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Pharmacokinetic Profile of Phytoestrogens: An Overview

P. Sunita*[1], S.Jha[2], S.P.Pattanayak[2]

[1]*Govt. Pharmacy Institute, Govt of Jharkhand, Ranchi-834 009*
[2]*Dept. of Pharmaceutical Sciences, Birla Institute of Technology (BIT), Mesra, Ranchi - 835 215, India.*
**Corresponding author. Tel: +919334740543*
E-mail: priyashree_lenka@yahoo.com

ABSTRACT
This review brings up regarding the plant derived compounds with estrogenic activity. The authors correctly emphasize the need of foods containing phyto-oestrogens in view of its significant pharmacokinetic profile. This is particularly essential in the light of the current wave of enthusiasm for vegetarian food in general and phyto-oestrogens in particular. Phytoestrogens are plant-derived hormone-like diphenolic compounds of dietary origin. These compounds are weakly estrogenic could play a multidirectional health benefits. They are present in the plant as glycosidic conjugates, some of which contain further chemical modifications. In the gastrointestinal tract, the conjugates undergo hydrolysis catalyzed by enzymes in the intestinal wall and by gut bacteria. On entering the systemic circulation, the phytoestrogens may undergo extensive metabolism to other compounds through reactions involving demethylation, methylation, hydroxylation, chlorination, iodination, and nitration. Besides that the review concludes with the discussion of the several factors affecting gut metabolism of phyto-oestrogen.

KEY WORDS: Gut metabolism; Pharmacokinetic profile; Phytoestrogens.

INTRODUCTION

Epidemiologic studies suggest that consumption of legumes protects against the development of colon, breast, and prostate cancers (1–4). Soybeans (soy) are among the most widely consumed legumes. Soy-derived chemopreventive compounds include isoflavones (daidzein and genistein) (4), a protease inhibitor (Bowman-Birk protease inhibitor) (5), phytosterols (6), saponins (7), and inositols (8). Of these components, metabolites of isoflavones (9–12) and of the Bowman-Birk protease inhibitor have been detected in animals and humans after soy ingestion. Isoflavone consumption is inversely associated with breast cancer risk in premenopausal Chinese women in Singapore (3) and with prostate cancer risk in men of Japanese ancestry in Hawaii (2). Legumes are also a major source of protein for vegetarians, a group with low risk for many cancers (1). The prevalence of prostate carcinoma is lower among Japanese in Japan than among US whites and blacks (13), which may be attributed to a higher intake of soy phytoestrogens by the Japanese (9). Isoflavones exhibit estrogenic activities and may act as estrogen agonists or antagonists (14). Daidzein and genistein inhibit cell proliferation (15) and induce cell differentiation (16). Genistein inhibits angiogenesis (17), is a specific inhibitor of tyrosine kinase (18), and inhibits topoisomerase II (15, 19). Although these isoflavones may play important roles in the reduction of cancer risk, adverse reproductive effects have been observed in sheep. These effects were found to be associated with the ability of sheep to metabolize daidzein (and its precursor, formononetin) to equol (20). Because metabolism and adverse reproductive effects of isoflavones vary greatly among species, it is extremely difficult to extrapolate results from animals to humans. Therefore, it is important to investigate the metabolism and disposition of isoflavones in humans (12, 21, 22).

The absorption, distribution, metabolism and excretion of isoflavones, lignans and other classes of phytoestrogen are not completely defined in humans and more systematic studies are required. Most of the available information on pharmacokinetics concerns the isoflavonoids daidzein and genistein and to a lesser extent, the lignans enterodiol and enterolactone

Generally speaking, isoflavonoids and lignans are ingested largely as glycosides, which undergo hydrolysis, possibly in the stomach, under the action of acid, or in the lower gut, under the action of the gut microflora. The deglycosylated (aglycone) compounds may be further metabolised by the gut bacteria and/or absorbed. Once absorbed, these compounds are rapidly and extensively re-conjugated,
largely with UDP-glucuronic acid, and excreted in the bile or urine. Biliary conjugates are hydrolysed by the gut bacteria and/or excreted in the faeces or further metabolised and/or reabsorbed (enterohepatic circulation) or degraded.

It is clear that the gut microflora play a crucial role in determining the absorption, metabolism, re-absorption (enterohepatic circulation), degradation and excretion of ingested isoflavonoid and lignan phytoestrogens and their metabolites. There is considerable inter-individual variation in metabolite profiles, which are both qualitative and quantitative. Variation seems to be particularly marked for the conversions of daidzein to equol and enterodiol to enterolactone and is due largely to
the gut microflora.

DIETARY SOURCES OF PHYTOESTROGENS

Several classes of dietary phyto-oestrogens have been identified, with two of the major subclasses (the lignans andisoflavones) currently stimulating interest from a nutritional and health perspective. The recent identification of a novel more-potent phyto-oestrogen in hops 8-prenylnaringenin; (23) suggests that our attention has been too focused and should in the future encompass other naturallyoccurring oestrogen-like compounds present in plants. Dietary phyto-oestrogens are present in plant foods and are synthesized from phenylpropanoids and simple phenols (24, 25, 26). Lignans are present in many fibre-rich foods, and although the nutritional properties of flaxseed, the richest identified source of the lignan precursor secoisolariciresinol, is receiving some attention, only limited data on the levels of lignans in food and their biological effects *in vitro* and *in vivo* are currently available (27). Most of the consumer attention has focused on the isoflavones, in part because of the epidemiological evidence which is suggestive of a potential role for soya bean, a major source of isoflavones, in explaining the wide differences in rates of some hormone-related diseases between Asian countries and the West (28). Soyabean proteins contain significant levels of isoflavones, predominantly daidzein and genistein. The chemical composition of the isoflavones in soyabean foods is either as different types of glycoside conjugates (β-glucosides, malonylglucosides, acetylglucosides) or as the unconjugated aglycone form (29). Glycetin conjugates are also frequently found in soyabean proteins at low levels (30), but high concentrations of glycetin conjugates are found in the hypocotyledon or germ (31). Levels of isoflavones are highly variable between soyabeans due to environmental factors and the variety of soyabean (32), and it is not surprising, therefore, that large ranges in isoflavone content have been reported within and between soyabean products (US Department of Agriculture, 1998).

Estrogenicity

These heterocyclic phenols are structurally similar to the mammalian oestrogen. oestradiol-17 β. A common chemical characteristic of these compounds is the presence of a phenolic ring, a prerequisite for binding to the oestrogen receptor. The principal compounds within these classes of phyto-oestrogens have been shown to have weak oestrogenic activity, ranging from 1/500 to 1/1000 the activity of oestradiol-l7β, and to produce typical and predictable estrogenic responses when administered to animals (33, 34, 35, 36). Estrogenic compounds can be agonistic or antagonistic to oestradiol-17 β when they act simultaneously at target tissues. Antagonistic compounds normally compete for oestradiol-17 β receptors but fail to stimulate the nucleus to respond fully. This partial oestrogen agonistic and/or antagonistic behaviour is a common feature of many weak oestrogens (37). In animal models and *in* ***vitro*** experimental systems the isoflavones appear to act as anti-oestrogens, with primary anti-oestrogenic effects mediated via competition with oestradiol-17P β for the oestrogen receptor. These compounds are weak oestrogens; the activity of genistein, for example, is 1000-fold less than that of oestradiol. However, certain foods contain comparatively large amounts so that urinary excretion and plasma concentrations may exceed levels of endogenous oestrogens by several orders of magnitude. Urinary excretion of the principal oestrogen, oestrone glucuronide, ranges between 2 and 27 kg/d during the menstrual cycle (38), while the excretion of isoflavones on 60 g TVP diet/d was 0.4-7.5 mg/d (39).

METABOLISM AND ABSORPTION OF INGESTED PHYTOESTROGENS

In general, phytoestrogens are ingested as conjugates and are thought to require hydrolysis prior to absorption. Hydrolysis is thought to occur in the stomach & lower gut. Microflora-mediated reduction, demethylation and dehydroxylation reactions occur prior to absorption. Absorbed phytoestrogens are then reconjugated in the liver and intestinal epithelium by glucuronosyl and sulpho-transferases. Plasma concentrations of free phytoestrogens are low.

Isoflavones

Following ingestion, the glycosidic forms of the isoflavone are hydrolysed to their unconjugated forms, largely under the action of the gut microflora, viaglucuronidase enzymes. Some acid hydrolysis may occur in the stomach (40), although there is some disagreement about this (41). There is also evidence to suggest that the human small intestine and liver contain β-glucosidase activity capable of efficiently hydrolysing some, but not all, naturally occurring flavonoid and isoflavonoid glycosides (42), thus providing hydrolysis independent of the gut-bacteria or stomach acid. Hydrolysis results in the formation of the biologically active aglycones, which, due to their lower hydrophilicity and lower molecular weight, are more readily absorbed than the parent glycosides. Absorption of intact glycosides may occur (43) but this may be limited to a few certain flavonoids. Absorption takes place mainly in the small and large intestine. Prior to absorption, the isoflavones may be further metabolised by the gut microflora, with genistein being converted to the hormonally inert *p*-ethyl-phenol and daidzein being reduced to the estrogenically active isoflavan equol and non-estrogenic *O*-desmethylangolensin (*O*-DMA). Once absorbed, the isoflavones are efficiently reconjugated, either with glucuronic acid or, to a lesser extent, sulphate. In addition, some sulphoglucuronides may be formed. Conjugation takes place in either the liver (44, 45), via hepatic UDPglucuronosyl transferase or sulphotransferase enzymes, or within the intestinal epithelium, which has also been shown to possess glucuronosyl transferase and sulphotransferase activity (46). As a consequence, very little free isoflavonoid is present in the circulation.

The pathways of isoflavone metabolism are even more complex than described above. Studies have revealed several diphenolic metabolites, representing intermediates in the biotransformation of daidzein and genistein, resulting from either microfloral or host-mediated metabolism. Identified metabolites include 6'OH-DMA, dihydrogenistein, dehydro-*O*-DMA and two isomers of tetrahydrodaidzein (33 47, 48, 49) (Figure 1). It has been demonstrated that some aglycones may undergo CYP-mediated metabolism (50). Incubations of

genistein in the presence of human recombinant CYP1A11, 1A2, 1B11 or 2E1 each resulted in the formation of one predominant and two minor unidentified metabolic species. On the other hand, CYP3A4 catalysed the formation of two unique products. All metabolites were thought to be hydroxylation products. Similarly, transformation of the flavonoids galangin to kaempferol then to quercetin in rat liver microsomes is thought to be CYP-mediated, with the latter step being attributed specifically to CYP1A11 (51). Additionally, metabolism studies in cultured (T47D, breast cancer) cells have shown that biochanin A and daidzein undergo methylation *and* hydroxylation reactions as well as sulphate ester formation (52).

Generally speaking, acute studies have shown that no more than 30% of an ingested dose of isoflavone is recovered from the urine and faeces in diphenolic form. It has been suggested that this low recovery is the result of extensive microbial degradation of the isoflavone nucleus, producing simpler phenols such as the quantitatively important genistein metabolite, *p*-ethyl-phenol. However, assessment of the extent of such degradation awaits the synthesis of a suitable stable labelled tracer (38).

***Biochanin A* (BCA)**

BCA is a dietary isofl avone present in legumes, most notably red clover, and in many herbal dietary supplements. BCA has been reported to have chemopreventive properties and is metabolized to the isofl avone genistein (GEN), BCA conjugates, and GEN conjugates. The metabolites may contribute to the chemopreventive effects of BCA. Moon et al 2006 (53) evaluated the pharmacokinetics and metabolism of BCA in rats. Male Sprague-Dawley rats were administered BCA by intravenous injection (1 and 5 mg/kg), by intraperitoneal injection (5 and 50 mg/kg), and orally (5 and 50 mg/kg). Plasma and bile samples were enzymatically hydrolyzed in vitro to determine conjugate concentrations for BCA and GEN. Equilibrium dialysis was used to determine protein binding. The BCA and GEN concentrations in plasma, urine, and bile were determined by liquid chromatography-tandem mass spectrometry (LC/MS/MS). The pharmacokinetic parameters of BCA were analyzed by noncompartmental analysis. Significant levels of BCA conjugates and GEN conjugates were detected in plasma and bile. Both BCA and GEN were found to have a high clearance and a large apparent volume of distribution; the bioavailability of both was poor (<4%). Although BCA can be regarded as a prodrug of GEN and is rapidly converted into the demethylated metabolite GEN in vitro and in vivo, (54, 55) probably under the catalysis of cytochrome P450 (CYP) enzymes, (56 , 57) its biological effects observed in vivo are not identical to those of GEN. For example, BCA can signifi cantly suppress the tumor growth of the human gastrointestinal cancer cells HSC-45M2 and HSC- 41E6 transplanted in athymic nude mice, but GEN cannot, (58) suggesting that BCA or its metabolites, other than those derived from GEN, also exert significant in vivo effects

BCA was extensively metabolized to GEN in human subjects after ingestion of herbal products containing BCA. Although *O* -demethylation of BCA has been attributed to metabolism by gut microfl ora, (59) hepatic microsomal enzymes can perform the same transformation. (53, 60) Conversion of BCA to GEN in rat liver microsomes was found to be rapid and saturable. Conversion of BCA into GEN also occurs in rat intestinal microsomes. (61) Multiple CYP isoforms, including CYP1A2 in liver microsomes and extrahepatic CYP1B1, participate in this demethylation reaction. These isoforms also generate other hydroxylated metabolites, (53,61) including 3 ' -HO-BCA, 6-HO-BCA, and 8-HO-BCA. The main conjugation reaction of BCA in rat liver and intestinal microsome preparations was glucuronidation (50). Glucuronide conjugates of BCA are likely to be 7-OH and 5-OH glucuronic acid derivatives. In the human breast cancer cell line MCF-7, sulphate conjugates represent the major metabolite of BCA.

Lignans

Ingested lignans also undergo bacterial hydrolysis and metabolism. Colonic fermentation results in the removal of glucose residues (attached to the phenolic or side-chain -OH groups), demethylation and dehydroxylation of the parent plant lignans to form what are often referred to as the mammalian lignans. Accordingly, matairesinol is converted to enterolactone, and secoisolariciresinol to enterodiol, which are both diphenol compounds. Enterodiol may be further metabolised (oxidised) to enterolactone in the gut (62) (Figure 2). Once absorbed, the lignans are conjugated with glucuronic acid (38, 45)

Coumestans

The metabolism of the coumestans has not been well characterised.

Prenylisoflavonoids

Little is known about the metabolism of prenylisoflavonoids.

Gastrointestinal Tract Hydrolysis

Deconjugation by Intestinal Lactase Phlorizin Hydrolase (LPH) Lactase phlorizin hydrolase is a membrane spanning enzyme on the luminal side of the brush border of the small intestine. LPH is responsible for the hydrolysis of lactose, the main carbohydrate in mammalian milk. Structure studies have shown a second active site that is capable of hydrolyzing β glycosylceramide (another component of milk) and phlorizin, a dihydrochalcone glycoside (57, 63). Structural similarities among dihydrochalcones, flavonoids, and isoflavonoids led to the discovery that LPH has a role in the hydrolysis of the β glycosides of these compounds (64, 57).

After absorption, phytoestrogens, like estrogens, are prone to enterohepatic circulation. They are excreted in the bile, deconjugated by intestinal flora, reabsorbed, reconjugated by the liver, and excreted in the urine (65).

DISTRIBUTION

Very little information is available on the tissue distribution of phytoestrogens. Foetal exposure to phytoestrogens has been reported. Lignans, genistein, daidzein and metabolites have been detected in neonatal umbilical cord, amniotic fluid in levels similar to maternal plasma.

There are few data concerning the tissue distribution of isoflavones. Yueh and Chu 1977 (66) reported relatively high

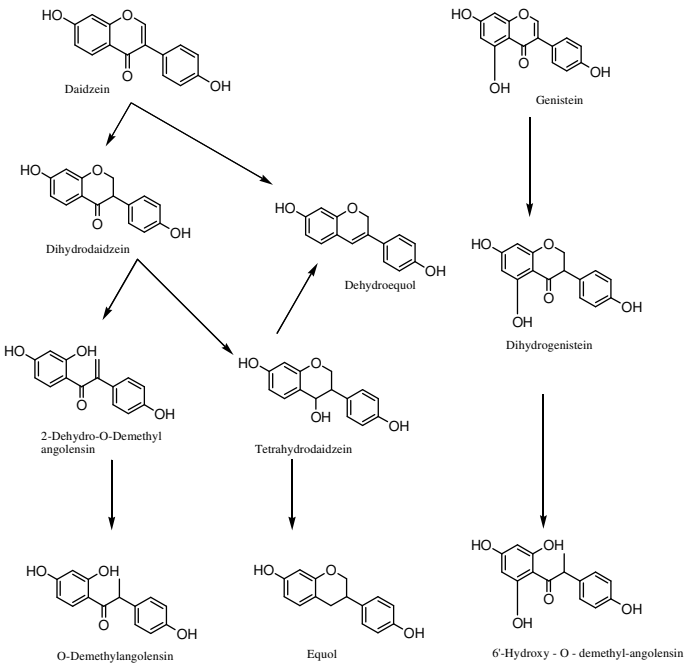

Fig. 1. *Proposed metabolic pathways of catabolism of daidzein and genistein based on urinary isoflavonoid metabolites found so far in human urine. Pathway through dehydroequol remains to be substantiated.*

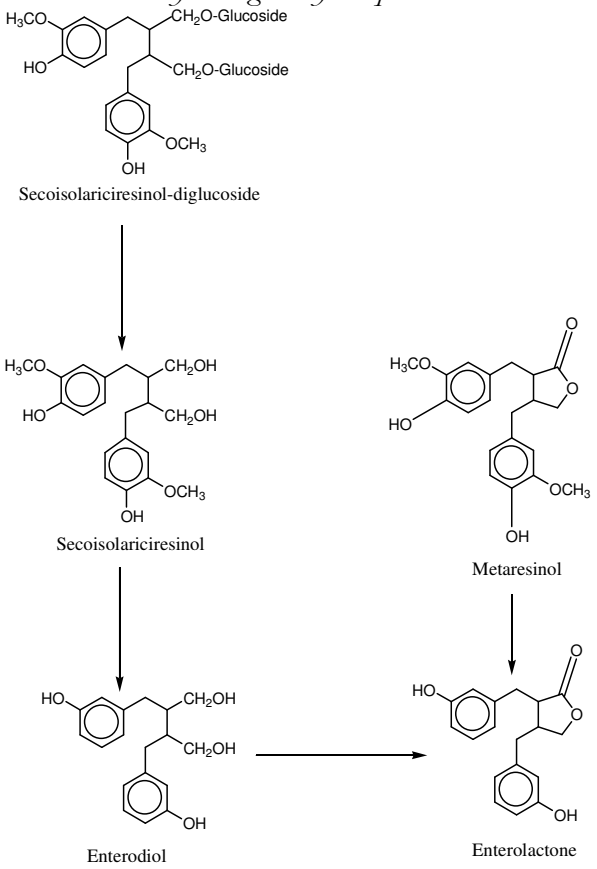

Fig. 2: *formation of enterolactone and enterodiol by human faecal flora. secoisolariciresinol diglucoside is metabolised to enterodiol through hydrolysis of the sugar moiety, dehydroxylation, and demethylation.*

levels of daidzein in the plasma, liver, lung and kidney in rats, 15 min after i.v. injection. Lower levels were found in skeletal muscle, spleen and heart and low levels in testis and brain. More recent studies have shown that following i.p. administration to rats, genistein rapidly appears in brain tissue and then in microdialysate fluid from the corpus striata along with its metabolite *p*-ethyl-phenol, indicating that isoflavones can cross the blood brain barrier.

EXCRETION AND ENTEROHEPATIC CIRCULATION

Conjugates of isoflavonoids and lignans undergo urinary elimination via the kidneys or are excreted in the bile. Various bacterial metabolites of isoflavones and lignans may be detected in both urine and faeces and the pattern of excretion follows that of food intake. Thus, in Western populations, where the typical diet is relatively low in isoflavones, urinary levels of isoflavones tend to be lower than the levels of lignans. Concentrations of total dietary estrogens are relatively low in most subjects consuming omnivorous diets without soy-foods. In contrast, vegetarians and individuals consuming macrobiotic diets tend to have higher urinary excretion of phytoestrogens, particularly lignans. Conjugates identified in human urine include those of formononetin, methylequol, daidzein, dihydrodaidzein, *O*-DMA, genistein, 3',7-dihydroxyisoflavan, equol, matairesinol, lariciresinol, isolariciresinol, seicoioslariciresinol and tentatively identified 7α-OH matairesinol and 7'-OH enterolactone. Conjugates that are excreted in the bile may be deconjugated by the gut bacterial microflora, and further metabolised or reabsorbed and returned to the liver via the portal blood. Elimination via the faeces is thus largely determined by the degree of enterohepatic circulation, which may result in prolonged exposure to these compounds .

Clinical findings

According to King and Bursill (67), 1998 Plasma and urinary kinetic profiles for genistein and daidzein, determined in six adult men, following a single soybean flour based meal, showed that maximum plasma concentrations were reached 7 and 8 hours following consumption, and elimination half-lives were ~ 5 and 6 hours for daidzein and genistein, respectively. Although urinary excretion of daidzein was greater than for genistein, the ratios of the plasma AUCs (areas under the curve) for the respective isoflavones were similar to the ratio of concentrations present in the soybean meal, indicating that their bioavailabilities were similar. This finding apparently conflicts with those of (67). However, a study by Setchell et al 1998(38), employing pure isoflavonoid compounds administered as a single bolus, similarly demonstrated that the apparent bioavailabilities, determined from plasma appearance and disappearance curves, of daidzein and genistein were similar. Peak plasma concentrations were generally attained between 6-8 hours after ingestion and plasma half-lives were ~7.9 hours.

Husband et al 1999(68) briefly described the acute and chronic pharmacokinetic profile of Promensil, an isoflavone supplement containing 40 mg of total isoflavones(genistein [4 mg], daidzein [3.5 mg], biochanin A [24.5 mg], and formononetin [8.0 mg]) (biochanin A and formononetin are the 4'-methyl-ether precursors of genistein and daidzein, respectively), in eight male and eight female subjects, previously maintained on low isoflavone diets. After acute dosing (1 tablet), all four isoflavones appeared in the plasma within 15 min and reached peak levels between 5-6 hours. Daidzein and genistein were present in much higher concentrations than their methylated precursors, indicating rapid demethylation of these compounds. Nonetheless, formononetin and biochanin A were detectable in plasma and urine at all times. Concentrations of all isoflavones remained above basal levels after 24 hours. Following chronic dosing (2 tablets/d x 14d), plasma and urine levels of isoflavones were 2-4 times higher that the peak levels achieved after a single dose, indicating accumulation. Interestingly, Zhang et al 1999 (69) have shown that microsomal UDP-glucuronosyl transferase from rat has a greater affinity for genistein than for daidzein *in vitro*. However, it remains unclear as to the relevance of this finding to humans. Logically, preferential glucuronidation of genistein should promote its excretion. Excretion patterns indicated enterohepatic circulation. Most of the isoflavone-derived excretion in faeces occurred on the second and third days following ingestion (4.5% and 2.5% of ingested daidzein and genistein, respectively).

Urinary excretion of isoflavones increases with increased soy intake, but absorption, as reflected by urinary excretion, may be saturable at high doses (70, 37, 38).

Lignans identified in human urine include enterolactone,enterodiol, lariciresinaol and isolariciresinol (41). Nesbitt et al 1999(71) examined the urinary and plasma profiles of the lignans, enterolactone and enterodiol, following a single (day 1) and repeated (days 1- 7 or 8) administration of 5, 15 or 25 mg of raw or processed (in the form of bread or a muffin) flaxseed to nine young women, during the follicular phase of their menstrual cycle, who were otherwise maintained on a low-lignan, low-fibre diet. Urinary excretion of lignans was dose-dependent and unaffected by processing. Enterodiol was the predominant lignan, although there was considerable variation in the ratio of enterolactone:enterodiol found among different individuals. The greater time required for the lignans to achieve peak plasma concentrations, compared to isoflavonoids, was also observed by Morton (72, 73). Increased serum concentrations of daidzein and genistein were observed, in male subjects, 30 min after consumption of a cake containing soybean flour and cracked linseed. Peak concentrations were reached between 5.5-8.5 hours after ingestion. In contrast, increases in plasma levels of enterolactone and enterodiol were not observed until 8.5 hours following consumption. After 24 hours, plasma concentrations of both lignans and isoflavonoids remained above pre-dose levels.

Franke and Custer, 1996; Franke et al, 1998(74, 75) monitored the appearance of isoflavones in breast milk in a single individual, following soybean challenge (containing 0.85 mg/kg daidzein and 1.1 mg/kg genistein). Appearance of isoflavones followed that in urine but with a slight delay and concentrations were dose-dependent Maximum levels were

attained 10-14 hours after ingestion. This biphasic pattern had also been observed in plasma and urine and was thought to be a reflection of the process of enterohepatic circulation. The pattern of metabolites found in the breast milk reflected that observed in plasma.

Human metabolism and excretion of isoflavones and lignans are subject to considerable inter-individual variation (38). Kelly et al 1993(76) found moderate variation in the urinary excretion of daidzein, genistein and glycitein and more marked variation in the excretion of *O*-DMA, 6'OH-*O*-DMA and, particularly, equol, following soy challenge. Several studies have suggested that ~ only one third of the population are capable of equol production (77, 78, 79). As a consequence, higher concentrations of precursor compounds appear in the urine of low equol excretors and inverse relationships have been established between equol and daidzein (Setchell, 1984,) and equol and *O*- DMA and 6'OH-*O*-DMA excretion (76). Presumably, low equol excretors are also subject to increased plasma levels of precursor compounds. A study by Nesbitt etal 1999 (71) showed that 2 of 9 female subjects produced little or no enterolactone following ingestion of lignans from flaxseed and (78) reported considerable differences in the ratios of urinary enterolactone and enterodiol in 30 women, following ingestion of flaxseed. These data suggested inter-individual variation in the ability to oxidise enterodiol to enterolactone.

ROLE OF GUT MICROFLORA IN PHYTOESTROGEN METABOLISM

The large inter-individual variation in phytoestrogen metabolism and proportions of metabolites excreted is largely a consequence of inter-individual differences in the bacterial flora involved in metabolising these compounds (and the preferred metabolic pathway). The importance of the gut microflora in the metabolism of phytoestrogens has been amply demonstrated. Setchell et al 1984 (78) showed that incubation of textured vegetable protein with cultured human faecal bacteria resulted in the formation of equol while Chang and Nair 1995 (80) demonstrated the metabolism of daidzein to dihydrodaidzein, benzopyran-4,7-diol, 3-(4-hydroxyphenol) and equol and of genistein to dihydrogenistein, when fermented with human faecal bacteria under anaerobic conditions. Antibiotic treatment in humans has been shown to decrease the excretion of bacterial metabolites (81) and germ-free rats fed soy were found to excrete daidzein and genistein but not their metabolites, equol or *O*-DMA, in urine. Colonisation of the same rats with bacterial flora from a human subject capable of converting daidzein to equol resulted in substantial excretion of equol, but not *O*-DMA. Germ-free rats, colonised with flora from a nonequol-producing human, produced no detectable urinary equol and only trace amounts of *O*-DMA, when fed with soy. Lignan metabolites, enterolactone and enterodiol, were detected in germ-free rats only when they were contaminated with human microflora, thus also confirming the role of gut bacteria in lignan metabolism. Furthermore, when enterodiol was administered to bile-fistula rats (in which enterohepatic circulation is interrupted) via i.p. injection, no enterolactone metabolite was detected in plasma (82, 38,41). No increases in either enterolactone or enterodiol were seen in the plasma of iliostomy patients, following rye bread consumption, presumably due to the absence of sufficient bacterial activity for the metabolic conversion of the parent plant lignans (83).

From the results of a study monitoring the gut microflora metabolism of isoflavones in 14-20 subjects, Hendrich et al 1998 (84) have suggested that there may be 16 three phenotypes (low, moderate and high) with respect to the ability of their faeces to degrade daidzein and genistein. While, these workers did not specifically measure equol production, it is possible that these phenotypes may also represent populations of good, poor and mederate equol producers.

At birth, the gut is sterile, but within a week it begins to develop a microflora, the profile of which continues to change from infancy to adulthood (85). Initial colonisation is determined by factors such as the composition of maternal gut flora, the mode of delivery (conventional or caesarean birth), hygiene, environment and genetics. The intestine of the new-born has a higher redox potential than that of the adult. Consequently, the first colonisers must be capable of oxidative metabolism and typically include *Enterobacteria*, *Streptococci* and *Staphylococci*. These facultative bacteria rapidly metabolise oxygen to provide a lower redox potential, thereafter allowing strictly anaerobic bacteria, such as *Bifidobacteria*, *Clostridia* and *Bacteroides,* to flourish. The infant microflora is also influenced by the method of feeding. Faecal populations are more diverse in formula fed babies than in breast-fed babies (86). Bacterial enzyme activities increase with age and are greatly influenced by the adoption of an adult diet (87). The influence of the diet is greater on gut microflora of previously breast-fed babies than on formula-fed babies. In humans, the upper third of the small intestine contains very low levels of bacteria, but this changes to a colon like flora in the lower third (88). The distal part of the small intestine and the large intestine contain substantial numbers (104-107 bacteria/g wet wt) of *Lactobacilli, Bacteroides* and *Bifodobacteria* (89). Although several groups of bacteria are known to possess β-glucosidase activity, including *Lactobacilli, Bacteroides* and *Bifidobacteria* (90). Whereas the proximal end of the small intestine contains very few of these bacteria. Some bacteria present in the large intestine also possess β -glucuronidase and arylsulphatase activity, which can liberate aglycones from conjugates excreted in the bile and render them available for reabsorption (88). However, incubation studies with human faeces suggest that human intestinal bacteria from some, but not all, individuals can further metabolise and degrade soybean isoflavones to a considerable extent (91), thus preventing their reabsorption from the lower bowel. In support of this, strains of *Clostridia,* present in the lower gastrointestinal tract of some individuals, have been shown to be capable of cleaving the C ring of certain flavonoids, which are analogous to isoflavonoids in structure, to produce monophenolic compounds anaerobically (92). Consequently, the intestinal microflora profile can have a profound effect both on the magnitude and pattern of isoflavone bioavailability.

FACTORS AFFECTING GUT MICROFLORA - Various physiological, pathological and environmental factors are likely

to influence gut bacterial profile, including hygiene, antibiotic use, bowel disease, stress, gut motility, gastric pH, mucin secretion, bile secretion, diet and intestinal transit time. Sex, genetics and ethnicity may also play their roles (86, 82, 93).

Diet

Human studies have revealed several important diet-related differences in the metabolism of phytoestrogens by the gut microflora. For example, consumption of less fat and more carbohydrate, as a proportion of total energy intake, has been correlated with greater equol production, particularly in women (77, 78). The reason for this is uncertain but it is possible that complex carbohydrates stimulate fermentation in the large bowel, resulting in increased breakdown of daidzein to equol (44, 82). Furthermore, low-fat diets are associated with decreased β-glucuronidase activity in the intestinal contents which may consequently impede the absorption of relatively more fat-soluble deconjugated compounds (48).

Dietary fibre has been shown to affect the absorption, reabsorption and excretion of estrogens and phytoestrogens by influencing the β-glycosidase and β- glucuronidase activities of the intestinal microflora. The bulking effect of dietary fibre, which results in the dilution of gut microflora activity, and the hydrophobic bonding, particularly of non-conjugated compounds, are both thought to contribute to a reduction in absorption and reabsorption of isoflavones (94, 95). Vegetarians generally have higher faecal weights than omnivores, and a lower faecal bacterial β-glucuronidase activity (48). The implication is that high dietary fibre could result in the partial disruption of the enterohepatic circulation of phytoestrogens and endogenous estrogens (which are also subject to enterohepatic circulation). The implication is that bioavailability of estrogens and phytoestrogens may be reduced in these individuals. Higher dietary fibre intakes have also been associated with excretors, rather then nonexcretors of equol, in females (78).

Sex

Claims have been made that there are sex differences in the urinary excretion of isoflavones. Data reported by Lu and Anderson 1998 (96) suggested that recovery of isoflavone conjugates in urine was greater in women than in men, following soy challenge. Furthermore, Lu et al 1995 (97) suggested that chronic soy consumption could differentially modulate the metabolism and disposition of isoflavones in men and women. Zhang et al 1999 (98) reported sex differences in urinary glycitein, but not daidzein and genistein, following ingestion of soymilk by individuals with a moderate excreting phenotype. However, the power of these studies was small and other studies have given no such indication of any sex differences. Kirkman et al 1995 (99) reported that men excreted higher ratios of enterolactone:enterodiol than did women, suggesting a sex difference in the colonic bacterial metabolism of lignans. No difference in the urinary excretion of isoflavonoids was observed. However, the number of subjects used in this study limits the conclusion.

Chemical structure and resistance to degradation

Isoflavones and flavonoids that possess a 5-OH group, such as genistein, but not daidzein, are much more susceptible to C ring cleavage by rat intestinal bacteria (100). Whether the same is true with human faecal bacteria is unclear, although selective C-ring cleavage of certain flavonoid compounds by certain strains of Clostridium isolated from human faecal flora has been show. Less faecal degradation should result in greater levels of and/or prolonged exposure to lignans and isoflavones in the circulation and increased urinary isoflavone excretion, although specific data are lacking.

Hepatic metabolism

Biotransformation of phytoestrogens in the liver, involving phase I and II metabolism may be subject to the influence of various genetic and environmental factors, including exposure to drugs and dietary components (101).

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