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

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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to act as an antioxidant in vivo; however, following our recent study (J O'Reilly, TAB Sanders and H Wiseman, unpublished results), it is currently less clear whether quercetin really can act as an antioxidant in vivo.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.

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 to the bioavailability of these plant compounds.
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, 1997a). 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, 1997a; Hollman et al. 1997a,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, in contrast to the latter which is probably absorbed only from the colon (Hollman, 1997a). 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, 1997a).

The kinetics of the absorption and disposition of quercetin has been studied in human subjects (Hollman et al. 1996; Hollman, 1997a). After 2.9 h peak plasma levels of quercetin of 196 ng/ml were reached. The half-life of quercetin absorption was 0.7 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, 1997a). 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, 1997b).

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, 1997a; Hollman et al. 1997a). 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, 1997a; Hollman et al. 1997a).

Importance of quercetin bioavailability for health protective effects

In the Zutphen Elderly Study (Hertog et al. 1993a; 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 & Holman, 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 (Holman, 1997a; Holman et al. 1997ab). 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 of human subjects, omnivorous subjects usually have low levels of isoflavonoid excretion, and in individuals consuming a Western diet, urinary excretion of lignans 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 excretion of lignans has been found to be greater than those of isoflavones (Adlercreutz et al. 1995; Adlercreutz, 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).
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 crossover dietary intervention study in healthy male and female subjects, a diet high in isoflavones (textured-soyabean-protein product supplying 36 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 (8% of subjects) and bad (33%) excreters were able to excrete more than 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 excretors (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 isoflavone 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 bioavailability 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 29.2ng/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 1.6–6.9 ng/ml).

Analysis of human breast milk has detected conjugates of genistein and daidzein following the consumption of roasted soyabees (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 5μM compared with 30μM for tamoxifen and 8μM 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 0.2μM 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 3μM compared with 15μM for tamoxifen and 1μM 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
dratic concentrations (8-epi prostaglandin F2α, an F2-isoprostane, was
the biomarkers of oxidative damage measured included
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) concen-
trations, 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 bio-
availability 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 meno-
pausal 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.

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