British Journal of Nutrition (2003), **89**, Suppl. 1, S101–S108
DOI: 10.1079/BJN2002800

Phyto-oestrogens and osteoporosis: what is a safe dose?

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Hormone replacement therapy (HRT) for preventing loss of bone following the menopause is utilised by only 8–10% of possible users, largely due to a fear of increased risk of breast cancer. Plant oestrogen-like compounds (phyto-oestrogens) have been proposed as an alternative to HRT to prevent osteoporosis. One class of phyto-oestrogens (the isoflavones) is found in soya foods and red clover. The food industry is developing a wide variety of new foods containing soya to substantially increase isoflavone intake, as well as extracting isoflavones from soya and clover to use as additives to non-soya foods. Pharmaceutical companies are also preparing isoflavone extracts to be used in pill form. In each case the targeted delivery is ~50 mg of isoflavones/d. Is this dose of isoflavones safe? In this review of the current literature, it is concluded that isoflavones consumed orally and in doses below 2 mg/kg body weight per d should be considered safe for most population groups. Whether these doses are sufficient to prevent osteoporosis is a separate matter.

Phyto-oestrogens: Isoflavones: Blood levels: Mechanisms

Introduction

As populations in many countries have become increasingly older, diseases that particularly affect the elderly have become a significant part of the cost of health care. These include atherosclerosis, cancer (Polednak, 1994) and osteoporosis (Riggs & Melton, 1995). Of these, osteoporosis has a profound effect on the quality of life rather than increasing mortality. Deposition of bone reaches a maximum at the age of between 25 and 35 years and declines thereafter (Sambrook *et al.* 1993). The senile osteoporosis associated with ageing occurs in both men and women. However, at the menopause and during 2–4 years thereafter, the fall in circulating plasma oestrogens precipitates a rapid loss of bone in women and is associated with the appearance of a clinical form of osteoporosis in 25% of this group. Administration of steroid hormones as replacement therapy (HRT) prevents loss of bone following the menopause (Reid, 1999). However, it is utilised by a only small proportion of possible users, largely due to a fear of increased risk of breast cancer (Jolleys & Olesen, 1996).

Plant oestrogen-like compounds (phyto-oestrogens) have been proposed as an alternative to HRT to prevent osteoporosis (Scheiber & Rebar, 1999). One class of phyto-oestrogens (the isoflavones) is found in soya foods and red clover. The food industry is developing a wide variety of new foods containing soya to provide the opportunity for individuals to substantially increase isoflavone intake. In addition, others have extracted isoflavones from soya and red clover for use as additives to non-soya foods. In each scenario the targeted delivery is ~50 mg of isoflavones/d. What is the rationale for this decision and is this dose of isoflavones safe? This paper is a summary of a presentation on this topic made at the meeting of a European Concerted Action in Versailles, France, on 4–6 October 2001. It should be noted that it focuses on isoflavones rather than other phyto-oestrogens, since dose levels for other phyto-oestrogens have not yet been proposed.

Historical considerations

To what extent phyto-oestrogens were part of the hunter–gatherer diet of ancient man is not known. Domestication of the soyabean occurred in NE China in the eleventh century BC, 3000 years ago, and therefore may mark the introduction of isoflavones into the diet (Hymowitz, 1990). The value of the soyabean had been appreciated even earlier, appearing in the *Materia Medica* of the Chinese Emperor Cheng Neng in 2859 BC. Knowledge concerning the use of soya as a food spread slowly in SE Asia, to Korea (second century AD) and Japan (third to sixth century AD). Soyabeans did not appear in Europe and North America until the eighteenth century AD (Hymowitz, 1990). In the latter part of the nineteenth

Abbreviations: ER, oestrogen receptor; HRT, hormone replacement therapy; TPO, thyroid peroxidase; VENUS, Vegetal Estrogens in Nutrition and the Skeleton.

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century and the early twentieth century, the United States Department of Agriculture evaluated many soyabean strains to determine which would best adapt to the light/dark cycle at the different latitudes in the USA. Soyabeans are particularly useful to farmers in that they fix nitrogen and have been therefore used in crop rotation to sustain the quality of agricultural growing areas.

The commercial value of both the oil and the protein fraction of the soyabean is attributed to George Washington Carver. Soya oil is used extensively for human consumption, both as a cooking oil and for the manufacture of margarines. The protein fraction has been widely used as a cheap source of protein for the feeding of farm animals (pigs, chickens, cattle, etc.), pets (dogs and cats), rodents and other animals used in scientific research, and for fish (catfish, telapia). In the early part of the twentieth century, through the efforts of industrialists such as Henry Ford, attempts were made to create a wide range of products from the soyabean. A part of this research included soya food products for human use. This led to the appearance of commercially available protein products in the 1950s and to soya infant formulas in the 1960s. The interest in soya foods that followed research on the potential health effects of soya that appeared in the late 1980s and in the 1990s led many companies to generate new soya foods with increasing public acceptability.

While this recent interest in isoflavones has occurred, there has also been a concern about isoflavones and other phyto-oestrogens that goes back over 50 years. This arose from an understanding of the causes of an infertility syndrome in sheep that was observed by farmers in Western Australia. It was associated with consumption of a subterranean clover species by the sheep (Bennetts *et al.* 1946). The compounds in the clover causing the infertility were shown to be members of the isoflavones family (daidzein and genistein and formononetin and biochanin A, their 4′-methyl ethers, as well as the daidzein metabolite equol; Bradbury & White, 1951; Shutt & Braden, 1968). This raises the question, does consumption of the isoflavones in soya (or in clover) cause infertility in other species, particularly man? The simple answer is that, in the majority of other species, consuming isoflavones at dietary levels does not cause infertility. However, cheetahs (Setchell *et al.* 1987) and possibly certain birds (Leopold *et al.* 1976) may exhibit infertility. Most farm animals and those used for biomedical research have been fed diets with high concentrations of soya protein (and hence isoflavones) without serious concern about effects on reproduction rates. Indeed, the use of such isoflavone-rich diets has masked for many investigators what would otherwise be much greater effects of specific drugs, gene knockouts and other experimental manoeuvres designed to mimic human disease (Barnes, 1997; Brown & Setchell, 2001).

Choice of phyto-oestrogen dose

Much of the justification regarding the selection of a dose of 50 mg isoflavones/d (0·5–1·0 mg/kg body weight per d) is based on the presumed average intake of isoflavones in adults in China, Japan and Taiwan (Messina *et al.* 1994). This may range from 0 to 125 mg/d (0–2 mg/kg body weight per d). The 50 mg/d estimate was higher than that observed in a study in Japan (Messina, 1995). However, careful studies conducted in Japan and Shanghai, China, have determined that the median isoflavone intake is 30–40 mg/d (Kimira *et al.* 1998; Chen *et al.* 1999; Wakai *et al.* 1999; Nakamura *et al.* 2000). The consumption of soy products has fallen in Japan because of Westernisation of their culture, particularly in the younger generation. The Japanese food industry is producing new soya food products acceptable to teenagers, as well as products that can be used in a fast food scenario. Their goal is to have a daily isoflavones intake in the young of 10 mg, somewhat lower than the proposed daily intake in the USA and Western Europe.

Excitement and concerns regarding human health and phyto-oestrogen intake

Besides the potential use of isoflavones for the prevention of osteoporosis, several other health benefits for isoflavone-containing soya diets have been proposed (Barnes, 1998). These include their effects on breast and prostate cancers, atherosclerosis, hypertension, diabetes and neurodegeneration. Those pursuing potential adverse effects have drawn attention to several of these same issues, in particular the role of phyto-oestrogens in increasing the risk of breast cancer (Hsieh *et al.* 1998; Allred *et al.* 2001*a*,*b*; Ju *et al.* 2001) and neurodegeneration (White *et al.* 2000). In addition, concerns have been raised regarding thyroid disease (Divi *et al.* 1997; Fitzpatrick, 2000) and the effects of phyto-oestrogens on children receiving soya milk or infant soya formula (Setchell *et al.* 1997; Fitzpatrick, 2000). However, many of these claims have also been disputed (Klein, 1998; Chang & Doerge, 2000; Messina & Loprinzi, 2001). In the remainder of this paper, the benefits/toxicity data are discussed in the context of the doses of phyto-oestrogens that may be used in the prevention of osteoporosis.

Peripheral and target site concentrations of phyto-oestrogens following oral dosing

Knowing the concentrations of phyto-oestrogens and their metabolites that result from oral intake is crucial in assessing both the benefits and the adverse effects of phyto-oestrogens. The blood concentrations of isoflavones in Japanese men were first reported by Adlercreutz *et al.* (1993) to have a mean of 276 nM. Using fluoroimmunoassay techniques, higher values (407 nM for genistein and 118 nM for daidzein) were recently reported for Japanese women (Uehara *et al.* 2000). In clinical trials utilising two 20 g servings of soya protein daily (containing the equivalent of 42 mg isoflavone aglucones), the plasma concentrations 6·5 h after consuming the first serving were 800–1000 nM (Coward *et al.* 1996; Urban *et al.* 2001). This suggests that chronic exposure to isoflavones, as occurs in SE Asia, may lead to lower blood isoflavone concentrations for a given daily intake of isoflavones. This may result from deficiencies in intestinal lactase in most Asians, a familiar enzyme that has recently been

shown to be responsible for hydrolysis of isoflavone β-glucosides (Day *et al.* 2000).

Pharmacokinetics of phyto-oestrogens

Doses as high as 16 mg of genistein/kg body weight have been used in acute safety studies in healthy male volunteers (Busby *et al.* 2002). Half-lives of unconjugated genistein were independent of the dose and ranged from 2 to 5 h. Total genistein half-lives were longer and ranged from 3·5 to 8 h. Peak plasma genistein concentrations were reached 3–6 h after ingestion. Unconjugated genistein concentrations were 383 nM at the highest dose, whereas the total genistein concentrations were 27·5 μM. Comparable values were observed for daidzein. These values correspond to data (total genistein 36 μM, total isoflavones 81 μM) from a single subject who consumed 2–3 g/d of a 40% by weight genistein preparation (estimated intake 11–15 mg/kg) for 1 month (S Barnes, L Coward, M Kirk and M Smith, unpublished results). In another recent study, Setchell *et al.* (2001) showed that the times to the maximum plasma concentration for orally administered genistein and daidzein were 5·2 and 6·6 h, respectively. The corresponding β-glucosides were absorbed more slowly. It should be noted that the pharmaceutical form of a phyto-oestrogen preparation might have important effects on its pharmacokinetics, as occurs for most drug formulations. Therefore, individual formulations of phyto-oestrogens in pills and foods may behave differently.

In summary, for a 50 mg dose (~0·7 mg/kg per d), the expected plasma concentrations of the isoflavones will be approximately 1 μM, with only 1–2% being in the unconjugated form (and hence capable of being absorbed by tissues). Since there is a tenfold variation in blood isoflavone concentrations from patient to patient when the same dose is administered (Urban *et al.* 2001), values as high as 3–4 μM could occur in certain patients. Even so, unconjugated isoflavones will be less than 50 nM. Higher daily doses will lead to proportionately higher isoflavone concentrations. A dose of 2 mg/kg per d would produce a mean plasma concentration of approximately 3 μM, with outliers as high as 10 μM. Even so, the unconjugated isoflavone concentration will only be 100 nM. These values must be considered when interpreting data from cell culture experiments.

Mechanisms of action of phyto-oestrogens

The majority of scientists, whether considering the benefits or adverse effects of phyto-oestrogens, have presumed that phyto-oestrogens act on the oestrogen receptor (ER) system. This is a narrow point of view and ignores contributions to biological effects of many other mechanisms. The high affinities of phyto-oestrogen interactions (K_d 0·3–10 nM) with ER at first glance appear to dominate weaker mechanisms (Kuiper *et al.* 1997); however, at the intake levels that occur in a soya-rich diet, it would appear that the ER system would always be fully active. Since phyto-oestrogens are not oestrogenising at these doses, it is likely that other targets of phyto-oestrogens must exist. The potential for this can be demonstrated by considering the large number of known post-receptor steps that are involved in oestrogen stimulation of breast and uterine tissue growth (Barnes *et al.* 1999). Many of these steps involve the activation of protein kinases — since the isoflavone genistein is well known for its property of inhibiting tyrosine kinases (Akiyama *et al.* 1987), it is not surprising that isoflavones do not have overt oestrogen-like activity. It is possible that sheep and other susceptible animals do not rely on these kinases, or that the isoflavones do not inhibit phosphorylation. These questions will be resolved by the use of DNA and protein microarrays and/or proteomics–protein MS, where the global effects of individual phyto-oestrogens can be examined. Even studying a small selection of oestrogen-responsive genes, it has already been shown that physiological oestrogens, plant oestrogens and synthetic oestrogens differentially regulate gene expression in the same tissue (Diel *et al.* 2000). Using an oligonucleotide microarray approach, it has been recently shown for the developing rat uterus that while the pharmacological oestrogen 17α-ethinyl-oestradiol and the synthetic oestrogen bisphenol A have largely similar effects, genistein had much fewer genes whose expression was changed in common (Naciff *et al.* 2002). In total, genistein led to changes in expression of 227 genes; it is noteworthy that for two-thirds of these genes expression was decreased. Furthermore, in a model of endometrial carcinoma in rats, genistein was able to increase gene expression of oestrogen-sensitive genes without effects on tumour growth (Diel *et al.* 2001). These observations render largely invalid interpretation of much of the data on the oestrogenic potential of phyto-oestrogens obtained using single reporter gene assays (Willard & Frawley, 1998) or changes of mRNA expression of genes such as *pS2* (Jorgensen *et al.* 2000).

Besides ER-dependent and tyrosine kinase-dependent processes, phyto-oestrogens have antioxidant activity (Chin-Dusting *et al.* 2001), much like many other polyphenols. In some cases the antioxidant effect occurs in the nM range. Furthermore, there appears to be a positive synergy between phyto-oestrogens and other antioxidants (Hwang *et al.* 2000; Patel *et al.* 2001*a*). This may be important in disease processes involving oxidative stress, e.g. in reducing LDL oxidation in atherosclerosis. Besides protecting lipid-carrying proteins, phyto-oestrogens may also prevent the oxidation of critical enzymes in the signal transduction pathways through protection of cysteine groups. Since this is not governed by their oestrogen-like structures, but rather their antioxidant properties, their overall effect may appear to be like that of an oestrogen or an anti-oestrogen.

Other important targets of phyto-oestrogens include apoptosis (Pagliacci *et al.* 1994; Davis *et al.* 1998) and cell adhesion (Patel *et al.* 2001*b*). However, in most cases they require concentrations above 20 μM (almost three orders of magnitude higher than the free phyto-oestrogen level in subjects consuming phyto-oestrogen-rich diets). Cell adhesion effects may be crucial in the attachment of circulating inflammatory cells to the endothelial cell wall (Patel *et al.* 2001*b*) and subsequent invasion into the tissue space, as well as in the process of metastasis.

Inhibition of specific metalloproteinases by isoflavones (Shao *et al.* 1998) may also contribute to this latter effect.

Unlike physiological oestrogens that have a fully conjugated steroid nucleus, phyto-oestrogens can undergo substantial modification of the parent molecule. While the parent phyto-oestrogens may have an overall shape (their ring systems are organised so that the two hydroxyl groups are 110–120 nm apart) that allows them to fit into the promiscuous ER ligand-binding site (Pike *et al.* 1999; Barnes, 2001), reduction or ring cleavage in the heterocyclic ring of the isoflavones would introduce chiral centres that would drastically alter their binding. Full cleavage of the phenyl B-ring to form metabolites such as 2-(4-hydroxyphenyl)propionic acid would have an even greater effect (Coldham *et al.* 1999). These metabolites may in fact be those that reach target tissues and create the physiological phyto-oestrogen effect. Indeed, as only 10–20% of an administered dose of genistein is excreted in the form of known metabolites, there is tremendous scope for further investigation of this point.

Oestrogenising effects of phyto-oestrogens

Until recently, infants on soya infant formula received a higher daily dose of isoflavones (4–6 mg/kg body weight per d) than adults (Setchell *et al.* 1997). This has led to speculation, indeed assertion, that infants would be oestrogenised in negative ways or that their development would be impaired (particularly male infants). Although evidence for this at the clinical level is yet to be presented, manufacturers have reconstituted soya infant formulas with soya protein preparations with much lower isoflavone contents.

As noted earlier, commercial lab chow diets have a very high phyto-oestrogen content due to the use of soya. Rats bred on these diets consume 1–8 mg of isoflavones/kg body weight per d, depending on the diet (Barnes *et al.* 1990). This could be interpreted that reproduction in the rat is not affected by phyto-oestrogens. However, there have been reports of diets that were associated with reproduction problems (Gallo *et al.* 1999), although this may be restricted to isoflavone intakes greater than 1000 ppm (Casanova *et al.* 1999). Infertility, as observed in sheep or cheetahs (Leopold *et al.* 1976; Setchell *et al.* 1987), does not occur at dietary levels. However, it should be noted that the metabolism of isoflavones in the rat is markedly different from that in man and the blood concentrations of daidzein and genistein are low for the doses administered compared with those in man (Barnes *et al.* 2002). Instead, the concentration of the daidzein metabolite equol is six to seven times higher (Bayer *et al.* 2001; Barnes *et al.* 2002). It remains to be seen in the rat whether the 'oestrogenic' responses in some of the many reported experiments come from equol or the isoflavones daidzein and genistein. Also, it could also be argued that the low oestrogenic response to soya phyto-oestrogens in rodents is due to this extensive metabolism and hence the low blood phyto-oestrogen values.

In summary, more effort must be placed on careful examination of the potential oestrogenising effects of phyto-oestrogens in man rather than in animals. There has been a recent study on a cohort of 30- to 40-year-olds who were fed soya infant formula (Strom *et al.* 2001). The growth characteristics of this group could not be distinguished from those of other 30–40-year-olds. This is analogous to the lack of effect of soya diets on growth rate and final body weight of many animals. On the other hand, an increase in length of menstruation was reported that reached statistical significance. Further, more careful studies should be directed at investigating this and related points.

Breast, endometrial and prostate cancers

The effects on hormonally dependent cancers remain the most controversial aspects of including phyto-oestrogens in the diet. On the one hand, the rates of each of these cancers are many times lower in SE Asia, where phyto-oestrogen intake is high, compared with the USA and Western Europe, where phyto-oestrogen intake is low (Shimizu *et al.* 1991; Mant & Vessey, 1994). In support of the soya/phyto-oestrogen prevention hypothesis, rats placed on soya-containing diets or soya-free diets supplemented with genistein had lower numbers of chemically inducible mammary tumours (Barnes *et al.* 1990; Hawrylewicz *et al.* 1995; Lamartiniere *et al.* 1995; Fritz *et al.* 1998; Gotoh *et al.* 1998), although this has been disputed (Cohen *et al.* 2000). Interestingly, there is a window of exposure early in life that is important for this effect (Lamartiniere *et al.* 1995; Fritz *et al.* 1998). SE Asian women who emigrate to the USA later in life sustain a difference in breast cancer rates from Americans whereas their daughters do not (Shimizu *et al.* 1991). This emphasises the importance of events in early life (such as exposure to phyto-oestrogens) that may be critical to the risk of breast cancer in man (Colditz & Frazier, 1995).

Proponents of both beneficial and adverse effects of isoflavones have used interaction of isoflavones with ER to support their cases. Isoflavones administered by injection to perinatal rats cause a more rapid maturation of the breast and other signs of oestrogenic action (Brown & Lamartiniere, 1995) but lower the risk of mammary adenocarcinomas in adult life (Lamartiniere *et al.* 1995). This route of administration also leads to the appearance of uterine adenocarcinomas (Newbold *et al.* 2001). However, orally administered isoflavones at levels up to 30 mg/kg body weight per d do not cause these toxicity effects, and the risk of uterine cancer in isoflavone-consuming SE Asians is substantially lower than in Americans or Europeans (Mant & Vessey, 1994).

It should be noted that when phyto-oestrogens are used to prevent osteoporosis in postmenopausal women, they are administered to a group who have not been significantly been exposed to phyto-oestrogens earlier in life. Would this increase the risk of breast cancer, as has been shown to occur in those receiving HRT? The answer is that we simply do not know. The increased risk of breast cancer from HRT use has been calculated at 1–2% per annum (Ross *et al.* 2000). Therefore, in theory, use of phyto-oestrogens for 20–30 years could lead to a 20–60% increased risk of breast cancer if phyto-oestrogens behave quantitatively in the same way as HRT. However, there is no clinical evidence available to support

or deny this possibility, although in a recent review Messina & Loprinzi (2001) concluded that soya and its phyto-oestrogens do not affect the risk of breast cancer or alter survival in breast cancer patients. Indeed, some argue that the apparent relationship between HRT and breast cancer is in doubt (Bieber & Barnes, 2001). Women at a high risk for osteoporosis will interpret these apparent risks differently from those with a family history of breast cancer. The latter may avoid all forms of oestrogens until safety can be proven definitively.

Experiments using rodent models reveal that, in intact animals that already have had at least one mammary tumour, a soya diet with isoflavones reduces the number of mammary tumours that occur subsequently (Hawrylewicz *et al.* 1995). However, if human MCF-7 breast cancer cells are implanted in ovariectomised, immunocompromised mice, the tumour cells can be induced to grow if genistein is included in the diet (Hsieh *et al.* 1998). The tumour cells in mice on an isoflavone-free diet did not grow. A similar result has since been obtained using soya protein (Allred *et al.* 2001*b*; Ju *et al.* 2001). What is the significance of this result? Several points can be made that may have influenced the outcome. First, ovariectomy in mice leads to complete removal of circulating oestrogens. This explains why the tumour cells in the animals on the isoflavone-free diet did not grow at all. This is not analogous to the loss of ovarian oestrogen synthesis after the menopause, since women still synthesise oestrogens at peripheral sites (Nevton *et al.* 1986). Second, the lack of immune response in the mice may have altered the mechanisms by which isoflavones prevent tumour cell growth. Third, MCF-7 cells represent a highly selected cell type that is not necessarily truly representative of human breast cancer.

Hypothyroidism and thyroid cancer

The discovery that isoflavones are substrates for thyroid peroxidase (TPO), being converted into 6,8,3′-triiodoisoflavones, was an interesting finding (Divi *et al.* 1997). It has its counterpart with the chlorination of isoflavones (and many other phenol-containing compounds) by HOCl generated during respiratory bursts in neutrophils (Boersma *et al.* 2001). It was speculated that isoflavone iodination would lead to either a fall in conversion from T_3 to T_4 or an overcompensation by the thyroid gland, resulting in increased thyroid activity (Divi *et al.* 1997). The latter might in turn cause thyroid hypertrophy or thyroid carcinogenesis. Although experiments carried out in animals on genistein-containing diets resulted in lowered TPO activity measured *in vitro*, there was no physiological effect on thyroid size, thyroid histology or thyroid function in the animals (Chang & Doerge, 2000). The conclusion is that the thyroid has substantial capacity to manufacture thyroid hormones that is not easily compromised by dietary substances. In this regard it should be noted that TPO *in vitro* is much more sensitive to a wide range of other polyphenols present in fruit and vegetables (Divi & Doerge, 1996). This would imply that a high fruit/vegetable diet would be associated with hypothyroidism or increased risk of thyroid cancer. A recently reported epidemiological study found a twofold reduction in thyroid cancer in association with soya intake (Horn-Ross *et al.* 2002).

Neurodegeneration and Alzheimer's disease

Concern regarding a possible association of tofu intake and brain atrophy came from the results of a 35-year epidemiological study in Japanese living in Hawaii (White *et al.* 2000). Although of great interest, the study was limited by the dietary assessment being carried out in 1965 and then in 1971, whereas examination of memory loss and of brain size did not occur until 1991. The nature of the diet in the intervening years is subject to speculation. Intervention studies on the effects of soya on short-term memory in the young suggest that soya enhances memory function (File *et al.* 2001), a finding also reported for high-dose oestrogen therapy (Asthana *et al.* 2001). Experiments carried out in rats have revealed that soya isoflavones increase the level of mRNA for neurotrophic factor (Pan *et al.* 1999) and reduce the phosphorylation of tau, a microtubule-associated protein that is hyperphosphorylated and forms paired helical filaments in patients with Alzheimer's disease (Kim *et al.* 2000).

Potential risks in the use of phyto-oestrogens

The synthetic isoflavone ipriflavone (7-isopropoxyisoflavone) is being studied in clinical intervention trials for its effectiveness in the prevention of osteoporosis, where it is used at a dose of 600 mg/d (~8–9 mg/kg per d). Ipriflavone caused lymphocytopaenia in 13·2% of the women that largely reversed to normal within 2 years after cessation of treatment (Alexandersen *et al.* 2001). It is of interest, therefore, that a single 16 mg/kg dose of genistein caused a grade 2 leukopaenia in one out of six subjects studied (Busby *et al.* 2002). Other changes observed in the acute dose-escalation study (Busby *et al.* 2002) included effects on blood lipase and hypophosphataemia in several subjects. It remains to be seen what dose of genistein used in chronic studies would cause similar effects. Since genistein can cause double-strand breaks in DNA in haematopoietic mononuclear cells isolated from blood of healthy adults *in vitro* (Kulling *et al.* 1999), there is a concern that this may occur in human subjects treated with isoflavones. The University of North Carolina group is conducting a safety study using a daily dose of 300–600 mg of genistein (SH Zeisel, personal communication) to examine this question.

In summary, a daily dose of 50 mg of isoflavones consumed orally should be considered safe for most population groups. It may also be reasonable to extend this limit of safety to 2 mg/kg body weight per d (150 mg/d) in clinical trials where there is careful monitoring of the participating patients. This higher dose may prove to be necessary to prevent osteoporosis.

Acknowledgements

This review has been carried out with financial support from the Commission of the European Communities,

Food and Agroindustrial Research programme CT-98-4456 'Dietary exposure to phyto-oestrogens and related compounds and effects on skeletal tissues (VENUS)'. It does not reflect its views and in no way anticipates the Commission's future policy in this area.

Research in the author's laboratory was supported in part by grants from the American Institute for Cancer Research (91B47R), the National Cancer Institute (5R01 CA-61668), the United Soybean Board (7312), and the National Institutes of Health Office of Dietary Supplements and the National Center for Complementary and Alternative Medicine for the Purdue–UAB Botanicals Center for Dietary Supplements Research (P50 AT00477-01).

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