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## The key importance of soy isoflavone bioavailability to understanding health benefits

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## Abstract

Research over the past two decades has provided significant epidemiological and other evidence for the health benefits of the consumption of soy-based foods. A large number of dietary intervention studies have examined the effects of soy isoflavones on risk factors for cardiovascular disease and hormone-dependent cancers. However, these report large variability in outcome measures, very limited reproducibility between studies and in some cases, controversy between results of clinical trials using dietary soy or soy protein and isoflavone supplementation. This highlights a major gap in our understanding of soy isoflavone uptake, metabolism, distribution, and overall bioavailability. There are many potential factors that may influence bioavailability and a better knowledge is necessary to rationalize the inconsistencies in the intervention and clinical studies. This review focuses attention on our current state of knowledge in this area and highlights the importance of metabolism of the parent soy isoflavones and the critical role of gut microbiota on the bioavailability of these compounds and their metabolites.

## Keywords

bioavailability, gut microbiota, soy isoflavones, equol, daidzein, genistein, CMMB

## Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

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“The key importance of soy isoflavone bioavailability to understanding health benefits”

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## **ABSTRACT:**

Research over the past two decades has provided significant epidemiological and other evidence for the health benefits of the consumption of soy-based foods. A large number of dietary intervention studies have examined the effects of soy isoflavones on risk factors for cardiovascular disease and hormone-dependent cancers. However, these report large variability in outcome measures, very limited reproducibility between studies and in some cases, controversy between results of clinical trials using dietary soy or soy protein and isoflavone supplementation. This highlights a major gap in our understanding of soy isoflavone uptake, metabolism, distribution, and overall bioavailability. There are many potential factors that may influence bioavailability and a better knowledge is necessary to rationalize the inconsistencies in the intervention and clinical studies. This review focuses attention on our current state of knowledge in this area and highlights the importance of metabolism of the parent soy isoflavones and the critical role of gut microbiota on the bioavailability of these compounds and their metabolites.

Key words: bioavailability; gut microbiota; soy isoflavones; equol; daidzein; genistein;

## **INTRODUCTION**

Among Asian populations with a high intake of soy, epidemiological evidence has demonstrated a lower incidence of cardiovascular disease (Adlercreutz, 1990), hormone-dependent cancers of the breast and prostate (Yu et al., 1991), colon cancer (Rose et al., 1986), menopausal symptoms (Clarkson, 2000) and osteoporosis (Adlercreutz et al., 1992). These effects have been extensively

reviewed and will not be reiterated here (Wu et al., 1998, Larkin et al., 2001, Murkies et al., 1998, Tham et al., 1998, Setchell and Cassidy, 1999, Bingham et al., 1998, Knight and Eden, 1996, Kurzer and Xu, 1997, Adlercreutz, 1995, Messina et al., 1994, Cassidy et al., 2000, Cassidy, 1996). However, it should be noted that there is also a strong association between dietary fat intake and cancers of the breast, prostate and colon (Rose et al., 1986). Although native Asian women and men have the lowest rates of breast (Henderson and Bernstein, 1991) and prostate cancer (Yu et al., 1991, Ross et al., 1995, Giovannucci, 1995), respectively, migration to Western countries and the adoption of a more Western diet, increases the incidence of these cancers amongst migrant Asians to an occurrence similar to Western populations (Ziegler et al., 1993, Whittemore et al., 1995). Similarly, while a cross-sectional study in Japan revealed an inverse association between soy intake and serum total cholesterol concentration (Nagata et al., 1998), an increase in the incidence of cardiovascular disease is reported for migrant Japanese (Kim et al., 1998). Thus diet, and in particular soy and its constituent isoflavones, have been implicated as affording some protection against the development of these hormone dependent cancers and cardiovascular disease.

In response to the epidemiological data, many soy dietary intervention studies have been conducted in an attempt to elucidate the mechanisms involved. However, these report large variability in outcome measures and there is limited reproducibility between studies (Lichtenstein, 1998). In 1999, the U.S. Food and Drug Administration approved a health claim for the cholesterol-lowering effects of soy protein, largely based on a meta-analysis of 38 clinical trials that reported significant decreases in total and LDL cholesterol and triglycerides with soy protein intake compared with animal protein consumption (Anderson *et al.*, 1995). This prompted a burst of investigation to elucidate the specific roles of isoflavones and soy protein in this hypocholesterolemic response; however, recent meta-analysis of the latter research reported inconclusive results (Yeung and Yu, 2003). Direct comparisons between studies are confounded by variations in endogenous hormone levels (Potter et al., 1998) and

baseline lipids of subjects (Gardner et al., 2001) as well as the dietary sources of soy, isoflavone concentration (Steinberg et al., 2003), intervention duration and study design (Merz-Demlow et al., 2000), all of which may impact on the results.

Isoflavones have both estrogenic activity and antioxidant capacity, related to their structural similarity to 17 $\beta$ -estradiol (Tham et al., 1998). These activities are relevant to the potential roles of isoflavones in reducing cancer and cardiovascular disease risk, as free radicals, as well as high endogenous hormone levels have been linked with the development of these conditions (Zheng and Zhu, 1999). The isoflavones are able to bind to estrogen receptors (ER) and elicit either a weak estrogenic (agonistic) or anti-estrogenic (antagonistic) effect, depending on the levels of endogenous estrogens present (Baghurst, 1997, Wang and Kurzer, 1998) and the tissue and the ER subtype (Kuiper et al., 1998, Setchell and Cassidy, 1999). Isoflavones have a much higher affinity for ER- $\beta$  than ER- $\alpha$  (Kuiper et al., 1997, Nikov et al., 2000), thus tissues with higher expression of ER- $\beta$  might be more responsive to isoflavones (Anderson et al., 1999). The estrogen receptor activity of isoflavones may play a major role in their effects against cancers of tissues that express estrogen receptors (Cassidy, 1996) and in the alleviation of menopausal symptoms (Wilcox et al., 1990, Persky et al., 2002). The isoflavones also demonstrate good antioxidant activity in various systems (Zheng and Zhu, 1999, Mitchell et al., 1998) in both aqueous and lipophilic phases (Ruiz-Larrea et al., 1997, Harper et al., 1999), attributed to a number of antioxidant mechanisms (Mitchell et al., 1998, Arora et al., 1998, Harper et al., 1999, Zheng and Zhu, 1999, Mitchell and Collins, 1999). The inhibition of lipid peroxidation, particularly of LDL, by isoflavones may be an important mechanism by which they positively influence lipid profiles.

For improvements in lipid levels, it appears that the soy protein matrix of isoflavones and intact soy protein may be more beneficial than either component alone (Potter 1998; Steinberg et al 2003)

Although the role of the individual soy components in their influence on lipids has not been fully elucidated, soy protein may affect hepatic metabolism of cholesterol or lipoproteins (Potter, 1998) or up-regulate LDL receptors (Anderson, 2003), while the isoflavones may act via their estrogenic and antioxidant activities. These latter effects of isoflavones in reducing lipid levels and LDL oxidation have been reported extensively elsewhere and will not be re-iterated here (Vitolins et al., 2001). Although less researched than the cardiovascular area, the estrogenic, antioxidant and anti-cancer activities of the isoflavones have all been implicated in reducing the occurrence or development of hormone-dependent cancers and are reported elsewhere (Adlercreutz et al 1995; Zheng and Zhu 1999; Slavin et al 1997). In relation to soy-derived foods, the large inter-individual variability in clinical and physiological effects may depend greatly on isoflavone bioavailability and the soy matrix in which they are contained.

To unravel some of these inconsistencies a much improved understanding of soy isoflavone uptake, metabolism, tissue distribution, and overall bioavailability is needed. Bioavailability, a term borrowed from pharmacology, has variable meanings in a nutritional context. Here we use the term broadly to include a full range of digestive and metabolic factors that influence the amount and type of isoflavone compounds that reach the systemic circulation. There are many factors that can influence bioavailability and this review focuses attention on our current state of knowledge of the area and highlights the importance of metabolism of the parent soy isoflavones and the critical role of gut microbiota on isoflavone bioavailability.

## **SOY – FOOD SOURCES AND ISOFLAVONE COMPOSITIONS**

To understand isoflavone bioavailability we need to have a good understanding of the form in which isoflavone compounds are found in foods, including those that have been processed post-harvest. Isoflavones are not widely distributed in plants, occurring almost exclusively in legumes (Coward et

al., 1993) with soybeans being the richest source of the plant precursors of the endogenous isoflavones, daidzein (4',7-dihydroxyisoflavone), genistein (4',5,7-trihydroxyisoflavone) and glycitein (4',7-dihydroxy-6-methoxyisoflavone) (Figure 1). Isoflavones are synthesised as part of the phenylpropanoid pathway, which has multiple branches common to legume and non-legume plants and from which other flavonoids are also synthesised (Parr and Bolwell, 2000, Yu et al., 2000, Hollman, 2001); however, their occurrence is limited because isoflavone synthase, the enzyme required to convert their flavanone precursors, is unique to legumes and only a few other species (Rolfe, 1988, Yu et al., 2000). This enzyme is developmentally and tissue-specifically regulated and may be induced by environmental stresses, particularly as the natural roles for isoflavones are in plant-microbial interactions, including disease resistance (Ebel, 1986, Yu et al., 2000). Formononetin and biochanin A, the 4'-O-methoxylated isoflavone derivatives and precursors to daidzein and genistein, respectively, occur in alfalfa and clover seeds and sprouts, and in chick peas, garbanzo beans, black bean seeds and some pulses (Wang and Murphy, 1994a, Murphy et al., 1999, Shoff et al., 1998, King et al., Franke et al., 1994).

Soybeans are composed of the fleshy cotyledons, the part of the seed that forms the first plant leaves; the hypocotyl, the part of the axis of the plant embryo below the cotyledon and the hull, the dry outer covering of the seed (Price and Fenwick, 1985). The majority of isoflavones are concentrated in the hypocotyl, where daidzein, glycitein and their respective conjugates account for more than 95% of total isoflavones (Price and Fenwick, 1985). Glycitein and its three derivatives occur exclusively in the hypocotyl (Wang and Murphy, 1994a), while genistein is found both in the hypocotyl and cotyledon, predominating in the latter (Erdman Jr et al., 2004, Price and Fenwick, 1985).

Total isoflavone content of soybeans varies widely and can be affected by crop year, soil conditions, local climate, genetics and stage of maturity (Wang and Murphy, 1994a, Simonne et al., 2000, Franke

et al., 1994). In addition, subsequent processing and storage conditions will also affect isoflavone yield (Wang et al., 1990, Eisen et al., 2003, Wang and Murphy, 1994b). Amongst different varieties, total isoflavone concentrations have been reported to range from 0.1 to 5 mg total isoflavones (aglycones + conjugates) per gram of soybean (Franke et al., 1995, Coward et al., 1993, Barnes et al., 1994, Wang and Murphy, 1994a, Simonne et al., 2000). Genistein is generally present at higher levels than daidzein and glycitein in soybeans and most soy-derived foods (Setchell et al., 2001, Franke et al., 1995). The variability in soy bean isoflavone composition is also reflected in other soy-derived foods; for example, soy milk produced from soy beans from different regions of the U.S, varies in total isoflavone content by up to approximately 70% (Murphy et al., 1999).

In traditional Asian diets, soy is consumed in many forms including soybeans, soybean sprouts, toasted soy protein flours, soy milk, tofu and fermented soybean products such as miso, tempeh, soybean paste, natto and soy sauce (Coward et al., 1993, Wang and Murphy, 1996). Soy consumption per individual in most Asian countries is reported to be about 35 g per day (Coward et al., 1993); this equates to a daily intake of between 25 and 100 mg total isoflavones (aglycone equivalents) (Messina, 1999, Coward et al., 1993, Setchell et al., 2001) and between 8 and 12 g soy protein (Erdman Jr et al., 2004). In Western countries, soy intake is more commonly in the form of soybean protein products including flours, grits, isolates, concentrates and textured soy proteins (Wang and Murphy, 1996) with the average daily exposure to isoflavones is less than 1 mg (aglycone equivalents) (Adlercreutz et al., 1991, Messina et al., 1994, Wei et al., 1995). Greater soy intake is reflected in plasma and urinary isoflavone concentrations, which are higher in Japanese men and women than Western populations (Adlercreutz et al., 1991) and are also higher in vegetarians than omnivorous subjects (Adlercreutz et al., 1993).



The isoflavones are present in four isomeric forms: aglycones and three glucoside conjugates, the  $\beta$ -, acetyl-, malonyl- and glucoside- conjugates (Wang and Murphy, 1994a, Xu et al., 2000) (Figure 2). The aglycones have no sugar residue attached, whereas the glucoside conjugates have a sugar group attached at the position 7 of the A ring. In whole soybeans, the isoflavones occur mostly as 6''-O-malonylglucoside conjugates, with the  $\beta$ -glucosides (daidzin and genistin) being the second most abundant isoflavone derivatives (Coward et al., 1993). In whole soybeans and other soy protein products, 97 – 98% of the isoflavones are present as their esterified conjugates (Wang and Murphy, 1994a); however, the glucoside composition of soybean-derived foods varies, being determined by processing conditions (Coward et al., 1998). Hot or acidic extraction procedures cause decarboxylation of the 6''-O-malonylglucoside conjugates in whole soybeans to produce the 6''-O-acetylglucosides conjugates, which can further undergo ester hydrolysis to form the  $\beta$ -glucosides (Coward et al., 1993, Simonne et al., 2000, Murphy et al., 1999, Barnes et al., 1994). While this does not necessarily change the total amount of isoflavones extracted, the ratio of conjugated forms varies between soy foods (Coward et al., 1998). Soy milk and tofu are produced from soybeans via hot aqueous extraction which results almost entirely in the formation of  $\beta$ -glucosides (Barnes et al., 1994, Coward et al., 1998, King and Bursill, 1998, Eisen et al., 2003). In fermented soy products, unconjugated aglycones, resulting from the action of the  $\beta$ -glucosidases of the fermentation organisms, are the predominant chemical forms (Murphy et al., 1999, Coward et al., 1993, Fukutake et al., 1996). Compared with whole soybeans, soy flour and soybean seeds generally contain slightly less total isoflavone content (Franke et al., 1995, Franke et al., 1994), while soy nuts, produced from dried late-harvest soy beans, can have higher levels (Fukutake et al., 1996). Soy germ products contain higher concentrations of daidzein and glycitein than genistein due to the lower levels of genistein occurring in the hypocotyl (Zhang et al., 1999).

Isoflavones are associated with the soluble components of soybean, most probably soluble proteins (Wang and Murphy, 1996) and therefore, high protein soy ingredients contain similar isoflavone concentrations compared with unprocessed soybeans (Eisen et al., 2003). While ethanol extraction of soy flour to produce soy protein concentrate removes most of the isoflavones (Coward et al., 1998, Coward et al., 1993), soy protein isolate produced from hot water extraction of soy flour retains most of the isoflavones, reflecting their strong protein binding and low aqueous solubility, and maintains the same pattern of conjugation as soybean, (Coward et al., 1993). Soy protein concentrate prepared by aqueous extraction has a reported total isoflavone content (isoflavones + conjugates) of up to 2.7 mg/g, which is comparable to that of soy flour and many Asian soybean products (Coward et al., 1993), although the relative levels of protein-bound daidzein or genistein can vary (Barnes et al., 1994, Franke et al., 1998). Alcohol-extracted soy protein and soy isolates contain lesser amounts of total isoflavones (Coward et al., 1993).

## **ISOFLAVONE BIOAVAILABILITY**

Isoflavone bioavailability is a measure of the amount of these compounds that becomes available for tissue distribution where they can exert physiological effects. Thus, an understanding of bioavailability is important in assessing the potential health benefits of isoflavones and may assist in the interpretation of the high variability of results in clinical trials. The pharmacokinetics of absorption, distribution, metabolism (bioconversion in the gut and biotransformation in the liver) and elimination all contribute to the bioavailability and subsequent effectiveness of the isoflavones (Wiseman, 1999, Rowland et al., 2003). However, most pharmacokinetic and bioavailability studies of isoflavones in humans have been limited to the determination of plasma and urinary concentrations of specific isoflavones and their metabolites due to the ethical and practical difficulties of tissue measurements. This does not provide comprehensive understanding of bioavailability and distribution, as plasma isoflavone concentrations

simply represent the balance between absorption, distribution and urinary and biliary excretion (King, 1998). In addition, the use of only plasma and urinary measurements of isoflavones does not differentiate between the contributions of the intestine, liver or other organs in isoflavone metabolism (Liu and Hu, 2002). The processes involved in isoflavone bioavailability, summarized in Figure 3 will be more thoroughly examined in the following sections.

## **ISOFLAVONE ABSORPTION**

After soy intake, the isoflavone glycosides are poorly absorbed in the small intestine because of their hydrophilicity and large molecular weight (Xu et al., 1995, Liu and Hu, 2002). Initial hydrolysis is thus necessary to release the free aglycones which are rapidly absorbed via passive diffusion across the intestinal brush border (Scalbert and Williamson, 2000) with high permeability (Liu and Hu, 2002). Glycosidase activity can occur in the food itself (via enzymes of endogenous origin or added during processing), in the cells of the gastrointestinal mucosa or the enzyme can be secreted by the colon microbiota (Scalbert and Williamson, 2000). Isoflavones are detectable in plasma as soon as 30 minutes after soy intake (King and Bursill, 1998) with an initial peak 1 hour post-meal (Franke et al., 1999, Richelle et al., 2002). This early increase may be due to the presence of a small proportion of aglycones available in the soy meal (King and Bursill, 1998), but also suggests that hydrolysis and initial absorption occur readily in the duodenum and proximal jejunum within the first hour of digestive processing (Watanabe et al., 1998, Setchell et al., 2001, Rowland et al., 2003).

A number of mammalian  $\beta$ -glucosidases have been identified in the small intestine, including a broad specificity cytosolic  $\beta$ -glucosidase enzyme and the membrane-bound lactase phlorizin hydrolase (LPH) enzyme (Day et al., 1998). LPH is present on the luminal side of the brush border in the small intestine and can deglycosylate genistein-7-glucosidase and daidzein-7-glucosidase within the gut lumen to

release the more hydrophobic aglycones which can then diffuse into the epithelial cells (Day et al., 2000). This activity suggests that the intestinal mucosa plays an important role in the deglycosylation of isoflavones (Day et al., 1998, Scalbert and Williamson, 2000) and confirms the view that the isoflavone absorption begins in the proximal small intestine (Xu et al., 1995) and occurs along its length (Setchell et al., 2003b). The cytosolic  $\beta$ -glucosidase enzyme has been identified in the small intestine, liver and kidney of mammals (Day et al., 1998), with the small intestine having a faster rate of hydrolysis of the 7-glucosides of daidzein and genistein than the liver (Day et al., 2000). However, intestinal hydrolysis of isoflavones would require initial uptake of the glycoside form, and although this has been demonstrated for other flavonoids (Paganga and Rice-Evans, 1997), it has not been conclusively shown for isoflavones (Setchell et al., 2002b). Isoflavone glycosides that are not absorbed in the small intestine will pass through to the colon, where bacterial  $\beta$ -glycosidases can hydrolyse them, removing the sugar moiety for energy conversion (Parodi, 1999).

Plasma genistein is consistently reported as being higher than that of daidzein after soy intake (King and Bursill, 1998). This may simply reflect the generally higher levels of genistein and its conjugates compared with daidzein and its conjugates in most soy foods (Setchell et al., 2001), but has also been reported when the intakes of daidzein and genistein are equivalent (Setchell et al., 2003b). However, Xu, Wang and others (2000) reported similar plasma concentrations of daidzein and genistein after intake of soy milk powder that contained more genistein than daidzein. Experiments with pure compounds and stable isotopically-labelled isoflavones suggest that genistein is more bioavailable, with greater systemic exposure than daidzein (Setchell et al., 2001, Setchell et al., 2003b). A higher clearance rate of daidzein and its high volume of tissue distribution also contribute to its consistently lower serum concentrations compared with genistein (Setchell et al., 2003a). In addition, LPH has shown a higher catalytic efficiency in hydrolysis of genistein than of daidzein (Day et al., 2000), a factor that may also contribute to the higher absorption of genistein.

## ISOFLAVONE METABOLISM

After initial absorption, the isoflavones undergo extensive first-pass metabolism, which accounts for their low bioavailability (Chen et al., 2003). During phase II biotransformation, the hydroxyl groups of the isoflavones provide sites for glucuronidation and sulfation by glucuronosyl-transferases and sulfotransferases in the liver (Xu et al., 1994) and/or intestine (Setchell et al., 2001). The isoflavones undergo classical enterohepatic circulation and are conjugated in the liver, similar to steroid metabolism (Winter and Bokkenheuser, 1987). The glucuronide and sulphate conjugates (see Figure 4) can be transported via the systemic circulation to tissues, from where they will eventually be excreted via the kidneys, or they can be secreted in bile and returned to the intestine (Xu et al., 1995). After deconjugation by intestinal bacteria, isoflavone aglycones can be reabsorbed, then returned to the liver via the portal vein for re-conjugation and either further enterohepatic circulation or renal excretion (Winter and Bokkenheuser, 1987).

Recent evidence suggests that the intestine and subsequent enteric recycling may play a more significant role in isoflavone metabolism and bioavailability than previously realised (Liu and Hu, 2002, Chen et al., 2003). In rats, the portal vein contains predominantly 7-O-glucuronide isoflavones (Barnes et al., 1996), suggesting that a the primary site of glucuronidation is the intestinal wall (Coldham and Sauer, 2000). This was confirmed by evidence that in rats MRP (multi-drug resistance-related protein) conjugated and the small intestine efficiently secreted glucuronidated isoflavones into the intestinal lumen (Liu and Hu, 2002). Chen, Lin and co-authors (2003) reported that *in vitro*, significant amounts of genistein were glucuronidated and sulphated by intestinal cells and these products were then excreted into both the apical and basolateral sides of the enterocyte. These authors further suggested that upper intestinal isoflavone metabolism could surpass that of the liver. Thus, the intestinal conjugation of isoflavones, their secretion back into the intestinal lumen and further

reabsorption and reconjugation constitutes enteric recycling (Liu and Hu, 2002, Chen et al., 2003), which, in combination with enterohepatic recycling, significantly prolongs systemic exposure to isoflavones (Turner et al., 2003).

In circulation, the aglycones represent only a small fraction of the total plasma isoflavones (Shelnutt et al., 2002). The glucuronides are the predominant metabolites of isoflavones (Zhang et al., 2003, Spencer et al., 1999), followed by the sulphated conjugates (Adlercreutz et al., 1993) with other conjugates including sulfoglucuronides (Adlercreutz et al., 1993). In addition, isoflavones also bind to plasma proteins (Coldham and Sauer, 2000). After soy intake, the percentage of total plasma daidzein present as glucuronides (Zhang et al., 2003) and sulphates (Shelnutt et al., 2002) is greater than that of genistein. For estrogens, the sulphate conjugates are excreted slowly compared with the glucuronide conjugates, and can serve as a source of biologically active estrogens when hydrolysed in target tissues (Adlercreutz et al., 1987). However, the sulphate conjugates of both daidzein and genistein are cleared faster than the glucuronides (Shelnutt et al., 2002). In addition, while the concentration of daidzein sulphate in plasma is much higher than genistein sulphate, it is cleared faster, but these two compounds show similar urinary recovery (Shelnutt et al., 2002); this may reflect a greater tissue distribution of daidzein sulphate.

Maximal plasma concentrations of daidzein and genistein are generally reached between 6 and 8 hours after soy intake in humans (Setchell et al., 2003b, Setchell and Cassidy, 1999, King and Bursill, 1998, Setchell et al., 2003a, Xu et al., 1994, Xu et al., 1995); daidzein often reaches its peak concentration later but has a faster plasma disappearance rate than genistein (Shelnutt et al., 2002). The half-lives of plasma elimination are dependent on the conjugate; these are 3 - 9 hours for daidzein and 8 - 11 hours for genistein after intake of soy foods or pure isoflavone glycosides (Watanabe et al., 1998, Shelnutt et

al., 2002, Setchell et al., 2003b), but 9 and 7 hours for pure daidzein and genistein aglycone administration respectively (Setchell et al., 2001).

Whether returned to the intestinal lumen via excretion in bile or by enterocytes, the isoflavone conjugates are deconjugated by enzymes in the intestinal wall ( $\beta$ -glucuronidases) or bacterial enzymes ( $\beta$ -glucuronidases and sulfatases) (Winter and Bokkenheuser, 1987, Chen et al., 2003). Aglycones that are not reabsorbed will reach the colon, along with any conjugates from liver or intestinal biotransformation that are not deconjugated (Liu and Hu, 2002) and the fraction of isoflavone that is neither hydrolysed nor absorbed in the small intestine initially (Decroos et al., 2005). Between 30 and 50% of estrogen metabolites are excreted in bile and about 80% of biliary conjugates are reabsorbed (Adlercreutz et al., 1987) with less than 10% excreted in the faeces (Thompson, 1994). Similarly for isoflavones, measurement of intact isoflavones has accounted for only between 15 and 30% of the ingested dose and faecal excretion is also low (Xu et al., 1995). Thus, the majority of unaccounted isoflavone dose must be metabolised in the intestine (Rowland et al., 2003) and/or more extensive metabolism must take place in the tissues, liver and circulation (Spencer et al., 1999).

In the colon, secondary metabolites are more easily absorbed (Chen et al., 2003) and colonic microbiota further degrade the isoflavones to simpler compounds which may involve splitting of the heterocyclic oxygen containing ring (Hollman, 2001, Scalbert and Williamson, 2000). Both daidzein and genistein can be further metabolized to secondary metabolites via the intermediates dihydrodaidzein and dihydrogenistein, respectively. Daidzein can be metabolised by reduction to equol or ring cleavage to O-desmethylangolensin (ODMA) and genistein to p-ethyl phenol or 6'-hydroxy-O-desmethylangolensin (6'ODMA), although, of these secondary metabolites, only equol is biologically active (Hutchins et al., 1995, Setchell et al., 2002a). Metabolism to equol and ODMA appears to be inversely related, suggesting two alternative pathways for daidzein metabolism (Kelly et al., 1995). In

addition, a number of other minor metabolites of both daidzein and genistein have been identified in plasma, urine and faeces (Kelly et al., 1993, Heinonen et al., 1999, Chang and Nair, 1995, Joannou et al., 1995). The higher molecular weight and lower water solubility of genistein may promote excretion of genistein conjugates in bile and provide more opportunity for bacterial degradation (Xu et al., 1994), whereas daidzein appears to be less subjected to bacterial metabolism *in vivo*. Thus, daidzein is more likely to be absorbed and therefore potentially more bioavailable (Decroos et al., 2005). It has also been suggested that the carbonyl moiety of genistein is protected by hydrogen bonding to the adjacent hydroxyl group, thus rendering it less reactive in contrast to the carbonyl moiety of daidzein that may be readily metabolised by reduction and dehydration to equol (Coldham et al., 2002).

## **DISTRIBUTION, ELIMINATION AND RECOVERY OF ISOFLAVONES**

Isoflavones have been quantified in plasma, urine, bile and faeces, as well as in human saliva, breast aspirate and prostatic fluid (Morton et al., 1997). They have also been shown to accumulate in breast tissue and milk (Franke et al., 1998, Franke and Custer, 1996, Pumford et al., 2002, Maubach et al., 2003) and to cross the blood brain barrier and placenta (Setchell and Cassidy, 1999, Adlercreutz et al., 1999). In men from soy-consuming countries, levels of isoflavones are higher in prostatic fluid than those of Western populations and concentrated approximately 2-fold relative to plasma (Morton et al., 1997). Setchell and co-authors (2001) have estimated a large volume of distribution for the isoflavone aglycones and secondary metabolites indicating that they have the potential to modulate physiological actions at a range of tissues. Busby and colleagues (2004) determined that the volume of distribution of free plasma daidzein and genistein was nearly twice that of their respective conjugates. The aglycones are also cleared from the plasma much more rapidly than conjugated isoflavones, suggesting that free isoflavones enter and perhaps are sequestered in tissues.



Isoflavones are excreted in urine almost exclusively as acidic conjugates, mainly glucuronides, with lesser amounts of sulphates and sulphoglucuronides (Adlercreutz et al., 1993). After soy intake, urinary excretion of daidzein and genistein is typically highest 7 - 8 hours post-meal (Watanabe et al., 1998, Lu et al., 1995). King and Bursill (1998) reported that mean excretion rates for genistein and daidzein increased progressively reaching a peak 6 – 12 hours after the meal and Watanabe and others (1998) found that a plateau was reached 8 - 12 hours after intake. The majority of the urinary excretion of daidzein and genistein occurs within the first 24 hours after soy ingestion (Lu et al., 1995, Setchell et al., 2003b). Although King and Bursill (1998) suggested a constant elimination rate between 11 and 35 hours after a meal, Watanabe and colleagues (1998) showed that during 48 hours post soy intake, subjects characteristically showed two or three peaks of daidzein and genistein excretion, and attributed this to enterohepatic circulation.

In contrast to the higher plasma concentrations of genistein compared with daidzein, most studies report greater urinary excretion of daidzein (Xu et al., 1994, Franke et al., 1999). In addition, a higher proportion of daidzein occurs in urine in the unconjugated form (Adlercreutz et al., 1993). It has been suggested that the lower molecular weight (254 vs. 270) and greater water solubility of daidzein could account for its higher urinary excretion (Xu et al., 1994), while the lower hydrophilicity of genistein may promote its excretion in bile (King and Bursill, 1998). The proportional urinary recovery of isoflavones, relative to the amount ingested, is generally quite low, reported to be between 10 and 50% (Cassidy et al., 2000, Hendrich et al., 1998), possibly indicating significant colonic bacterial degradation and/or metabolism to other unidentified compounds (Xu et al., 1994, Lampe et al., 1998). Hendrich and co-authors (1998) suggested that biliary excretion is likely to be the main limiting factor with respect to the percentage of isoflavones that are systemically available after intake. In addition, cytochrome p450 enzymes appear to play an important role in the oxidative metabolism of the soy isoflavones and might explain their low recoveries (Kulling et al., 2000). Total faecal excretion of

isoflavones is typically less than 5% (Xu et al., 1994, Xu et al., 1995, Watanabe et al., 1998), predominantly in the unconjugated form with less than 10% being conjugated (Adlercreutz et al., 1995).

## **PRODUCTION OF EQUOL**

The conversion of daidzein to equol may be physiologically important as equol has significantly greater antioxidant activity (Mitchell et al., 1998, Arora et al., 1998, Wiseman and O'Reilly, 1997, Vedavanam et al., 1999) and estrogenic activity (approximately 100-fold higher) on binding to the ER (Sathyamoorthy and Wang, 1997) compared with daidzein. A case-control study found a substantial reduction in breast cancer risk among women with high excretion of isoflavones, with those excreting equol having the greatest reduction (Ingram et al., 1997). Similarly, Akaza and co-authors (2002) reported that the percentage of male equol-producers in a case-control study was significantly lower among patients with prostate cancer compared with controls. Further, the greatest increases in the menstrual cycle follicular phase, a change associated with lower breast cancer risk, were found in two subjects who also had the highest urinary equol excretion in a soy supplementation study (Cassidy et al., 1994). However, as highlighted in a recent review paper (Atkinson et al., 2005), most studies that have associated equol production with health benefits have done so with only weak, if any, statistical significance. Furthermore, the review highlighted many studies that report no differences in health effects or biomarkers for pathologies based on an individual's ability to produce equol. It is clear that more research needs to be conducted in this area as currently the evidence is lacking and inconclusive.

Equol is exclusively produced by intestinal bacteria (Decroos et al., 2005), but not all people can metabolise daidzein to equol. It is consistently reported that among subjects of Caucasian background, between 30 and 40% of individuals excrete equol after consuming soy products (Lampe et al., 2001, Slavin et al., 1998, Lampe et al., 1998); however this proportion is much higher in Asian countries of

between 45 and 60% (2002). The presence of equol in urine or plasma has been used by researchers to classify subjects with analysis of outcomes in relation to equol-producing ability (Rowland, 1999, Kelly et al., 1995, Lampe et al., 1998, Karr et al., 1997, Setchell et al., 2002a).

Equol is unique in having a chiral centre due to the absence of a double bond in the heterocyclic ring, resulting in two distinct optically active isomers. The naturally occurring enantiomer from endogenous conversion of daidzein is S(-)equol (Muthyala et al., 2004). The R and S isomers differ conformationally, with the S(-) form being more non-planar (Setchell et al., 2002a), suggesting a higher affinity of this isomer for estrogen receptors (Barnes and Peterson, 1995). It has recently been confirmed that S(-)equol has a high binding affinity and strong preference for ER- $\beta$ , whereas R(+)-equol has lower affinity and a preference for ER- $\alpha$  (Muthyala et al., 2004). The non-planar structure of S(-)equol also gives it greater flexibility for conformational changes, which may allow it to penetrate into cell membranes with greater ease than other the more rigid isoflavones (Arora et al., 1998). This is important in terms of the use of isoflavone supplements, since consuming a racemic mixture of equol from a synthetic source would not be expected to have the same physiological effects as the enantiomer product of endogenous metabolism of daidzein.

The pharmacokinetics of equol are similar to the other isoflavones; however, equol has a slower plasma clearance (Setchell et al., 2002a, Lampe et al., 2001) and a longer half-life (Kelly et al., 1995) than daidzein, with maximal plasma levels generally reached between 24 hours and 3 days post-intake (Setchell et al., 2001, Kelly et al., 1995). Metabolism of daidzein to equol is time-dependent (Setchell et al., 2003a) with a lag time in its appearance of at least 6 - 8 hours after intake of a bolus dose, consistent with its colonic origin (Setchell et al., 2001, Setchell et al., 2003b). Equol is readily absorbed from the gastrointestinal tract, more efficiently through the colon wall compared with daidzein (Decroos et al., 2005), and conjugated to glucuronic acid in the liver (Axelson et al., 1982).

Hepatic metabolism may be more important for compounds like equol, that are mainly absorbed from the large intestine (Chen et al., 2003) and this may contribute to longer pharmacokinetics. The formation of equol may be dependent on initial levels of daidzein (Tsangalis et al., 2002) and equol bioavailability is reportedly greater after ingestion of daidzein glucoside rather than the aglycone, possibly due to the longer transit time of the former (Zubik and Meydani, 2003). Once formed, equol appears to be metabolically stable, undergoing no further biotransformation, other than phase II metabolism (Setchell et al., 2002a); however, it has been recently suggested that equol is further metabolised, possibly in the liver, leading to catecholic structures of either ring A or B (Adlercreutz et al., 2004). Although glycitein metabolism is poorly understood, recent literature implies that equol-like metabolites may be produced from glycitein. Heinonen and co-authors (2003) identified glycitein metabolites in human urine after soy consumption and found significant amounts of 4',6,7-trihydroxyisoflavone, a compound differing from equol only in the presence of an extra hydroxyl group.

Equol also remains elevated in the urine for longer after a soy challenge compared with daidzein and genistein (Lampe et al., 2001), with maximum urinary excretion reported between 24 and 72 hours or more after intake (Kelly et al., 1995, Kelly et al., 1993, Axelson et al., 1982, Xu et al., 1994). It is excreted in urine almost exclusively as the monoglucuronide conjugate (Axelson et al., 1982). Watanabe and co-authors (1998) calculated the percent metabolic conversions of daidzein to O-DMA and equol as 4 and 7%, respectively and reported the faecal excretion of equol was much higher 5 – 6 days compared with 4 days after intake, suggesting that much of the faecal equol represents biliary excretion.

## ROLE OF THE GUT MICROBIOTA IN ISOFLAVONE BIOAVAILABILITY

The essential role of the gut microbiota in isoflavone absorption and metabolism has been demonstrated through the use of antibiotics which dramatically decrease plasma isoflavone concentrations and urinary excretion of the bacterial metabolites (Winter and Bokkenheuser, 1987, Rowland, 1999, Adlercreutz, 1998). Additionally, studies using “germ-free” rats show the absence of isoflavone absorption, which is revived after inoculation with human gut flora (Bowey et al., 2003). The enzymes most pertinent to isoflavone uptake are the  $\beta$ -glucosidases, necessary for glycoside hydrolysis and aglycone absorption, as this initial hydrolysis to the aglycone appears to be the rate-limiting step in isoflavone absorption (Izumi et al., 2000, Setchell et al., 2001, Steer et al., 2003). In the gastrointestinal tract,  $\beta$ -glucosidase enzymes are produced by several groups of bacteria including *Lactobacilli*, *Bacteroides* and *Bifidobacteria* (Steer et al., 2003, Xu et al., 1995), of which the latter two comprise the majority of microorganisms in the human gastrointestinal tract (Friend and Chang, 1984). Following initial absorption, endogenous  $\beta$ -glucuronidases and sulfatases are required for reabsorption of the hepatic conjugates and biliary excretion (Xu et al., 2000). The relative contribution of intestinal and bacterial enzymes has not been established; however both appear to play an important role in isoflavone bioavailability.

The extent to which compounds are metabolised by gut bacteria depends on a range of factors, notably, the region of the gut from which the compound is absorbed, the distribution and type of bacteria and the availability of the necessary enzyme (Hawksworth et al., 1971). The microbiota of the large intestine is acquired after birth, with a pattern resembling adult flora established after weaning (Isolauri, 2001, Salminen et al., 1998). The species composition that develops is largely controlled by diet (Salminen et al., 1998), which especially affects gut microbial enzyme activities during the transition to a more diversified diet, between 6 and 12 months (Mykkanen et al., 1997). Bacterial numbers and composition vary considerably along the human gastrointestinal tract, with numbers

increasing along the length of the small intestine to approximately  $10^8$  per mL of contents at the ileocecal region (Salminen et al., 1998). The large intestine usually contains more than 400 species of bacteria (Parodi, 1999), typically  $10^{12}$  bacteria per gram contents (Salminen et al., 1998, Gibson, 1998). In the proximal small intestine (duodenum and jejunum), where absorption is at its peak, the microbiota is dominated by species of *Streptococcus*, *Lactobacillus* and *Bifidobacterium*, while in the distal small intestine (ileum) and colon, *Bacteroides* and *Bifidobacterium* species dominate (Parodi, 1999, Turner et al., 2003). Of the gut microbiota, enterococci (found in high levels in the colon) have the highest  $\beta$ -glucosidase activity, followed by *Lactobacilli*, *Bacteroides* and *Bifidobacteria*. *In vitro*, *Bifidobacteria* can metabolise the isoflavone glycosides and further metabolism of daidzein to equol is correlated with its  $\beta$ -glucosidase activity (Tsangalis et al., 2002).

Recently, much research has been directed towards the elucidation of the bacteria responsible for the production of equol from daidzein. It appears that more than one bacterial species is involved in the metabolism of daidzein to equol (Hur et al., 2000, Decroos et al., 2005) with strains of bifidobacteria (Tsangalis et al., 2002), streptococci, ruminococci, bacteroides (Ueno and Uchiyama, 2001), enterococci and lactobacilli (Decroos et al., 2005) as well as *Escherichia coli* (Hur et al., 2000), have all been identified as having this capability. Thus, the presence or absence of equol will depend on an individual's microbiota composition and bacterial enzyme expression (Turner et al., 2003). Urinary excretion of equol seems to be inversely related to that of daidzein and ODMA (Kelly et al., 1993, Kelly et al., 1995, Slavin et al., 1998, Lampe et al., 2001), suggesting that daidzein is preferentially metabolized to either ODMA or equol, depending on gut microbiota and/or other inherent characteristics. Decroos and co-authors (2005) reported that equol and O-DMA are indeed formed by different bacteria, but that these species can co-exist. Thus, equol-excretor status may be a marker of a particular colonic microbial profile (Lampe et al., 2001) although it is not known whether this can be modulated by diet. The relationship between an individual's ability to produce equol and their inherent

gut microbial balance is not known but may have significant impact in relation to potential health benefits of soy consumption (Atkinson et al., 2005).

## **FACTORS AFFECTING ISOFLAVONE BIOAVAILABILITY**

A number of factors can influence the absorption of food components, including dietary habits, the food matrix, intestinal fermentation and transit time (Zubik and Meydani, 2003). In isoflavone bioavailability studies, the soy food used and its isoflavone composition are important determinants of the resulting isoflavone pharmacokinetics and potential physiological effects. The influence of diet is important due to interactions between dietary components and because diet has a strong effect on composition of the gut microbiota, which in turn plays a crucial role in isoflavone bioavailability. Inherent and genetic characteristics which determine pathways of absorption and metabolism will also contribute to variability in isoflavone bioavailability. A better understanding of these differences will assist in the interpretation of outcomes of dietary studies. Information regarding genetic and inherent influences as opposed to dietary-induced effects on isoflavone bioavailability and any consequent health effects is lacking and would benefit from further investigation.

## **EFFECTS OF ISOFLAVONE CONJUGATION ON BIOAVAILABILITY**

The relative proportions of different isoflavone conjugates in soy may have an effect on resulting bioavailability. It is not known what effects 6"-O-substitution has on the susceptibility of the isoflavone conjugates to intestinal hydrolysis and absorption; however, differences in bioavailability and metabolism dependent on the nature of their chemical form would be anticipated (Barnes et al., 1994). There are differences in the location of absorption of these conjugates, as the aglycones are absorbed readily from the upper small intestine, the  $\beta$ -glucoside conjugates from the distal small intestine after hydrolysis to the aglycone, and the malonylglucoside and acetylglucoside conjugates

from the large intestine after hydrolysis (Coward et al., 1998). This could affect the subsequent bioavailability of the aglycones as absorption efficiency and the distribution of conjugating enzymes differs between different areas of the gastrointestinal tract (Liu and Hu, 2002). More specifically, the proportions of daidzein, genistein and glycitein, will also greatly affect the resulting isoflavone bioavailability and overall physiological effects, due to their different chemical structures and *in vivo* properties.

Most soy-containing foods consumed in Western diets are made from soy protein which contains glycosidic isoflavones, as opposed to fermented soy products in which aglycones predominate (Setchell et al., 2001). Although there are differences in the kinetics of plasma absorption and excretion after consumption of  $\beta$ -glucosides compared with aglycones, there does not appear to be an overall difference in systemic bioavailability using a measure of area under the curve (Steer et al., 2003). The aglycones are absorbed faster and with greater maximum concentration; however, plasma levels do not remain elevated for as long (Izumi et al., 2000), while the glycoside conjugates take longer both to reach maximum plasma concentration and to be cleared from the plasma (Setchell et al., 2001, Steer et al., 2003). These differences in pharmacokinetics would presumably affect tissue exposure and overall bioavailability; however, the nature of these effects has not yet been established. In addition, the conjugation may also determine the extent to which the isoflavones are metabolised to secondary metabolites and Zubik and Meydani (2003) suggested that the longer transit time for glucosides may provide more opportunity for bacterial metabolism (Zubik and Meydani, 2003). The food matrix will also influence subsequent bioavailability, with isoflavones from supplements likely to be absorbed at a faster rate than those ingested in a food matrix (Richelle et al., 2002).



## LEVEL AND DURATION OF ISOFLAVONE INTAKE

In a dose-dependent analysis using pure isoflavone supplements, Setchell and co-authors (2003a) reported no effect of isoflavone dose on the time to maximum plasma concentration, but there was a decreased fractional absorption when the dose was doubled from 0.4 to 0.8 mg/kg body weight, indicating that a plateau was reached and that absorption may be rate-limited. Similarly, intake of soy nuts at three different amounts resulted in similar half-lives, clearance and volume of distribution, independent of dose (Setchell et al., 2003b). Although Hendrich and others (1998) reported that human bioavailability of isoflavones is linearly related to dose within a broad range, it is possible that non dose-dependent plasma kinetics at higher intakes may result in saturable plasma levels (Setchell and Cassidy, 1999, Setchell et al., 2001). However, once absorbed, dietary polyphenols including isoflavones are not expected to saturate metabolic pathways, but dose will determine the primary site of metabolism (Scalbert and Williamson, 2000). Large doses will be metabolised primarily in the liver, while smaller doses may be metabolised predominantly by intestinal mucosa with the liver playing a secondary role (Scalbert and Williamson, 2000). Apart from the effects of first-pass metabolism reducing overall bioavailability, these two sites differ in terms of the types of reactions that predominate, which may affect metabolite production. In the liver, oxidative metabolism predominates, whereas the gut is active in reductive reactions (Rowland, 1986) and the metabolism of daidzein to equol occurs almost exclusively via intestinal metabolism (Rowland, 1999). It is not known how subsequent bioavailability and tissue distribution is affected by the location of isoflavone metabolism.

Long-term soy intake may also affect isoflavone bioavailability if enzymes important for isoflavone bioavailability are induced by prior exposure. Bacterial  $\beta$ -glucuronidase has been demonstrated to be inducible by glucuronide conjugates (Silvi et al., 1999) and this may be similar for other enzymes. Four weeks of soy milk ingestion increased the absorption half-lives of daidzein and equol as well as

the proportion of free unconjugated isoflavones (Lu et al., 1995), which may result in greater isoflavone bioavailability. Hendrich and colleagues (1998) reported that more frequent doses prolong plasma clearance time. However, there does not appear to be an effect of long-term intake (up to 10 weeks) of soy foods on absolute plasma concentrations (Wiseman et al., 2004).

## **EFFECTS OF GENETICS AND DIET ON GUT MICROBIOTA**

The species composition of gut microbiota and total activity of enzymes vary widely between individuals (Salminen et al., 1998, Day et al., 1998) and host genetic polymorphism has been demonstrated for a number of enzymes (Scalbert and Williamson, 2000). The expression of  $\beta$ -glucuronidases in human cells is often regulated during development (Scalbert and Williamson, 2000). LPH, which has been implicated as having a major role in intestinal absorption of isoflavone aglycones, is also primarily responsible for hydrolysis of lactose and deficiency of this enzyme causes lactose intolerance (Day et al., 1998). This condition affects approximately 5% of Europeans and 90% of Africans and Asians in adulthood (Scalbert and Williamson, 2000). For those individuals who are deficient in LPH, isoflavone absorption in the small intestine may be reduced which would result in more isoflavones reaching the colon for microbial metabolism (Day et al., 2000), including the conversion of daidzein to equol. Furthermore, Thadepalli and co-authors (1979) reported differences between the gastrointestinal microbiota of North Americans and Western Europeans compared with South Indian and Guatemalan individuals.

Human intestinal microbiota composition is relatively stable (Lampe et al., 1998) and differences in normal dietary patterns do not appear to influence the composition of the intestinal microbiota extensively. However, diet and antibiotics can substantially modify the metabolic activity of bacteria including that of  $\beta$ -glucosidases and  $\beta$ -glucuronidases (Parodi, 1999, Persky et al., 2002) and enterohepatic circulation of nutrients (Goldin et al., 1982, Gorbach and Goldin, 1992). For instance,

vegans have lower faecal  $\beta$ -glucuronidase activity (Parodi, 1999) and transition to a vegan diet in adults alters faecal bacterial enzyme activities (Mykkanen et al., 1997). Dietary induced changes in gut microbiota and enterohepatic circulation may subsequently affect isoflavone bioavailability. In addition, dietary substrates such as fibre can also modulate intestinal and faecal characteristics including transit time, bulk and water content (Parodi, 1999) and conversely, these faecal characteristics can influence substrate availability, redox potential in the colon (Gibson, 1998) and accessibility of bacteria for dietary substrates (Parodi, 1999).

The principal substrates for colonic bacterial growth are dietary carbohydrates that have not been digested in the upper intestinal tract (Gibson, 1998, Yue and Waring, 1995). Most genera of the large intestinal microbiota are saccharolytic and obtain energy by fermentation of such dietary carbohydrates, including non-starch polysaccharides and resistant starch (Parodi, 1999). Recently, much research has been focused on modulation of gut microbiota from either oral intake of probiotic bacteria or ingestion of a prebiotic, which can induce the activities of specific endogenous probiotic gut microbiota, with expected subsequent effects on isoflavone bioavailability.

Prebiotic effects in the gastrointestinal tract may be particularly relevant to equol production as the metabolism of daidzein to equol relies exclusively on gut microbiota (Decroos et al., 2005). Although Kelly and co-workers (1993) suggested that genetics may play a role in the ability to metabolise daidzein to equol, gut microbiota may be a co-determinant. If this is the case, it may be possible to alter bacterial composition via dietary intervention in a manner favourable to equol production. An individual's ability to produce equol has been associated with low dietary fat intake (Lampe et al., 2001) and with a greater intake of carbohydrate (Lampe et al., 2001), non-starch polysaccharides (Rowland, 1999) and dietary fibre (Lampe et al., 1998). In particular, the type and amount of carbohydrate available to intestinal microbiota may be important for equol-producing capacity

(Rowland, 1999, Lampe et al., 2001) and experiments using an *in vitro* colonic fermentation system found that a high carbohydrate environment increased fermentation and the rate of conversion of daidzein to equol (Setchell and Cassidy, 1999). Thus, increased intake of carbohydrate, either acutely or via habitual diet, may also increase equol production *in vivo*. *In vivo* effects on the metabolism of daidzein to equol could influence the potential health protective effects of soybean isoflavones if indeed equol has greater impacts on physiological function (Wiseman, 1999). It still remains to be established whether equol-producing ability is indicative of a microbial balance predisposed to health effects or whether the microbiota can be modified by diet to enhance isoflavone bioavailability and/or equol production and any consequent health effects (Atkinson et al., 2005). However, the latter has not been conclusively established and again highlights the need for further research for more conclusive results related to soy isoflavones, isoflavone bioavailability and human health outcome measures.

## CONCLUSIONS

The substantial evidence supporting the potential for beneficial effects of soy consumption has led to much wider use of traditional soy products in Western societies and to the development of new isoflavone and soy-enriched foods and supplements. However, the high level of variability in clinical outcome measures means that it is very difficult to unravel the various effects and to shed light upon the mechanisms by which isoflavones may act and influence health outcomes. This high level of variability is in large part due to the inter-individual variation in the bioavailability of the isoflavones. This review has focused on the central importance of understanding the factors influencing isoflavone bioavailability. The process by which the isoflavones are distributed to tissues and hence exert their physiological effects has been shown to be a complex one with many components. These include uptake and absorption, first pass metabolism and then enterohepatic circulation via conjugation in the liver. The role of biotransformation to various conjugates together with the level and duration of isoflavone consumption have been shown to be of importance. Furthermore, the effects of habitual diet

on gut microbiota may be one of the most important factors affecting isoflavone bioavailability and thus, modulation of physiological effects. One of the attractive features of a focus on background diet is that it is an easily modified condition. In addition, the role of gut microbiota may be particularly important in the production of the daidzein metabolite equol, which may confer more health benefits than its precursor. Much more research needs to be carried out in this area, in order to understand how soy can have health benefits in the broader population.

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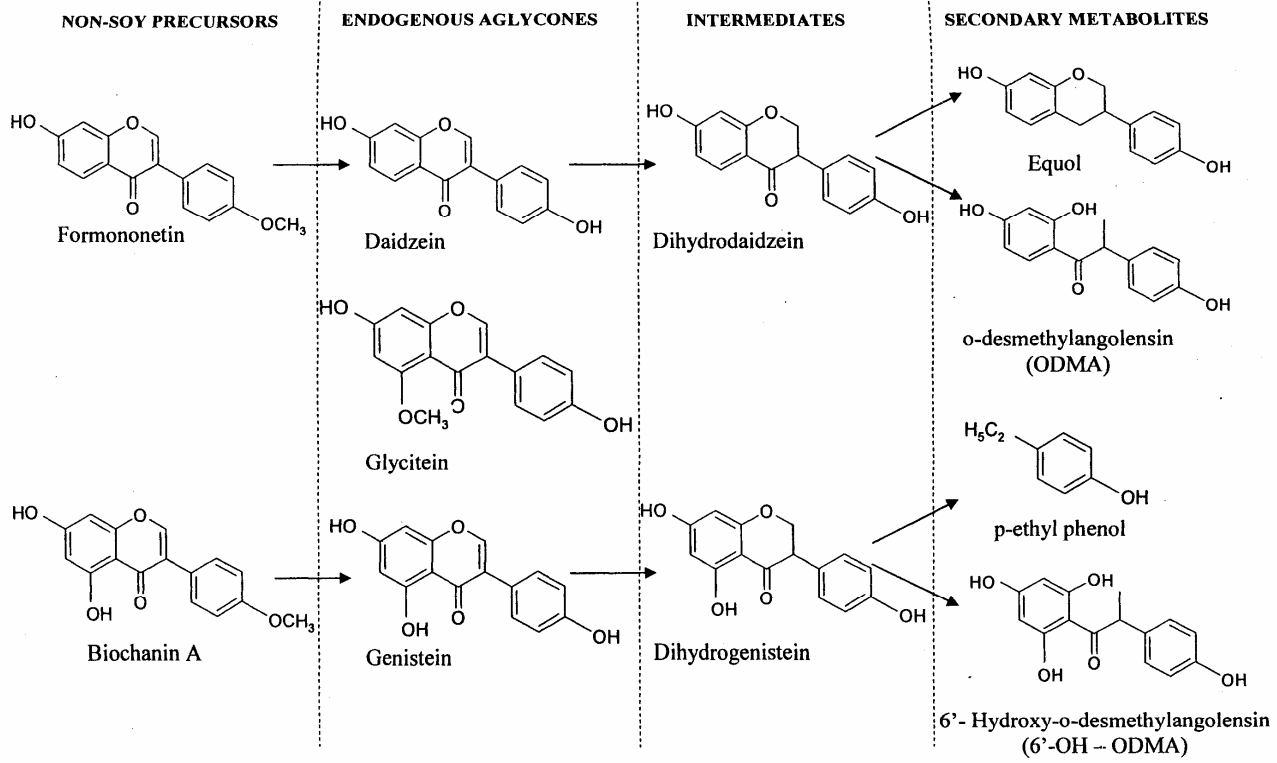
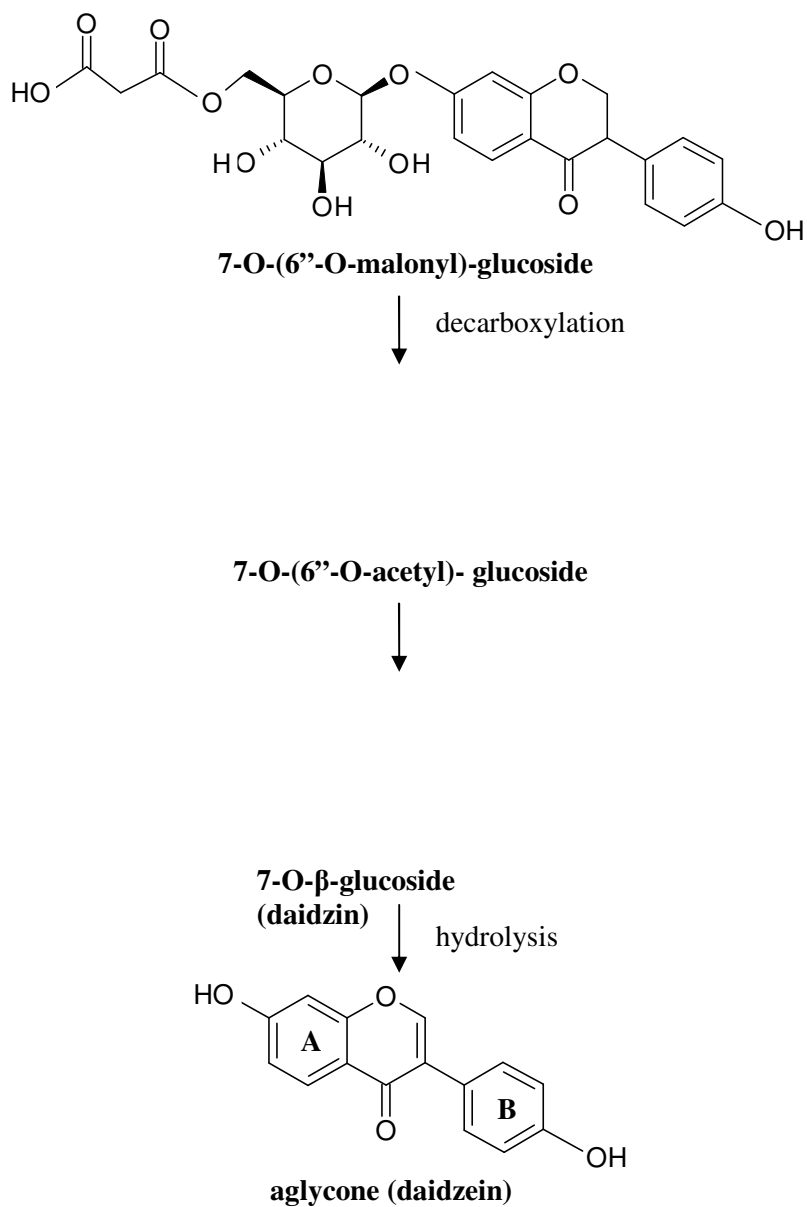


Figure 1 : Isoflavone Structures



*Figure 2 The four different isomers of daidzein in soy foods. Analogous structures of genistein and glycitein are also present in soybeans.*



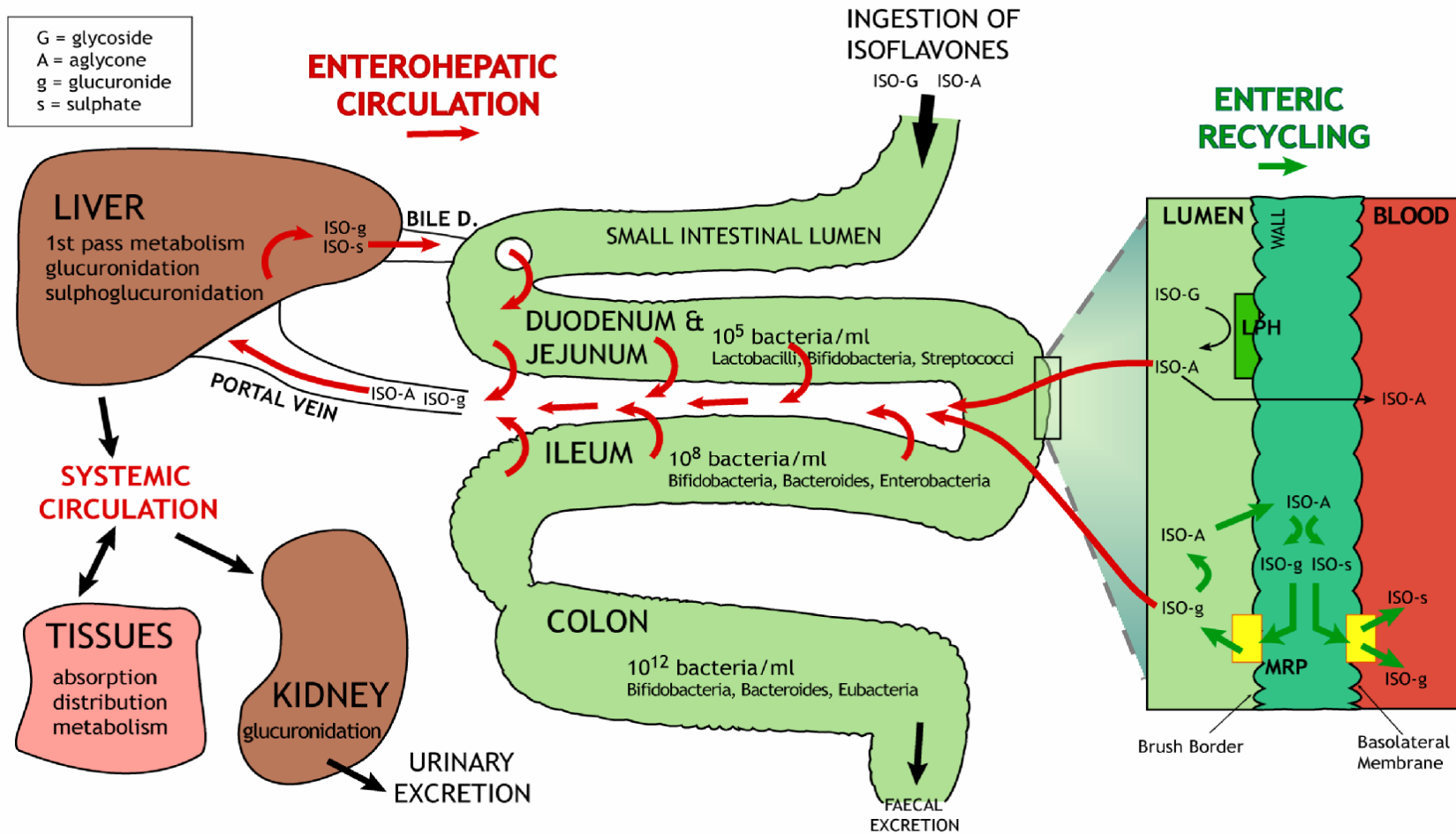
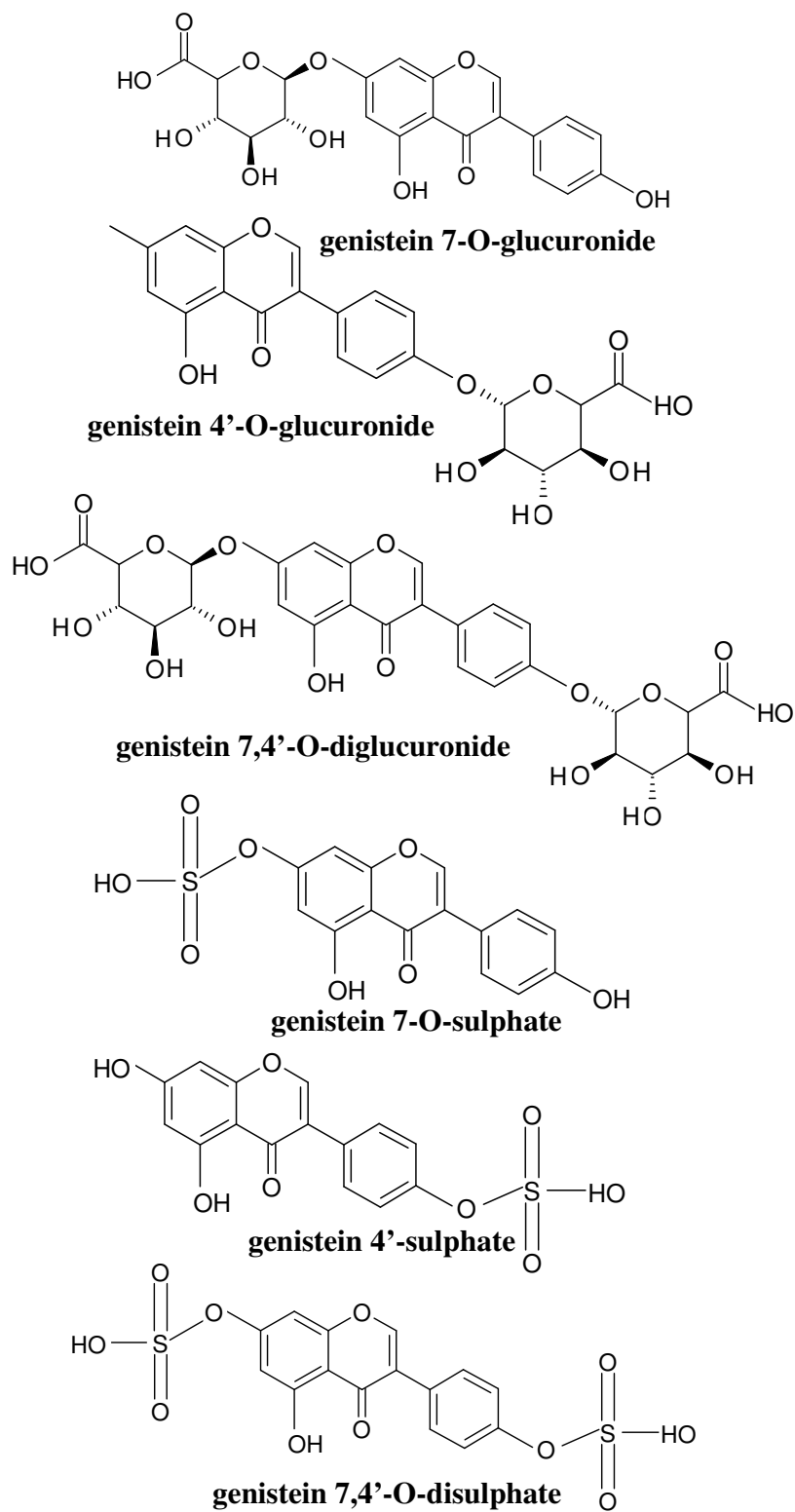


Figure 3 Diagram depicting the metabolism of isoflavones



*Figure 4 Glucuronide and sulphate conjugates of genistein. Analogous structures of daidzein are also present in the circulation.*