoestrogènes pourraient avoir un rôle protecteur dans le développement du cancer du sein. Les données expérimentales des études sur le rôle protecteur exercé par les phytoestrogènes dans la prévention du cancer de la prostate sont moins convaincantes.

Il est évident que ces composés sont absorbés, mais il y a une grande variabilité individuelle dans leur métabolisme. Des travaux plus approfondis devraient chercher à étudier le métabolisme des phytoestrogènes *in vivo* pour expliquer la grande variabilité individuelle dans l'excrétion de ces composés.

* Present address: School of Biological Sciences, University of Surrey, Guildford GU2 5XH.

Il est nécessaire de mesurer les effets indésirables de phytoestrogènes lorsqu'ils sont consommés à des concentrations naturelles dans l'alimentation. Les effets physiologiques des phytoestrogènes peuvent également avoir des effets bénéfiques sur le métabolisme osseux et la maladie ischémique de coeur, mais ces hypothèses demandent une étude approfondie.

Edible plant material contains numerous weakly oestrogenic diphenolic compounds, termed phyto-oestrogens. Fig. 1. shows the major classes of phyto-oestrogens, which are the isoflavones, lignans, coumestans and resorcyclic acid lactones. Most work on the effects of phyto-oestrogens in relation to human health has centred on the isoflavones and lignans.

STRUCTURE AND SOURCES

Isoflavones are intrinsic plant compounds, the amount present being dependent on many factors, including those associated with growth (Hanson et al. 1965) and genetic background (Morley & Francis, 1968). Concentrations may be increased as a direct response to microbial or insect damage and it has been speculated that these diphenols possess anti-fungal activity (Naim et al. 1974). They are associated also with flower pigments (Clevenger, 1964) and play a role in lignification (Francis & Hume, 1971). Lignans are widely distributed as minor constituents of some plant species (Haworth & Slinger, 1940), and are defined as compounds possessing a 2,3-dibenzylbutane structure (Rao, 1978). The mammalian lignans, enterodiol and enterolactone, differ significantly in chemical structure from plant lignans in that they have a phenolic hydroxyl group substituted on the meta position of the aromatic ring (Setchell et al. 1981).

Several plants contain high concentrations of phyto-oestrogens; for example, linseed (or flaxseed) is a rich source of the lignan precursor, secoisolariciresinol, while soyabean-protein products contain high concentrations of isoflavones (Table 1; Price & Fenwick, 1985). Phyto-oestrogens occur naturally in plants either as highly-polar glycoside conjugates, or in the unconjugated form; for example, textured vegetable
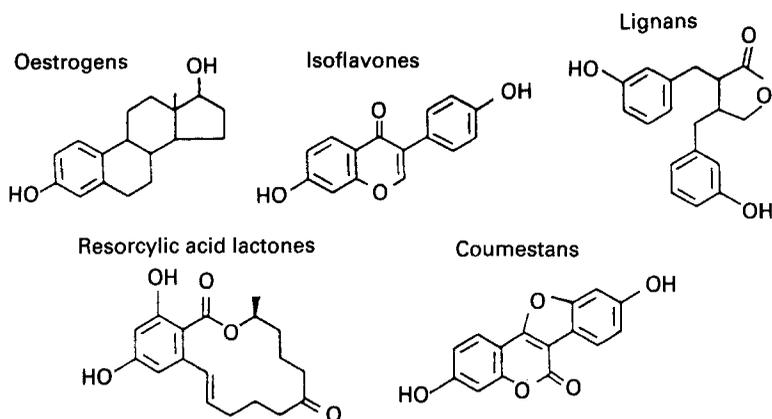

Fig. 1. Common classes of phyto-oestrogens.

Table 1. *Total isoflavone and lignan concentrations in foods (µg/g)*

Textured soyabean*	1136
Soyabean flakes*	501
Soyabean flakes†	366
Soyabean dessert†	232
Soyabean milk: 1†	20
2†	18
Prosobee*	39
Isomil*	42
Linseed meal‡	675
Linseed meal§	808
Linseed flour‡	527
Linseed oil§	17
Oat bran‡	7
Oatmeal§	2
Wheat‡	5
Rye‡	2
Rye§	6
Asparagus‡	3
Carrot‡	4
Lentil‡	18
Pear‡	2

* Setchell *et al.* (1987*b*).
† Jones *et al.* (1989).
‡ Thompson *et al.* (1991).
§ Axelson *et al.* (1982).

protein (TVP) and tofu (soyabean curd) contain high levels of conjugated isoflavones, such as daidzin and genistin, while in fermented soyabean products such as miso, approximately 90% of the isoflavones are present in the unconjugated form, e.g. daidzein and genistein (Coward *et al.* 1993). When ingested, these compounds are metabolized by the bacteria in the large gut to form equol (Setchell *et al.* 1984).

OESTROGENICITY

These heterocyclic phenols are structurally similar to the mammalian oestrogen, oestradiol-17β. A common chemical characteristic of these compounds is the presence of a phenolic ring, a prerequisite for binding to the oestrogen receptor. The principal compounds within these classes of phyto-oestrogens have been shown to have weak oestrogenic activity, ranging from 1/500 to 1/1000 the activity of oestradiol-17β, and to produce typical and predictable oestrogenic responses when administered to animals (Shutt & Cox, 1972; Martin *et al.* 1978; Verdeal *et al.* 1980; Juniewicz *et al.* 1988). Oestrogenic compounds can be agonistic or antagonistic to oestradiol-17β when they act simultaneously at target tissues. Antagonistic compounds normally compete for oestradiol-17β receptors but fail to stimulate the nucleus to respond fully. This partial oestrogen agonistic and/or antagonistic behaviour is a common feature of many weak oestrogens (Jordan, 1990). In animal models and *in vitro* experimental systems the isoflavones appear to act as anti-oestrogens, with primary anti-oestrogenic effects mediated via competition with oestradiol-17β for the oestrogen receptor.

These compounds are weak oestrogens; the activity of genistein, for example, is 1000-fold less than that of oestradiol (Shutt & Cox, 1972). However, certain foods contain comparatively large amounts so that urinary excretion and plasma concentrations may exceed levels of endogenous oestrogens by several orders of magnitude (Table 2). Urinary excretion of the principal oestrogen, oestrone glucuronide, ranges between 2 and 27 μg/d during the menstrual cycle (Setchell, 1985), while the excretion of isoflavones on 60 g TVP diet/d was 0·4–7·5 mg/d (Cassidy et al. 1994).

EPIDEMIOLOGY

Soyabean consumption is higher in countries where the risk of hormone-dependent cancers is low. Cross-sectional studies which have measured urinary excretion of phyto-oestrogens show that urinary isoflavone (daidzein, genistein and equol) excretion is substantially greater in Japanese volunteers consuming a traditional diet compared with levels in adults living in Boston and Helsinki (Table 2). The limited plasma data that are currently available also suggest that levels of biologically-active isoflavones (free and sulphate fraction) are ten to twenty times higher in Japanese men than in Finnish males (Table 2). Urinary lignan (enterodiol and enterolactone) excretion is higher in vegetarian subjects than in those consuming an omnivorous diet (Table 2). In addition, recent data from Adlercreutz et al. (1995), show that faecal excretion of phyto-oestrogens is substantially higher in vegetarian subjects than in a group of omnivores (Table 2).

The incidence of breast cancer and prostate cancer in countries of the Western world is significantly greater than that in Oriental countries, where the incidence of developing the disease is 5–8-fold less (Muir et al. 1987). However, the incidence of breast and prostate cancer in Asian migrants and their offspring approaches the incidence rates of indigenous Westerners (Muir et al. 1987). These findings indicate that the majority of hormone-dependent cancers occur as a result of environmental influences, probably diet, and that they are potentially preventable.

The differences in breast-cancer incidence rates between Asian populations and Western countries may possibly be related to the action of phyto-oestrogens as protective agents in the diet. British intake of one class of phyto-oestrogens, the isoflavones, has been estimated to be <1 mg/d (Jones et al. 1989), while the average consumption of these compounds in Asian countries is 50–100 mg/d (Barnes et al. 1990; Adlercreutz et al. 1991a; Coward et al. 1993). Table 2 shows that Japanese excrete up to twenty times more equol in urine than Western populations, and have higher circulating levels of isoflavones in plasma than Western populations (Table 2).

Table 3 shows that three of five epidemiological trials have reported a protective effect of soyabean products against the development of breast cancer. In the two prospective trials, Nomura et al. (1978) found an inverse association between intake of miso soup (a fermented soyabean paste), and a trend toward an inverse association between intake of tofu (a soyabean curd), and subsequent risk of breast cancer. Hirayama (1986) also showed that a high intake of soyabean-paste soup was associated with a reduced risk in Japanese women. One case–control study (Lee et al. 1991) found a significant inverse association in premenopausal women between breast cancer risk and soyabean-protein intake, although two other case–control studies found no relationship (Table 3).

Table 3 shows that there is less consistency between soyabean consumption and risk of prostate cancer. In one Japanese study, there was no significant association between

soyabean-paste soup and prostate cancer risk, although protective effects were observed for leafy green vegetables (Hirayama, 1979). In addition, Oishi *et al.* (1988) found no significant relationship between intake of miso soup and prostate cancer when they compared cancer patients with patients with hyperplastic disease and hospital controls. Severson *et al.* (1989) showed a trend towards a protective effect with tofu, but no effect with miso soup in a group of 7999 men in Hawaii followed for almost 20 years. In this study the number of subjects with the highest intake was small and the association reached only borderline significance ($P=0.054$).

ANIMAL STUDIES

In the last 50 years, several important examples of the way in which dietary intake of phyto-oestrogens can influence reproductive physiology in animals have been reported. The earliest documented example was the recognition of an infertility syndrome in sheep, referred to as 'clover disease' (Bennetts *et al.* 1946; Shutt, 1976). This condition resulted from ewes grazing on pastures rich in *Trifolium subterraneum*, a species of clover rich in the isoflavone precursor, formononetin. The intestinal metabolism of this isoflavone leads to the formation of equol, and this weak oestrogen is absorbed and attains high circulating concentrations that subsequently cause permanent histological damage to the ovaries and uterus of the animal.

Setchell *et al.* (1987a) showed that the addition of soyabean protein to the commercial diet of the captive cheetah (*Acinonyx jubatus*) was the cause of infertility and veno-occlusive liver disease. The effectiveness of isoflavones in the cheetah is explained by the poor capacity of the feline liver to conjugate steroids with glucuronic acid, an important route of deactivation. As a result, the unconjugated isoflavones predominate in the circulation.

Compounds in soyabean also appear to be active in animal models of breast cancer. Hawrylewicz *et al.* (1991) showed that soyabean-protein isolate decreased tumours by 50% in an N-methyl-N-nitrosourea (NMU) model of breast cancer. Troll *et al.* (1980) showed a 50% reduction in experimentally-induced X-ray irradiated mammary tumours in rats consuming a powdered soyabean diet. More recently, Barnes *et al.* (1990) showed that soyabean ingestion led to a reduction in mammary tumour growth in both the NMU and the dimethyl-benz(α)anthracene rat models of breast cancer. In these models, tumour formation was negatively correlated with total dietary isoflavone concentrations and, in particular, with dietary intake of genistein and urinary isoflavone excretion levels. In addition, the results from a follow-up study found that a soyabean product from which the isoflavones had been chemically removed (Arcon F) had no effect on mammary carcinogenesis (Barnes *et al.* 1990), again providing evidence that isoflavones exert anti-oestrogenic effects *in vivo*.

More recently, Lamartiniere *et al.* (1995) showed that administration of genistein to rats in the perinatal period was sufficient to cause a marked latency in the appearance of mammary tumours after administration of 7,12-dimethylbenz(α)anthracene at 50 d of age.

Only limited data on the effects of soyabean on prostate cancer induced in animal models are available. Sharma *et al.* (1992) showed in a rat model that a soyabean diet reduced the severity and incidence of prostatitis in the lateral lobe of the prostate. Soyabean appears to exert anti-oestrogenic effects in neonatally-oestrogenized mice. A

Table 2. Urinary (µg/d), faecal (µg/d) and plasma (ng/ml) phyto-oestrogen concentrations

Group studied	Diet consumed	n	Country	Enterodiol Mean	Enterodiol Range	Enterolactone Mean	Enterolactone Range	Daidzein Mean	Daidzein Range	Equol Mean	Equol Range	Genistein Mean	Genistein Range
Urine													
Post-menopausal women	Omnivore*	5	USA			880	540–1320						
	Vegetarian*	8	USA			640	170–5010						
	Omnivore†	7	Finland	263	96–410	745	224–2370						
	Omnivore‡	10	USA	97	57–211	685	334–2178			34	ND–96·8		
	Vegetarian‡	10	USA	121	42–429	948	373–2780			34	ND–107		
	BC patients‡	7	USA	63	ND–230	310	83–528			27	ND–70		
	Omnivore§	36	UK	62	0·2–533	156	4–931	30	3–8	3	0–61	22	5–166
	Traditional‖	10	Japan	211		417		660		629		929	(n 3)
	Traditional¶	10	Japan					660		629			
	Omnivore¶	21	USA					55		15		9	
	Omnivore¶	12	Finland			1		10		11		38	
	Omnivore**	4	Finland	815				43		17			
	Vegetarian**	4	Finland	864		18		358		17		324	
Premenopausal women	Omnivore*	7	USA			570	310–1050						
	Vegetarian*	6	USA			5000	122–24000						
	Omnivore†	3	Finland	283	147–495	807	544–1156			17			
	Omnivore††	9	USA	85		611		81		25			
	Omnivore††	12	Finland	61		733		56		24			
	Lactovegetarian††	9	USA	224		1243		320		16			
	Lactovegetarian††	11	Finland	111		1088		70		210			
	Macrobiotic††	12	USA	2000		5269000		880		19			
	Omnivore**	10	Finland	755		1		56		19		49	
	Vegetarian**	10	Finland	483		2		467		3·7		451	
	Control‡‡	6	UK	20		622		1·4		2·7		2·7	
	60 g TVP‡‡	6	UK	30		288		1800		835		630	
Men	Omnivore†	2	Finland	144	82–205	1650	1242–2057	33	3–160	10	4–16	49·8	6–319
	Omnivore§§	10	Finland	133	1·2–779	700	54–1603	559		726			
	Traditional‖	9	Japan	121		328							
Faeces													
Women (all ages)	Omnivore‖‖	9	Finland	45		450		12		4		3	
	Vegetarian‖‖	9	Finland	145		977		66		65		51	
Premenopausal	Control¶¶	13	USA	24		191		31		2		16	
	Flaxseed¶¶	13	USA	773		3069		20		4		9	

Table 2. Urinary (μg/d), faecal (μg/d) and plasma (ng/ml) phyto-oestrogen concentrations (continued)

Group studied	Diet consumed	n	Country	Enterodiol			Enterolactone			Daidzein			Equol			Genistein		
				F+S	Gluc	Total	F+S	Gluc	Total	F+S	Gluc	Total	F+S	Gluc	Total	F+S	Gluc	Total
Plasma																		
Post-menopausal women	Omnivore**	4	Finland	0·1	0·6	0·8	2·1	7·8	9·9	0·3	1·4	1·6	0·02	0·4	0·4	0·2	2·4	2·5
	Vegetarian**	4	Finland	1·3	3·9	5·2	15·7	59·6	75·3	1·4	11·3	12·7	0·1	0·3	0·4	0·6	15·4	16
	Omnivore***	1	Finland	0·1		6·7	1·6		37·9	0·06		2·5	0·02		0·06			
	2 week clover (Trifolium sp.)†††	23	Australia									49·1			34·2			
	2 week soyabean†††	23	Australia									68·3			31·1			
	2 week linseed†††	23	Australia			106·3			117·5									
Premenopausal women	Omnivore**	10	Finland	0·1	0·5	0·6	1·6	6·7	8·3	0·2	1·4	1·6	0·02	0·4	0·4	0·2	1·9	2·1
	Vegetarian**	10	Finland	1·2	4·1	5·3	8·8	17·9	26·7	1·5	13·6	15·1	0·1	0·3	0·4	0·5	11·6	12·1
Men	Omnivore‡‡‡	6	France	ND			3·9		19·7									
	Omnivore§§§	14	Finland							0·2	0·5	1·6	0·02	0·1	0·2	0·1	1·4	1·7
	Traditional§§§	14	Japan							3·3	23·3	27·2	0·2	0·9	1·3	2·1	45·1	74·7

BC, breast cancer; TVP, textured vegetable protein; ND, not detected; F+S, free and sulphate fraction (active); Gluc, glucuronides.

* Adlercreutz et al. (1981)
† Fotsis et al. (1982)
‡ Adlercreutz et al. (1982)
§ Cassidy et al. (1990)
‖ Adlercreutz et al. (1991a)
¶ Adlercreutz et al. (1992)
** Adlercreutz et al. (1993a)
†† Adlercreutz et al. (1986)

‡‡ Cassidy et al. (1994)
§§ Adlercreutz et al. (1991b)
‖‖ Adlercreutz et al. (1995)
¶¶ Kurzer et al. (1995)
*** Finlay et al. (1991)
††† Dehennin et al. (1982)
‡‡‡ Morton et al. (1994)
§§§ Adlercreutz et al. (1993b)

Table 3. *Epidemiological studies examining the role of soyabean products in breast and prostate cancer risk* (Adapted from Messina *et al.* 1994)

Study	Soyabean product	Estimate of relative risk	Findings	Reference
Breast cancer				
Case–control	Soyabean	NC	NS	Hirohata *et al.* (1985)
Prospective	Miso soup	NC	Decreased risk*	Nomura *et al.* (1978)
	Tofu	NC	Decreased risk*	
Prospective	Miso soup	0·46	Decreased risk	Hirayama (1986)
Case–control†	Soyabean protein	0·43	Decreased risk	Lee *et al.* (1990)
	Soyabean: total protein	0·29	Decreased risk	
Case–control	Soyabean protein	1·0	No change in risk	Yuan *et al.* (1995)
	Soyabean: total protein	1·0	No change in risk	
Prostate cancer				
Cohort	Miso soup	0·76	NS	Hirayama (1979)
Case–control	Miso soup	1·29	NS (cancer patients *v.* benign condition)	Oishi *et al.* (1988)
		0·64	NS (cancer patients *v.* hospital controls)	
Cohort	Tofu	0·35	NS ($P=0·054$)	Seversen *et al.* (1989)
	Miso soup	1·24	NS	

NC, could not be calculated.

* Decreased risk was significant only for baseline period 1971–1975.

† Premenopausal only.

soyabean diet reduced the prostatic growth inhibition due to diethylstilbestrol and, in preliminary studies, delayed the development of dysplastic changes in the prostate (Makela *et al.* 1995). However, the homology between this animal model and human prostatic cancer remains to be established.

MECHANISMS OF ACTION AND METABOLISM

On ingestion the phyto-oestrogen conjugates daidzin and genistin are hydrolysed in the large gut under the action of intestinal bacteria (Setchell *et al.* 1984) to release the unconjugated daidzein and genistein. The extent of absorption may be dependent on the chemical form of the isoflavones, in that the unconjugated isoflavones, because of their amphiphilic properties, may be more readily absorbed and, therefore, more bioavailable than the highly-polar conjugated species. The isoflavone metabolite, equol, is thought to be derived from isoflavone precursors.

Absorbed phyto-oestrogens are transported to the liver via the hepatic portal vein, where they are rapidly conjugated with glucuronic acid, and to a much lesser extent with sulphuric acid, by the liver (Axelson & Setchell, 1980; Setchell *et al.* 1981, 1984; Fig. 2). Thus, in both blood and urine, phyto-oestrogens are found primarily as conjugates. In plasma, the free and sulphate fractions are biologically active, while the glucuronide fraction is considered to be biologically inactive (Adlercreutz *et al.* 1993*a*). In faeces, phyto-oestrogens are predominantly found in the unconjugated form, with less than 10% present in the conjugated state (Fig. 2). Quantification of phyto-oestrogens in biological
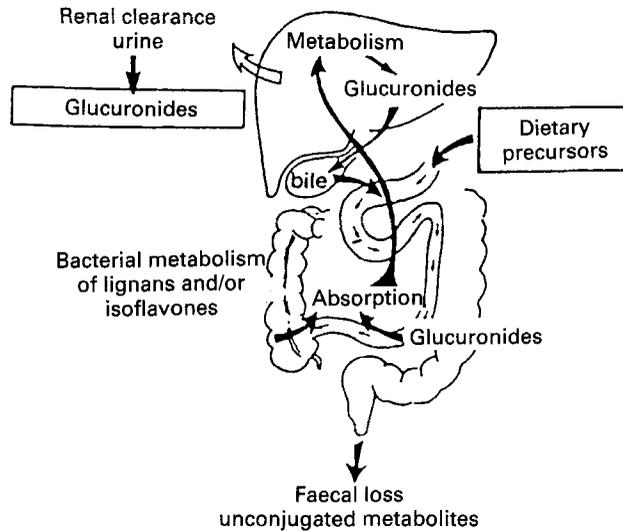
Fig. 2. Physiology of phyto-oestrogens.

fluids is generally performed by gas chromatography–mass spectrometry, following extensive extraction, hydrolysis, and derivatization of the compounds (Setchell *et al.* 1983; Adlercreutz *et al.* 1993*a*).

Under controlled conditions, Cassidy *et al.* (1994) showed that a challenge of 45 mg conjugated isoflavones/d, as 60 g TVP/d over a complete menstrual cycle, resulted in a 1000-fold increase in isoflavone excretion (Table 1). Total urinary isoflavone levels ranged from 1 to 17 µg/d during the control diet, while levels increased to between 0·4 and 8 mg/d on the TVP diet. There was marked individual variability in metabolism of the isoflavones. Only two subjects excreted substantial levels of the metabolite, equol, while the other four subjects absorbed and excreted the precursors, daidzein and genistein (Cassidy, 1991; Cassidy *et al.* 1994). Quantitatively, daidzein excretion was more important than genistein and this finding has been reported also by other investigators (Kelly *et al.* 1993; Xu *et al.* 1994). Individual variability in isoflavone metabolism had been observed previously by Setchell *et al.* (1984). Kelly *et al.* (1993) also showed some individual variability in ability to deconjugate and to absorb dietary isoflavones in twelve subjects following ingestion of a 40 g soyabean-flour cake over two consecutive days. Inter-individual variation in isoflavone excretion between twelve female volunteers challenged for 1 d, on three separate occasions with either 0·7, 1·3 or 2·0 mg isoflavones/kg body weight was substantial (Xu *et al.* 1994).

Cassidy *et al.* (1994) reported an average recovery of total urinary isoflavones of between 2 and 13% of intake, following consumption of 45 mg isoflavones/d as 60 g TVP. Xu *et al.* (1994) showed average 24 h urine recoveries of daidzein and genistein to be 21 and 9% respectively at all three doses of isoflavones. Only 1–2% of ingested isoflavones was found in faeces. No equol was detected in their urine samples. Therefore, the absorption, excretion and plasma concentrations of isoflavones may be dependent on the dose given, with urinary and plasma isoflavone levels increasing with increasing dose. At all three doses, urinary isoflavone levels were significantly greater in the first 12 h after

dosing than in the subsequent 12 h period; plasma concentrations were significantly increased 6·5 h after dosing (Xu *et al.* 1994).

It is evident, therefore, that these compounds are absorbed. However, currently there is limited information on their bioavailability and further investigation is required to establish the reasons for the wide individual variability in excretion of these compounds.

BIOLOGICAL EFFECTS IN HUMANS

Studies by Cassidy *et al.* (1994, 1995) have examined the physiological effects of phyto-oestrogens in humans. Isoflavones, given as various soyabean products, modified hormonal status in healthy premenopausal women. Fifteen premenopausal women were studied over a 9-month period, 4–6 months of which were spent in a metabolic suite, and 2 or 3 months under closely-monitored conditions. Consumption of 45 mg conjugated isoflavones as TVP (60 g/d; *n* 6) over a 1-month period significantly prolonged the length of the menstrual cycle, specifically the length of the follicular phase. The delay in menstruation was accompanied by a delay in peak luteal-phase progesterone concentrations. In addition, the mid-cycle surges of the gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were suppressed ($P<0.05$ and $P<0.01$ respectively; Cassidy *et al.* 1994, 1995). A small number of subjects (*n* 3) consumed half the dose (25 mg) as unconjugated isoflavones, 50 g miso/d. Similar biological effects were observed with this diet as with 45 mg conjugated isoflavones, although the findings of the study were not statistically significant. However, peak luteal phase progesterone concentrations were significantly delayed ($P<0.05$). The results of these studies suggest that the chemical form of dietary isoflavones influences the observed physiological effects, with unconjugated isoflavones being potentially more efficient. None of these changes were observed when soyabean was replaced with Arcon F, an isoflavone-free soyabean product, in five subjects, providing convincing evidence that phyto-oestrogens are the biologically active components of soyabean products.

Several other studies with free-living subjects have examined the biological effects of phyto-oestrogens in women. Wilcox *et al.* (1990) supplemented the diets of a group of post-menopausal women to evaluate the oestrogenic effects of several sources of phyto-oestrogens. They showed significant changes in vaginal cytology and suppression in FSH after 6 weeks on the diet. Phipps *et al.* (1993) showed that 10 g flaxseed powder/d over three menstrual cycles in eighteen premenopausal women had little effect on their sex steroid hormone metabolism. In trials with free-living post-menopausal women, 60 g soyabean/d over a 4-week period suppressed LH levels, while 40 g linseed/d over a 6-week period caused a significant suppression of both LH and FSH (A. Cassidy, M. Faughnan, R. Hughes, C. Fraser, A. Cathcart, N. Taylor and S. Bingham, unpublished results).

These findings suggest that the phyto-oestrogens present in soyabean potentially exert a weak oestrogenic effect on the hypothalamic–pituitary–gonadal axis and potentially may be beneficial with respect to risk factors for breast cancer. Oestrogen exposure and the cumulative number of menstrual cycles a woman experiences over her pre-menopausal years are two important risk factors for breast cancer (Henderson *et al.* 1985). Mean cycle length in Western countries, where risk of breast cancer is high, is 28–29 d, while average cycle length is 32 d in Japan, where risk of breast cancer is 4-fold lower (Henderson *et al.* 1985). Breast-cell division is 4-fold lower during the follicular

phase of the cycle (Ferguson & Anderson, 1981; Anderson *et al.* 1982), so women with longer menstrual cycles, therefore, will have longer follicular phases, resulting in reduced cell division over their premenopausal years. Tamoxifen, which is successfully used in breast-cancer therapy, at a daily dose of 20 mg, has been shown to similarly interfere with the hypothalamic–pituitary–gonadal axis, resulting in a decrease in circulating levels of LH and FSH in breast-cancer patients (Golder *et al.* 1976; Willis *et al.* 1977; Jordan *et al.* 1987).

Several trials have examined the biological effects of lignans in groups of free-living men. Shultz *et al.* (1991) showed that a daily 13·5 g supplement of linseed over 6 weeks had no effect on plasma testosterone, free testosterone or sex-hormone-binding globulin levels in a group of six young men. Hughes *et al.* (1994) showed that a higher level of linseed (40 g/d) resulted in a significant decrease in FSH, total cholesterol and LDL-cholesterol levels in a group of middle-aged men over 4 weeks. Although male serum testosterone and dihydrotestosterone showed a trend towards a decrease on the linseed diet there was no observed change in total urinary androgen output. Only one dietary-intervention trial with isoflavones in men has been conducted. In a group of middle-aged men 60 g TVP/d did not result in any significant hormonal modifications (A. Cassidy, M. Faughan, R. Hughes, C. Fraser, A. Cathcart, N. Taylor and S. Bingham, unpublished results).

OTHER POTENTIAL MECHANISMS OF ACTION

In addition to phyto-oestrogens, soyabeans contain several other potential anti-carcinogens, including protease inhibitors, phytosterols, saponins and inositol hexaphosphate (Barnes *et al.* 1990). Substantial literature has been published on the anti-cancer activity of the Bowman-Birk trypsin inhibitor (Kennedy, 1995); however, Barnes *et al.* (1990) showed that autoclaved soyabean, which inactivates the trypsin inhibitor, was as effective as non-autoclaved soyabean in reducing the number of tumours in two standard experimental animal models of breast cancer. They also showed that the Bowman-Birk trypsin-inhibitor preparations typically contain high concentrations of isoflavones.

Initial interest in the role of phyto-oestrogens in cancer prevention has centred on their anti-oestrogenic effects. However, results of several *in vitro* studies suggest that isoflavones, specifically genistein, do not inhibit cell growth by classical anti-oestrogenic mechanisms.

At high concentrations, genistein acts as a potent inhibitor of proliferation of both oestrogen-receptor-negative and -positive breast-cancer cells (Peterson & Barnes, 1991). Genistein also specifically inhibits the activity of the epidermal-growth-factor receptor (EGFR) tyrosine kinase with the value for the concentration required for 50% inhibition (IC$_{50}$) of about 0·7 μg/ml. Serine and threonine kinases require concentrations >30 μg/ml for inhibition (Akiyama *et al.* 1987). However, genistein does not inhibit the activation of growth-factor receptors in tumour cells, because genistein did not block EGFR autophosphorylation in DU-145 prostate cancer cell lines (Peterson & Barnes, 1993). Neither daidzein (Sargeant *et al.* 1993) nor genistin (Arrora *et al.* 1995) exhibit anti-tyrosine kinase activity. Genistein also inhibits *in vitro* angiogenesis and endothelial-cell proliferation (Fotsis *et al.* 1993). In contrast, enterolactone, equol and daidzein were twenty to fifty times less potent (Fotsis *et al.* 1993). This may be an important finding; however, the half-maximal effect for inhibition of angiogenesis required approximately

40·5 μg genistein/ml, which is an order of magnitude higher than the levels required to inhibit cancer-cell growth, and almost two orders of magnitude higher than estimates of blood isoflavone concentrations reported in humans consuming soyabean products.

Genistein inhibits the activity of topoisomerase II *in vitro* with IC_{50} values of approximately 30 μg/ml (Okura *et al.* 1988; Markovitz *et al.* 1989; Constantinou *et al.* 1990). Slight inhibition occurs at low genistein concentrations (3 μg/ml) but complete inhibition requires concentrations >50 μg/ml. *In vitro* studies also show that genistein inhibits topoisomerase II activity by stabilizing the DNA–topoisomerase II complex (Yamashita *et al.* 1991). Stabilization of this complex in tumour cells leads to double- and single-strand breaks in cellular DNA, leading to growth inhibition or cell death (Gerwirtz, 1991). However, at IC_{50} values for growth inhibition, little DNA damage is observed, suggesting that genistein does not inhibit cell growth through inhibition of topoisomerase II activity (Okura *et al.* 1988; Markovitz *et al.* 1989; Constantinou *et al.* 1990).

Isoflavones may exert some of their anti-cancer effects through an antioxidant mechanism. Genistein has been shown to decrease the production of reactive oxygen species (ROS) by tumour cell types and cells of the immune system. Genistein at 0·5–8 μg/ml can directly inhibit agonist-stimulated ROS production (Akimura *et al.* 1992; Tanimura *et al.* 1992; Utsumi *et al.* 1992; Wei *et al.* 1993).

The relevance of these *in vitro* studies in understanding the potential chemopreventative effects of phyto-oestrogens is unknown. Many of these studies were carried out using high concentrations of phyto-oestrogens and few of the previously described mechanisms in tumour cells are sensitive to physiological serum concentrations of genistein. However, limitations in the existing analytical methods need to be resolved (K. D. R. Setchell, personal communication). For example, mean total genistein levels in plasma were 16 ng/ml in a group of vegetarians, while a group of Japanese men secreted on average 75 ng genistein/ml (Table 2), whereas concentrations used in the *in vitro* studies were in the microgram range.

The majority of *in vitro* tissue-culture experiments have been carried out with established human cancer cell lines, although these experiments will not address the role of phyto-oestrogens on normal cell proliferation. Thus, the physiological significance of the inhibitory effects of isoflavones on the growth of untransformed, normal cells needs to be examined.

POTENTIAL ADVERSE EFFECTS

In order to evaluate any potential risks offered by phyto-oestrogens, their potency and sites of action, when consumed at natural dietary concentrations, need to be investigated. Each phyto-oestrogen needs to be fully characterized in terms of its site of action, balance of agonistic and/or antagonistic properties, natural potency and short-term and long-term effects. It has been suggested that differences in sensitivity to phyto-oestrogens is dependent on differences in metabolism, particularly in conjugative capacity.

Phyto-oestrogens are known to induce infertility in livestock (Price & Fenwick, 1985), so it is important to determine if there are potential effects on fertility in humans. Several studies have examined the effects of phyto-oestrogens in the reproductive tract and brain of experimental animals. These studies show that phyto-oestrogens in a similar way to

other oestrogens, have the capacity to induce developmental toxicity, but most of these studies have concentrated on the actions of the most-potently-oestrogenic isoflavone, coumestrol (Burroughes *et al.* 1985, 1990; Whitten *et al.* 1992; Medlock *et al.* 1995).

It is well established that exogenous oestrogens increase the risk of endometrial cancer (Smith *et al.* 1975). Tamoxifen, an anti-oestrogen drug which has successfully been employed in the treatment of breast cancer, has also been associated with oestrogenic side effects, such as thrombo-embolic phenomena and endometrial proliferation in post-menopausal women (Fornander *et al.* 1989; Fisher *et al.* 1994). Phyto-oestrogens also exert partial agonist and/or partial antagonist activity, but there is insufficient epidemiological evidence to suggest that the agonist properties of the compounds may cause an increased risk of endometrial hyperplasia, or endometrial carcinoma. Miyazawa (1976) suggested that there was a high prevalence of endometriosis in Japanese women, but this finding was based on one cross-sectional study of gynaecological admissions to hospitals in Hawaii, in which fewer than four cases of endometriosis were Japanese.

OTHER POTENTIAL HEALTH BENEFITS

The physiological effects of plant oestrogens are also likely to have effects on bone metabolism and on heart disease. There is substantial evidence from studies on experimental animals and human subjects that substituting soyabean protein for animal protein in the diet reduces serum concentrations of total and LDL-cholesterol (Sirtori *et al.* 1993), but the mechanism by which this occurs is unknown. There are well-documented effects of oestrogens on lipid metabolism, thus the observed effects of phyto-oestrogens in lowering blood cholesterol levels may be relevant to ischaemic heart disease, of which total cholesterol, LDL-cholesterol, and LDL-cholesterol:HDL-cholesterol are known to be important risk factors (Cardiovascular Review Group Committee on Medical Aspects of Food Policy, 1994). Independent of a possible role of isoflavones in the reduction of serum cholesterol levels, limited studies of cultured vascular cells suggest that some of the cellular processes involved in lesion formation are inhibited by genistein. Genistein is a potent inhibitor of tyrosine kinase activity *in vitro* (Akiyama *et al.* 1987) and is, therefore, capable of blocking growth factor action. For example, genistein (27µg/ml) inhibited platelet-derived growth factor-induced thymidine incorporation and receptor autophosphorylation (Hill *et al.* 1990). It has been shown also to inhibit thrombin formation, and platelet activation *in vitro* (Sargeant *et al.* 1993; Wilcox & Blumenthal, 1995). If genistein has the same effects *in vivo*, this compound has the potential to slow the development or progression of atherosclerotic disease by modifying coagulation responses. However, currently this is speculative and future research should assess its potential therapeutic effects for vascular disease *in vivo*.

The weak oestrogenic action of isoflavones may be potentially beneficial in the prevention of osteoporosis; however, there are few studies to support this hypothesis. Anderson *et al.* (1995) compared the potential agonistic effects of genistein on the retention of cancellous bone tissue in rats with premarin, an oestrogen with known bone-retaining properties. Genistein at a dose of 1 mg/d had a similar effect in maintaining trabecular bone tissue to that of premarin (5 µg/d), but at higher doses (3·2 and 10 mg/d) it had no retentive effect on bone mass. In addition, an isoflavone derivative, ipriflavone, directly inhibited bone resorption in a human model of osteoblast (Cheng *et al.* 1995).

High levels of circulating gonadotrophins have been associated with post-menopausal symptoms, e.g. hot flushes (Aitken *et al.* 1974; Casper *et al.* 1979; Tataryn *et al.* 1979). The observed decreases in LH and FSH levels in the free-living post-menopausal women consuming TVP and linseed (A. Cassidy, M. Faughnan, R. Hughes, C. Fraser, A. Cathcart, N. Taylor and S. Bingham, unpublished results) suggest that phyto-oestrogens have the potential to alleviate post-menopausal symptoms. Recently, Murkies *et al.* (1995) showed that a daily supplement of 45 g soyabean flour significantly reduced the number of hot flushes within 6 weeks in a group of post-menopausal women.

CONCLUSIONS

Epidemiological evidence, results from human and animal studies, and some *in vitro* work suggest that phyto-oestrogens may be protective against the development of hormone-dependent cancers. It is evident that these compounds are absorbed, and from the available data, have potential biological effects in humans. Isoflavones, 50–100 mg/d, are typically consumed by humans in the Far East (Barnes *et al.* 1990; Adlercreutz *et al.* 1991; Coward *et al.* 1993) where hormone-dependent cancer rates are low. Phyto-oestrogens, therefore, may protect against hormone-dependent cancers but more research needs to be conducted.

Future research should examine the metabolism and absorption of these compounds *in vivo* to help explain the wide individual variability in excretion of these compounds, even under controlled dietary conditions. More feeding trials need to be conducted to examine the biological effects of lignans in humans, and to confirm the importance of the chemical form in which the isoflavones are ingested. Evidence of a direct effect at the breast or prostate needs to be demonstrated in clinical trials. The mechanisms of action of the phyto-oestrogens needs to be established by performing further *in vitro* studies using non-transformed normal cells at physiologically-relevant concentrations of the compounds. The biological relevance of these *in vitro* studies could then be predicted.

REFERENCES

Adlercreutz, H., Fotsis, T., Bannwart, C., Wahala, K., Brunow, G. & Hase, T. (1991*b*). Isotope dilution gas chromatographic–mass spectrometric method for the determination of lignans and isoflavonoids in human urine, including the identification of genistein. *Clinica Chimica Acta* **199**, 263–278.

Adlercreutz, H., Fotsis, T., Bannwart, C., Wahala, K., Makela, T., Brunow, G. & Hase, T. (1986). Determination of urinary lignans and phytoestrogen metabolites, potential antiestrogens and anticarcinogens, in the urine of women on various habitual diets. *Journal of Steroid Biochemistry* **25**, 791–797.

Adlercreutz, H., Fotsis, T., Heikkinen, R., Dwyer, J. T., Goldin, B. R., Gorbach, S. L., Lawson, A. M. & Setchell, K. D. R. (1981). Diet and urinary excretion of lignans in female subjects. *Medical Biology* **59**, 259–261.

Adlercreutz, H., Fotsis, T., Heikkinen, R., Dwyer, J. T., Woods, M., Goldin, B. R. & Gorbach, S. L. (1982). Excretion of the lignans enterolactone and enterodiol and of equol in omnivorous and vegetarian postmenopausal women and in women with breast cancer. *Lancet* **ii**, 1295–1299.

Adlercreutz, H., Fotsis, T., Kurzer, M. S., Wahala, K., Makela, T. & Hase, T. (1995). Isotope dilution gas chromatographic–mass spectrometric method for the determination of unconjugated lignans and isoflavonoids in human feces, with preliminary results in omnivorous and vegetarian women. *Analytical Biochemistry* **225**, 101–108.

Adlercreutz, H., Fotsis, T., Lampe, J., Wahala, K., Makela, T., Brunow, G. & Hase, T. (1993*a*). Quantitative determination of lignans and isoflavonoids in plasma of omnivorous and vegetarian women by isotope dilution gas chromatography–mass spectrometry. *Scandinavian Journal of Clinical and Laboratory Investigation* **53**, Suppl. 215, 5–18.

Adlercreutz, H., Hamalainen, E., Gorbach, S. & Goldin, B. (1992). Dietary phyto-oestrogens and the menopause in Japan. *Lancet* **339**, 1233.

Adlercreutz, H., Honjo, H., Higashi, A., Fotsis, T., Hamalainen, E., Hasegawa, T. & Okada, H. (1991*a*). Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *American Journal of Clinical Nutrition* **54**, 1093–1100.

Adlercreutz, H., Markkanen, H. & Watanabe, S. (1993*b*). Plasma concentrations of phyto-oestrogens in Japanese men. *Lancet* **342**, 1209–1210.

Aitken, J. M., Davidson, A., England, P., Govan, A. D. T., Hart, D. M., Kelly, A., Lindsay, R. & Moffatt, A. (1974). The relationship between menopausal vasomotor symptoms and gonadotrophin excretion in urine after oophorectomy. *Journal of Obstetrics and Gynaecology* **81**, 150–154.

Akiyama, T., Ishida, J., Nakagava, S., Ogawara, S., Watanabe, S., Itoh, N., Shiyba, M. & Fukami, Y. (1987). Genistein, a specific inhibitor of tyrosine-specific protein kinases. *Journal of Biological Chemistry* **262**, 5592–5595.

Akiyama, K., Utsumi, T., Sato, E. F., Klostergaard, J., Inoue, M. & Utsumi, K. (1992). Role of tyrosyl phosphorylation in neutrophil priming by tumour necrosis factor-α and granulocyte colony stimulating factor. *Archives of Biochemistry and Biophysics* **298**, 703–709.

Anderson, J. J., Ambrose, W. W. & Garner, S. C. (1995). Orally dosed genistein from soy and prevention of cancellous bone loss in two ovariectomized rat models. *Journal of Nutrition* **125**, 799S Abstr.

Anderson, T. J., Ferguson, D. J. P. & Raab, G. M. (1982). Cell turnover in the 'resting' human breast: influence of parity, contraceptive pill, age and laterality. *British Journal of Cancer* **46**, 376.

Arora, P. D., Ma, J., Min, W., Cruz, A. & McCulloch, C. A. G. (1995). Interleukin-1-induced calcium flux in human fibroblasts is mediated through focal adhesions. *Journal of Biological Chemistry* **270**, 6042–6049.

Axelson, M. & Setchell, K. D. R. (1980). Conjugation of lignans in human urine. *FEBS Letters* **122**, 45–53.

Axelson, M., Sjovall, J., Gustafsson, B. E. & Setchell, K. D. R. (1982). Origin of lignans in mammals and identification of a precursor from plants. *Nature* **298**, 659–660.

Barnes, S., Grubbs, C., Setchell, K. D. R. & Carlson, J. (1990). Soyabeans inhibit mammary tumor growth in models of breast cancer. In *Mutagens and Carcinogens in the Diet*, pp. 239–253 [M. W. Pariza, editor]. New York: Wiley-Liss.

Bennetts, H. W., Underwood, E. J. & Shier, F. L. (1946). A specific breeding problem of sheep on subterranean clover pastures in Western Australia. *Australian Veterinary Journal* **22**, 2–12.

Burroughes, C. D., Mills, K. T. & Bern, H. A. (1985). Prolonged vaginal cornification and other changes in mice treated neonatally with coumestrol, a plant estrogen. *Journal of Toxicology and Environmental Health* **15**, 51–61.

Burroughes, C. D., Mills, K. T. & Bern, H. A. (1990). Long-term genital tract changes in female mice treated neonatally with coumestrol. *Reproductive Toxicology* **4**, 127–135.

Cardiovascular Review Group Committee on Medical Aspects of Food Policy (1994). *Nutritional Aspects of Cardiovascular Disease*. London: H.M. Stationery Office.

Casper, R. F., Yen, S. S. C. & Wilkes, M. M. (1979). Menopausal flushes: a neuroendocrine link with pulsatile luteinising hormone secretion. *Science* **205**, 823–825.

Cassidy, A. (1991). Plant oestrogens and their relation to hormonal status in women. PhD Thesis, University of Cambridge.

Cassidy, A., Bingham, S. & Setchell, K. (1994). Biological effects of isoflavones present in soy in premenopausal women: Implications for the prevention of breast cancer. *American Journal of Clinical Nutrition* **60**, 333–340.

Cassidy, A., Bingham, S. & Setchell, K. (1995). Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. *British Journal of Nutrition* **74**, 587–601.

Cassidy, A., Bingham, S., Setchell, K. & Watson, D. (1990). Plant oestrogen excretion in a group of postmenopausal women. *Proceedings of the Nutrition Society* **50**, 105A.

Cheng, S. L., Zhang, S. F., Nelson, T. L., Warlow, P. M. & Civitelli, R. (1995). Stimulation of human osteoblast differentiation and function by ipriflavone and its metabolites. *Calcified Tissue International* **55**, 356–362.

Clevenger, S. (1964). Flower pigments. *Scientific American* **210**, 84–92.

Constantinou, A., Kiguchi, K. & Huberman, E. (1990). Induction of differentiation and strand breakage in human HL-60 and K-562 leukemia cells by genistein. *Cancer Research* **50**, 2618–2624.

Coward, L., Barnes, N. C., Setchell, K. D. R. & Barnes, S. (1993). The isoflavones genistein and daidzein in soy based foods from American and Asian diets. *Journal of Agriculture and Food Science* **41**, 1961–1967.

Dehennin, L., Reiffsteck, A., Jondet, M. & Thiber, M. (1982). Identification and quantitative estimation of a lignan in human and bovine semen. *Journal of Reproduction and Fertility* **66**, 305–309.

Ferguson, D. J. P. & Anderson, T. J. (1981). Morphological evaluation of cell turnover in relation to the menstrual cycle in the 'resting' human breast. *British Journal of Cancer* **44**, 177–181.

Finlay, E. M. H., Wilson, D. W., Adlercreutz, H. & Griffiths, K. (1991). The identification and measurement of 'phyto-oestrogens' in human saliva, plasma, breast aspirate or cyst fluid, and prostatic fluid using gas chromatography–mass spectrometry. *Journal of Endocrinology* **129**, Suppl., 49 Abstr.

Fisher, B., Costantino, J. P., Redmond, C. D., Fisher, E. R., Wickerham, D. L. & Cronin, W. M. (1994). Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. *Journal of the National Cancer Institute* **865**, 527–537.

Fornander, T., Rutqvist, L. E. & Cedermark, B. (1989). Adjuvant tamoxifen in early breast cancer, occurrence of new primary cancers. *Lancet* **i**, 117.

Fotsis, T., Heikkinen, R., Adlercreutz, H., Axelson, M. & Setchell, K. D. R. (1982). Capillary gas chromatographic method for the analysis of lignans in human urine. *Clinica Chimica Acta* **121**, 361–371.

Fotsis, T., Pepper, M., Adlercreutz, H., Fleischmann, G., Hase, T., Montesano, R. & Schweigerer, L. (1993). Genistein, a dietary-derived inhibitor of angiogenesis. *Proceedings of the National Academy of Sciences, USA* **90**, 2690–2694.

Francis, C. M. & Hume, I. D. (1971). The relationship between lignification and flavonoid production in subterranean clover. *Australian Journal of Biological Science* **24**, 1–5.

Gerwirtz, D. A. (1991). Does bulk damage to DNA explain the cytostatic effects of topoisomerase II inhibitors. *Biochemical and Biophysical Research Communications* **42**, 2253–2258.

Golder, M. P., Phillips, E. A., Fahmy, D. R., Preece, P. E., Jones, V., Henk, J. M. & Griffiths, K. (1976). Plasma hormones in patients with advanced breast cancer treated with Tamoxifen. *European Journal of Cancer* **12**, 719–723.

Hanson, C. H., Loper, G. M., Kohler, G. O., Bickoff, E. M., Taylor, K. W., Kehr, W. R., Stanford, E. H., Dudley, J. W., Pedersen, M. W., Sorensen, E. L., Carnahan, H. L. & Wilsie, C. P. (1965). Variation in coumestrol content of alfalfa, as related to location, variety, cutting, year, stage of growth and disease. *United States Department of Agriculture, Agricultural Research Service technical bulletin* no. 1333, pp. 1–72. Washington, DC: US Department of Agriculture, Agricultural Research Service.

Haworth, R. D. & Slinger, F. H. (1940). The constituents of natural phenolic resins. Part XVII. A synthesis of 1-matairesinol. *Journal of the Chemical Society* 1098–1101.

Hawrylewicz, E. J., Huang, H. H. & Blair, W. H. (1991). Dietary soya bean isolate and methionine supplementation affect mammary tumour progression in rats. *Journal of Nutrition* **121**, 1693–1698.

Henderson, B. E., Ross, R. K., Judd, H. L., Krailo, M. D. & Pike, M. C. (1985). Do regulatory ovulatory cycles increase breast cancer risk? *Cancer* **56**, 1206–1208.

Hill, T. D., Dean, N. M., Mordan, L. J., Lau, A. F., Kanemitsu, M. Y. & Boynton, A. L. (1990). PDGF-induced activation of phospholipase C is not required for induction of DNA synthesis. *Science* **248**, 1660–1663.

Hirayama, T. (1979). Epidemiology of prostate cancer with special reference to the role of diet. *National Cancer Institute Monograph* **53**, 149–155.

Hirayama, T. (1986). A large scale cohort study on cancer risks by diet – with special reference to the risk reducing effects of green-yellow vegetable consumption. In *Diet, Nutrition and Cancer*, pp. 41–53 [Y. Hayashi, M. Nagao, T. Sugimura, S. Takayama, L. Tomatis, L. W. Wattenberg and G. N. Wogan, editors]. Tokyo: Japanese Scientific Society Press.

Hirohata, T., Shigematsu, T., Nomura, A. M. Y. & Horie, A. (1985). Occurrence of breast cancer in relation to diet and reproductive history: A case–control study in Fukuoka, Japan. *National Cancer Institute Monograph* **69**, 187–190.

Hughes, R., Cassidy, A. & Bingham, S. (1994). Hormonal effects of lignans in a group of men. *Proceedings of the Nutrition Society* **53**, 230A.

Jones, A. E., Price, K. R. & Fenwick, G. R. (1989). Development and application of a high-performance liquid chromatographic method for the analysis of phytoestrogens. *Journal of the Science of Food and Agriculture* **46**, 357–364.

Jordan, V. C. (1990). The only true antiestrogen is a no estrogen. *Molecular and Cellular Endocrinology* **74**, C91.

Jordan, V. C., Fritz, N. F. & Tormey, D. C. (1987). Endocrine effects of adjuvant chemotherapy and long-term Tamoxifen administration on node-positive patients with breast cancer. *Cancer Research* **47**, 624–630.

Juniewicz, P. E., Pallante Morell, S., Moser, A. & Ewing, L. L. (1988). Identification of phytoestrogens in the urine of male dogs. *Journal of Steroid Biochemistry* **31**, 987–994.

Kelly, G. E., Nelson, C., Waring, M. A., Joannou, G. E. & Reeder, A. Y. (1993). Metabolites of dietary (soya) isoflavones in human urine. *Clinica Chimica Acta* **223**, 9–22.

Kennedy, A. R. (1995). The evidence of soybean products as cancer prevention agents. *Journal of Nutrition* **125**, 733S–743S.

Lamartiniere, C. A., Moore, J., Holland, M. B. & Barnes, S. (1995). Neonatal genistein chemoprevents mammary carcinogenesis. *Proceedings of the Society for Experimental Biology and Medicine* **208**, 120–123.

Lee, H. P., Gourley, L., Duffy, S. W., Esteve, J., Lee, J. & Day, N. E. (1991). Dietary effects on breast cancer risk in Singapore. *Lancet* **337**, 1197–1200.

Makela, S. I., Pylkkanen, L. H., Santti, R. S. S. & Adlercreutz, H. (1995). Dietary soybean may be antiestrogenic in male mice. *Journal of Nutrition* **125**, 437–445.

Markovitz, J., Linassier, C., Fosse, P., Couprie, J., Jaquemin-Sablon, A., Pecq, J. B. & Larson, A. K. (1989). Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. *Cancer Research* **49**, 5111–5117.

Martin, P. M., Horwitz, K. B., Ryan, D. S. & McGuire, W. L. (1978). Phytoestrogens interaction with estrogen receptors in human breast cancer cells. *Endocrinology* **103**, 1860–1867.

Medlock, K. L., Branham, W. S. & Sheehan, D. M. (1995). Effects of coumestrol and equol on the developing reproductive tract of the rat. *Proceedings of the Society for Experimental Biology and Medicine* **208**, 67–71.

Messina, M. J., Persky, V., Setchell, K. D. R. & Barnes, S. (1994). Soy intake and cancer risk: A review of the in vitro and in vivo data. *Nutrition and Cancer* **21**, 113–131.

Miyazawa, K. (1976). Incidence of endometriosis among Japanese women. *Obstetrics and Gynaecology* **48**, 407–409.

Morley, F. H. & Francis, C. M. (1968). Varietal and environmental variations in isoflavone concentrations in subterranean clover. *Australian Journal of Agricultural Science* **19**, 15–26.

Morton, M. S., Wilcox, G., Wahlqvist, M. L. & Griffiths, K. (1994). Determination of lignans and isoflavonoids in human female plasma following dietary supplementation. *Journal of Endocrinology* **142**, 251–259.

Muir, C., Waterhouse, J., Mack, T., Powell, J. & Whelan, S. (1987). *Cancer Incidence in Five Continents*, vol. 5, *International Agency for Research on Cancer Scientific Publication* no. 88. Lyon, France: IARC Scientific Publications.

Murkies, A. L., Lombard, C., Strauss, B. J. G., Wilcox, G., Burger, H. G. & Morton, M. S. (1995). Dietary flour supplementation decreases postmenopausal hot flushes – effect of soy and wheat. *Maturitas* **21**, 189–195.

Naim, M., Gestetner, B., Zilkah, S., Birk, Y. & Bondi, A. (1974). Soybean isoflavones. Characterisation, determination and antifungal activity. *Journal of Agricultural and Food Chemistry* **22**, 169–170.

Nomura, A., Henderson, B. E. & Lee, J. (1978). Breast cancer and diet among the Japanese in Hawaii. *American Journal of Clinical Nutrition* **31**, 2020–2025.

Oishi, K., Okada, K., Yoshida, O., Yamabe, H., Ohno, Y., Hayes, R. B. & Schroeder, F. H. (1988). A case–control study of prostatic cancer with reference to dietary habits. *Prostate* **12**, 179–190.

Okura, A., Arakawa, H., Oka, H., Yoshinara, T. & Monden, Y. (1988). Effect of genistein on topoisomerase activity and on the growth of (Val 12) Ha-ras-transformed NIH 3T3 cells. *Biochemical and Biophysical Research Communications* **157**, 183–189.

Peterson, G. & Barnes, S. (1991). Genistein inhibition of the growth of human breast cancer cells – independence from estrogen receptors and multidrug resistance gene. *Biochemical and Biophysical Research Communications* **179**, 661–667.

Peterson, G. & Barnes, S. (1993). Isoflavones inhibit growth of human prostate cancer cell lines without inhibiting epidermal growth factor receptor phosphorylation. *Prostate* **22**, 335–345.

Phipps, W. R., Martini, M. C., Lampe, J. W., Slavin, J. L. & Kurzer, M. S. (1993). Effect of flaxseed ingestion on the menstrual cycle. *Journal of Clinical Endocrinology and Metabolism* **77**, 1215–1219.

Price, K. R. & Fenwick, G. R. (1985). Naturally occurring oestrogens in foods – A review. *Food Additives and Contaminants* **2**, 73–106.

Rao, C. B. S. (1978). *The Chemistry of Lignans*. Waltair, India: Andhra University Press.

Sargeant, P., Farndale, R. W. & Sage, S. O. (1993). The tyrosine kinase inhibitors methyl 2,5-dihydroxy-cinnimate and genistein reduce thrombin-evoked tyrosine phosphorylation and $Ca2+$ entry in human platelets. *FEBS Letters* **315**, 242–246.

Setchell, K. D. R. (1985). Naturally occurring non-steroid estrogens of dietary origin. In *Estrogens in the Environment* II, *Influences on Development*, pp. 69–86 [J. A. MacLachan, editor]. New York: Elsevier.

Setchell, K. D. R., Borriello, S. P., Hulme, P. & Axelson, M. (1984). Nonsteroidal estrogens of dietary origin: possible roles in hormone-dependent disease. *American Journal of Clinical Nutrition* **40**, 569–578.

Setchell, K. D. R., Gosselin, S. J., Welsh, M., Johnston, J. O., Balistreri, W. F., Kramer, L. W., Dresser, B. L. & Tarr, M. J. (1987a). Dietary oestrogens – a probable cause of infertility and liver disease in captive cheetah. *Gastroenterology* **93**, 225–233.

Setchell, K. D. R., Lawson, A. M., Conway, E., Taylor, N. F., Kirk, D. N., Cooley, G., Farrant, R. D., Wynn, S. & Axelson, M. (1981). The definitive identification of the lignans trans-2,3-bis(3-hydroxybenzyl)-y-butyrolactone and 2,3-bis(3-hydroxybenzyl)-butane-1,4-diol in human and animal urine. *Biochemical Journal* **197**, 447–458.

Setchell, K. D. R., Lawson, A. M., McLaughlin, L. M., Patel, S., Kirk, D. N. & Axelson, M. (1983). Measurement of enterolactone and enterodiol, the first mammalian lignans, using stable isotope dilution and gas chromatography. *Biomedical Mass Spectrometry* **10**, 227–235.

Setchell, K. D. R., Welsh, M. & Lim, C. K. (1987b). High-performance liquid chromatographic analysis of phytoestrogens in soy protein preparations with ultraviolet, electrochemical and thermospray mass spectrometric detection. *Journal of Chromatography* **386**, 315–323.

Severson, R. K., Nomura, A. M. Y., Grove, J. S. & Stemmermann, G. N. (1989). A prospective study of demographics, diet and prostate cancer among men of Japanese ancestry in Hawaii. *Cancer Research* **49**, 1857–1860.

Sharma, O. P., Adlercreutz, H., Strandberg, J. D., Zirkin, B. R. & Coffey, D. S. (1992). Soy of dietary source plays a preventative role against the pathogenesis of prostatitis in rats. *Journal of Steroid Biochemistry and Molecular Biology* **43**, 557–564.

Shultz, T. D., Bonorden, W. R. & Seaman, W. R. (1991). Effect of short term flaxseed consumption on lignan and sex hormone metabolism in men. *Nutrition Research* **11**, 1089–1100.

Shutt, D. A. (1976). The effect of plant oestrogens on animal reproduction. *Endeavour* **35**, 110–113.

Shutt, D. A. & Cox, R. I. (1972). Steroid and phytoestrogen binding to sheep uterine receptors *in vitro*. *Journal of Endocrinology* **52**, 299–310.

Sirtori, C. R., Even, R. & Lovati, M. R. (1993). Soyabean protein diet and plasma cholesterol: From therapy to molecular mechanisms. *Annals of the New York Academy of Sciences* **676**, 188–201.

Smith, D. C., Prentice, R. & Thompson, D. J. (1975). Association of exogenous estrogen and endometrial carcinoma. *New England Journal of Medicine* **293**, 1164–1167.

Tanimura, M., Kobuchi, H., Utsumi, T., Yoshioka, T., Kataoka, S., Fujita, Y. & Utsumi, K. (1992). Neutrophil priming by granulocyte colony stimulating factor and its modulation by protein kinase inhibitors. *Biochemical Pharmacology* **44**, 1045–1052.

Tataryn, I. V., Meldrum, D. R., Lu, K. H., Frumar, A. M. & Judd, H. L. (1979). LH, FSH and skin temperature during the menopausal hot flush. *Journal of Clinical Endocrinology and Metabolism* **49**, 152–154.

Thompson, L. U., Robb, P., Serraino, M. & Cheung, F. (1991). Mammalian lignan production from various foods. *Nutrition and Cancer* **16**, 43–52.

Troll, W., Wiesner, R., Shellabarger, C. J., Holtzman, S. & Stone, J. P. (1980). Soybean diet lowers breast tumour incidence in irradiated rats. *Carcinogenesis* **1**, 469–472.

Utsumi, T., Klostergard, J., Akimura, K., Edashige, K. & Sato, E. F. (1992). Modulation of TNF-alpha-priming and stimulation dependent superoxide generation in human neutrophils by protein kinase inhibitors. *Archives of Biochemistry and Biophysics* **294**, 271–278.

Verdeal, K., Brown, R. R., Richardson, T. & Ryan, D. S. (1980). Affinity of phytoestrogens for estradiol-binding proteins and effect of coumestrol on growth of 7,12-dimethylbenz(α)anthracene-induced rat mammary tumors. *Journal of the National Cancer Institute* **64**, 285–290.

Wei, H., Wei, L., Frenkel, K., Bowen, R. & Barnes, S. (1993). Inhibition of tumour-promoter induced hydrogen peroxide formation by genistein in vitro and in vivo. *Nutrition and Cancer* **20**, 1–12.

Whitten, P. L., Lewis, C. & Naftolin, F. (1992). A phytoestrogen diet induces the premature anovulatory syndrome in lactationally exposed female rats. *Biology of Reproduction* **49**, 1117–1121.

Wilcox, G., Wahlqvist, M. L., Burger, H. G. & Medley, G. (1990). Oestrogen effects of plant derived foods in postmenopausal women. *British Medical Journal* **301**, 905–906.

Wilcox, J. N. & Blumenthal, B. F. (1995). Thrombotic mechanisms in atherosclerosis: Potential impact of soy proteins. *Journal of Nutrition* **125**, 631S–638S.

Willis, K. J., London, D. R., Ward, H. W. C., Butt, W. R., Lynch, S. S. & Rudd, B. T. (1977). Recurrent breast cancer treated with the antiestrogen tamoxifen: correlation between hormonal changes and clinical course. *British Medical Journal* **i,** 425–428.

Xu, X., Wang, H.-J., Murphy, P. A., Cook, L. & Hendrich, S. (1994). Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. *Journal of Nutrition* **124,** 825–832.

Yamashita, Y., Kawada, S. & Nakano, H. (1991). Induction of mammalian topoisomerase II dependent DNA cleavage by non-intercalative flavonoids genistein and orobol. *FEBS Letters* **288,** 46–50.

Yuan, M.-J., Wang, Q.-S., Ross, R. K., Henderson, B. E. & Yu, M. C. (1995). Diet and breast cancer in Shanghai and Tianjin, China. *British Journal of Cancer* **71,** 1353–1358.

Printed in Great Britain