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Dietary phyto-oestrogens: molecular mechanisms, bioavailability and importance to menopausal health

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Following the high-profile studies on hormone replacement therapy which provided little evidence in support of the drug therapy improving future health, there remains a growing demand for dietary solutions for maintaining health and preventing disease as women age. Although interest in the relative importance of phyto-oestrogens to human health has increased dramatically over the last decade, the effective dose for health benefits and hypothetical issues on safety remain to be resolved. Plausible mechanisms and epidemiological data are available to support the concept that phyto-oestrogen-rich diets exert physiological effects, but optimal doses and sources of these compounds have still not been elucidated for specific health benefits. In addition, much of the current mechanistic data are difficult to interpret as the experiments have incorporated levels of phyto-oestrogens that may not be achievable *in vivo* and have to date only used aglycones and glycosides of the pure compounds rather than examining the biological effects of gut and liver metabolites. The present review will concentrate on the isoflavone subclass of phyto-oestrogens, as, to date, these compounds have received most attention from both a commercial and research perspective.

Phyto-oestrogens: Menopausal women: Disease prevention: Isoflavones

Introduction

Phyto-oestrogens are multi-faceted compounds; however, to date, much of the interest in their biological activity has related to oestrogen receptor (ER)-mediated mechanisms, but the relative importance of non-oestrogenic mechanisms of action in defining their relative importance to human health has been gaining momentum, particularly in relation to women's health. The well-publicised results of two large-scale hormone replacement therapy (HRT) trials, the Women's Health Initiative in the USA and the Million Women's study in the UK, showing evidence of an increased risk of combined HRT on breast cancer, heart disease, stroke and venous thromboembolism (Rossouw *et al.* 2002; Banks *et al.* 2003) have led to the conclusion that HRT will not protect future health although short-term use remains beneficial for severe menopausal symptom relief (McPherson, 2004). Since the justification for long-term HRT can no longer be applied for disease prevention, women continue to seek alternative 'natural' options, such as phyto-oestrogens, to improve their quality of life and reduce their risk of disease. However, many are unaware of the limited scientific evidence of safety and efficacy of such natural therapies.

Traditionally, phyto-oestrogens have been considered to be weakly oestrogenic and it is well established that serum levels of isoflavones following consumption of a modest intake of soya foods can reach the low micromolar level, about 100–1000 times that of oestradiol. Therefore, even if these compounds have a weak potency, they have the potential to exert biological effects *in vivo*; such effects have been reported in several trials using a range of different endpoints (Cassidy *et al.* 1994, 1995; Nestel *et al.* 1999; Davis *et al.* 2001; Djuric *et al.* 2001). The biological action of phyto-oestrogens is complex and their ultimate cellular actions are determined by many factors including the relative levels of ERα and β, the diverse mixture of coactivators and co-repressors present in any given cell type, and the nature of the response elements with which the receptors interact and modulate gene expression (Montano & Katzenellenbogen, 1997). It is thus not surprising that the resulting effects observed from available *in vitro* and *in vivo* experiments are inconsistent, since the biological effects vary depending on the phyto-oestrogen compound studied, cell line used, and the species and tissue under examination. Numerous other biological effects independent of the ER (for example; antioxidant capacity, antiproliferative and anti-angiogenic effects) have been ascribed to phyto-oestrogens, and many of these mechanisms are common to other plant phenolics (Setchell & Cassidy, 1999).

Abbreviations: ER, oestrogen receptor; HRT, hormone replacement therapy.

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Molecular mechanisms of action

Oestrogen receptor-mediated mechanisms of action

Until recently the predominant research on the mechanisms of action of phyto-oestrogens has concentrated on their ER-mediated effects. The oestrogenic activity of isoflavones was first described in the 1940s when infertility of sheep in Western Australia was caused by ingestion of clover rich in the isoflavone precursors, formononetin and biochanin A (Bennetts *et al.* 1946). These animal data, together with their similar spatial conformation to mammalian oestrogens (Fig. 1) and ability to bind to ER and alter oestrogen-regulated genes (Markiewicz *et al.* 1993), stimulated interest in the oestrogenic proprieties of isoflavones.

Traditionally, isoflavones have been considered to be weakly oestrogenic compared with 17-β oestradiol, but with divergent estimates of oestrogenicity depending on the assay system used. *In vitro*, concentrations of phyto-oestrogens equivalent to humans consuming a moderate phyto-oestrogen intake stimulate cell growth in oestrogen-positive, but not oestrogen-negative cells. In contrast, very high concentrations (possibly achievable from supplement intake) inhibit cell growth in both ER-positive and -negative cell lines (Zava *et al.* 1997; Sathyamoorthy *et al.* 1998; Miodini *et al.* 1999). Nevertheless, the estimates of oestrogenicity suggest that these compounds have the capacity to exert physiological effects *in vivo* because serum levels of phyto-oestrogens following the consumption of soya will exceed endogenous oestrogen levels by several orders of magnitude (Axelson *et al.* 1984; Adlercreutz & Mazur, 1997). In addition, isoflavones may be more available to tissues by binding less tightly to serum proteins than oestrogens (Nagel *et al.* 1998) and tissue-selective effects are possible given the higher binding affinity of isoflavones to ERβ compared with ERα (Kuiper *et al.* 1997, 1998) and the different tissue distribution of this receptor sub-type (Couse *et al.* 1997; Nilsson *et al.* 1998; Cassidy & Faughnan, 2000). Oestrogens and isoflavones also have wide differences in transcriptional activity which results not only from their differences in binding affinities but also from differences in their ability to recruit co-regulators and trigger transcriptional functions of ERα and ERβ (An *et al.* 2001). The isoflavone gensitein has 1000-fold greater potency at triggering transcriptional activity with ERβ than ERα (Pike *et al.* 1999; An *et al.* 2001). Therefore, understanding the role of ERβ in different tissues is critical in further understanding the role of isoflavones in specific diseases.

Non-oestrogen receptor-mediated mechanisms of action

Many mechanisms of action of isoflavones occur without direct interaction with ER including influencing cell signalling, cell division and growth and gene expression. Specifically, the isoflavone genistein *in vitro* inhibits enzymes involved in oestrogen and androgen metabolism, and inhibits tyrosine kinase activity, angiogenesis and DNA repair enzymes (Akiyama *et al.* 1987; Kao *et al.* 1998; Kim *et al.* 1998). However, *in vitro* many of these inhibitory effects occur only at levels that exceed 25 μmol (Barnes *et al.* 2000). This contrasts to peak genistein levels of about 5 μmol following *in vivo* soya consumption, and most of this genistein will be conjugated with glucuronic acid and therefore less biologically active. It is therefore unclear how relevant such *in vitro* findings are to man.

Isoflavones possess antioxidant properties; however, to date most studies have focused predominantly on the antioxidant effects of the isoflavone precursor, genistein (Wei *et al.* 1993; Rimbach *et al.* 2004). It has been demonstrated that genistein is a more effective antioxidant than daidzein, and this is probably attributable to the presence of a third hydroxyl group in the C-5 position. Interestingly, the gut metabolite of daidzein, equol, is a more potent antioxidant than either daidzein or genistein or the parent glycosides, suggesting that the absence of the 2,3-double bond in conjunction with a loss of the

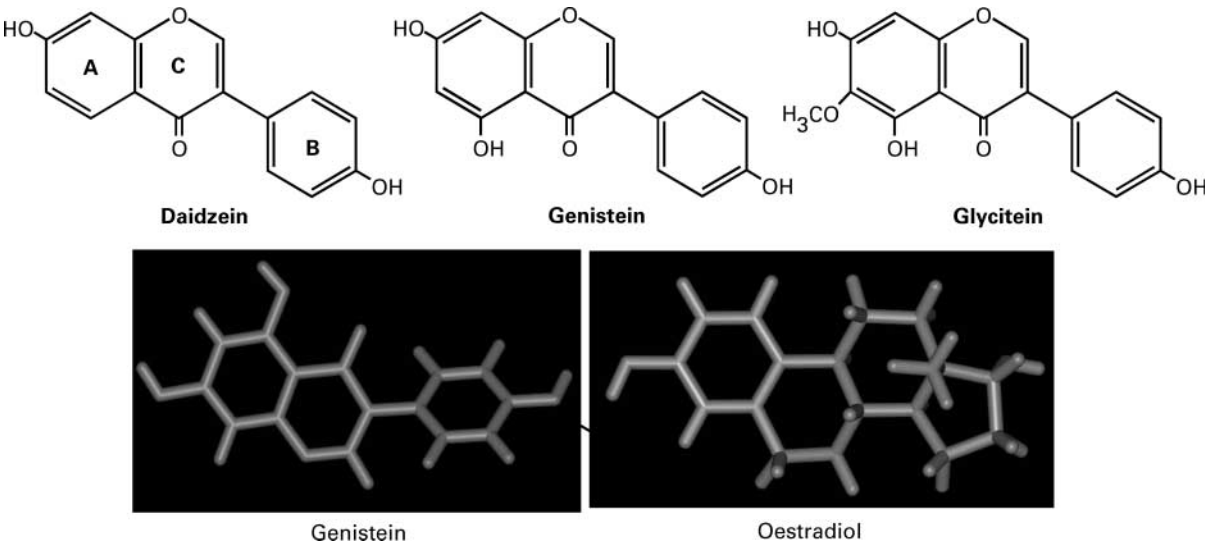

Fig. 1. The chemical structures of the isoflavone aglycones, daidzein, genistein and glycitein and a three-dimensional comparison of the structure of genistein and the female hormone oestradiol.

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4-oxo group enhances antioxidant properties (Arora *et al.* 1998). Antioxidant activity, assessed by the trolox equivalent antioxidant capacity (TEAC) assay also suggests that equol is a more potent isoflavone compared with genistein and daidzein (Mitchell *et al.* 1998). Proposed molecular mechanisms responsible for their antioxidant potential include the ability to scavenge radicals, chelate metals, inhibit H_2O_2 production and stimulate antioxidant enzymes, including catalase (Fig. 2). The ability of isoflavones to scavenge hydroxyl, superoxide, NO, diphenylpicrylhydrazyl, galvinoxyl, and lipid-derived radicals has also been investigated with no significant scavenging effects on these radicals at concentrations up to 1·0 mM for a range of isoflavones (Guo *et al.* 2002*a*,*b*). However, at a concentration of 5 nM, both genistein and daidzein resulted in modest increases in intracellular-reduced glutathione levels in human endothelial cells, while cellular α-tocopherol and uric acid remained unchanged following isoflavone treatment. These data suggest that the free radical-scavenging activities of the isoflavones tested may not substantially contribute to their antioxidant properties, and the ability of genistein and daidzein to increase cellular reduced glutathione may make a more significant contribution to their biological action. Recent data suggest that sulfation of genistein, with the associated loss of hydroxyl groups, decreases its beneficial activity on platelet aggregation and inflammation, as well as cell adhesion and chemotaxis (Rimbach *et al.* 2004; Turner *et al.* 2004).

Food sources

The most extensively studied class of the phyto-oestrogens, the isoflavones, occurs largely in soyabeans and a few other legumes (Coward *et al.* 1993). To date, twelve different soyabean isoflavone isomers have been identified. Most dietary sources contain a mixture of derivatives based on the isoflavone aglycones, daidzein, genistein and glycitein (Fig. 1). As well as the aglycone form, isoflavones may be present in soya foods as glucosides, acetyl glucosides or malonyl glucosides. Typically, soyabeans and soya foods contain more genistein than daidzein (Murphy *et al.* 1999).

Although all soyabean-derived protein extracts and foods available for human consumption contain significant levels of isoflavones, there is large variability in concentration and profile among these products that depends on species, geographical and environmental conditions, and the extent of industrial processing of the soyabeans (Coward *et al.* 1993). However, even for a given brand of soya product, recent data suggest significant variation in isoflavone levels over time (Setchell & Cole, 2003). These data reinforce the importance of accurately defining the isoflavone content of foods or supplements used in clinical intervention trials and question the validity of setting up databases with phyto-oestrogen content of foods designed to estimate isoflavone content of the diet.

There are numerous commercial phyto-oestrogen supplements available, which are predominantly promoted for their value in treating postmenopausal conditions. These are made from a variety of sources, including concentrated soyabean extracts, or an extract of red clover. However, to date there are limited data examining the relative clinical effectiveness of specific preparations, and analytical data suggest that quality assurance is a significant issue with commonly available isoflavone supplements (Setchell *et al.* 2001). In particular, since dietary supplements and some foods enriched in phyto-oestrogens contain comparatively high amounts of these compounds, consumers may be exposed to high concentrations and the relative risk:benefit from such consumption over the short and longer term warrants further investigation in clinical trials.

In general, Western populations consume low levels of isoflavones because few foods included in the typical Western diet contain soya protein, the fraction with which isoflavones are associated. The average daily dietary intake

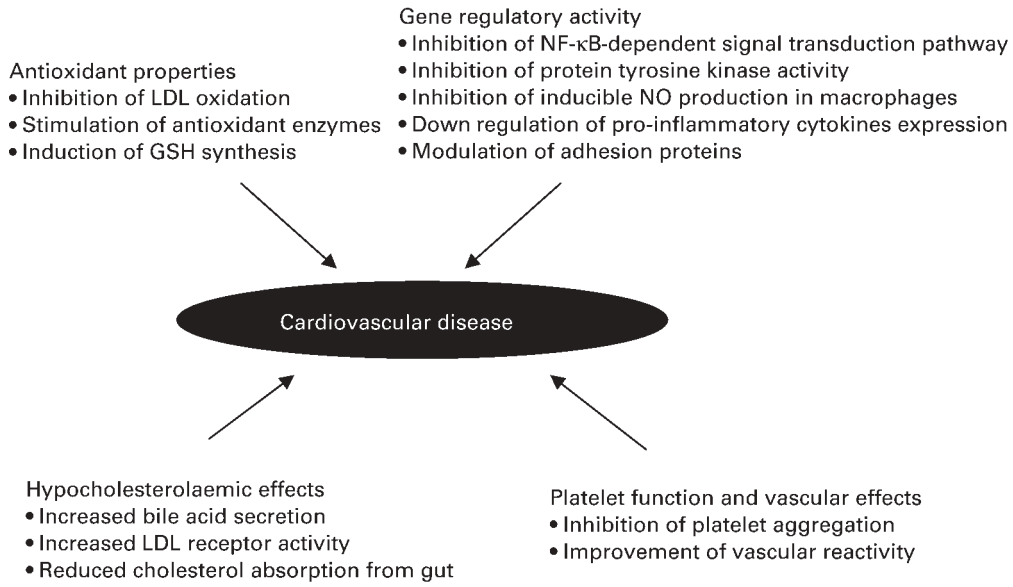

Fig. 2. Proposed molecular mechanisms of action of isoflavones on CVD risk.

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of isoflavones among Western populations is negligible (< 1 mg/d; Setchell & Cassidy, 1999) and the lack of these dietary phytoprotectants is viewed as one explanation for the disparity in disease incidence rates between Western and Asian populations.

Soya has been a traditional staple in Far Eastern countries for generations, and the lower incidences of osteoporosis, breast cancer, and menopausal symptoms among women who consume soya as a dietary staple have been suggested in part to be due to the high intake of isoflavones (Axelson *et al.* 1984; Setchell *et al.* 1984; Adlercreutz *et al.* 1986). In the early 1990s estimates of intakes in Asian countries were in the region of 100 mg isoflavones/d but these data are now acknowledged as overestimates. As the methods of analysis of levels in foods have been improved there has been a move towards a consistent form of reporting of intake in aglycone equivalents (converted from glucosides using the appropriate ratios of molecular weights). More recent estimates of the amounts of soya food consumed in Japan indicate typical isoflavone intakes of 11–40 mg/d (reported as aglycone equivalents to standardise doses between different foods) in adults (Nagata *et al.* 1998; Munro *et al.* 2003). This would convert to 18–63 mg/d as the glucoside using 1·61 as the conversion factor based on the ratios of genistein, daidzein and glycitein in the most commonly consumed foods (Beecher *et al.* 1999). However, it is difficult to determine the precise isoflavone intake in countries such as China, Korea, Indonesia and Japan. Food and eating trends are changing rapidly, intake levels may vary between urban and rural areas, and intake is affected by generational and lifestyle differences. However, data from several studies suggest that intakes of 60 mg isoflavones (expressed aglycone equivalents)/d is not uncommon, with intake levels ranging from 32 to 66 mg aglycone equivalents in several recent studies (Seow *et al.* 1998; Chen *et al.* 1999; Wakai *et al.* 1999). The most widely used soya products, soya oil, soya sauce, and soya lecithin, do not have significant levels of isoflavones, and this is also the case for aqueous alcohol-washed soya proteins (Coward *et al.* 1993).

The equol phenotype

Equol does not naturally occur in plants but is a specific bacterial metabolite, which is found in high concentrations in urine and plasma following the consumption of isoflavone-rich foods (Axelson *et al.* 1982, 1984). Our recent data, using stable isotopes of the pure compounds, showed conclusively that it is a metabolite specifically formed following daidzein consumption (Setchell *et al.* 2003*b*).

The consistent observation that all adults do not synthesise equol in response to challenges from soya foods or isoflavones has led to the realisation that there are two distinct sub-groups of the population, defined as 'equol producers' and 'non-equol producers'. The factors governing equol production remain poorly understood (Lampe *et al.* 1998; Rowland *et al.* 1999, 2003; Setchell *et al.* 2002*b*), but emerging data from clinical studies suggest that the ability to produce equol following the ingestion of soya isoflavones may be a significant factor in the clinical effectiveness to soya diets. This factor has not previously been considered in the design of dietary intervention studies examining the effectiveness of soya diets (Setchell *et al.* 2002*b*). The greater efficacy of soya diets in subjects who can make equol and the paucity of data on the bacteriology involved in its production present challenges for developing strategies to convert non-equol producers into equol producers, as this metabolite may be important in explaining the potential efficacy of isoflavones.

Equol production may enhance the action of isoflavones as it has a lower affinity for serum proteins, greater affinity for ER compared with its precursors, daidzein and dihydrodaidzein and exerts superior antioxidant activity (Shutt & Cox, 1972; Hodgson *et al.* 1996; Arora *et al.* 1998). Equol exists in two enantiomeric forms, S and R equol, and recent data suggest that only the S isomer is present in man (Setchell *et al.* 2002*a*).

The hormonal activity of equol was first evident in human subjects when high inter-individual variation in the excretion of equol was observed in volunteers who were taking 45 mg isoflavones/d (Cassidy *et al.* 1994, 1995). Following this consumption of isoflavones from soya, a significant increase in menstrual cycle length was observed, a finding that was not observed when soya devoid of isoflavones were fed. Follicular phase length was correlated with urinary equol excretion, adding weight to the evidence that equol is a more potent isoflavone. A significant amount of literature exists on the biological and clinical effectiveness of soya in relation to heart disease, bone health, menopausal symptoms and hormone-dependent cancers, with wide variability in responses reported. This variability in response may be related to subjects' ability to produce equol as it is well established that only 30–40 % of any given population group studied can produce equol (Cassidy *et al.* 1994, 1995; Rowland *et al.* 2003; Atkinson *et al.* 2005). However, in two recent studies there have been inconsistent results. No significant differences in serum hormone concentrations were observed in postmenopausal women in relation to equol phenotype (Frankenfeld *et al.* 2004), while in a group of premenopausal women the equol producers had lower serum concentrations of oestrogen and androgens (Duncan *et al.* 2000).

An inability to produce equol may be related to an absence of appropriate enzymes in the intestinal microflora or absence of bacterial species capable of producing equol (Adlercreutz *et al.* 1981; Setchell *et al.* 1984). Its formation is exclusively related to intestinal microflora as germ-free rats do not excrete equol (Adlercreutz *et al.* 1981; Axelson *et al.* 1982) and the absence of equol from infant blood samples following soya infant formula ingestion add weight to the need for an active microflora for its formation (Setchell *et al.* 1997).

The metabolism of isoflavones in animals and man is complex and is a combination of both mammalian and gut microbial processes (Setchell & Cassidy, 1999; Rowland *et al.* 2003). Factors that may be important in influencing the large interindividual variation in the metabolism and excretion of isoflavones, particularly with respect to equol, are complex but relate to the composition of diet and the human gut microflora (Lampe *et al.* 1998; Setchell & Cassidy, 1999; Rowland *et al.* 2000). Prospective intervention studies in equol-producing subjects are required

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to further elucidate factors governing the conversion of daidzein to equol and to determine the potential role of prebiotics in influencing the ability of individuals to convert daidzein to equol in the large gut. In addition, determining its potential clinical significance merits further investigation in human intervention studies.

Bioavailability

Bioavailability of phyto-oestrogens is based on data from absorption, metabolism, distribution and excretion studies conducted both in human subjects and animals. Following the consumption of either pure compounds, isoflavone-rich extracts, or foods or beverages rich in isoflavones, the parent compounds and their metabolites can be detected in the plasma and urine of human volunteers. Most of the available pharmacokinetic data on phyto-oestrogens relate to levels attained in plasma and urine of specific isoflavones and their metabolites; for example, daidzein and genistein.

After ingestion, isoflavones are hydrolysed by intestinal glucosidases, which release the aglycones daidzein, gensitein and glycitein (Fig. 3). These may be absorbed or further metabolised to many specific metabolites including equol and *p*-ethyl phenol (Axelson *et al.* 1984; Bannwart *et al.* 1984; Kelly *et al.* 1993; Joannou *et al.* 1995). Because there are currently no guidelines on optimal levels of isoflavones and there are limited data on their bioavilability from foods (Xu *et al.* 1994; Setchell *et al.* 2001, 2003*a*,*b*), dietary intakes in clinical studies examining the risks and benefits of isoflavones for human health have to date been empirically derived. The daily intake of about 50 mg isoflavone glucosides/d which has predominantly been used in clinical intervention studies appears to be largely based on our earlier observation that daily consumption of soya foods containing 45 mg isoflavone glucosides caused endocrine modulation of the menstrual cycle in healthy premenopausal women (Cassidy *et al.* 1994, 1995). More recently there has been a tendency to use relatively large dietary intakes of soya isoflavones derived from foods or supplements far exceeding typical consumption levels in Asian countries (Nagata *et al.* 1998; Chen *et al.* 1999; Wakai *et al.* 1999). However, the rationale for these higher intakes remains unclear and is not based on knowledge of their pharmacokinetic behaviour. As with pharmacological compounds, demonstrating efficacy of soya and its isoflavones requires knowledge of their bioavailability, but, to date, there is limited information on how this varies among subjects and whether it is influenced by age or other factors.

Although it is well established that infants, in contrast to adults, are unable to metabolise isoflavones (Setchell *et al.* 1997), to date there are limited data available examining the effect of age on isoflavone metabolism and absorption in later life. This is particularly important since much of the current interest in relation to isoflavones relates to the health of postmenopausal women. However, to date, many studies investigating the biological effects of these compounds have been carried out in premenopausal women (Cassidy *et al.* 1994, 1995). Furthermore, the influence of sex is contentious, with several studies suggesting that urinary isoflavone kinetics are not related to sex (Setchell *et al.* 1984; Kelly *et al.* 1993; Kirkman *et al.* 1995; Lampe *et al.* 1998), while others are suggestive of a sex difference in absorption and metabolism of these compounds (Lu & Anderson, 1998).

In Asian countries where soya is consumed as a staple it remains to be determined if the chemical composition of the soya food alters absorption and metabolism and thus potential biological efficacy of soya isoflavones. Asian populations have traditionally consumed primarily fermented soya protein products, and since these foods contain a higher proportion of aglycone isoflavones, it has been suggested that they may be more bioavailable since these aglycone isoflavones do not require hydrolysis in the intestine before absorption. There are preliminary suggestions that urinary recoveries of daidzein and genistein following the ingestion of fermented soya foods may be greater (Cassidy *et al.* 1995; Hutchins *et al.* 1995), but this contrasts with recent data on the pure compounds which suggests no differences in the apparent bioavailability of pure daidzein and gensitein tablets when consumed as either aglycones or glycosides (Zubik & Meydani, 2003). Although in their purified form, daidzein and genistein aglycones are more rapidly absorbed into the systemic circulation (Izumi *et al.* 2000; Setchell *et al.* 2001), other data suggest that the overall systemic bioavailability of the pure aglycone compounds was lower compared with their glycoside forms (daidzin and genistin) (Setchell *et al.* 2001). However, whether the same effects are observed when subjects are fed different soya foods containing isoflavones in the conjugated or unconjugated form remains to be investigated.

In human intervention trials investigating the biological effects of these compounds in relation to human health,

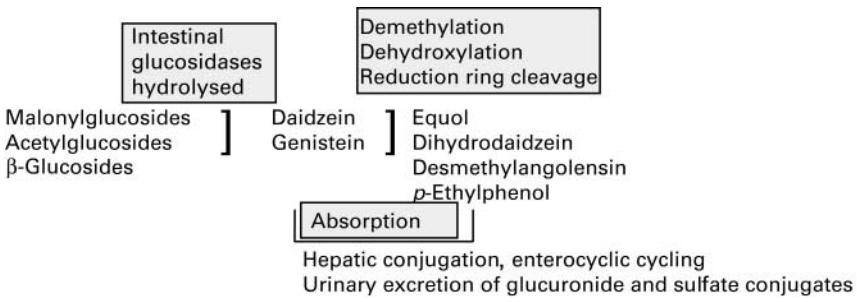

Fig. 3. Absorption and metabolism of isoflavones in man.

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urinary phyto-oestrogens are frequently used as a biomarker of their potential bioavailability from foods or supplements rich in these compounds, in part because urine is an easier biological sample to collect, and because urine contains 100-fold higher concentrations of isoflavones (Cassidy *et al.* 1994; Setchell *et al.* 2001, 2003*b*). Our recent data from pharmacokinetic studies using stable isotopes of the pure isoflavone compounds, daidzein and gensitein, suggest that the collection of random 'spot' urine samples does not correlate with serum measurements, but 24 h urine collections monitored for completeness with an exogenous marker correlate well with serum assessment of systemic bioavailability of these compounds (Setchell *et al.* 2003*b*). Additionally, quantitative assessment in urine provides information on the extent of intestinal metabolism of isoflavones and provides information on subject compliance within soya intervention trials.

Knowledge of the pharmacokinetics of phyto-oestrogens is essential before making recommendations regarding long-term efficacy in clinical studies, as recent research suggests significant differences in bioavailability between foods rich in phyto-oestrogens, and supplements (Setchell *et al.* 2003*c*; Faughnan *et al.* 2004). In addition, the dose administered, food matrix and the chemical form of the compound appear to exert effects on the bioavailability (Faughnan *et al.* 2004). Maintenance of a steady-state serum level should be optimal for clinical effectiveness of these compounds and on the basis of current pharmacokinetic data, this would be best achieved by divided doses of the soya food or supplement throughout the day, rather than by a single dose. The available absorption and metabolism data for these compounds suggest that levels attained in plasma do not increase in a linear fashion with increased intake (Setchell *et al.* 2003*c*; Faughnan *et al.* 2004), suggesting limited advantage in consuming high levels of these compounds through functional foods and supplements, but data on the levels attained in plasma following mega-dosing of these compounds warrant further investigation. Importantly, increases in the concentrations of isoflavones and its associated metabolites in plasma and urine do not necessarily mean that they have additional effects *in vivo*.

Potential health effects

The international variation in CVD, osteoporosis, menopausal symptoms, breast and prostate cancer has stimulated interest in the role of isoflavones in the diet as potentially protective components. In Asia, where urine and plasma levels of isoflavones are high, these conditions are rare (Adlercreutz *et al.* 1986). However, to date, clinical studies that have examined the potential of isoflavones to cause physiological effects in human subjects have been limited to epidemiological studies, or to dietary intervention trials that have examined effects on menopausal symptoms, cardiovascular function, and endocrine regulation of the menstrual cycle. Overall, these dietary studies have shown effects that may be interpreted as beneficial, but it is difficult to tease out the precise contribution that isoflavones play in the overall endpoints measured. In particular, we have insufficient data to ascertain the optimal dose of isoflavone necessary to exert specific clinical effects (Setchell & Cassidy, 1999).

Breast cancer

Interest in the potential role of phyto-oestrogens in reducing risk of breast cancer stemmed from the intriguing epidemiological data showing low breast cancer incidence in Asian countries where soya is more frequently consumed. Associations between consumption of isoflavone-containing foods (soya) and breast cancer risk have been inconsistent and were recently reviewed (Peeters *et al.* 2003); however, in a recent prospective cohort study in Japan, consumption of isoflavones from foods was inversely related to risk of breast cancer, with the greatest reduction of risk for postmenopausal women (Yamamoto *et al.* 2003).

Although sex steroid hormones play a central role in breast carcinogenesis, evidence from *in vitro* and animal studies suggest that phyto-oestrogens may inhibit the development of mammary tumours through their role in regulating the synthesis, metabolism and signal transduction of steroid hormones (Barnes, 1998; Messina & Loprinzi, 2001). The molecular mechanisms involved in potentially explaining the cancer-preventative properties of isoflavones are not completely understood and may only be partially mediated by the alteration of ER-dependent pathways, as isoflavones can exert hormonal and anti-oestrogenic effects either with or without direct interaction with ER; *in vitro* isoflavones inhibit enzymes involved in oestrogen metabolism, inhibit aromatase and inhibit 17β oxidoreduction of oestrogens (at relatively low micromolar concentrations) (Ibrahim & Abulhajj, 1990; Kao *et al.* 1998; Le Lain *et al.* 2001). Low concentrations of isoflavones also inhibit hydroxysteroid dehydrogenase (Makela *et al.* 1998; Wahala & Alho, 2002). This is together with the other described mechanisms of action including inhibition of tyrosine kinase, DNA topoisomerases, angiogenesis and antioxidant effects (Setchell & Cassidy, 1999). It is thought that oestrogens, which drive the growth of oestrogen-sensitive mammary tumours, are generated locally; thus the effects of isoflavones on oestrogen metabolism at the tissue level may be important but have yet to be investigated.

The established relationship between breast cancer and steroid hormone status (Clemons & Goss, 2001), the structural similarity of isoflavones to endogenous oestrogens (Fig. 1) and their ability to bind to ER have led to significant interest in the potential mechanisms by which phyto-oestrogens may reduce the risk of breast cancer. This stimulated interested in human intervention studies to evaluate the effect of intervention with soya on hormone levels (Cassidy *et al.* 1994, 1995). These studies showed physiological effects of soya-rich diets on the endocrine regulation of the menstrual cycle and cycle length and led to further investigations which suggested significant effects of soya intervention on urinary oestrogen metabolism (Xu *et al.* 1998; Duncan *et al.* 1999, 2000). All of these effects on hormonal regulation and menstrual cycle length may potentially relate to decreased breast cancer risk.

However, to date, there are limited data from human studies to support a protective effect on breast tissue in healthy women. Several human studies have investigated

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the effect of changes in mammographic parenchymal patterns as a biomarker of the effect of isoflavones and soya on breast cancer risk. In one cross-sectional study there was a significant trend towards a higher mammographic density with increasing intake of soya foods in women aged 45–74 years (Jakes *et al.* 2002). However, two intervention studies, feeding pure isoflavone compounds, showed no significant change in mammographic density when fed either as a 100 mg isoflavone supplement daily over a 1-year period to premenopausal women (Maskarinec *et al.* 2003) or to older women (aged 45–65 years) fed a red clover supplement for a 1-year period. A recent *in vivo* examination on the effect of soya intervention (soya protein for 1 year) on breast tissue health in a group of premenopausal women was suggestive of a reduction in fibrocystic disease of the breast (Fleming, 2003), data which require further investigation to determine if soya and its associated isoflavones have a similar beneficial effect in atypia and breast cancer. A recent study showed that red clover extract does not cause any oestrogenic increase in breast density, which would indicate that it is unlikely to cause an increased risk of breast cancer (Powles, 2004).

One of the most contentious issues in phyto-oestrogen research relates to the potential role these compounds play in the prevention of breast cancer and safety of use in women with a history of breast cancer. Several short-term human studies have generated some data of potential concern. In one, consumption of 60 g soya supplement (45 mg isoflavones) increased the number of breast epithelial cells in a group of premenopausal women, while in another, consumption of a soya protein isolate (38 mg isoflavones/d) was associated with increased secretion of breast fluid and the appearance of hyperplastic cells (Petrakis *et al.* 1996; McMichael-Phillips *et al.* 1998). Both of these observations would be consistent with increased cell proliferation. In isolation these observations pose concern for increased risk of tumour development in women consuming phyto-oestrogen-rich diets, even though this view is not consistent with the epidemiological data. Data from animal models suggest that a life-long diet rich in phyto-oestrogen-rich foods may confer the greatest protective effects, and this increased resistance to developing experimentally induced breast cancer was observed in neonatal and prepubertal rats and also in the offspring of mothers who were fed isoflavones while lactating (Lamartiniere *et al.* 1995; Lamartiniere, 2002). Unquestionably, further studies are needed to address the potential safety issues, particularly for women who are at high risk for developing breast cancer as the use of a weak oestrogen may hypothetically be harmful to patients after a hormone-dependent cancer at the stage of micrometastases.

Particular concerns have been expressed for breast cancer patients taking tamoxifen, the widely prescribed long-term adjuvant treatment for breast cancer, and the potential for isoflavones-containing foods or supplements to interfere with the efficacy of this drug. The available *in vitro* data on phyto-oestrogens and tamoxifen are confusing and suggest differential effects depending on the concentrations of the two molecules present (Messina & Loprinzi, 2001). Two animal studies suggest a combination of tamoxifen and genistein enhance the efficacy of the treatment. In a rat model, the combination of genistein and tamoxifen synergistically inhibited the development of mammary cancer while in another rat model system, the combination of tamoxifen and soya reduced tumour development by almost 50 % more than either treatment alone (Messina & Loprinzi, 2001). Further investigation of such drug–phyto-oestrogen interaction merits further research.

Endometrial cancer

Dietary factors may play an important role in explaining the international variability in incidence rates of endometrial cancer which vary more than 10-fold worldwide (Schaffer, 1997). Phytochemicals that elicit oestrogenic effects are of increasing interest in relation to their possible influence on the physiology of the reproductive tract (Wade *et al.* 2003).

Studies in animals have found that whilst commercially available oestrogen preparations increase uterine weight, the isoflavone genistein has the opposite effect, potentially suggesting that consumption of phyto-oestrogens in the diet would not increase the risk of endometrial cancer. In a non-human primate model, treatment with soya protein isolate for 6 months (dose equivalent to 148 mg/d in man) did not induce proliferation in endometrial tissue (Foth & Cline, 1998). Epidemiological data are supportive of the animal data, where a study of a group of multi-ethnic women in Hawaii suggested that high soya consumers had a decreased risk of endometrial cancer (Goodman *et al.* 1997). A recent case–control study also suggested that isoflavone intake was inversely related to risk of endometrial cancer (Horn-Ross *et al.* 2003) and this association was stronger in postmenopausal women. However, in another study legume intake was shown to be associated with a slight increase in risk of endometrial cancer in Chinese women (Shu *et al.* 1991). However, many of these studies were not specifically designed to address the potential role of soya food intake in relation to endometrial cancer risk. A recent population-based case–control study which was specifically designed to assess the potential role of soya food intake in relation to endometrial cancer risk suggests that habitual consumption of soya foods, measured as either soya protein intake or soya isoflavones, is associated with a significant reduction in risk of endometrial cancer particularly among women with a higher BMI or waist:hip ratio (Xu *et al.* 2004).

The responsiveness of endometrial genes to phyto-oestrogens (genistein and daidzein) has been examined *in vivo* in the rat endometrium with data suggesting that genistein had specific effects on the transcription of a gap junction connexin gene (Cx26; Heikaus *et al.* 2002). In several reported human intervention studies, with a range of health endpoints, there was no observed effect of soya or phyto-oestrogen supplementation on endometrial histology (Duncan *et al.* 2000; Scambia *et al.* 2000; Upmalis *et al.* 2000; Clifton-Bligh *et al.* 2001). In addition, several studies specifically investigated the effects of isoflavone consumption on endometrial thickness. In one study, a double-blind randomised placebo-controlled trial conducted in sixty-two postmenopausal women who were fed 72 mg soya isoflavones daily, there was no observed effect on either endometrial thickness or on the pulsatility index of the uterine or cerebral arteries (Penotti *et al.*

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2003). A smaller intervention in a group of perimenopausal women fed 50 mg red clover extract daily also showed no effect on the endometrium using Ki-67 as a proliferative index biomarker (Hale *et al.* 2001). However, a recent study examined the effect of soya isoflavones together with oestrogen treatment (fed as soya protein isolate containing 120 mg isoflavones/d for 6 months) and showed that this combined treatment did not protect the endometrium from oestradiol-induced hyperplasia in postmenopausal women (Murray *et al.* 2003).

Cognition

It has been known for some time that phyto-oestrogens can cross the blood–brain barrier (Chang *et al.* 2000; Doerge *et al.* 2001; Lephart *et al.* 2002), and in animal models isoflavone stimulated biomarkers important for cognitive function and improved performance on a radial maze task (Lund *et al.* 2001; Lephart *et al.* 2002). Therefore the impact of phyto-oestrogens on cognitive performance is currently of significant interest and was recently reviewed (Hill & Dye, 2003).

Previous data from an epidemiological study suggested a positive association between tofu consumption and cognitive decline in middle-aged Japanese-Americans with a dose-dependent increase of up to 2·8-fold in risk of developing vascular dementia when two to three or more servings of tofu were consumed per week (White *et al.* 1996). However, although age, education and history of prior stroke explained 27·8 % of the variance in cognitive function test scores, tofu intake only accounted for 0·8 % (White *et al.* 1996). To date, human intervention studies investigating the effects of phyto-oestrogens on cognitive function have been equivocal; the largest study, a double-blind randomised placebo-controlled trial in postmenopausal women, observed no effect following a 1-year intervention of 99 mg isoflavones (aglycone equivalents) on a range of measures of cognitive function (Kreijkamp-Kaspers *et al.* 2004). In two other smaller intervention studies in postmenopausal women there was a suggestion of beneficial effects on cognitive function following intervention with soya isoflavone supplements. In one short-term study, postmenopausal women fed 60 mg isoflavones as a soya supplement showed improvements in cognitive performance following the intervention (Duffy *et al.* 2003). In addition a 6-month study, where 110 mg soya isoflavone supplement (fed as 55 mg twice daily) was fed had favourable effects on cognitive function, particularly verbal memory (Kritz-Silverstein *et al.* 2003). A small randomised controlled study (soya containing 100 *v.* 0·5 mg/d over 10 weeks) was suggestive of a significant influence of isoflavone intake on cognitive function in a group of young volunteers and showed sex differences in cognitive ability (File *et al.* 2001) but time of day effects on cognitive function were not controlled (Hill & Dye, 2003).

Menopausal symptoms

One area of active research relates to the potential for phyto-oestrogens to alleviate symptoms of the menopause. The epidemiological observation that there are marked differences in hot flushes in menopausal women in Europe and Asian countries which may relate to their soya exposure, together with data from prospective and cross-sectional studies from Japan (Nagata *et al.* 1999, 2001) suggesting that soya intake is negatively correlated with the number of hot flushes, have resulted in significant research activity. In a case–control study there was a trend towards a decrease in hot flushes with increased intake of isoflavones, although this did not reach statistical significance (Somekawa *et al.* 2001). However, the lowest quartile of intake ranged up to 35 mg/d with no comparison of intakes akin to levels of exposure in Europe, and may have been above the threshold necessary to experience benefit.

Numerous short-term studies have attempted to evaluate the effect, using a range of isoflavone supplements, traditional soya foods or isoflavone-enriched soya foods. These intervention studies, conducted in both peri- and postmenopausal women, have generated variable results, but in general isoflavone supplements appear to be relatively ineffective in managing hot flushes, whilst isoflavone-rich foods appear to have a beneficial effect that exceeds that of the placebo (there is a well-established strong placebo effect on menopausal symptoms from HRT studies) but the response is significantly less impressive than the effects observed with HRT (Kang *et al.* 2002; Kronenberg & Fugh-Berman, 2002). However, most of the studies have been conducted over a short time scale, used limited endpoint assessment, and did not clearly define the dose administered.

Given the growing interest in alternatives to HRT, and paucity of data on efficacy, several larger-scale studies have recently reported on the effects of isoflavone supplements on menopausal symptom relief. In one, a randomised placebo-controlled study compared the efficacy and safety of two dietary isoflavone supplements made from red clover extract. Symptomatic menopausal women (*n* 246; experiencing 8·1 flushes per d) consumed either 82 or 57 mg red clover extract preparations daily for 12 weeks. Although the data showed no significant reduction in hot flush count in either treatment group compared with placebo at 12 weeks, the 82 mg/d dose did appear to reduce hot flushes more rapidly (Tice *et al.* 2003). In another multicentre randomised controlled study, 80 mg soya isoflavones daily for 12 weeks had no advantage over placebo on severity of menopausal symptoms, although there was some improvement in psychological symptoms in the group consuming both isoflavones and melatonin (Secreto *et al.* 2004).

Although together these data suggest that isoflavones do not exert a clinically important effect on hot flushes and other symptoms of menopause, severity of flushes may be an important determinant of efficacy. A meta-analysis of available studies examined the relationship between frequency of hot flushes before study and efficacy of the phyto-oestrogen intervention. These data suggest that hot flush frequency at the beginning of the study explained about 46 % of the treatment effect (Messina & Hughes, 2003) and suggest that patients with frequent hot flushes may get greater benefit by including soya in their diet. Another area which warrants further research relates to drug interactions with phyto-oestrogens, and one specific area

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that warrants investigation relates to potential interactions with HRT preparations.

Most of the studies that have been performed, however, have been short-term and the question of whether consuming phyto-oestrogen-rich diets before entering the menopause would be more effective is unknown. This is more akin to the Japanese experience, where women have consumed isoflavones for all their fertile years.

Renal health

Although it is well established that consumption of soya retards the development and progression of chronic renal disease (Ranich *et al.* 2001; Velasquez & Bhathena, 2001), it remains unclear if these renal protective effects are related to the protein content, the isoflavone content, a combination of these factors, or some other component of soya.

Dietary intervention studies have shown that consumption of soya-based protein reduces proteinuria and attenuates renal functional or structural damage in animal models and human subjects with various forms of chronic renal disease (Ranich *et al.* 2001). However, it remains unclear which component or combination of components of soya protein are responsible for these effects. Data from animal studies have shown that soya protein improved peripheral insulin sensitivity and lowered fasting glucose and insulin levels, suggesting a mechanism for renal protection (Lavigne *et al.* 2000). In addition, the amino acid component of soya, high in arginine and glycine, may induce a low postprandial insulin:glucagon ratio that may be associated with low serum cholesterol (Ranich *et al.* 2001). The diverse cellular actions of isoflavones lends support to their potential protective effect on renal function (Velasquez & Bhathena, 2001), including their effects on lipoprotein metabolism (Crouse *et al.* 1999), and vascular atherosclerosis (Honore *et al.* 1997). In addition, the observed blood pressure-lowering effect of isoflavones may translate into a reduced glomerular blood flow and hydraulic pressure, and this may offer protection against glomerular injury (Lafferty & Brenner, 1990). More recently we have observed, using metabonomics technology, that soya isoflavone intervention modifies osmolyte levels in a direction which suggests they may improve glomerular function and kidney function (Solanky *et al.* 2003).

In human subjects, short-term incorporation of soya protein in the diet (3 weeks) has been associated with lower renal plasma flow, glomerular filtration rate and fractional clearance of albumin. In a randomised cross-over study in patients with a variety of nephritic syndromes a soya diet lowered albumin and blood lipid levels (D'Amico & Gentile, 1993). However, in a study of obese patients with type 2 diabetes, consumption of a soya-based diet for 8 weeks reduced lipoprotein status but had no effect on glomerular filtration rate or proteinuria (Anderson *et al.* 1998). In several animal studies it has been shown that soya protein preserves glomerular morphology, prevents proteinuria, and prevents glomerular hyperfiltration (Lafferty & Brenner, 1990; Maddox *et al.* 2002). More recently in a study with obese Zucker rats it was shown that soya blunted the rate of progression of glomerular injury as evidenced by a delay in the development of proteinuria and significantly less glomerular injury (Maddox *et al.* 2002).

The long-term effects of soya protein have yet to be fully understood. However, animal studies indicate that chronic soya protein intake preserves the function of damaged kidneys significantly better than animal protein (Ranich *et al.* 2001). It has been suggested that incorporating soya into the diet may have therapeutic benefits in diseases such as diabetic nephropathy by slowing the deterioration of renal function and decreasing proteinuria.

A recent study in end-stage renal disease patients on dialysis showed that patients who ingested isoflavone-rich diets had higher levels of genistein and daidzein than healthy subjects, and levels remained high for several days due to a lack of renal excretion. The half-life of both compounds was also significantly longer in the end-stage renal disease patients than in healthy subjects (Fanti *et al.* 1999, 2003). Long-term studies are therefore required to evaluate safety and efficacy of phyto-oestrogens in renal disease progression and in patients with renal failure.

Cardiovascular health

Epidemiological studies suggest that differences in diet may explain the lower incidence of CVD in Japan compared with other industrialised countries such as the USA or the UK. The high dietary intake of dietary isoflavones is thought to be in part responsible (Cassidy, 1996, 2003; Adlercreutz & Mazur, 1997; Setchell, 1998). Potential anti-atherogenic effects of isoflavones include a reduction in LDL-cholesterol, modulation of pro-inflammatory cytokines, cell-adhesion proteins and NO formation, protection of LDL against oxidation, inhibition of platelet aggregation and an improvement in vascular reactivity (Fig. 2).

Although the hypocholesterolaemic effect of soya has been recognised from animal studies for almost a century (Anderson *et al.* 1995; Anthony *et al.* 1996) the relative importance of isoflavones in this mechanism remains a contentious issue and data from a recent meta-analysis suggest the isoflavone component may not be as important as initially thought (Weggemans & Trautwein, 2003). The effect of soya and its isoflavones on lipoprotein status have been extensively reviewed previously (Demonty *et al.* 2003; Hermansen *et al.* 2003). However, any potential beneficial effects on lipid profiles may be only one component of protective responses since isoflavones have also been shown *in vitro* to inhibit the process of coagulation, improve blood flow, exert anti-inflammatory effects, act as antioxidants, or may exert direct effects on the arterial wall (Cassidy & Griffin, 1999).

One of the greatest health benefits associated with a high phyto-oestrogen diet may relate to the effects on blood vessels. ERβ plays an essential role in the regulation of blood pressure and vascular function (Rubanyi *et al.* 2002; Watanabe *et al.* 2003), and the presence of equal proportions of ERα and ERβ in the endothelial wall of blood vessels (Register & Adams, 1998; Adams *et al.* 2002) together with the stronger binding affinity of isoflavones to ERβ (Kuiper *et al.* 1997) support this hypothesis. Impaired endothelial function is associated with hypertension, dyslipidaemia and diabetes

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in women (Sader & Celermajer, 2002) and is considered to be an important predictor of the risk of cardiovascular events (Halcox *et al.* 2002). Animal data suggest that isoflavones increase blood vessel dilatation and improve blood flow in rhesus monkeys (Honore *et al.* 1997; Williams & Clarkson, 1998). More recently, studies on the effects of isoflavones, from soya foods and supplements, on vascular function have been conducted, with available data on effects on blood pressure and endothelial function reviewed in Tables 1 and 2 (Hodgson *et al.* 1999; Nestel *et al.* 1999; Washburn *et al.* 1999; Simons *et al.* 2000; Vigna *et al.* 2000; Hermansen *et al.* 2001; Teede *et al.* 2001, 2003; Yildirir *et al.* 2001; Bloedon *et al.* 2002; Chiechi *et al.* 2002; Hale *et al.* 2002; Jayagopal *et al.* 2002; Jenkins *et al.* 2002; Rivas *et al.* 2002; Blum *et al.* 2003; Cuevas *et al.* 2003; Howes *et al.* 2003; Steinberg *et al.* 2003).

Dietary supplementation with soya which has a high content of isoflavones appears to reduce blood pressure in both men and postmenopausal women (Table 1), particularly when consumed from soya sources. Several studies have examined the effects on isoflavones from red clover on blood pressure, and only one reported improvements in postmenopausal women with type 2 diabetes (Table 1). The available data on the effects of isoflavones on endothelial function are equivocal, with four studies suggesting no effect, while five have reported an improvement in endothelial function (Table 2).

Further studies are required to elucidate the relative importance of isoflavones on vascular function and importantly to address effects on more robust morbidity and mortality endpoints. However, recent data showing an effect of isoflavones (from red clover) on arterial stiffness (Teede *et al.* 2003), which is considered to be predictive of future cardiovascular events (van Popele *et al.* 2001; Boutouyrie *et al.* 2002), suggest that this is an important area for future research.

Bone health

Although data from rodent studies clearly demonstrate that soya isoflavones are effective in reducing bone loss and increasing bone formation, two long-term studies using ovarectomised monkeys have failed to show an effect of soya isoflavones on bone (Anderson & Garner, 1998). It is possible that the responsiveness to bone may differ between species as it is well established that there are significant

Table 1. Effects of isoflavones on blood pressure

Reference	Study design	Sex	Duration (months)	Isoflavone source and dose (mg/d)	Change of SBP and DBP following intervention (mmHg)
Bloedon *et al.* (2002)	Single dose (*n* 24)	Women, postmenopausal	1 d	Supplement: 2, 4, 8 or 16	−16/−13
Chiechi *et al.* (2002)	Parallel group (*n* 187)	Women, postmenopausal	6	Soya foods: 47	−3/0
Hale *et al.* (2002)	Parallel group (*n* 29)	Women, postmenopausal	0·5	Soya isoflavone concentrate: 80	NC
Hodgson *et al.* (1999)	Placebo-controlled cross-over (*n* 59)	Mixed	2	Supplement (red clover): 55	NC
Jayagopal *et al.* (2002)	Placebo-controlled cross-over (*n* 32), type 2 diabetics	Women, postmenopausal	3	Soya protein: 132	−2/−1
Jenkins *et al.* (2002)	Placebo-controlled cross-over (*n* 41), hyperlipidaemic	Mixed	1	Soya protein: 10, 73	NC
Nestel *et al.* (1999)	Placebo-controlled cross-over (*n* 21)	Women	1	Supplement: 80	NC
Rivas *et al.* (2002)	Parallel group (*n* 40), hypertensive	Mixed	3	Soya milk: 143	−18/−16
Simons *et al.* (2000)	Placebo-controlled cross-over (*n* 20)	Women, postmenopausal	2	Supplement: 80	NC
Teede *et al.* (2001)	Parallel group (*n* 179)	Mixed	3	Soya protein isolate: 118	−3·9/−2·4
Vigna *et al.* (2000)	Parallel group (*n* 77)	Women, postmenopausal	3	76	−3/0
Washburn *et al.* (1999)	Placebo-controlled cross-over (*n* 51)	Women, perimenopausal	1·5	Soya protein: 34	0/−5
Howes *et al.* (2003)	Placebo-controlled cross-over (*n* 16), type 2 diabetics	Women, postmenopausal	4	Supplements (red clover): 50	−3·7/−1·5
Teede *et al.* (2003)	Placebo-controlled (*n* 80)	Mixed	1·5	Supplements (red clover): 80	NC
Hermansen *et al.* (2001)	Placebo-controlled cross-over (*n* 22), type 2 diabetics	Women, postmenopausal	1·5	Soya protein: 165	NC

SBP, systolic blood pressure; DBP, diastolic blood pressure; NC, not changed.

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Table 2. Effects of isoflavones on endothelial function

Reference	Study design	Sex	Duration (months)	Isoflavone source and dose (mg/d)	Endothelial function
Simons *et al.* (2000)	Double-blind placebo-controlled (*n* 20)	Women, postmenopausal	2	Supplement: 80	No effect
Teede *et al.* (2001)	Double-blind placebo-controlled (*n* 179)	Mixed	3	Soya protein isolate: 54	No effect
Yildirir *et al.* (2001)	Hypercholesterolaemic (*n* 20)	Men	1·5	Soya protein	Improvement
Hale *et al.* (2002)	Placebo-controlled (*n* 29)	Women, postmenopausal	0·5	Soya isoflavone concentrate: 80	No effect
Blum *et al.* (2003)	Double-blind placebo-controlled (*n* 24), hypercholesterolaemic	Women, postmenopausal	1·5	Soya protein supplement: 85	No effect
Steinberg *et al.* (2003)	Double-blind cross-over (*n* 28)	Women, postmenopausal	1·5	Soya protein: 2 and 107	Improvement
Howes *et al.* (2003)	Double-blind placebo-controlled cross-over (*n* 16), type 2 diabetics	Women, postmenopausal	4	Supplements (red clover): 50	Improvement
Teede *et al.* (2003)	Double-blind placebo-controlled (*n* 80)	Mixed	1·5	Supplements (red clover): 80	Improvement
Cuevas *et al.* (2003)	Double-blind cross-over (*n* 18), hypercholesterolaemic	Women, postmenopausal	1	Soya protein isolate	Improvement

species differences in the metabolic handling of isoflavones (Lundh, 1995; Latonnelle *et al.* 2002).

Epidemiological evidence is supportive of a role for isoflavones in preventing bone loss since the incidence of hip fractures is lower in Asia than in most Western communities (Tobias *et al.* 1994). However, these differences in osteoporosis-related fractures may be accounted for by other factors including, for example, skeletal size (Cummings *et al.* 1994; Ho, 1996). To date the available data from observational studies and short-term intervention trials have produced variable results, and evaluation of the existing data is complex given the differences in study designs, sources of isoflavones, dose administered and endpoints measured. The human data have recently been reviewed (Branca, 2003; Setchell & Lydeking-Olsen, 2003) and a summary of available data is shown in Table 3 (Murkies *et al.* 1995; Dalais *et al.* 1998; Potter *et al.* 1998; Alekel *et al.* 2000; Scambia *et al.* 2000; Upmalis *et al.* 2000; Wangen *et al.* 2000; Arjmandi, 2001; Clifton-Bligh *et al.* 2001; Hsu *et al.* 2001; Scheiber *et al.* 2001; Anderson *et al.* 2002; Chiechi *et al.* 2002; Morabito *et al.* 2002; Uesugi *et al.* 2002; Chen *et al.* 2003; Atkinson *et al.* 2004; Brooks *et al.* 2004; Gallagher *et al.* 2004; Lydeking-Olsen *et al.* 2004). These available data suggest that when soya foods containing significant levels of isoflavones are substituted in the diet of postmenopausal women, bone resorption is reduced (Branca, 2003; Setchell & Lydeking-Olsen, 2003). There also appears to be a threshold of intake required for a measurable change in bone mineral density. These data are suggestive of beneficial effects on biochemical markers of bone turnover; however, whether these data translate into long-term effects on bone density or, more importantly, fracture risk remains to be established. More long-term studies are therefore required, with fracture as an endpoint measure to determine effective doses and relative importance of isoflavones for potentially preventing osteoporosis.

Skin ageing

Interest in the potential anti-photocarcinogenic and anti-photoageing effects of isoflavones has been emerging predominantly in relation to topical application of the phyto-oestrogens and, more recently, in relation to potential skin benefits following the ingestion of isoflavones (Wei *et al.* 2003). Numerous *in vitro* mechanisms of action including protection from oxidative and photodynamically damaged DNA, down regulation of UVB-activated signal transduction cascades and antioxidant activities suggest in particular that the isoflavone genistein has potential anti-cancer properties (Wei *et al.* 2003). Genistein significantly suppressed UV light-induced oxidative DNA damage in purified DNA and cultured cells and inhibited UVB-induced c-*fos* and c-*jun* proto-oncogene expression in mouse skin.

In addition, data from animal experiments suggest that genistein, administered either topically or orally, inhibits skin carcinogenesis and cutaneous ageing induced by UV light in mice and photodamage in man (Wei *et al.* 2003). Topical application of genistein protects the skin from photodamage by inhibiting UVB-induced acute and chronic photodamage in mouse skin (Shyong *et al.* 2002; Wei *et al.* 2003). In human subjects, topical administration of genistein before UV exposure also protected human skin against UVB-induced photodamage (Wei *et al.* 2003). The skin naturally uses antioxidants to protect it from photodamage and topical use of isoflavones may favourably supplement sunscreen protection and provide an additional anticarcinogenic protection (Pinnell, 2003). Further studies are required in human subjects to elucidate the potential protective effect of isoflavones on skin health following the ingestion of isoflavones rather than topical application.

Safety issues

It is inevitable that advocating the increased consumption of compounds that have the potential to exert 'oestrogenic'

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Table 3. Human intervention studies on isoflavones and bone biomarkers or bone density

Reference	Study design (no. of subjects)	Duration (months)	Isoflavone source and dose (mg/d)	Bone biomarkers	Bone density
Short-term interventions (<6 months)					
Murkies *et al.* (1995)	Parallel-arm (*n* 58)	3	Soyabean flour (74 mg)	BR ↓	–
Dalais *et al.* (1998)	Cross-over (*n* 45)	3	Soya foods (53 mg)	–	BMD (NS), increase BMC (5·2 %) *v.* 4 % control group
Scheiber *et al.* (2001)	Open design (no placebo) (*n* 42)	3	Soyabean foods (60 mg)	No effect on BR, BF ↑	–
Scambia *et al.* (2000)	RCT, cross-over (*n* 39)	1·5	Soya extract (50 mg)	No effect	–
Wangen *et al.* (2000)	RCT, cross-over (*n* 17)	3	Soya protein isolate (8, 65, 130 mg)	No effect	–
Upmalis *et al.* (2000)	RCT, double-blind	3	Soya isoflavone extract (50 mg)	No effect	–
Uesugi *et al.* (2002)	RCT, cross-over (*n* 23)	1	Soya isoflavone extract (62 mg)	BR ↓	–
Brooks *et al.* (2004)	Parallel (*n* 46)	4	Soya flour (?)	No effect	–
Wong (2000)	Open pilot (*n* 6)	1·5	Soya isoflavones (60 mg)	No effect	–
Pansini *et al.* (1997)	(*n* 40)	3	Soya protein isolate (60 mg)	BR ↓	–
Arjmandi (2001)	(*n* 142)	3	Soya protein (30–40 mg)	BR ↓, no effect on BF	–
Lu *et al.* (2002)	(*n* 12)	4	Soya milk (112 mg)	BF ↑, BR ↑	–
Medium-term interventions (≥6 months)					
Potter *et al.* (1998)	RCT, double-blind (*n* 66)	6	Soya protein isolate (56 and 90 mg)	–	↑ Lumbar BMD with 90 mg dose, no effect with 56 mg
Alekel *et al.* (2000)	RCT, double-blind (*n* 69)	6	Soya protein isolate (4 and 80 mg)	–	↑ BMC (80 mg dose only)
Clifton-Bligh *et al.* (2001)	RCT, double-blind (*n* 46)	6	Red clover (28, 57 and 85 mg)	No effect	↑ BMD (57 and 85 mg doses) no effect with 28 mg
Hsu *et al.* (2001)	RCT (*n* 37)	6	Isoflavone supplement (300 mg)	–	No effect
Chiechi *et al.* (2002)	RCT (*n* 187)	6	Soya foods	BF ↑, no effect on BR	
Gallagher *et al.* (2004)	RCT (*n* 65)	9	Soya protein isolate (4, 52, 96)	No effect	No effect
Longer-term interventions (>12 months)					
Yoles *et al.* (2003)	RCT, double-blind (*n* 98)	12	Tofu pills	–	↑ BMD with high dose, no effect with 344 mg dose
Chen *et al.* (2003)	RCT, double-blind (*n* 203)	12	Soya extract (40, 80)	–	↑ Femoral neck, no effect on spine, hip 80 mg, no effect with 40 mg
Morabito *et al.* (2002)	Parallel RCT (*n* 90)	12	Genistein (54)	BF ↑, BR ↓	↑ BMD
Atkinson *et al.* (2004)	RCT, double-blind (*n* 177), pre-and postmenopausal	12	Red clover (43·5)	BF ↑, no effect on BR	Smaller decrease lumbar spine BMC and BMD *v.* placebo; no effect on hip BMC or BMD
Lydeking-Olsen *et al.* (2004)	RCT (*n* 89)	24	Soya milk (76)	No effect	↓ Lumbar spine bone loss
Kreijkamp-Kaspers *et al.* (2004)	RCT, double-blind (*n* 175)	12	Soya protein isolate (99)	–	No effect

BR, bone resorption; –, not measured; BMD, bone mineral density; BMC, bone mineral content; BF, bone formation; RCT, randomised controlled trial.

effects would raise issues of safety. Phyto-oestrogens are multifaceted compounds, and only one of a series of their potential mechanisms relates to their weak oestrogenic nature. Substantial literature on the potential genotoxicity, carcinogenicity, reproductive and development toxicity of soya isoflavones exists and has recently been reviewed (Munro *et al.* 2003). However, the extrapolation of such animal and *in vitro* data to human exposure data remains difficult to interpret.

Numerous dietary intervention studies have been conducted in men, premenopausal and postmenopausal women, in which a range of isoflavone-rich foods or supplements have been ingested. Duration of exposure has ranged from 1 month to 6 months with intakes of between 3 and 131 mg aglycone equivalents/d (Gooderham *et al.* 1996; Baum *et al.* 1998; Crouse *et al.* 1999; Samman *et al.* 1999; Dewell *et al.* 2002; Munro *et al.* 2003). In these human intervention studies no adverse effects have been reported. These data provide supportive evidence for the safety of chronic intake of isoflavones at this level of expsoure. However, it is critical that markers of potential adverse effects are monitored in human clinical trials addressing the hypothetical benefits of these compounds in sub-groups of the population.

Although no clinical studies have to date examined the effect of the combined treatment of HRT and isoflavones, data from non-human primate studies suggest that it does not produce adverse effects. In ovarectomised non-human primates a combination of HRT and soya isoflavones produced a greater decrease in cardiovascular risk factors than either HRT or soya isoflavone alone (Wagner *et al.* 1997). In studies on ovarectomised macaque monkeys, soya protein isolate in combination with oestradiol did not increase uterine weight, nor did it affect a range of morphometric, histopathological or immunohistochemical parameters measured in mammary gland and endometrial tissues over a 6-month intervention (Foth & Cline, 1998). This was in contrast to the effects observed with oestradiol alone.

Assessment of safety is a critical element in the design of future studies, in particular addressing the effects of high levels of intake following long-term exposure.

Summary

Studies conducted to date in human subjects clearly confirm that isoflavones can exert hormonal effects. These effects may be of benefit in the prevention of many of the common diseases and conditions observed in Western populations (such as breast cancer, menopausal symptoms, osteoporosis and CVD) where the diet is typically devoid of these biologically active naturally occurring compounds. However, inter-comparisons of available data are difficult given the wide range of food products, supplements and doses used in existing studies. In addition, biological effects are potentially dependent on many factors including dose, duration of use, metabolism and intrinsic oestrogenic state, and many of the available studies are short term and rely on intermediate biomarkers as endpoint measures rather than 'hard' disease endpoints. There is a great need for long-term prospective studies and clinical trials to derive empirical proof of the efficacy and safety of isoflavones and to fully explore their potential role in preventative medicine.

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