The bioavailability of non-nutrient plant factors: dietary flavonoids and phyto-oestrogens

Helen Wiseman

Department of Nutrition and Dietetics, King's College London, Campden Hill Road, Kensington, London W8 7AH, UK

The bioavailability in human subjects of non-nutrient plant factors, including dietary flavonoids and phyto-oestrogens, is of great importance relative to their reported health protective effects. These effects include protection against heart disease, and also in the case of the phyto-oestrogens, hormone-dependent cancers. Epidemiological studies have shown flavonoid intake (mostly quercetin) to be inversely associated with mortality from CHD. Quercetin is a potent antioxidant *in vitro*, and protection against the oxidative damage to LDL implicated in atherogenesis has been suggested as a possible mechanism. Human subjects can absorb significant amounts of quercetin (particularly in the glucoside form) and it would appear to be sufficiently bioavailable to act as an antioxidant *in vivo*; however, following our recent study (J O'Reilly, TAB Sanders and H Wiseman, unpublished results), it is currently less clear whether quercetin really can act as an antioxidant *in vivo*. The isoflavone phyto-oestrogens genistein and daidzein are much less effective antioxidants than quercetin *in vitro*, however, they are well-absorbed by human subjects and appear to be sufficiently bioavailable to act as antioxidants *in vivo*. In our recent study (O'Reilly *et al.* 1998) lower plasma isoprostane concentrations and increased resistance of LDL to oxidation were observed following the high-isoflavone dietary phase compared with the low-isoflavone dietary phase. Considerable inter-individual variation in isoflavone metabolite excretion has been observed, in particular the production of equol (the gut bacterial metabolite of daidzein; a more potent antioxidant and more oestrogenic than daidzein), and this appears to be influenced by habitual diet. Further studies on the bioavailability of these non-nutrient plant factors and related influencing factors are clearly still required.

Phyto-oestrogens: Flavonoids: Bioavailability: Antioxidants: Heart Disease: Cancer

Non-nutrient plant factors, including the dietary flavonoids and phyto-oestrogens, are currently being investigated to determine their importance in the prevention of major diseases, including heart disease and cancer. Flavonoids, in particular the flavonol quercetin (Cook & Saman, 1996; Hertog & Hollman, 1996; Wiseman, 1998), and phyto-oestrogens (Adlercreutz *et al.* 1995; Adlercreutz, 1996; Cassidy, 1996; Wiseman, 1996, 1997, 1998; Bingham *et al.* 1998; Williams & Rutledge, 1998) such as the isoflavones genistein and daidzein, are the main focus of the present article. Some members of the flavonoid family, including the isoflavonoids and some of the flavones and flavanones, also belong to the phyto-oestrogen family (non-steroidal oestrogen-mimics derived from plants), which also includes a number of chalcones, coumestans, stilbenes and lignans (Wiseman, 1998). In general, most flavonoids are present in plants in their glycoside form, whereas the unconjugated (aglycone) forms demonstrate far greater oestrogenic activity (Miksicek, 1995). The present article thus examines the importance of obtaining a measure of the bioavailability of such non-nutrient plant factors in order to assess fully their biological effects.

Factors influencing bioavailability of dietary factors

An estimate of the absolute bioavailability (expressed as a percentage) of a non-nutrient plant factor from a food source is derived from knowledge of the proportion of molecules that enter the blood circulation intact after consumption and following their passage across the gut wall and their first passage through the liver. Bioavailability is thus not indicated entirely by the extent of absorption. Absorption, distribution, metabolism (bioconversion in the gut and biotransformation in the liver) and elimination all contribute

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Corresponding author: Dr Helen Wiseman, fax +44 (0)171 3334185, email Helen.Wiseman@kcl.ac.uk

to the bioavailability and subsequent effectiveness of a non-nutrient plant factor following ingestion. Bioavailability thus quantifies the exposure of the body (not including the liver or the gut) to the non-nutrient plant factor in question (Hollman, 1997*a*). Absolute bioavailability can be determined experimentally by administering the flavonoid, for example, both orally and intravenously, and then calculating the ratio of plasma levels with time (from the areas under the curve). The studies in human subjects described here did not include intravenous administration of flavonoids or phyto-oestrogens, and thus only provide information on the relative bioavailability of the non-nutrient plant factor in different foods (Hollman, 1997*a*; Hollman *et al.* 1997*a,b*). The bioavailability of dietary flavonoids and phyto-oestrogens is clearly important in assessing the likely importance of these compounds to human health. Until recently, relatively little information has been available on the absorption and subsequent distribution, metabolism and excretion in human subjects of non-nutrient plant factors (including the flavonoids and phyto-oestrogens) which will invariably influence their bioavailability. It was originally suggested that flavonoids derived from foods could not be absorbed from the small intestine because, with the exception of catechins, they are found attached to sugars (as glycosides) which were considered to be non-absorbable from the intestine (Hollman, 1997*a,b*). It was considered possible for the aglycones (free flavonoid without a sugar molecule) to be taken up from the gut, but no enzymes capable of liberating free aglycones are present in the intestinal wall (or secreted into the gut). Hydrolysis of the mostly β-glycosidic bonds that attach flavonoid to sugars takes place only in the colon, where it is mediated by the micro-organisms that can also extensively degrade the flavonoid molecule (Hollman, 1997*a,b*).

Bioavailability of the flavonol quercetin

Flavonols include quercetin, found in onions (*Allium cepa*), and to a lesser extent in French beans (*Phaseolus vulgaris*), broad beans (*Vicia faba*), apples, apricots, cherries, grapes, wine, fruit juices, black tea and green tea (Hertog *et al.* 1992, 1993*b,c*; Crozier *et al.* 1997). Green tea is a major dietary source of the catechins, which include catechin, epicatechin, epigallocatechin and epigallocatechin gallate (Cook & Saman, 1996; Hollman, 1997*b*). Grapes and other highly-pigmented fruits are good sources of anthocyanidins, which are responsible for the colour and include cyanidin and pelargonidin (Hollman, 1997*b*).

A dietary study with healthy ileostomy patients (thereby avoiding losses caused by colonic bacterial degradation) has shown that human subjects can in fact absorb significant amounts of quercetin, and that glycosides can be absorbed from the small intestine (Hollman *et al.* 1995, 1997*b*). Absorption of quercetin glucosides was 52 %, absorption of pure quercetin was 24 % and that of quercetin rutinoside was 17 % (Hollman *et al.* 1995, 1997*b*). This not only shows that the glycone form of quercetin can be absorbed, but also that absorption of the glucoside is greater than that of both the aglycone and the rutinoside; thus, absorption is enhanced by conjugation with glucose. A number of flavonoid glycosides, including quercetin rutinoside (rutin),

other quercetin glycosides (as yet unidentified) and phloridzin, have all been detected in the plasma of unsupplemented subjects, in the range 0·5–1·6 µM (Paganga & Rice-Evans, 1997).

The exact influence of the sugar group on the absorption of quercetin glycosides has been investigated, and the much more rapid and greater rise in plasma quercetin levels following ingestion of the glucoside compared with the rutinoside suggests that the former may be rapidly absorbed directly from the small intestine, in contrast to the latter which is probably absorbed only from the colon (Hollman, 1997*a*). A role for the Na–glucose co-transporter (located in the brush-border membranes of the small intestine) in the absorption of quercetin 4′-glucoside has been proposed (Hollman, 1997*a*).

The kinetics of the absorption and disposition of quercetin has been studied in human subjects (Hollman *et al.* 1996; Hollman, 1997*a*). After 2·9 h peak plasma levels of quercetin of 196 ng/ml were reached. The half-life of quercetin absorption was 0·87 h, and those of the distribution and elimination phases were 3·8 and 16·8 h respectively. The plasma concentration decreased to approximately 10 ng/ml after about 48 h, suggesting that quercetin glycosides from onions are absorbed and eliminated at quite a gradual rate (Hollman *et al.* 1996; Hollman, 1997*a*). Quercetin has also been shown to be extensively metabolized; less that 1·5 % of the ingested quercetin excreted in urine had an intact flavonoid structure (Hollman *et al.* 1995, 1997*b*).

The accumulation in plasma and excretion in urine of flavonol glucosides following the consumption of lightly-fried yellow onions by healthy subjects has been investigated and has been found to be greater for isorhamnetin-4′-O-β-glucoside compared with quercetin-4′-O-β-glucoside, indicating again that flavonols are absorbed as glucosides, and demonstrating that small structural differences can have a major effect on absorption and excretion (Aziz *et al.* 1998).

In order to determine the relative bioavailability of quercetin from different food sources, the time-course of the plasma quercetin concentration has been measured following single doses of onions or apples (Hollman, 1997*a*; Hollman *et al.* 1997*a*). Black tea is another good dietary source of quercetin; the major glycosides present in tea leaves are the 3-rutinoside (50 %), the 3-glucoside (30 %) and the 3-galactoside (20 %). However the low quercetin concentrations of actual tea infusions meant that pure quercetin rutinoside, selected as the representative tea glycoside of quercetin, was best administered in capsule form. The bioavailability of pure quercetin rutinoside and of the form of quercetin found in apples was 30 % relative to that of the form of quercetin found in onions. This suggests that the type of sugar group of the glycoside has a great influence on absorption (Hollman, 1997*a*; Hollman *et al.* 1997*a*).

Importance of quercetin bioavailability for health protective effects

In the Zutphen Elderly Study (Hertog *et al.* 1993*a*; Keli *et al.* 1996) the mean baseline flavonoid intake was 25·9 mg

and the major sources of intake were tea (61 %), onions (13 %) and apples (10 %). Flavonoid intake, which was analysed in tertiles, was significantly inversely associated with mortality from CHD. This study also provided evidence for flavonoid-mediated protection against stroke; dietary flavonoid content (in particular quercetin) was inversely associated with stroke incidence (Keli et al. 1996). The average intake of quercetin in The Netherlands is 16 mg/d and this is provided mostly by tea (48 %), onions (29 %) and apples (7 %) (Hertog et al. 1993b). The contribution of quercetin to the total flavonol plus flavone consumption has been determined to be 68–73 % in free-living subjects from fourteen different countries eating a variety of diets (average total flavonoid intake 27·6 mg/d), and the major sources of intake were (%): tea 37, onions 26, vegetables 14, fruit 22, red wine 1 (de Vries et al. 1997). However, flavonoid intake did not appear to influence cancer mortality in the Zutphen Elderly Study (Hertog et al. 1994), and this is in contrast to the anti-carcinogenic effects observed in animal models and in human cancer cells in vitro (Hertog & Hollman, 1996).

It thus appears possible that consumption of quercetin-rich foods can result in significant plasma quercetin levels that could contribute to the plasma antioxidant capacity. The long half-life of elimination (24 h) is independent of the type of glycoside of quercetin and suggests that quercetin will accumulate in plasma; steady-state levels of 0·2 μM can be achieved (Hollman, 1997a; Hollman et al. 1997a,b). This concentration can be achieved after consumption of onions containing 30 mg quercetin. This corresponds to the intake in the highest tertile in the Zutphen Elderly Study, where risk of CHD was substantially reduced (Hertog et al. 1993a).

A number of mechanisms have been proposed for the protection conferred against CHD by flavonoids, including their antioxidant activity. Oxidative damage to LDL, particularly to the apoprotein B molecule, is considered to be an important stage in the development of atherosclerosis (Steinberg et al. 1989). Flavonoids such as quercetin are effective inhibitors of in vitro oxidative modification of LDL by macrophages or by Cu ions (De Whalley et al. 1990). The concentration of quercetin reported in plasma (0·2 μM) may be able to protect LDL against oxidative modification. Indeed, quercetin can inhibit LDL oxidation in vitro, with a value of 0·5 μM for the inhibitory concentration giving 50 % maximal oxidation (J O'Reilly and H Wiseman, unpublished results). It is interesting, therefore, that our recent randomized crossover study in human subjects does not appear to indicate any overall changes in the biomarkers of oxidative damage measured, including resistance of LDL to oxidation, between the low- and high-flavonoid dietary phases (J O'Reilly, TAB Sanders and H Wiseman, unpublished results).

Bioavailability of the isoflavone and lignan phyto-oestrogens

The isoflavonoids include the isoflavone phyto-oestrogens genistein and daidzein, which occur mainly as glycosides of the parent aglycone in soyabeans, and consequently in a wide range of soyabean-derived products, and to a lesser extent in other legumes (Reinli & Block, 1996). Soya foods traditionally made from soyabeans include both fermented and non-fermented foods (Golbitz, 1995). Some alcoholic beverages such as beer have been shown to contain significant amounts of isoflavonoid phyto-oestrogens (Lapcik et al. 1998). Lignans are non-flavonoid dietary phyto-oestrogens which are found as the plant precursors (secoisolariciresinol and matairesinol) of the mammalian lignans enterodiol and enterolactone. Secoisolariciresinol and matairesinol are found in linseed and sesame seed respectively, and both are found also in a number of whole grains (Thompson, 1994; Slavin et al. 1997).

Following ingestion, daidzin and genistin are hydrolysed in the large intestine by the action of bacteria to release genistein and daidzein. Daidzein can be metabolized by the bacteria in the large intestine to form equol (oestrogenic) or O-desmethylangolensin (non-oestrogenic), whereas genistein is metabolized to the non-oestrogenic p-ethyl phenol. Inter-individual variation in ability to metabolize daidzein to equol could thus influence the potential health protective effects of soyabean isoflavones (see p. 143). The lignan phyto-oestrogen precursors in plant material, matairesinol and secisolariciresinol, are converted by gut bacteria to the two main mammalian lignans (health factors) enterolactone and enterodiol respectively (Thompson, 1994). Matairesinol undergoes dehydroxylation and demethylation directly to enterolactone, whereas secisolariciresinol is converted to enterodiol, which can then be oxidized to enterolactone (Adlercreutz, 1996). After absorption, enterolactone and enterodiol are excreted in urine (see later), although relatively limited studies of the metabolism of lignans in human subjects have been carried out. A study in ileostomists found (in the absence of sufficient bacterial activity for conversion of plant lignans to mammalian lignans) no increase in plasma levels of mammalian lignans (Pettersson et al. 1996).

The urinary excretion of phyto-oestrogens can be used as a measure of intake, and hence possible exposure of tissues to bioavailable phyto-oestrogens which could confer protection against cancer. In assessing exposure to the protective effects of phyto-oestrogens, urinary excretion rates should be considered in combination with the plasma levels attained. A very low urinary excretion of lignans and equol (isoflavan metabolite of the isoflavone daidzein) has been observed in post-menopausal breast cancer patients compared with vegetarians (Adlercreutz et al. 1995; Adlercreutz, 1996). In human subjects, omnivorous subjects usually have low levels of isoflavonoid excretion, and in individuals consuming a Western diet, urinary levels of lignans have been found to be greater than those of isoflavones (Adlercreutz et al. 1995; Adlercreutz, 1996).

Urinary excretion of the lignans enterodiol and enterolactone was elevated in subjects consuming a carotenoid-vegetable diet and a cruciferous-vegetable diet compared with the basal (vegetable-free) diet, suggesting these vegetables may provide a source of mammalian lignan precursors (Kirkman et al. 1995). In addition, men excreted less enterodiol and more enterolactone compared with women, suggesting there may be a sex difference in the bacterial metabolism of lignans in the colon (Kirkman et al. 1995). Urinary excretion of genistein, daidzein, equol and

O-desmethylangolensin was found to be greater in subjects when they consumed a soyabean-rich diet compared with vegetable-rich (carotenoid v. cruciferous) and basal (vegetable-free) diets (Kirkman et al. 1995). Young American women from different ethnic groups have been found to predominantly excrete different types of phyto-oestrogens in their urine, and presumably this reflects cultural influences on the diets consumed (Horn-Ross et al. 1997). Urinary isoflavonoid excretion has been reported to be dose-dependent at low to moderate levels of soyabean consumption (Karr et al. 1997).

In a study of plasma isoflavonoid levels in Japanese men compared with Finnish men, the means of the total daidzein, genistein, O-desmethylangolensin and equol levels were approximately 17-, 44-, 33- and 55-fold higher for the Japanese subjects compared with the Finnish subjects (Adlercreutz et al. 1993). In a study of post-menopausal Australian women consuming soyabean flour, mean plasma levels of daidzein and equol of 68 and 31 ng/ml respectively were observed (Morton et al. 1994). In some subjects, however, levels (ng/ml) as high as 148 for genistein, 43 for equol and 312 for daidzein were reached (Morton et al. 1994). Furthermore, only 33 % of subjects were able to metabolize daidzein to equol. In addition, combined levels of enterolactone and enterodiol of 500 ng/ml were reached following linseed supplementation (Morton et al. 1994). In a study of the short-term metabolism of isoflavonoid and lignan phyto-oestrogen, male subjects ingested a cake containing soyabean flour and cracked linseed (Morton et al. 1997). Increased serum concentrations of both genistein and daidzein were observed within 30 min of consumption, with maximum concentrations reached by 5·5–8·5 h following consumption. Maximum plasma concentrations were 87·5–190 ng/ml for genistein and 83·3–129 ng/ml for daidzein; none of the subjects was able to metabolize daidzein to equol. The metabolism of the plant lignans to the mammalian lignans was slower, with increased plasma levels of enterolactone and enterodiol found 8·5 h after consumption (Morton et al. 1997).

The plasma and urinary kinetics of daidzein and genistein have been determined in men following a single soyabean meal (soyabean-flour-based meal; King & Bursill, 1998). The maximum plasma concentrations were approximately 3 μmol/l at approximately 7 h and 4 μmol/l at 8 h for daidzein and genistein respectively, and the corresponding elimination half-lives were approximately 5 and 6 h (King & Bursill, 1998). A slow rise in plasma isoflavone concentrations was observed, which suggests that absorption is taking place following hydrolysis in the small and large intestines of the glycone forms of the isoflavones to their aglycone forms (King & Bursill, 1998). Although the urinary excretion of daidzein was greater than that of genistein (recoveries of approximately 62 and 22 % respectively), the ratio of the areas under the plasma concentration v. time curves for genistein and daidzein was equal to the ratio of the concentrations of the respective isoflavones in the soyabean meal. Overall, this study thus suggests that despite the differences in urinary excretion, the bio-availabilities of genistein and daidzein are in fact similar (King & Bursill, 1998).

A variable metabolic response to isoflavones has been reported for subjects who consumed soyabean flour over 2 d (Kelly et al. 1995). Urinary levels of genistein, daidzein, equol and O-desmethylangolensin were elevated 8-, 4-, 45- and 66-fold respectively over baseline following consumption. Considerable inter-individual variation in metabolic response was found, with the peak levels of equol showing the greatest variation (Kelly et al. 1995). An inverse relationship between equol and O-desmethylangolensin was observed, suggesting inter-individual variation in the dominant metabolic pathway for daidzein metabolism. This may be important in relation to cancer risk, as equol is much more oestrogenic than daidzein or O-desmethylangolensin (Kelly et al. 1995). The reasons for the wide inter-individual variation in isoflavone metabolite excretion following the consumption of soyabean have not been fully elucidated; however, in a recent study female equol excreters have been reported to consume a higher percentage of energy as carbohydrate and also greater amounts of plant protein and NSP (Lampe et al. 1998). In our recent randomized cross-over dietary intervention study in healthy male and female subjects, a diet high in isoflavones (textured-soyabean-protein product supplying 56 mg/d) and a diet low in isoflavones (textured-soyabean-protein product supplying 2 mg/d) were each consumed for 2 weeks separated by a 3-week washout period. We found that good equol producers (35 % of subjects and defined as >1000 nmol/24 h) excreted about 200-fold more equol in their urine than the poor equol producers, and consumed significantly less fat and more carbohydrate and also greater amounts of NSP compared with the poor equol producers (Bowey et al. 1999).

Chronic soyabean ingestion (as soyabean milk) appears to modulate the metabolism and disposition of ingested isoflavones in young females, and an increase in the production of equol was observed in some of the subjects (Lu et al. 1996). In contrast, in males, chronic soyabean exposure did not alter the pathways of isoflavone metabolism, but altered the time-courses of excretion (Lu et al. 1995).

Metabolism by the gut microflora is an important factor influencing the disposition of chemicals in the gut, and can result in activation of substances to more-biologically-active products. The bioavailability of soyabean isoflavones has been shown to depend on the gut microflora in women (Xu et al. 1995). Differences in faecal excretion greatly influenced isoflavone bioavailability; urinary recovery of ingested isoflavone phyto-oestrogens was more than twice as high in high v. low faecal excreters (Xu et al. 1995). Gut bacterial enzymes such as β-glucuronidases can hydrolyse isoflavone conjugates to aglycones (rapidly re-absorbed). However, intestinal microflora can also extensively metabolize and degrade isoflavones and other flavonoids, thus preventing their re-absorption from the colon. If an individual possesses bacteria that are not effective in iso-flavone metabolism and degradation then more isoflavones would be absorbed, which would explain the positive association between high faecal isoflavones and greater total urinary recovery of isoflavones (Xu et al. 1995).

The presence of different populations of microflora in the human gut may influence the bioavailability of soyabean

isoflavone phyto-oestrogens. Our recent dietary intervention study with soyabean isoflavones has demonstrated considerable inter-individual variation in isoflavone phyto-oestrogen metabolism (Bowey et al. 1999: see p. 142) and significant effects on gut bacterial activity and gastrointestinal function (E Bowey, H Adlercreutz, I Rowland, TAB Sanders and H Wiseman, unpublished results). Studies on the types of human gut microflora involved in phyto-oestrogen bio-availability are needed. The metabolism of daidzein to equol by certain types of gut bacteria has important implications for daidzein bioavailability, because equol producers are likely to have lower levels of bioavailable daidzein. Equol has its own particular health benefits and concerns (i.e. it is a more potent oestrogen and antioxidant).

Importance of bioavailability to the health protective action of phyto-oestrogens

The incidence of breast and prostate cancer is much greater in Western countries than in Far Eastern countries, where there is an abundance of dietary phyto-oestrogens (Adlercreutz et al. 1995; Adlercreutz, 1996; Cassidy, 1996; Bingham et al. 1998). Lower levels of prostate cancer are also reported for vegetarians who consume large amounts of plant material (rich in lignans). Levels of isoflavonoids and lignans have been measured in prostatic fluid from males from different parts of the world (Morton et al. 1997) to determine the levels of bioavailable (and thus bioactive) phyto-oestrogens in body tissues. The highest mean levels of daidzein and its metabolite equol were found in males from Hong Kong (70 and 29·2 ng/ml respectively) and China (24·2 and 8·5 ng/ml respectively), compared with those in males from the UK (11·3 and 0·5 ng/ml respectively) and Portugal (4·6 and 1·72 ng/ml respectively). In contrast, the highest levels of lignans were found in prostatic fluid from men from Portugal (enterolactone 162 ng/ml, enterodiol 13·5 ng/ml). The levels of enterolactone and enterodiol were similar in prostatic fluid from men from the other countries investigated (enterolactone 20·3–32·9 ng/ml, enterodiol 1·6–6 ng/ml).

Analysis of human breast milk has detected conjugates of genistein and daidzein following the consumption of roasted soyabeans (France & Custer, 1996). Thus, concentrations of daidzein and genistein of 20–28 and 8–14 ng/ml respectively have been found in a subject who naturally consumed a diet rich in soyabean products and from another subject following a soyabean challenge. A preferential excretion of the main metabolites (of daidzein), equol and O-desmethylangolensin, over the parent compounds was observed, when compared with the patterns in urine and in faeces. This may be due to the higher isoflavone:metabolite found in plasma compared with urine and faeces (milk is produced by secretory processes of blood). The lower cancer rates in soyabean-consuming populations may thus be due to exposure to isoflavones at the critical developmental period just following birth, via the mother's milk, rather than from adult consumption of soyabean products (Franke & Custer, 1996; Franke et al. 1998).

Free radicals may play a role in human cancer developments; free radicals have been shown to possess many characteristics of carcinogens (Wiseman & Halliwell,

1996). Furthermore, membrane function is now understood to be of vital importance to many cellular processes, including the role of membrane enzymes and receptors in cell growth and signalling (Wiseman, 1998). Protection of membrane function against the free radical-mediated process of membrane lipid peroxidation that can result in oxidative membrane damage is clearly important. The reported antioxidant properties of soyabean isoflavones (Wiseman, 1996; Wiseman et al. 1998) could thus contribute to their proposed anti-cancer action. In model membrane systems, the daidzein metabolite equol was a more effective antioxidant than genistein or daidzein itself. Equol had a concentration giving 50 % maximal inhibition of membrane lipid peroxidation of 5 µM compared with 30 µM for tamoxifen and 8 µM for 4-hydroxytamoxifen (Wiseman & O'Reilly, 1997; Wiseman et al. 1998). Tamoxifen is an anti-oestrogen drug widely used in the treatment of breast cancer and currently being assessed for the prevention of breast cancer (Wiseman, 1994). Plasma values as high as 0·2 µM have been reported for equol (Morton et al. 1994), and although this appears to be considerably less than that required in vitro for effective membrane protection against oxidative damage, membrane accumulation in vivo to achieve effective concentrations is likely (see p. 144).

The isoflavones may also exert their protective effects by antagonizing the action of sex hormones and by interfering with cellular signalling mechanisms involving tyrosine kinase (Wiseman, 1996). Phyto-oestrogens demonstrate a structural similarity to the steroid nucleus of the female sex hormone oestrogen, which enables them to bind to oestrogen receptors (and androgen receptors) to produce effects ranging from agonism to antagonism of the endogenous hormone ligand (Miksicek, 1995). The molecular basis of agonism and antagonism at the oestrogen receptor has only recently been elucidated (Brzozowski et al. 1997). Phyto-oestrogens appear to block the growth-enhancing effects of oestrogen in the breast, including inhibition of the growth of breast cancer cells. Breast cancer is thought to have a multifactorial causation, ranging from gene profile to diet and lifestyle (Wiseman, 1994). Phyto-oestrogens can be considered to be acting as anti-oestrogens in the prevention of breast cancer in a similar way to tamoxifen (Wiseman, 1994).

Phyto-oestrogens can also imitate the protective action of oestrogen on the cardiovascular system and on bone. This action may contribute to their reported protective ability against heart disease and osteoporosis, and suggests their use as a natural supplement, as an alternative to hormone-replacement therapy in women (Clarkson et al. 1995). Soyabean protein incorporated into a low-fat diet can reduce cholesterol and LDL and raise HDL, and the oestrogenic isoflavones present are likely to contribute to this effect (Raines & Ross, 1995). The antioxidant action of phyto-oestrogens may contribute also to their cardio-protective properties. Isoflavones can protect LDL against oxidative modification in vitro. The inhibitory concentration giving 50 % maximal LDL lipid peroxidation for equol was 3 µM compared with 15 µM for tamoxifen and 1 µM for 4-hydroxytamoxifen (Wiseman & O'Reilly, 1997; Wiseman et al. 1998), and although this is greater than the

bioavailable level of equol of 0·2 μM that can be achieved (see p. 143), nevertheless, bioaccumulation into LDL particles and into the lipophilic atheromal plaques associated with the arterial wall is likely, as previously suggested for tamoxifen and 4-hydroxytamoxifen (Wiseman *et al.* 1993). Indeed, in our own recent dietary intervention study, subjects consumed a soyabean textured vegetable protein product with or without isoflavones (see p. 142), and the biomarkers of oxidative damage measured included plasma isoprostane (a lipid peroxidation product) concentrations (8-epi-prostaglandin $F_{2\alpha}$, an F_2-isoprostane, was measured), which showed a significant decrease, and resistance of LDL to oxidation which showed a significant increase (O'Reilly *et al.* 1998). The increased resistance of LDL to oxidation following consumption of soyabean products is in agreement with other similar studies (Tikkanen *et al.* 1998). Although total plasma cholesterol and apoprotein B concentrations were unaffected by the isoflavone content of the diet, HDL and apolipoprotein A-I (a novel biomarker of total oestrogenicity) concentrations were significantly higher following the high-isoflavone dietary period (Dean *et al.* 1998).

Recent developments in bioavailability of flavonoids and phyto-oestrogens: future trends

The relative bioavailabilities of non-nutrient plant factors in different food sources need to be determined before their exact contribution to the protection of human health can be assessed. Quantitative data for the separate quercetin glycosides of a food could be used to predict the bioavailability of the quercetin in it (Hollman, 1997a; Hollman *et al.* 1997a,b). To evaluate the risk:benefit ratio of dietary phyto-oestrogens in human subjects more information is required regarding their bioavailability and the role of gut microflora in modulating bioavailability in both high and low consumers. The differences in response between adults and children clearly need to be elucidated. Soyabean is a major source of dietary phyto-oestrogens, and is widely used in both human and animal feeds. The popularity of soyabean products as meat substitutes has increased markedly over the past decade, with the rise in the popularity of vegetarianism, and this trend is likely to continue. Furthermore, there is increased interest in the use of diets high in phyto-oestrogens to help alleviate menopausal symptoms. More information is needed, however, on the biological effects of increased phyto-oestrogen intake by menopausal women. No simple answers are yet available to the basic uncertainty of the bioavailability of ingested non-nutrient plant factors, including dietary flavonoids and phyto-oestrogens. Research in progress will provide clarification of these important issues within the next 3–5 years.

Acknowledgements

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