

## Bioavailability of phyto-oestrogens

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The term phyto-oestrogen encompasses isoflavone compounds, such as genistein and daidzein, found predominantly in soya products and the lignans, such as matairesinol and secoisolariciresinol, found in many fruits, cereals and in flaxseed. There is evidence that they have potential health benefits in man particularly against hormone-dependent diseases such as breast and prostate cancers and osteoporosis. This has led to intense interest in their absorption and bio-transformation in man. The metabolism of isoflavones and lignans in animals and man is complex and involves both mammalian and gut microbial processes. Isoflavones are present predominantly as glucosides in most commercially available soya products; there is evidence that they are not absorbed in this form and that their bioavailability requires initial hydrolysis of the sugar moiety by intestinal  $\beta$ -glucosidases. After absorption, phyto-oestrogens are re-conjugated predominantly to glucuronic acid and to a lesser degree to sulphuric acid. Only a small portion of the free aglycone has been detected in blood, demonstrating that the rate of conjugation is high. There is extensive further metabolism of isoflavones (to equol and *O*-desmethylandrolensin) and lignans (to enterodiol and enterolactone) by gut bacteria. In human subjects, even those on controlled diets, there is large interindividual variation in the metabolism of isoflavones and lignans, particularly in the production of the gut bacterial metabolite equol (from daidzein). Factors influencing absorption and metabolism of phyto-oestrogens include diet and gut microflora.

### Phyto-oestrogens: Isoflavones: Lignans: Bioavailability

#### Introduction

The term bioavailability has been the subject of much debate, particularly in relation to micronutrients. Early definitions concentrated on uptake from the gut, specifically the extent (completeness) and rate of absorption. Subsequently the importance of post-absorptive processes, namely distribution, metabolism and excretion, was recognised. Combined with absorption, these four elements can be considered as 'biodelivery'. Current definitions of bioavailability, however, have been extended to encompass concepts of 'bioefficacy'; that is, the fraction of the nutrient that meets tissue functional requirements. Thus Jackson (1997) has provided a working definition: 'bioavailability is the fraction of ingested nutrient utilized for normal physiological function'.

Extending the concept to non-nutrients such as phyto-oestrogens, bioavailability thus refers to the effectiveness

of a chemical in eliciting a response in a target tissue. For a true assessment of bioavailability, a sensitive functional marker is required (e.g. plasma homocysteine for folate). In the case of phyto-oestrogens, no such marker is available, and indeed, given the diversity of the biological activities of the compounds, there are likely to be different markers in different tissues.

For the present, therefore, bioavailability assessments must be based on data from absorption, metabolism, distribution and excretion (ADME) studies in man and animals. Even when taking this more limited interpretation of the term 'bioavailability', it is clear that ADME of phyto-oestrogens are not completely defined in man. Most of the available pharmacokinetics on phyto-oestrogens relates to levels attained in plasma and urine of specific isoflavones and their metabolites, e.g. daidzein and genistein, with less information on lignans and little or no data on coumestrol and glycitein.

**Abbreviations:** ADME, absorption, distribution, metabolism and excretion; VENUS, Vegetal Estrogens in Nutrition and the Skeleton.

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## Absorption of isoflavonoids

### *Rate and extent of absorption*

There have been a number of studies that have investigated the rate and extent of absorption of isoflavones by measuring plasma and urinary isoflavone concentrations in adults following the ingestion of a single dose of a soya product (Table 1). The relative importance of the limited animal data is difficult to interpret owing to the pharmacological doses administered. Human studies indicate rapid absorption of the phyto-oestrogens with peak concentrations occurring between 2 and 12 h after ingestion. Elevated isoflavone concentrations in the circulation have been reported within minutes of ingestion of soya foods (Morton *et al.* 1997; Rowland *et al.* 1999). Rowland *et al.* (1999) reported a significant increase in plasma isoflavones only 15 min after the ingestion of soya protein. Morton *et al.* (1997) demonstrated elevated blood levels of daidzein and genistein in four men within 30 min of the ingestion of a cake containing 15 g of soya protein and 15 g of flaxseed. On the other hand, lignan metabolites did not appear in plasma until 8.5 h after ingestion, suggesting a slower rate of absorption of this class of phyto-oestrogen.

In studies where molar equivalents of genistein and daidzein were consumed, the former achieved a higher plasma level than the latter. This is due to the higher volume of distribution ( $V_d$ ) and clearance rate for daidzein rather than a greater rate of absorption for genistein (Setchell *et al.* 2001, 2003). Urinary recovery of dose ingested provides a good indicator of the apparent fractional absorption. Available data (Table 1) clearly demonstrate that the fractional absorption of ingested daidzein is greater than that of genistein. The limited data on glycitein indicate a greater rate of absorption of the ingested dose than for other isoflavones found in food (Zhang *et al.* 1999). A dose-response effect has been noted in two studies using doses of between 0.4 and 1 mg isoflavones/kg body weight in fractional absorption (Setchell *et al.* 2003; M Faughnan *et al.* unpublished results). At a higher dose of 2 mg isoflavones/kg body weight plasma and urinary isoflavone concentrations did not increase in a dose-dependent manner, suggesting that the absorption of parent compounds may be rate-limited (M Faughnan *et al.* unpublished results). A dose-response effect was also noted in the urinary excretion of lignans over a 7 d period in nine healthy premenopausal women, with single doses ranging from approximately 5 to 25 mg (Nesbitt *et al.* 1999). To date, however, the measurement of intact isoflavones has accounted for only approximately 30 % of the ingested dose (see Table 1). Faecal excretion of these compounds is low (Table 1), indicating that the majority of the unaccounted isoflavone dose is metabolised in the intestine (see 'Metabolism in the intestinal tract'). The rate and extent of absorption of these metabolites are less well characterised than those of the parent compounds, as our understanding of the metabolism of these compounds along the intestinal tract is limited.

King & Bursill (1998) indicated that plasma and urinary isoflavone levels return to baseline 24 h after the ingestion of soya flour. However, studies that have sampled more

extensively have indicated that this is not the case, and baseline levels are not attained until approximately 48 h post ingestion (Setchell *et al.* 2001, 2003; M Faughnan *et al.* unpublished results). Setchell *et al.* (2001) highlighted the importance of sensitive methods in quantifying low levels of isoflavones, particularly in blood, when determining the pharmacokinetic behaviour of these compounds.

### *Hydrolysis of glucosides*

Isoflavones are present predominantly as glucosides in most commercially available soya products, with the exception of fermented soya products. Although another, related class of polyphenols, the flavonoids (such as rutin and quercetin), have been shown to be absorbed in their naturally occurring glycosidic forms (Hollman & Katan, 1998), this does not appear to be the case for the isoflavones since glucosides have not been identified in plasma. Indeed recent evidence (Setchell *et al.* 2002) clearly reveals that isoflavone glucosides are not absorbed intact across the enterocyte of healthy adults and that their bioavailability requires initial hydrolysis of the sugar moiety by intestinal  $\beta$ -glucosidases for uptake to the peripheral circulation. It is already well established that hydrolysis is necessary for these flavonoids to be absorbed across the intestinal wall (Day *et al.* 1998; Liu *et al.* 1998). In a Caco-2 TC7 monolayer system, genistin was found not to penetrate the enterocyte readily compared with the aglycone, genistein, which was readily permeable (Steensma *et al.* 1999). In support, the efficient uptake of genistein has also been confirmed in isolated rat small intestine (Andlauer *et al.* 2000). In this later study, only 1.3 % of genistin penetrated the isolated intestinal wall, indicating that the absorption of the intact glucoside is unlikely to be of significance *in vitro* (Andlauer *et al.* 2000).

Early studies suggested intestinal microfloral enzymes ( $\beta$ -glucosidases) present in several groups of bacteria including lactobacilli, bifidobacteria and bacteroides (Xu *et al.* 1995) were responsible for hydrolysis. However, current studies refute the exclusive involvement of the gut microflora. The main site of microflora activity is the distal ileum and colon and it is known that isoflavone absorption occurs extremely rapidly after ingestion (see above), suggesting that hydrolysis occurs rapidly in the upper gastrointestinal tract. In addition, germ-free rats (who have no intestinal microflora) excrete large quantities of daidzein and genistein in urine after the consumption of intact glucoside isoflavones in soya protein (Rowland *et al.* 1999).

King & Bursill (1998) suggested that the rapid elevation in plasma isoflavonoid concentrations after soya consumption might be the result of absorption of the aglycone fraction present in the soya meal. This was also stated by Lu *et al.* (1996), as they reported that isoflavones were detectable in female urine 1–2 h after soya milk ingestion. However, aglycones form only a small proportion of most soya-based products. Xu *et al.* (1995) and Piskula *et al.* (1999) suggested that gastric acid present in the stomach might be involved in releasing the aglycones. However, these conclusions were based on studies performed in rats which, unlike healthy human subjects, are

**Table 1.** Summary of human studies investigating the pharmacokinetic behaviour of phyto-oestrogens following a single oral bolus dose of isoflavones as food or pure compounds (Data are given as mean with standard error of the mean in parentheses. SEM is replaced by range where data are available)

Study	Subjects	Source	Dose	Duration of sampling	$t_{1/2}$ (h)	$C_{max}$ (nmol/ml)	$t_{max}$ (h)	AUC (nmol/ml per h)	Urine (% recovery)	Faeces (% recovery)
Xu <i>et al.</i> (1994)	n=12, pre	soya milk powder	0.7 mg/kg bw (D:G=1.3:1) 1.3 mg/kg bw 2.0 mg/kg bw	0-24 h, blood (0, 6 & 24 h)	D: 0.79 (0.011) G: 0.74 (0.127)	D: 7.4 (0.7)	D: 19.6 (1.8) G: 5.29 (1.1)	D: 48.6 (6.4) G: 27.6 (5.7) Gly: 55.3 (7.2)	total: 0.8 (0.3)	total: 1.0 (0.4) total: 2.8 (0.7)
King & Bursill (1998)	n=6, men	soya flour added to milk	0.67 mg D/kg 0.97 mg G/kg	0-24 h, each 12 h studied on separate days	D: 4.71 (1.1) G: 5.74 (1.3)	D: 7.4 (0.7) G: 8.0 (0.7)	not calculated	D: 6.0 (6.0) G: 22.0 (4.0)	not assessed	
Zhang <i>et al.</i> (1999)	n=7, pre; n=7, men	soya milk	1.1 mg/kg bw (D:G:Gly = 0.9:1.0:0.2)	0-48 h, blood (0, 6 & 24 h)	♀D: 1.04 (0.248) ♀G: 1.70 (0.419) ♀Gly: 56.8 (9) ♂D: 1.29 (0.190) ♂G: 1.78 (0.310) ♂Gly: 63 (9) ♀D: 1.63 (0.390) ♀G: 0.51 (0.189) ♀Gly: 207 (24) ♂D: 1.16 (0.165) ♂G: 0.47 (0.109) ♂Gly: 241 (27)					
Watanabe <i>et al.</i> (1998)	n=7, men	baked soya-bean powder (Kinako)	26.1 mg D plus 30.2 mg G	0-72 h, blood once daily, 24 h urines, faeces 0-3 d	D: 1.56 (0.340) G: 2.48 (0.650)	D: 8 G: 8	not calculated	D: 35.8 (27.3-64.6) G: 17.6 (9.7-47.9)	D: 4.4 (1.1-12.3) G: 2.5 (0.2-7.1)	
Sheinutt <i>et al.</i> (2000)	n=6, men; n=6, pre	soya protein isolate	1.0 mg D/kg bw, 0.6 mg G/kg bw	0-48 h					D — agl: 0.11 (0.03); glu: 21.2 (1.5); sul: 5.5 (0.8) G — agl: 0.01 (0.00); glu: 5.7 (1.0); sul: 1.1 (0.3)	

Continued

Table 1. Continued

Study	Subjects	Source	Dose	Duration of sampling	Blood parameters					
					$t_{1/2}$ (h)	$C_{max}$ (nmol/ml)	$t_{max}$ (h)	AUC (nmol/ml per h)	Urine (% recovery)	Faeces (% recovery)
Setchell <i>et al.</i> (2000)	n=6, pre	daidzein	50 mg	0-48 h	D: 9.3 (1.3)	D: 0.76 (0.120)	D: 6.6 (1.4)	D: 11.6	not assessed	not assessed
	n=4, pre	daidzin	50 mg	0-48 h	D: 4.6 (0.5)	D: 1.55 (0.240)	D: 9.0 (1.0)	D: 17.7		
	n=6, pre	genistein	50 mg	0-48 h	G: 6.8 (0.8)	G: 1.26 (0.274)	G: 9.3 (1.3)	G: 16.7		
	n=3, pre	genistin	50 mg	0-48 h	G: 7.0 (0.8)	G: 1.26 (0.470)	G: 9.3 (1.3)	G: 18.3		
	n=1, men	red clover	40 mg	0-48 h		D: 0.06 G: 0.13	D: 12 G: 2			
n=1, men	glycitin	25 mg	0-48 h	Gly: 8.9	Gly: 0.72	Gly: 4	Gly: 0.002			
Setchell <i>et al.</i> (2003)	n=8, pre	[ <sup>13</sup> C]daidzein	0.4 mg/kg bw	0-72 h	8.2	0.31		5.02	29.5	<LOD
			0.8 mg/kg bw		7.2	0.71		8.70	25.6	
	n=8, pre	[ <sup>13</sup> C]genistein	0.4 mg/kg bw		7.5	0.55		6.01	8.9	
			0.8 mg/kg bw		7.4	0.88		9.77	8.3	
M Faughnan and A Cassidy (unpublished results)	n=13, pre	soya milk	0.45 mg/kg bw	0-72 h	D: 7.3	D: 0.52		D: 5.67	total: 39 (24-62)	
	n=13, pre		0.90 mg/kg bw		G: 8.9	G: 1.20		G: 18.45		
					D: 7.6	D: 0.88		D: 9.39	total: 33 (18-47)	
	n=12, pre		1.80 mg/kg bw		G: 8.6	G: 2.03		G: 29.52		
				D: 7.6	D: 1.27		D: 15.22	total: 27 (15-41)		
				G: 8.2	G: 3.63		G: 45.87			

$t_{1/2}$ , half-life;  $C_{max}$ , maximum concentration;  $t_{max}$ , time of maximum concentration; AUC, area under curve; pre, premenopausal women; bw, body weight; D, daidzein; G, genistein; Gly, glycitein; agl, aglycone; glu, glucuronide; sul, sulphate; LOD, limit of detection.

known to harbour bacteria in the stomach, so bacterial hydrolysis could have been responsible. A more plausible site of hydrolysis in man is the small intestine. There is evidence (Day *et al.* 1998) that  $\beta$ -glucosidase activity is present in the brush border of the gut mucosa, capable of hydrolysing some, although not all, isoflavonoid and flavonoid compounds in foods. Until recently,  $\beta$ -glucosidase activity of the intestinal microflora was considered to be responsible for hydrolysis, but a number of membrane-bound  $\beta$ -glucosidases have now been identified in the small intestine (McMahon *et al.* 1997; Ioku *et al.* 1998). The activity of these enzymes is highly expressed in the jejunum, particularly for flavonoid compounds that contain the glucose moiety at position 7 of the molecule, as is the case with many isoflavones (Day *et al.* 1998). Thus it appears the major site of hydrolysis in man is likely to be the small intestine.

The mechanism by which these compounds cross the intestinal wall has not been elucidated. Setchell *et al.* (2003) proposed that absorption of phyto-oestrogens occurs by passive diffusion following the hydrolysis of any glucosides.

### Metabolism in the intestinal tract

It has been demonstrated that the isoflavonoid aglycones can undergo further fermentation by colonic microflora to produce a number of metabolites prior to absorption (Axelson *et al.* 1984; Setchell, 1998; Mazur *et al.* 1996; Wiseman, 1999). The metabolism of dietary isoflavones in man is shown in Figs. 1 and 2.

The appearance of these metabolites in plasma is time-dependent on their production in the colon. These metabolites appear in plasma several hours after soya products are consumed, presumably reflecting the time taken for unabsorbed daidzein, or daidzein in the enterohepatic circulation, to reach the colon. A classical example of the appearance of equol in serum is shown in Fig. 3, which demonstrates that peak equol concentration occurs at 36 h post ingestion. A number of studies have indicated that isoflavone metabolites, like equol, are excreted in the urine within 24 h after exposure (Xu *et al.* 1994; Lu *et al.* 1995*a,b*, 1996; King & Bursill, 1998). However, it is now known that sampling over this period is insufficient accurately to determine total equol excretion.

The lignans matairesinol and secoisolariciresinol are metabolised to enterolactone and enterodiol, respectively. In addition, enterodiol can be converted to enterolactone (Fig. 4; Borriello *et al.* 1985). This metabolism appears to be necessary for the absorption of these compounds due to the fact that negligible concentrations of the parent compounds occur in blood.

Metabolism may be important in relation to the biological efficacy of these compounds. For example, equol, a metabolite of daidzein, is more oestrogenic than its parent compound (Brieholt & Larsen, 1998; Kuiper *et al.* 1998). Recent data indicated a more favourable hormone profile in relation to breast cancer risk in women who were producers of equol (Duncan *et al.* 2000).

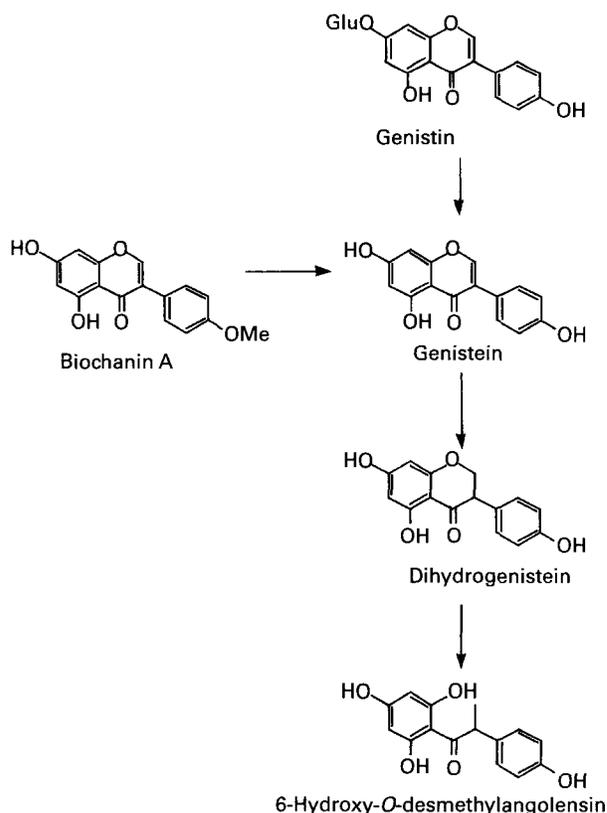


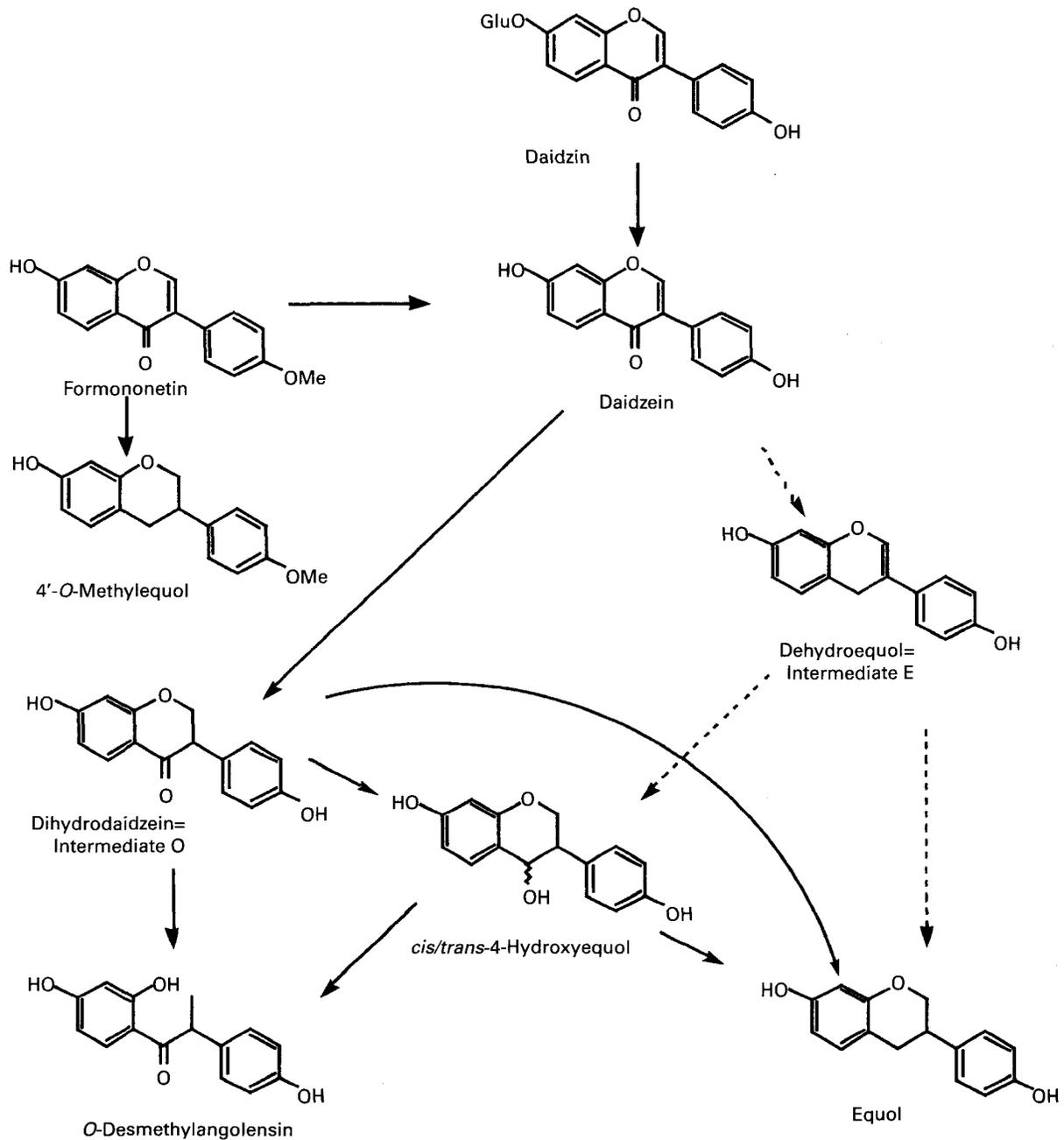
Fig. 1. Metabolism of biochanin A and genistein in man. (From Adlercreutz *et al.* 1987.)

### Evidence for the role of microflora in phyto-oestrogen metabolism

The crucial importance of the gut microflora in phyto-oestrogen metabolism has been convincingly demonstrated *in vitro* and *in vivo*. Incubation of soya protein with human faecal micro-organisms resulted in the formation of equol (Setchell *et al.* 1984). Similarly, Chang & Nair (1995) have shown conversion of daidzein to dihydrodaidzein, benzopyran-4,7-diol,3-(4-hydroxyphenyl) and equol by human faecal bacteria and also the conversion of genistein to dihydrogenistein. Setchell *et al.* (1981) reported reduced excretion of isoflavone metabolites in antibiotic-treated human subjects. The absence of equol and *O*-desmethylan-golensin in the urine of germ-free rats fed soya provides further confirmation of the role of the microflora in daidzein metabolism. When the rats were colonised with human faecal flora from an equol-producing subject, equol was detected in urine. Corresponding rats colonised by gut bacteria from a non equol-producing volunteer did not excrete the metabolite (Bowey *et al.* 2003). In the case of lignans, enterolactone and enterodiol were excreted only by rats harbouring gut microflora. The absence of lignan metabolites in the urine of germ-free rats confirms the crucial role of gut bacteria in their formation (Bowey *et al.* 2003).

### Range of metabolites

Isoflavones and their metabolites have been detected in the majority of biological fluids in animals and man



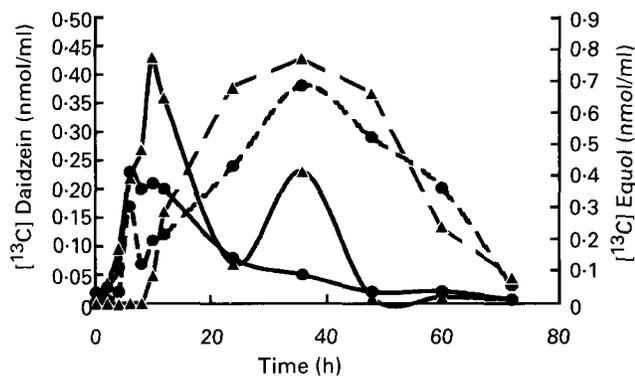
**Fig. 2.** Metabolic pathways of formononetin and daidzein in man. (From Adlercreutz *et al.* 1987; Setchell & Adlercreutz, 1988; Joannou *et al.* 1995; Heinonen *et al.* 1999.)

(Bannwart *et al.* 1984, 1987; Adlercreutz *et al.* 1993, 1995; Morton *et al.* 1994, 1997; Xu *et al.* 1994, 1995; King & Bursill, 1998; Lampe *et al.* 1998; Rowland *et al.* 2000). Most studies have focused on urine where the isoflavones are present in high concentrations, thus facilitating analysis.

The isoflavones identified in the urine, faeces and plasma of human adults are shown in Figs. 1 and 2 (Adlercreutz *et al.* 1987; Kelly *et al.* 1993; Joannou *et al.* 1995; Wähälä *et al.* 1998; Heinonen *et al.* 1999). These metabolites comprise the parent compounds that did not undergo any further

metabolism in the intestine, the major metabolites of daidzein (equol and *O*-desmethylangolensin produced by bacterial metabolism) and a number of intermediate products and hepatic metabolites (mostly hydroxylation products). In urine, these compounds are found predominantly as glucuronides, as is the case in plasma.

The most cited metabolite in the literature is equol, a metabolite of daidzein, which interestingly led to the discovery of its parent compound in the early 1980s (Axelson *et al.* 1982). More recently, minor metabolites such as dihydrodaidzein, tetrahydrodaidzein and dehydroequol



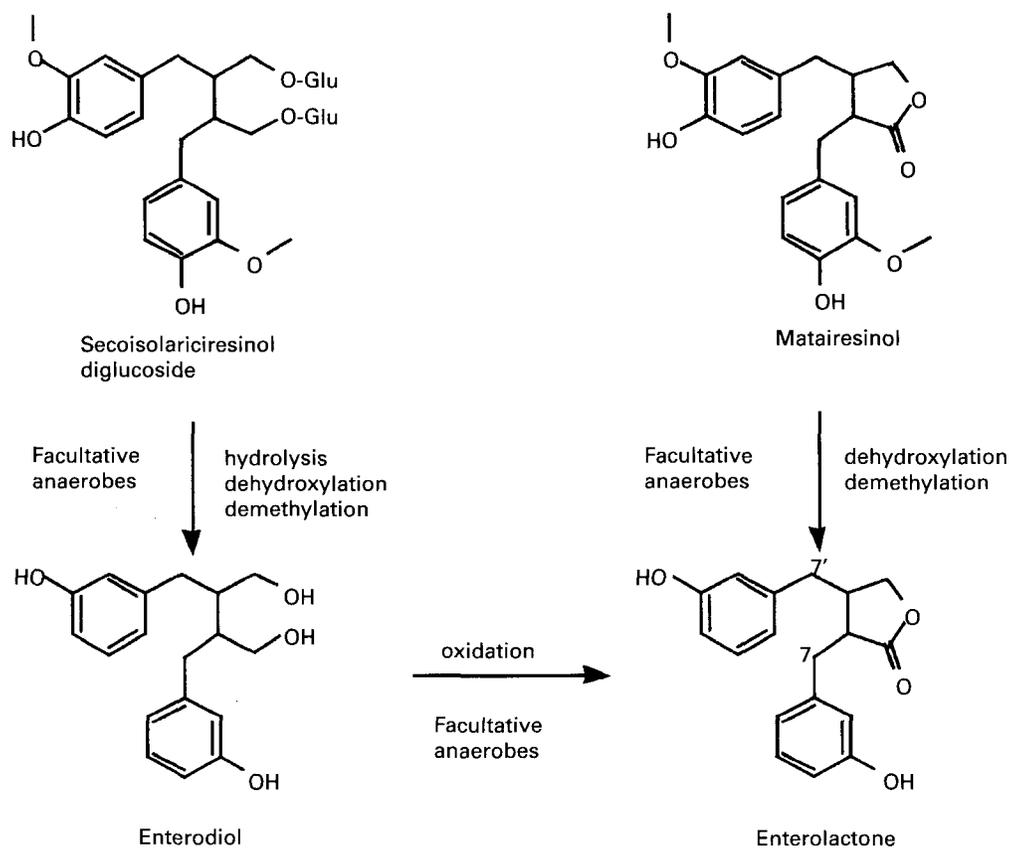
**Fig. 3.** Typical serum appearance/disappearance curve of [<sup>13</sup>C]daidzein and [<sup>13</sup>C]equol following the ingestion of a single oral bolus dose of 25 mg (0.4 mg/kg body weight) by two subjects. (▲), Subject 1; (●), subject 2; (—), daidzein; (---), equol.

have been identified and postulated as intermediates in the formation of equol and *O*-desmethylangolensin (Adlercreutz *et al.* 1987; Kelly *et al.* 1993; Joannou *et al.* 1995; Heinonen *et al.* 1999). The putative metabolite dehydro-*O*-desmethylangolensin, isolated by Kelly *et al.* (1993), has recently been shown to be an artifact formed during the silylation step of the analytical procedure (Wähälä *et al.* 1998; Heinonen *et al.* 1999).

In man, genistein is purported to be metabolised to dihydrogenistein and 6-hydroxy-*O*-desmethylangolensin (Fig. 1; Setchell & Adlercreutz, 1988; Kelly *et al.* 1993; Joannou

*et al.* 1995; Wähälä *et al.* 1998; Heinonen *et al.* 1999). Coldham *et al.* (1999) and Coldham & Sauer (2000) recently performed an extensive analysis of the metabolism of [<sup>14</sup>C]genistein in rats and have also studied the metabolism *in vitro* using hepatic preparations and caecal extracts. The major metabolite (produced by gut bacteria) was found to be 4-hydroxyphenyl-2-propionic acid. Other studies, in farm animals, have identified *p*-ethylphenol as an important bacterial metabolite of genistein, although firm evidence for its presence in human urine is lacking (Shutt, 1976; Verdeal & Ryan, 1979). However, animal data should be interpreted with caution given the fact that wide interspecies variability in the metabolism of isoflavones has been noted. Many rodents, unlike man, have been shown to very efficient at converting daidzein to equol, and therefore the relevance of using rodent models for investigating the metabolism of isoflavones in man is questionable.

Recent data have provided pharmacokinetic information on the methoxylated isoflavones glycitein, formononetin and biochanin A (Setchell *et al.* 2001) These data demonstrate that formononetin and biochanin A are efficiently demethoxylated to daidzein and genistein, respectively. This is consistent with the known metabolism of these two compounds (Lundh *et al.* 1988). In contrast to these and other data, Zhang *et al.* (1999) have demonstrated high levels of glycitein in plasma following the ingestion of glycitein, indicating low biotransformation of this compound. Recently, urinary analysis by GC-MS has demonstrated



**Fig. 4.** Metabolism of secoisolariciresinol diglucoside and matairesinol in man. (From Borriello *et al.* 1985.)

that 5'-hydroxy-*O*-desmethylangolensin and *cis*-4-hydroxy-equol are metabolites of glycitein (Heinonen *et al.* 1999).

The metabolism of lignans is poorly understood. Jacobs *et al.* (1999) recently identified a number of novel metabolites of enterolactone and enterodiol, but the relative importance of these metabolites in terms of biological efficacy has yet to be elucidated. Heinonen *et al.* (2001) have investigated the *in vitro* metabolism of several plant lignans including matairesinol and secoisolariciresinol, and shown that enterolactone and enterodiol are usually the main metabolites (Table 2). Incubation of secoisolariciresinol diglucoside with human faecal suspensions has recently been shown to generate a wide range of metabolites (Fig. 5; Wang *et al.* 2000; Owen *et al.* 2001).

### Conjugation

Phyto-oestrogens are found predominantly conjugated to glucuronic acid and to a lesser degree to sulphuric acid in plasma and urine (Adlercreutz *et al.* 1993; Coward *et al.* 1993). Only a small portion of the free aglycone has been detected in blood, demonstrating that the rate of conjugation is high (Setchell, 1998). Recent data have indicated that the percentage of circulating aglycones of daidzein and genistein was 8.42 and 3.71% respectively, within 2 h after ingestion of 50 mg of these compounds, decreasing to 2.74 and 1.59% thereafter (Setchell *et al.* 2002a). Conjugation with glucuronic acid and sulphuric acid is considered the main route for deactivation of these compounds. Captive cheetah that were fed a soya-based diet and subsequently suffered infertility and veno-occlusive liver disease were later discovered to be the one animal species that was unable to conjugate isoflavones (Setchell *et al.* 1987).

Conjugation occurs via UDP-glucuronosyl transferase and sulphotransferase in the liver and within the intestinal mucosa (Sfakianos *et al.* 1997). Consequently, free isoflavones are present at low concentrations in plasma. Conjugation of phyto-oestrogens to polar groups like glucuronic acids increases their solubility. The isoflavonoid conjugates excreted in bile sustain extensive enterohepatic circulation (Messina *et al.* 1994; Lee *et al.* 1995) due to their hydrolysis by bacterial  $\beta$ -glucuronidases and sulphatases in the gut and subsequent reabsorption. The conjugates are also removed by the kidney and excreted in urine (Messina *et al.* 1994).

Conjugation was initially thought to occur exclusively in the liver. However, it is now evident that the major site of conjugation is in the enterocyte of the intestinal wall during first-pass uptake. This had been demonstrated when enterolactone, enterodiol and equol were found predominantly conjugated to glucuronic acid in the portal venous blood in human subjects (Setchell, 1998) and confirmed later in rats when [ $^{14}$ C]genistein was infused into the duodenum (Sfakianos *et al.* 1997).

### Distribution

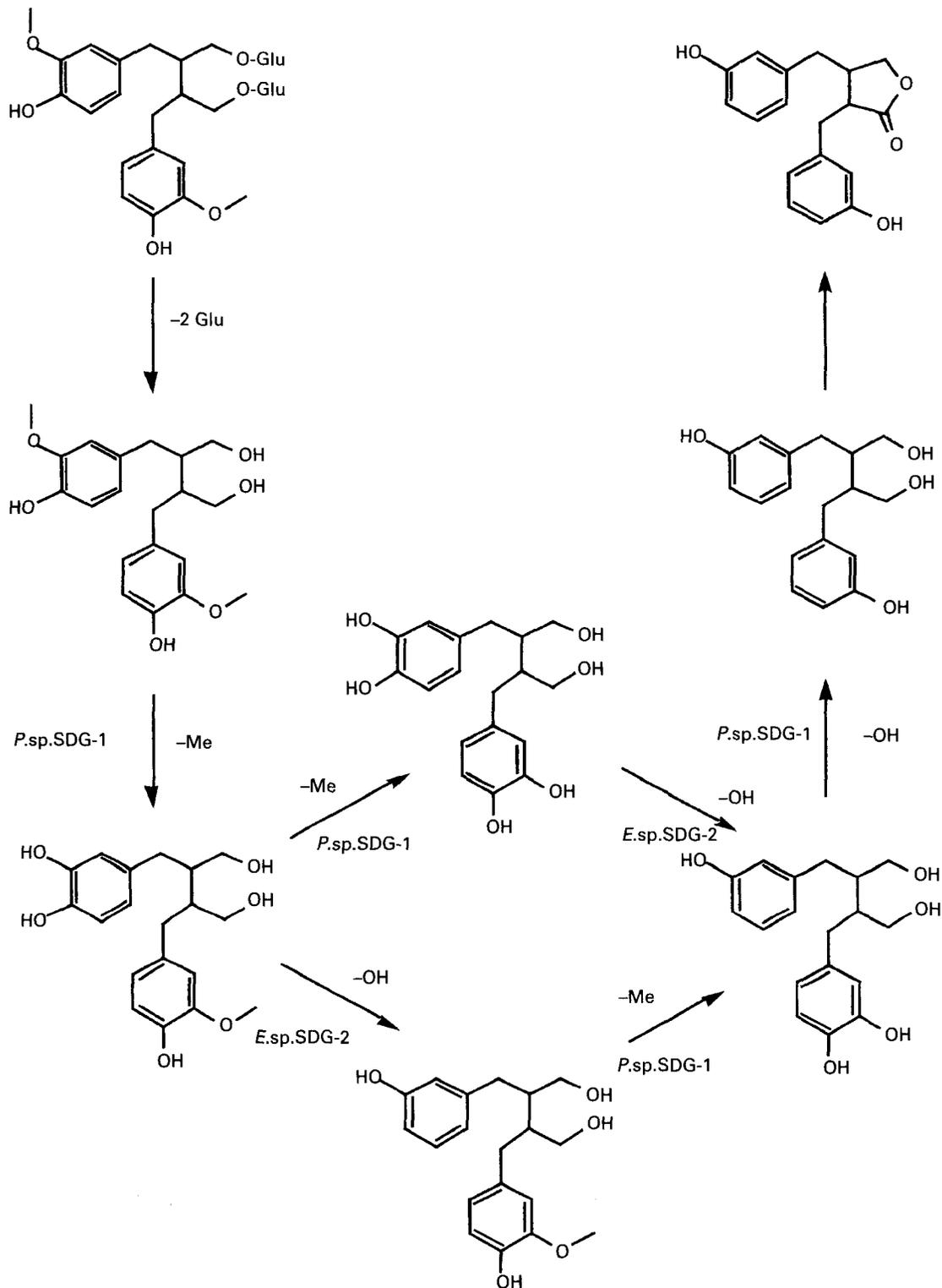
It is evident from existing data that isoflavones enter the bloodstream within 15–30 min of ingestion (Morton *et al.* 1997; Rowland *et al.* 1999). Plasma concentrations achieved following the consumption of approximately 50 mg isoflavones/d in an adult can range from 0.2 to 3.2 nmol/ml (50–800 ng/ml; Setchell & Cassidy, 1999). These levels exceed the circulating levels of plasma oestradiol, which range from 0.14 to 0.28 pmol/ml (40–80 pg/ml; Adlercreutz *et al.* 1993). Oestrogens have a strong binding affinity for serum proteins, such as albumin and sex hormone binding globulin. In contrast, phyto-oestrogens have a considerably weaker binding for such proteins so that, theoretically, there is a greater proportion of free isoflavones available for biological activity compared with endogenous oestrogens (Setchell & Cassidy, 1999).

The extent to which these compounds reach tissues and cells in man is unclear. However, there is recent evidence that high levels of these compounds can be found in breast cells in premenopausal women (Hargreaves *et al.* 1999) and prostatic fluids (Morton *et al.* 1997). Moreover, in animals, isoflavones have been shown to cross the blood-brain barrier (Setchell & Cassidy, 1999; Weber *et al.* 1999). The data indicate that high levels of these compounds are attainable at the cellular level where they may exert biological activity. Recent pharmacokinetic data have demonstrated that daidzein and genistein have a wide  $V_d$  in man that provides further evidence that phyto-oestrogens have a wide tissue distribution (Setchell *et al.* 2001). Setchell *et al.* (2001) demonstrated large  $V_d$  for daidzein and genistein of 236 and 161 litres, respectively. This higher  $V_d$  and faster clearance rate (half-life,  $t_{1/2}$ ) for daidzein explains why genistein levels are

**Table 2.** *In vitro* metabolism of various plant lignans, and potential precursors of enterolactone and enterodiol (Heinonen *et al.* 2001)

Compound	Metabolite(s)
Matairesinol	ENL (62%)
Secoisolariciresinol	ENL + END (72%), ENF
Pinoresinol	ENL + END (55%), ENF minor metabolite
Syringaresinol	ENL + END (4%), other dehydroxylated and demethylated analogues of ENL and END
7'-Hydroxymatairesinol	ENL + 7'-hydroxyENL (23%), other minor metabolites
Arctigenin	ENL (4%), other metabolites
Isolariciresinol	Mostly unchanged, four minor metabolites
Lariciresinol	ENL + END main metabolites, ENF minor metabolite

ENL, enterolactone; END, enterodiol; ENF, enterofuran.



**Fig. 5.** Metabolism of secoisolariciresinol diglucoside (SDG) by human faecal flora *in vitro*. *P.sp.SDG-1*, *Peptostreptococcus* species strain SDG-1; *E.sp.SDG-2*, *Eubacterium* species strain SDG-2. (From Owen *et al.* 2001; Wang *et al.* 2000.)

consistently higher in plasma/serum than daidzein levels when equivalent amounts of the two compounds are ingested and when soya protein products are consumed (Setchell *et al.* 2001).

In clinical trials  $t_{1/2}$  values of daidzein and genistein are between 6 and 8 h (Table 1; Setchell & Cassidy, 1999; Watanabe *et al.* 1998; M Faughnan and A Cassidy, unpublished results). Some data indicated a significantly shorter

$t_{1/2}$  for both daidzein and genistein (King & Bursill, 1998) but this is likely to be due to insufficient sampling (Setchell *et al.* 2001).

### Excretion

The main route of excretion of phyto-oestrogens is via the kidney. To date, urinary excretion has accounted for approximately 30% of the ingested doses, although there is wide interindividual variability (Table 1). The unaccounted dose recovered is probably due to the conversion of parent compounds to metabolites in the intestine that were not identified in urine.

Faecal excretion appears to be minimal, but very few human trials have investigated this issue. Human trials have indicated the faecal excretion of daidzein and genistein is between 1 and 4% of the ingested dose (Xu *et al.* 1995; Watanabe *et al.* 1998). Rodent models have indicated a greater faecal excretion of these compounds, as high as 20%, but this is probably due to the fact that rodents are high excretors of faecal steroids (King, 1998). Supplementation with 10 g flaxseed/d resulted in significant increases ( $P < 0.01$ ) in faecal lignan excretion from 727 to 12 871 nmol/d (Kurzer *et al.* 1995).

### Interindividual variation in metabolism of phyto-oestrogens

A number of groups have reported large interindividual differences in plasma levels and urinary excretion of both parent isoflavones and metabolites, even in subjects consuming controlled amounts of phyto-oestrogen-containing foods.

The studies listed in Table 1, which have specifically looked at excretion following a single oral bolus dose of isoflavones, have demonstrated a wide range of urinary excretion. For example, M Faughnan and A Cassidy (unpublished results) found urinary isoflavone recovery to extend from 15 to 60% across a range of intakes from 0.45 to 1.80 mg/kg body weight. This has also been noted during chronic feeding studies such as that of Karr *et al.* (1997), who observed a twelve-fold and fifteen-fold interindividual variation for genistein and daidzein excretion, respectively, within soya treatments. Rowland *et al.* (2000) reported a sixteen-fold range in total isoflavonoid excretion in urine after feeding soya to twenty-two human volunteers.

Much of the variability between individuals may in part be explained by differences in phyto-oestrogen metabolism. Overall, data indicate that approximately 33% of individuals in Western populations, where soya is not a common component of the diet, have the ability to produce equol. Excretion of equol and *O*-desmethylangolensin exhibited even greater variation between subjects than that of their parent compound, daidzein. Karr *et al.* (1997) detected a 180-fold difference in *O*-desmethylangolensin excretion, while Rowland *et al.* (2000) found a 600-fold variation in equol excretion. Setchell *et al.* (1984) found an increase of up to 1000 times baseline levels in equol excretion within 24 h in some subjects who were

administered 40 g of textured soya protein for five consecutive days.

Variations in lignan metabolism have also been reported. Nesbitt *et al.* (1999) showed that two out of nine subjects produced only small amounts of enterolactone after flaxseed ingestion. Similarly, Lampe *et al.* (1998) and Rowland *et al.* (2000) reported a wide range of excretion of enterolactone and enterodiols in human subjects.

### Factors influencing absorption, distribution, metabolism and excretion

Kelly *et al.* (1993) suggested that this variability in urinary excretion might be caused by inherent rather than dietary factors, as consistent concentrations of the various metabolites appeared in the urine of several subjects who were studied repeatedly over a number of years. However, a number of factors have been proposed to influence phyto-oestrogen bioavailability and these include intestinal microflora, gender, age, food matrix, chemical composition, early exposure and background diet (Kelly *et al.* 1993; Axelson *et al.* 1984; Setchell *et al.* 1984; Hutchins *et al.* 1995; Knight & Eden, 1995). These are discussed in more detail below.

#### Microflora

The importance of the microflora in the metabolism of isoflavonoids and lignans suggests that variation in microflora composition plays an important role in the interindividual variation of absorption and metabolism. It has been suggested that the inability of some individuals to metabolise daidzein to equol is due to the absence of certain bacterial species in the intestine, but this theory remains to be investigated systematically (Rowland *et al.* 1999; Setchell & Cassidy, 1999). Other factors that may affect metabolism are different dietary substrates that alter the microflora environment, e.g. pH, substrate availability and redox potential (Mallett & Rowland, 1988).

The inverse relationship noted between equol and *O*-desmethylangolensin production from daidzein in some studies suggests interindividual variation in the preferred pathways of metabolism of ingested isoflavones and subsequent excretion (Kelly *et al.* 1993, 1995; Hutchins *et al.* 1995; Karr *et al.* 1997). This may have important implications as equol is known to be more oestrogenic than *O*-desmethylangolensin or daidzein in *in vitro* model systems, has greater anti-oxidant activity (Rowland *et al.* 1999) and has a longer time in circulation (Lampe *et al.* 1998).

#### Gender

Setchell *et al.* (1984) reported the excretion of equol and other isoflavonoids after ingestion of soya to be sex-independent, as both females and males can excrete substantial amounts, a finding that was subsequently confirmed (Kelly *et al.* 1993; Kirkman *et al.* 1995; Lampe *et al.* 1998). However, there is some evidence that males and females respond differently to the chronic exposure of isoflavones, although there are considerable inconsistencies in the data. Lu & Anderson (1998) found that the recovery of

daidzein and genistein decreased and that of equol increased in females following the daily ingestion of soya milk for 1 month. In males daidzein and genistein recovery increased. These data indicate a gender difference in the response to chronic feeding but warrant further investigation.

#### Age

During the first few months of life it has been demonstrated that there is an inability to convert daidzein to equol (Setchell *et al.* 1997). This has been attributed to the presence of an immature gut microflora. The effect of age later on in life on the bioavailability of phyto-oestrogens is unknown.

#### Chemical composition of phyto-oestrogens

The chemical composition of isoflavones fed to man may be important in the metabolism of these compounds. Hutchins *et al.* (1995) found that chronic feeding of tempeh, a fermented soya product that contains predominantly aglycones, resulted in greater urinary recoveries of daidzein and genistein than a non-fermented soya (soya-bean pieces) product. This study indicated that the aglycones from fermented soya products might be more bioavailable. However, recent data, based on assessment of plasma concentrations over a 48 h period, demonstrated a greater bioavailability of pure glucoside conjugates of daidzein and genistein compared with aglycones (Setchell *et al.* 2001). This later finding is in agreement with flavonoid bioavailability trials (Hollman & Katan, 1998). In a recent study, Izumi *et al.* (2000) found that isoflavone aglycones were more bioavailable than their respective glucosides due to the fact that they attained higher peak concentrations. However, no attempt was made at calculating area under the curve, which would have been a more accurate assessment of bioavailability. The aglycones and glucosides were administered in two different matrices, making comparisons difficult. In addition, Setchell *et al.* (2001) found no equol production in individuals fed the aglycone compared with 30% of individuals producing equol when fed the glucoside conjugate.

The urinary recovery of the ingested dose is generally greater for daidzein than genistein and a number of authors have suggested that daidzein is more bioavailable than genistein (Xu *et al.* 1994; King, 1998). It is apparent from these data that genistein may be more subject to microbial metabolism than daidzein. Interestingly, a recent trial demonstrated that the recovery of glycitein was greater than that of daidzein, which in turn was greater than that of genistein (Zhang *et al.* 1999). However, there is no strong evidence indicating differing degrees of metabolism of these compounds. Also of interest, when the supplement Premensil (Novagen), which contains predominantly biochanin A and formononetin in the aglycone form, was fed to one individual, serum contained principally daidzein and genistein (Setchell *et al.* 2001). This demonstrates the excellent conversion of these methoxylated isoflavones into genistein and daidzein.

#### Food matrix

The effect of the food matrix remains to be investigated but is likely to have a major impact on the pharmacokinetics of these compounds. Supplements are likely to be absorbed at a faster rate compared with isoflavones ingested within a food matrix, as noted by Izumi *et al.* (2000).

#### Background diet

Adlercreutz *et al.* (1991) suggested that individuals who have high fat and meat intakes might harbour the gut microflora required for the conversion of daidzein to equol. This conclusion was reached on observing that excretion of equol, unlike other isoflavonoids, was positively correlated with total intakes of fat ( $P < 0.01$ ) and meat ( $P < 0.05$ ) and fat:fibre ( $P < 0.05$ ). Other data indicate that the intake of fibre has an adverse effect on the absorption of isoflavonoids (Tew *et al.* 1996). In contrast, other studies disagree with these findings and suggest that equol formation is more prevalent in individuals who consume carbohydrate as a high percentage of energy intake, have high dietary fibre intake and low fat:fibre (Lampe *et al.* 1998; Rowland *et al.* 1999), as this promotes fermentation in the colon and thus greater breakdown of daidzein to equol. It has also been shown in an *in vitro* colon model that a high-carbohydrate diet facilitates the conversion of daidzein to equol (Cassidy, 1991). These contradictory findings may be attributable to variations in age, diet and intestinal flora populations of the various individuals studied (Lampe *et al.* 1998).

#### Chronic exposure

Information on the effect of chronic feeding on the bioavailability of phyto-oestrogens is scant. Feeding 50 mg isoflavones/d for seven days had no significant effect on the pharmacokinetic behaviour of a single oral bolus dose of either [ $^{13}\text{C}$ ]daidzein or [ $^{13}\text{C}$ ]genistein (A Cassidy, unpublished results). In the study of Lu *et al.* (1995b, 1996), three of twelve women studied developed the ability to produce equol after 2 weeks of soya ingestion, suggesting that chronic soya ingestion may alter the metabolic pathways of daidzein and contribute to interindividual variation. This phenomenon was not observed in the male subjects. Chronic feeding of isoflavones (36 oz soya milk/d, three times daily) over a period of 1 month resulted in an increased recovery of equol in females ( $n = 3$ ; Lu *et al.* 1995b). These data indicate that, in women, chronic feeding may alter the metabolism of isoflavones. However, these trials have been carried out using very small numbers of subjects so it is difficult to draw firm conclusions.

#### Early exposure

Recent evidence has demonstrated that phyto-estrogens cross the placenta (Adlercreutz *et al.* 1999). Soya infant formulas contain considerable levels of isoflavones, with concentrations of between 32 and 77 mg/l, compared with 5–6  $\mu\text{g/l}$  found in breast milk (Setchell *et al.* 1997). Infant formulas can provide doses six- to eleven-fold

higher, on a body weight basis, compared with adults. Plasma isoflavones in 4-month-old infants reflected this higher dosing, with levels ranging from 654 to 1775  $\mu\text{g/l}$  (Setchell *et al.* 1997). Infants are unable to metabolise daidzein and genistein due to the inability of an immature intestinal microflora to biotransform these compounds.

### Conclusions

The bioavailability of phyto-oestrogens is clearly a crucial factor influencing the biological activity of these compounds. Most information is available for the isoflavonoid phyto-oestrogens derived from soya products, although work has focused on genistein and daidzein with less emphasis on glycitein. Less research has been conducted on the lignan phyto-oestrogens, reflecting perhaps the difficulties in quantifying their presence in foods.

There is considerable interindividual variation in absorption and metabolism of phyto-oestrogens, which may influence the biological effects of the compounds in man.

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