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Phyto-oestrogens through the life cycle

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The growing interest in the role of phyto-oestrogens in human health has prompted scientists to evaluate the risk : benefit which would result from consuming high levels of these compounds at different stages of the life cycle. These compounds have been shown to exert a wide range of hormonal and non-hormonal activities in animals and *in vitro*, and these activities suggest plausible mechanisms for potential health effects in human subjects consuming phyto-oestrogen-rich diets. In addition, experimental and epidemiological data are available supporting the concept that phyto-oestrogen-rich diets exert physiological effects *in vivo*; however, their relative importance to human health remains to be elucidated. Our understanding of factors involved in their absorption and metabolism, including the role of intestinal microflora, is limited, and these factors together with dose-related effects may well be important in determining clinical efficacy.

Phyto-oestrogens: Isoflavones: Lignans: Soyabean

The role of dietary phyto-oestrogens as protective substances naturally present in the diet is receiving considerable attention. However, despite substantial epidemiological and experimental evidence from animal models and *in vitro* test systems that are supportive of a potential protective role in human health, to date limited clinical data are available to support any of the hypothesized benefits of these compounds (Setchell & Cassidy, 1999). The phyto-oestrogens are a varied class of compounds, but most of the research attention has focused specifically on the isoflavone subclass. However, more recently novel phyto-oestrogens have been identified in other foods, e.g. in hops (*Humulus lupulus* L.; Milligan *et al.* 1999), and interest is growing in these compounds, given their greater oestrogenic activity than the established phyto-oestrogens, such as the isoflavones. To date our understanding of the physiological behaviour of these compounds following ingestion, including intestinal metabolism, absorption and metabolic fate is limited. Although evidence is available to support biological effects of phyto-oestrogens following ingestion, the clinical relevance of such effects and the doses required to exert potential influences on different short- and long-term health issues remains to be established.

Mechanisms of action

Numerous mechanisms of action for the dietary phyto-oestrogens have been proposed as a result of animal studies and *in vitro* experiments. However, it is important to put into perspective the relevance of these mechanisms to the *in vivo* human situation (Cassidy, 1997). Currently we have little understanding of the 'physiologically-relevant' range of dietary phyto-oestrogens in human subjects, since we do not know what levels are attainable within cells or the tissue distribution of these compounds following ingestion. However, if we evaluate the relevance of the various mechanisms based on current evidence, it is interesting that many of the proposed mechanisms (including antioxidant potential, anti-angiogenesis, anti-proliferative effects and inhibition of enzymes involved in steroid metabolism) may be difficult to realize at a 'physiologically-relevant' range of plasma levels (Setchell & Cassidy, 1999).

The main interest in dietary isoflavones initially related to their structural similarity to the mammalian oestrogen, 17β-oestradiol (Fig. 1), which indicates their ability to act potentially both as oestrogen agonists and antagonists (Shutt & Cox, 1972). It has been demonstrated that these compounds bind to the classical oestrogen receptor (oestrogen receptor α) and produce typical oestrogenic

Abbreviations: HRT, hormone-replacement therapy; SERMS, oestrogen receptor modulators.

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responses when administered to animals (Shutt & Cox, 1972). However, similarly to other weakly oestrogenic compounds, phyto-oestrogens have the potential to exert antagonistic effects, and this apparent paradoxical nature of these compounds may in part be explained by the identification of the novel oestrogen receptor (oestrogen receptor β; Kuiper *et al*. 1996). Not only do these two oestrogen receptors have a differential distribution among tissues (Fig. 2) but the isoflavone genistein has a 20-fold higher binding affinity for oestrogen receptor β than for oestrogen receptor α (Kuiper *et al*. 1998), suggesting that isoflavones may preferentially bind to oestrogen receptor β-expressing tissues and have the capacity to exert tissue-specific effects. Support for this tissue-specific potential has emerged from animal models in which treatment with the isoflavone genistein produced vasculo-protective effects unaccompanied by the uterotrophic outcome seen with 17β-oestradiol treatment in ovarectomized rats (Makela *et al*. 1999). These actions are being compared with those of a group of drugs collectively known as oestrogen receptor modulators (SERMS), e.g. raloxifene (Etinger *et al*. 1999). SERMS bind to oestrogen receptors, and although they mimic the effects of oestrogen in some tissues, in other tissues such as the breast and uterus, classical oestrogenic effects are not observed (Etinger *et al*. 1999). Hypothetically, therefore, these drugs have the capacity to exert oestrogenic effects in vascular and bone tissues, and anti-oestrogenic effects at the breast and reproductive tissue sites. Given the distribution of oestrogen receptors and the apparent greater binding affinity of isoflavones to oestrogen receptor β, the potential of phyto-oestrogens to act as SERMS needs to be investigated further.

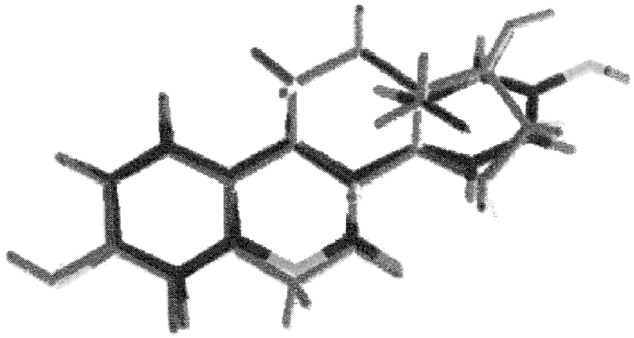

Fig. 1. Comparison of chemical structures of oestradiol and isoflavones. (From Jacobs, 1999.)

Substantial evidence suggests that the oestrogen receptor is only one mode of action by which phyto-oestrogens exert their clinical effects. These compounds appear to alter steroid metabolism through inhibition of the enzyme aromatase (Ibrahim & Abdul-Haj, 1990; Adlercreutz *et al*. 1993; Wang *et al*. 1993) and stimulation of sex hormone-binding globulin (Adlercreutz *et al*. 1992; Mousavi & Adlercreutz, 1993). Additionally, phyto-oestrogens act as antioxidants (Ruiz-Larrea *et al*. 1997; Tikkanen *et al*. 1998), have anti-proliferative potential (Peterson & Barnes, 1991; Brown *et al*. 1998) and exert anti-angiogenic effects (Fotsis *et al*. 1998). However, it is difficult to clarify the clinical relevance of these mechanisms without reference to the amounts of phyto-oestrogens attained within cells following ingestion.

Dietary sources, metabolism and bioavailability

Several classes of dietary phyto-oestrogens have been identified, with two of the major subclasses (the lignans and

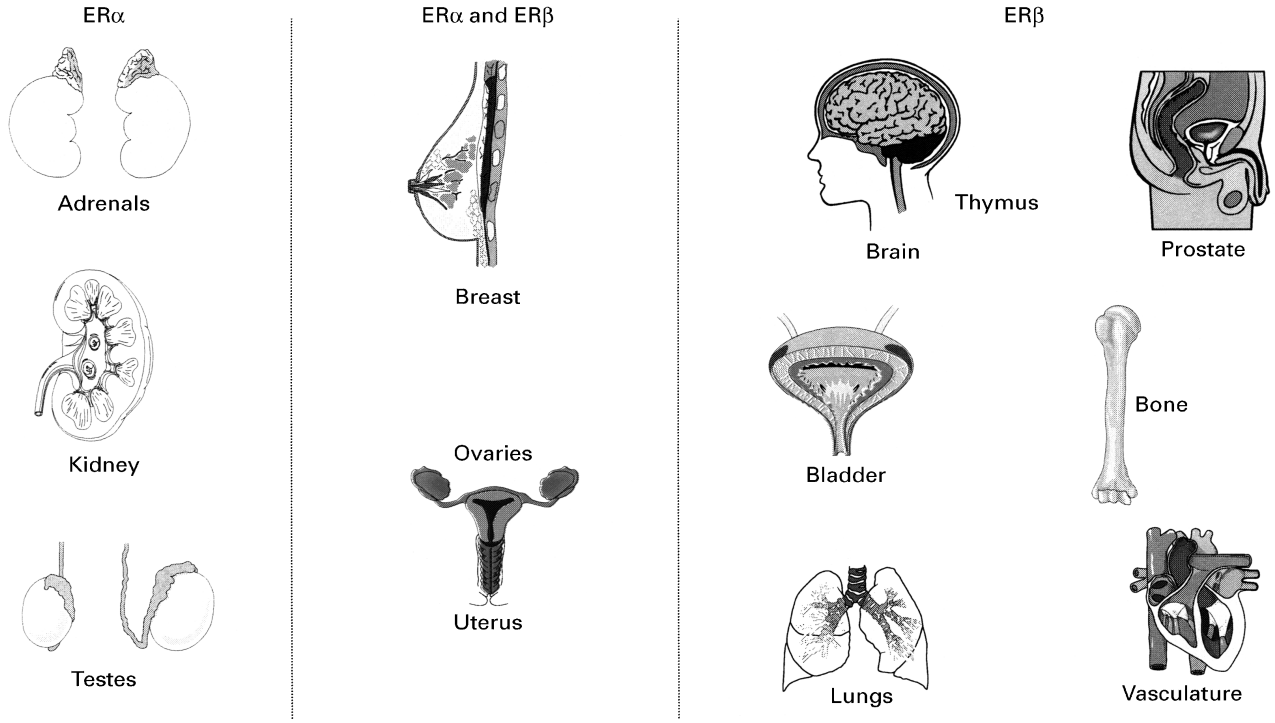

Fig. 2. Distribution of oestrogen receptors (ER) α and β in human tissues.

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isoflavones) currently stimulating interest from a nutritional and health perspective. The recent identification of a novel more-potent phyto-oestrogen in hops (8-prenylnaringenin; Milligan *et al*. 1999) suggests that our attention has been too focused and should in the future encompass other naturally-occurring oestrogen-like compounds present in plants.

Dietary phyto-oestrogens are present in plant foods and are synthesized from phenylpropanoids and simple phenols (Hahlbrock *et al*. 1981; Ayres & Loike, 1990; Rolfe, 1998). Lignans are present in many fibre-rich foods (Table 1), and although the nutritional properties of flaxseed, the richest identified source of the lignan precursor secoisolariciresinol, is receiving some attention, only limited data on the levels of lignans in food and their biological effects *in vitro* and *in vivo* are currently available (Setchell, 1995). Most of the consumer attention has focused on the isoflavones, in part because of the epidemiological evidence which is suggestive of a potential role for soya bean, a major source of isoflavones, in explaining the wide differences in rates of some hormone-related diseases between Asian countries and the West (Messina *et al* 1994). Soyabean proteins and foods (Table 1) contain significant levels of isoflavones, predominantly daidzein and genistein. The chemical composition of the isoflavones in soyabean foods is either as different types of glycoside conjugates (β-glucosides, malonylglucosides, acetylglucosides) or as the unconjugated aglycone form (Coward *et al*. 1993). Glycetin conjugates are also frequently found in soyabean proteins at low levels (Kudou *et al*. 1991), but high concentrations of glycetin conjugates are found in the hypocotyledon or germ (Naim *et al*. 1973). Levels of isoflavones are highly variable between soyabeans due to environmental factors and the variety of soyabean (Eldridge & Kwolek, 1983), and it is not surprising, therefore, that large ranges in isoflavone content have been reported within and between soyabean products (US Department of Agriculture, 1998)

Although we know that after ingestion the phyto-oestrogens are absorbed and metabolized, and the bio-transformation of ingested phyto-oestrogens occurs by the action of the intestinal microflora (Borriello *et al*. 1985), currently we have a limited understanding of factors which affect the bioavailability and pharmacokinetic profile of these compounds in human subjects. Only limited data exist as to the metabolites produced during intestinal metabolism (Joannou *et al*. 1995), and the specific bacteria involved are currently unknown (Setchell & Cassidy, 1999). Data currently available indicate that large intra- and inter-individual variability exists in the metabolic fate of these compounds (Setchell & Cassidy, 1999). For example, there is large variation in the excretion of isoflavones between individuals, and individuals appear to be either equol (metabolite of equol) or non-equol producers (Cassidy *et al*. 1994). The factors which determine equol or other metabolite production have yet to be elucidated, but some evidence suggests that a high-carbohydrate diet facilitates intestinal phyto-oestrogen metabolism (Setchell *et al*. 1984; Kelly *et al*. 1995; Lampe *et al*. 1998). Metabolism may well be an important indicator of clinical efficacy, since *in vivo* evidence suggests that equol is a more potent 'oestrogen' than its precursor daidzein (Shutt & Cox, 1972). Generally, measurement of phyto-oestrogens in biological fluids has focused only on daidzein, genistein and equol, and subsequently only a small percentage of the ingested compounds can be accounted for by urinary excretion in studies which have measured these compounds (Cassidy *et al*. 1994; King & Bursill, 1998). Many metabolites may have been identified only recently, or currently remain unidentified (Joannou *et al*. 1995). Furthermore, the effect of the food matrix and the chemical composition of the compounds (conjugate : aglycone) on their metabolic fate remains to be elucidated, and further information as to the effects of the absorption and metabolism of these compounds needs to be obtained.

Table 1. Examples of dietary sources of isoflavones and lignans in average portion sizes*

Food	Isoflavones (mg)	Food	Lignans (mg)
Soyabeans	25–143	Linseed (flaxseed)	13·5
Soyabean flour	0·8–59		
Textured vegetable protein	29–67	Oat bran	0·25
		Asparagus	0·46
Tofu	19	Carrot	0·21
Tofu yoghurt	5–85	Broccoli	0·20
Tempeh	4–38	Lentils	0·72
Miso	4–16	Pear	0·27
Soya sauce	Negligible	Sweet potato	0·36
Soya cheese	1–24	Kidney bean	0·20
Soya milk	3–53	Leek	0·15

*Adapted from the US Department of Agriculture isoflavone database (Thompson *et al.* 1996), using average portion sizes from Crawley (1992).

Early life: *in utero* and infancy

In recent years considerable attention has been paid to the phyto-oestrogen levels in soyabean infant formula and the potential biological effects in infants of exposure to phyto-oestrogen (Setchell, 1985). Soyabean-formula-fed infants have plasma concentrations of isoflavones which are 10-fold higher than those of adults (Setchell *et al*. 1997). These concentrations exceed circulating levels of oestradiol, but the biological relevance of this finding is unclear (Setchell *et al*. 1997). However, a more sensitive period of development is during fetal life. Recent evidence has demonstrated that phyto-oestrogens cross the placenta, indicating that these compounds have the potential to modify oestrogen metabolism *in utero* (Adlercreutz *et al*. 1999). Information on the effect of such exposure on development is limited, but there is no epidemiological evidence from Asian populations (who historically have consumed high levels of phyto-oestrogens and who are currently consuming between 20 and 50 mg/d; Nagata *et al*. 1998) to suggest that *in utero* exposure results in any future developmental effects. In fact, studies in which rats were exposed to isoflavones *in utero* indicated beneficial effects, with a subsequent reduction in the risk of developing mammary carcinoma in later life (Lamartinière *et al*. 1995; Murrill *et al*. 1996). These findings indicate that benefits of phyto-oestrogen exposure in later life may occur as a result of early exposure to these compounds. However, since this

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period of development is particularly sensitive in human subjects, long-term follow-up trials are required to fully elucidate the effect of *in utero* exposure to these compounds.

Women

The lower incidence of many hormone-dependent diseases, including CHD, menopausal symptoms, osteoporosis and breast cancer, in women in Asian countries where adults consume between 20 and 50 mg isoflavone/d (Nagata *et al*. 1998), along with the observed biological effects of these compounds (Cassidy *et al*. 1994, 1995), has led to speculation that phyto-oestrogens have potential health benefits in women, particularly in relation to menopausal health.

There is compelling evidence that phyto-oestrogen ingestion can exert biological effects in both premenopausal and post-menopausal women (Wilcox *et al*. 1990; Gavaler *et al*. 1991; Phibbs *et al*. 1993; Cassidy *et al*. 1994, 1995, 1998; Brzezinski *et al*. 1997; Xu *et al*. 1998). Isoflavone ingestion has been shown to have an endocrine-modifying effect through the suppression of follicle-stimulating hormone and luteinizing hormone in both premenopausal and post-menopausal women (Wilcox *et al*. 1990; Cassidy *et al*. 1994, 1995, 1998; Xu *et al*. 1998). In post-menopausal women isoflavone ingestion has resulted in the elevation of plasma sex hormone-binding globulin (Wilcox *et al*. 1990; Gavaler *et al*. 1991; Brzezinski *et al*. 1997), but this increase has not been evident in premenopausal women (Phibbs *et al*. 1993; Cassidy *et al*. 1994). Alterations in oestrogen metabolism have also been demonstrated following isoflavone ingestion (Xu *et al*. 1998).

However, although evidence is available to suggest the existence of biological effects following ingestion of phyto-oestrogen-rich diets, limited data are available defining the optimal intake leading to potential 'health effects'. Intakes of approximately 45 mg isoflavones/d are known to exert biological effects (Cassidy *et al*. 1994), yet a dose of 23 mg isoflavones/d caused no hormonal effect (Cassidy *et al*. 1995). Whether or not specific health benefits are related to intake needs to be addressed, and differential effects of phyto-oestrogen-rich foods need to be ascertained. Different 'optimal' doses may be required depending on the health issue of concern, and limited data from human trials have indicated that the intake required to exert effects on bone (Potter *et al*. 1998; Scheiber & Rebar, 1999) may exceed that required to alter lipoprotein metabolism and antioxidant activity (Anderson *et al*. 1995; Tikkanen *et al*. 1998; Crouse *et al*. 1999). The ultimate goal, therefore, in determining the optimal dose, particularly for menopausal women, will be to establish the dose that is likely to be most efficacious in addressing their specific health outcomes.

The changes that occur during the menopause, characterized by oestrogen deficiency due to decreasing ovarian oestrogen production, result in an increased risk of developing breast cancer, CHD, osteoporosis and menopausal symptoms. Hormone (oestrogen)-replacement therapy (HRT) is effective in symptom relief and has beneficial effects on the bone and vasculature, but is associated with a number of side effects including a slightly elevated risk of thrombosis, breast cancer and endometrial cancer (Colditz *et al*. 1995; Col *et al*. 1997). It is not surprising, therefore, that due to the weak oestrogenicity of phyto-oestrogens and their potential to act as SERMS they are being investigated as a potential natural alternative to conventional HRT (Clarkson, 1997).

Epidemiological data are supportive of a beneficial role of isoflavones in decreasing menopausal symptoms; the incidence of hot flushes ranges from 70 to 80 % in menopausal women in Europe compared with 18 % in China (Sturdee, 1997). A number of short-term clinical studies investigating the efficacy of isoflavone-rich foods have reported a significant reduction (40–56 %) in the incidence of hot flushes (Murkies *et al*. 1995; Brzezinski *et al*. 1997; Albertazzi *et al*. 1998), but all studies were characterized by a strong placebo effect, a problem which is also consistently seen in HRT trials. Studies using isoflavone extracts have yielded unconvincing data (Knight *et al*. 1998), suggesting that they may be less effective than the isoflavone-rich foods in alleviating menopausal symptoms. However, these studies were all of short duration, used variable doses of isoflavones, and used only limited assessments of hormonal status (Setchell & Cassidy, 1999). Further clinical studies need to be conducted in order to evaluate the efficacy of different doses of isoflavones for menopausal symptom relief.

The efficacy of HRT in ameliorating the bone loss associated with the menopause is well established (Felson *et al*. 1993). The idea that phyto-oestrogens may have beneficial effects on bone is gaining momentum, especially in the knowledge that bone is an oestrogen receptor β-expressing tissue (Kuiper & Gustafsson, 1997). Asian women have a considerably lower risk of suffering an oesteoporosis-related fracture than their Western counterparts (Tobais *et al*. 1994; Ho, 1996), and to date experimental studies using the ovarectimized rat model have demonstrated that isoflavones can prevent bone loss (Arjmandi *et al*. 1996, 1998; Anderson *et al*. 1998; Ishida *et al*. 1998). However, to date there have been only limited clinical studies in human subjects. One study reports that ingestion of isolated soyabean protein over a 6-month period by post-menopausal women resulted in an increase in bone mineral content and the density of the lumbar spine at a dose of 90 mg isoflavones/d (Potter *et al*. 1998). Studies in which biochemical markers of bone turnover were measured showed that consumption of 60–70 mg isoflavones/d over a 3-month period in post-menopausal women resulted in decreases in markers, including urinary excretion of D-pyridinoline and N-telopeptide, and urinary markers of bone metabolism (Scheiber & Rebar, 1999; F Pansini, G Bonaccorsi, P Albertazzi, D Costantino, A Valerio, C Negro, S Ferrazini, I Bonacuore, D De Aloysio, A Fontano, N Pansini and G Mollica, unpublished results). The mechanisms through which phyto-oestrogens prevent bone loss are still unclear, but one possibility is that these compounds modulate cytokine production, which in turn regulates ostoeclast activity since genistein has been shown to stimulate the production of transforming growth factor β in osteoclasts (Kim *et al*. 1998). Long-term studies are required to address the effect of phyto-oestrogen-rich diets on bone health, given the length of time required to observe

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physical changes in the bone and to elucidate the optimal dose required for bone health effects.

CHD is one of the leading causes of death in post-menopausal women in the UK (Williams, 1997). Endogenous oestrogen levels during the premenopausal years appear to confer protection from the disease, and during the peri- and post-menopausal years it has been suggested that HRT may reduce the risk of developing the disease by up to 44 % (Stampfer & Colditz, 1991). Epidemiological data are supportive of a cardio-protective influence from a diet high in phyto-oestrogens (Key *et al*. 1996; Nagata *et al*. 1998). Furthermore, evidence exists to indicate that phyto-oestrogens have favourable effects on factors that affect the two major processes involved in the development of the disease, atherosclerosis and thrombosis (Cassidy & Griffin, 1999). The lipid-lowering effect associated with the ingestion of soyabean protein in human subjects is well established (Anderson *et al*. 1995; Baum *et al*. 1998; Crouse *et al*. 1998; Potter *et al*. 1998; Scheiber & Rebar, 1999), but the extent of the effect is highly dependent on initial cholesterol levels. This phenomonen has been attributed in part to the isoflavone content of the soyabean in both animal and human trials (Anthony *et al*. 1996, 1997; Balmir *et al*. 1996; Anthony & Clarkson, 1998; Crouse *et al*. 1998), but interestingly the pure compounds exert no hypocholesterolaemic effect (Baum *et al*. 1998; Nestel *et al*. 1999), suggesting a food matrix effect. Animal studies have demonstrated that soyabean ingestion inhibits atherosclerotic plaque growth (Anthony & Clarkson, 1998; Kirk *et al*. 1998) and improves vascular reactivity (Honore *et al*. 1997; Williams & Clarkson, 1998). Human studies have also shown that isoflavone ingestion can improve systemic arterial compliance (Nestel *et al*. 1997), diastolic blood pressure (Washburn *et al*. 1999) and can reduce the susceptibility of LDL to oxidation (Ruiz-Larrea *et al*. 1997; Tikkanen *et al*. 1998; Scheiber & Rebar, 1999). Furthermore, genistein has been shown to inhibit platelet activation and aggregation (McNicol, 1993; Murphy *et al*. 1993), and to inhibit thrombin formation (Wilcox & Blumenthal, 1995; Tham *et al*. 1998) *in vitro*, processes that initiate the thrombotic process. The evidence, therefore, for a role for phyto-oestrogens, particularly soyabean protein and genistein, in the prevention of CHD is promising, although the optimal dose for exerting specific effects is currently unavailable.

Men

To date, the relevance of phyto-oestrogens to male health remains to be elucidated. Short-term dietary intervention studies have shown little evidence of hormonal effects of phyto-oestrogen-rich diets in men (Cassidy *et al*. 1998). However, despite the lack of evidence for any specific hormonal effects following ingestion of phyto-oestrogens in men, nevertheless these compounds may be beneficial to male health. The potential benefits of these compounds in relation to osteoporosis and CHD discussed earlier are also of course relevant to male health.

The incidence of clinical prostate cancer is 10- to 15-fold higher in American Caucasian men than in Japanese men, compared with only a 50 % higher rate of latent prostate cancer (Yatani *et al*. 1989). Evidence also suggests that the development of prostatic tumours in Japanese men is slower (Shibata *et al*. 1997), raising the question of whether dietary factors play a protective role. Analysis of plasma and prostatic fluid from Asian men showed that isoflavones concentrations were 2-fold higher in prostatic fluid than in plasma (Morton *et al*. 1997). These data have consequently led to speculation that dietary isoflavones may play a role in the reduced risk for prostatic cancer. *In vitro* studies have demonstrated that genistein and bichanin A can inhibit the growth of prostate cancer cell lines (Peterson & Barnes, 1993; Naik *et al*. 1994; Kyle *et al*. 1997) via inhibition of 5α-reductase and 17β-hydroxysteroid dehydrogenase (Evans *et al*. 1995; Makela *et al*. 1995), enzymes involved in androgen and oestrogen synthesis. In rat models soyabean and genistein ingestion have resulted in a reduction in the incidence of prostate cancer (Naik *et al*. 1994; Wang *et al*. 1995; Pollard & Luckert, 1997; Zhang *et al*. 1997; Schleicher *et al*. 1998; Zhou *et al*. 1988). The specific protective roles that phyto-oestrogens may play are most likely to be multifactorial, incorporating both oestrogen-dependent and -independent mechanisms, including induction of cell proliferation and expression of tyrosine-phosphorylated proteins (Griffiths *et al*. 1998).

Summary

Hypothetically, phyto-oestrogens have the potential to exert biological effects from fetal life through to old age. Early exposure of some populations to these compounds has raised a number of issues which have implications on future health, but animal data suggest that lifetime exposure may be beneficial to future cancer risk (Lamartinière *et al*. 1998). For women these compounds have the mechanistic potential to act as a natural alternative to HRT, and in men phyto-oestrogens may reduce the risk of prostatic cancer, osteoporosis and CHD; however, clinical data are not yet available to support specific health effects. Nevertheless, these compounds are known to exert biological effects, and mechanisms of actions have been demonstrated which suggest plausible health effects. However, as yet our understanding of the physiological behaviour of these compounds, the doses required to exert biological and specific health effects, and the influence of factors such as age and sex is limited.

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