ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION OF PHYTOESTROGENS: INTER-INDIVIDUAL VARIATION AND THE ROLE OF THE GUT MICROFLORA

SUMMARY

1.   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.

2.   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.

3.   Phytoestrogens and their metabolites appear to be widely distributed within body fluids, although extensive tissue distribution studies have not been performed. There is evidence of transfer of isoflavonoids to the foetal compartment at levels similar to those in maternal plasma and the occurrence of post-natal exposure through transfer in breast milk.

5.   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.

6.   As a consequence, the activity of the gut microflora may influence the bioavailabilities of phytoestrogens and/or their metabolites. Since biological activities/potencies of parents and metabolites, this could have implications for the determination of any ensuing biological response, beneficial or adverse.

7.   There have been relatively few studies undertaken that comprehensively describe the pharmacokinetics of ingested isoflavonoids and lignans. Data indicate considerable inter-individual differences in pharmacokinetic profiles. Such differences may be largely attributed to an individual's unique gut microflora, which is influenced by factors including diet, particularly fibre content, and intestinal transit

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time, hygiene, antibiotic use, bowel disease, stress, gut motility, gastric pH, mucin secretion, and bile secretion. Sex, genetics and ethnicity may also be determining factors.

8.   There is limited information available concerning the absorption, distribution and metabolism in the new-born but data suggest that infants can absorb significant levels of phytoestrogens from soy-based formulas. However, the pharmacokinetic handling of phytoestrogens by the neonate is likely to differ considerably from that of the adult.

METABOLISM AND ABSORPTION OF INGESTED PHYTOESTROGENS

Isoflavones
9.   Following ingestion, the glycosidic forms of the isoflavone are hydrolysed to their unconjugated forms, largely under the action of the gut microflora, via β-glucuronidase enzymes. Some acid hydrolysis may occur in the stomach (Kelly et al, 1993), although there is some disagreement about this (Piskula et al, 1999). 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 (Day et al, 1998), thus providing hydrolysis independent of the gut-bacteria or stomach acid.

10.   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 (Hollman et al, 1997) 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).

11.   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 (Bingham et al 1998; Setchell 1998; Knight and Eden, 1996, references therein), via hepatic UDP-glucuronosyl transferase or sulphotransferase enzymes, or within the intestinal epithelium, which has also been shown to possess glucuronosyl transferase and sulphotransferase activity (Sfakianos et al, 1997). As a consequence, very little free isoflavonoid is present in the circulation.

12.   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 (Kelly et al, 1993; Joannou et al 1995, Adlercreutz et al, 1987; Yasuda and Ohsawa, 1998**.** See Figure 1). It has been demonstrated that some aglycones may undergo CYP-mediated metabolism (Roberts-Kirchoff et al, 1999). Incubations of genistein in the presence of

human recombinant CYP1A1[1], 1A2, 1B1[1] 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 flavonoid galangin to kaempferol then to quercetin in rat liver microsomes is thought to be CYP-mediated, with the latter step being attributed specifically to CYP1A1[1] (Silva et al, 1997). 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 (Peterson et al, 1998).

Figure 1 (from Joannou et al, 1995).
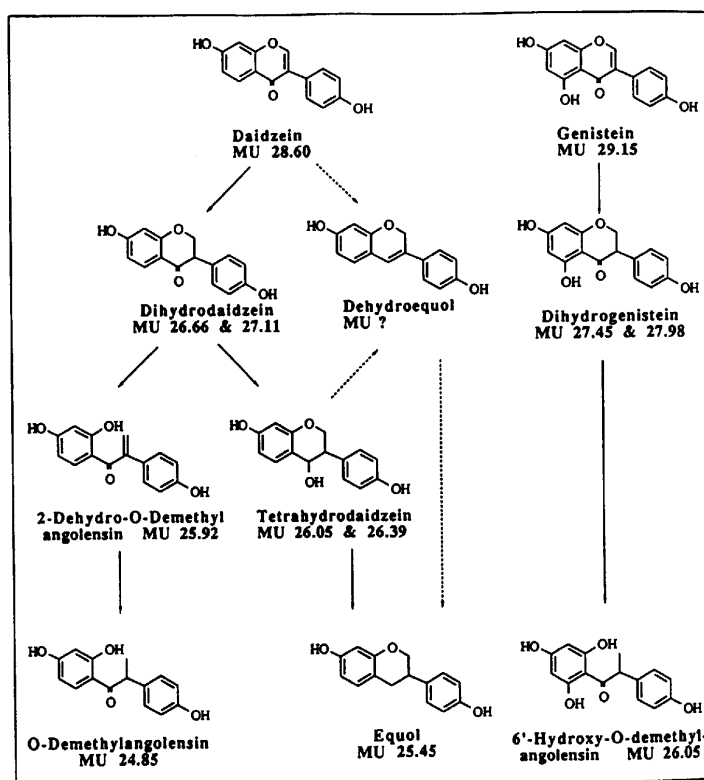
Scheme 3. Proposed metabolic pathways of catabolism of daidzein and genistein based on the urinary isoflavonoid metabolites found so far in human urine. Pathway through dehydroequol remains to be substantiated.

13.   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 (Setchell, 1998, references therein).

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[1] CYP1A1 and CYP1B1 are constitutively low or absent in liver

Lignans

14. 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 (Setchell and Adlercreutz, 1988; Kurzer and Xu, 1997 – see Figure 2). Once absorbed, the lignans are conjugated with glucuronic acid (Setchell, 1998 and references therein).

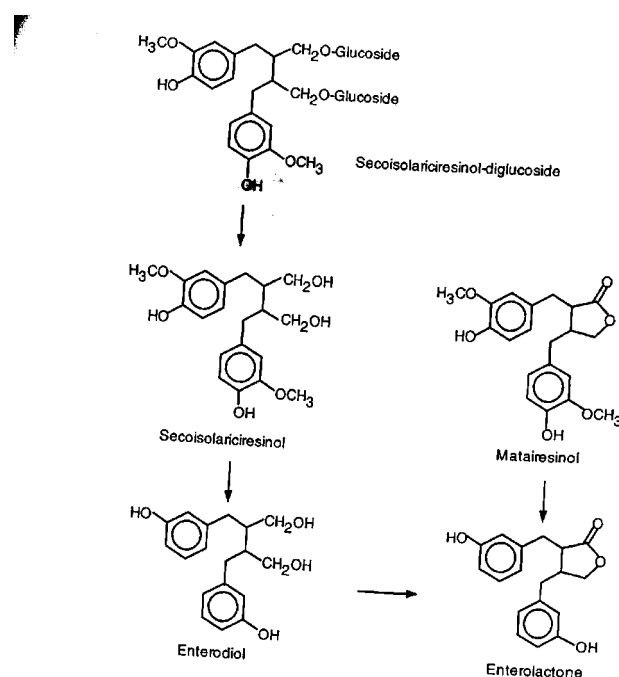**Figure 2 (From Kurzer and Xu, 1997).**

Figure 4 Formation of enterolactone and enterodiol by human fecal flora (modified from 28). Secoisolariciresinol diglucoside is metabolized to enterodiol through hydrolysis of the sugar moiety, dehydroxylation, and demethylation. Enterodiol can then be further oxidized to enterolactone. Matairesinol is converted to enterolactone by gut bacteria through dehydroxylation and demethylation.

Coumestans

15. The metabolism of the coumestans has not been well characterised.

EXCRETION AND ENTEROHEPATIC CIRCULATION

Excretion as an index of exposure

16. 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

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individuals consuming macrobiotic diets tend to have higher urinary excretion of phytoestrogens, particularly lignans. This is illustrated by data presented in Figure 3 (Adlercreutz et al, 1987). Intervention studies suggest that urinary excretion of isoflavones increases with increased soy intake, but absorption, as reflected by urinary excretion, may be saturable at high doses (Karr et al 1997; Bingham et al 1998: Setchell, 1998 and references therein).

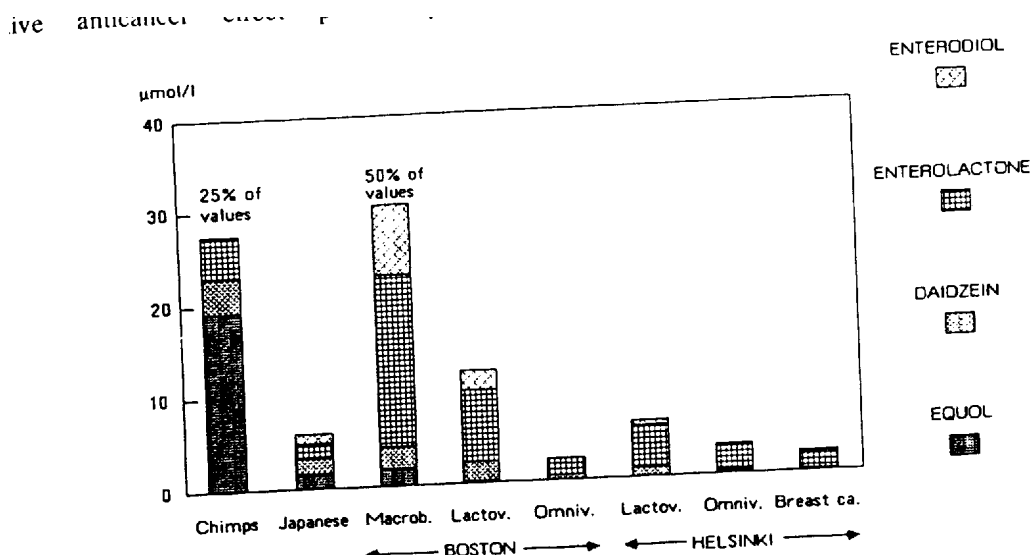**Figure 3 (From Adlercreutz et al, 1987).**

Fig. 2. Mean urinary concentration of daidzein, equol, enterolactone and enterodiol in chimpanzees, and in young women on various habitual diets. The values shown for chimpanzees are 25% of the actual values and those for the macrobiotics are 50% of the actual values.

Urinary concentration of total lignans and isoflavonoids in young macrobiotic (n=13), lactovegetarian (n=11) and omnivorous (n=10) Boston women and young lactovegetarian (n=12) and omnivorous (n=12) women living in Helsinki, and a group of breast cancer patients (n=12), also from Helsinki, compared with a group of young women from Japan consuming their traditional diet. A group of male chimpanzees in captivity (n=6) consuming their normal diet were also included. [2]

17.   Conjugates identified in human urine include those of formononetin, methyl-equol, daidzein, dihydrodaidzein, *O*-DMA, genistein, 3',7-dihydroxyisoflavan, equol, matairesinol, lariciresinol, isolariciresinol, seicoioslariciresinol and tentatively identified 7α-OH matairesinol and 7'-OH enterolactone (Adlercreutz et al, 1995 and references therein). 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.

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[2] In the text Adlercreutz et al (1987) state that the urinary concentrations of lignans and isoflavones are expressed as nmol/L. However, in the figure they are given as μmols/L.

DISTRIBUTION

18.   Lignans and isoflavones have been detected in urine, plasma, faeces, prostatic fluid, semen, bile, saliva, breast milk, and breast aspirate or cyst fluid. Daidzein, genistein, equol and *O*-DMA are the major isoflavonoid metabolites detected in the blood and urine of humans and animals (Adlercreutz et al, 1995; Knight and Eden, 1996 and references therein). Lignans identified in human urine include enterolactone, enterodiol, lariciresinaol and isolariciresinol (Adlercreutz et al, 1987, references therein).

19.   There are few data concerning the tissue distribution of isoflavones. Yueh and Chu (1977) reported relatively high 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 (unpublished but referred to by Setchell, 1998) 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.

20.   High levels of isoflavonoids (daidzein, genistein, *O*-DMA, equol) and low levels of lignans (enterolactone and enterodiol) have been found in healthy Japanese neonates, born to mothers consuming soy-rich diets during pregnancy (measured in maternal and cord plasma and amniotic fluid), indicating that these phytoestrogens can cross from the maternal to the foetal compartment. Concentrations in the neonates were similar to those found in maternal plasma (Adlercreutz et al, 1999). The metabolic handling of the isoflavonoids within the foetus/neonate is likely to be impaired, due to reduced glucuronidation capacity, which will affect elimination.

PHARMACOKINETICS AND BIOAVAILABILITY OF PHYTOESTROGENS

21.   Many studies have examined the excretion profiles of phytoestrogens following challenge with isoflavones or lignans. However, relatively few studies have examined the pharmacokinetics of these compounds in plasma.

Pharmacokinetics of isoflavonoid compounds
22.   The most complete pharmacokinetic study of soybean isoflavones so far is probably that reported by Watanabe et al (1998). Profile data from plasma, urine and faeces were collected from seven adult males, previously maintained on otherwise low isoflavone diets, following the ingestion of 60 g of baked soybean powder containing 103 µmol daidzein and 112 µmol as genistein. Data are illustrated in Figures 4-8 and Table 1. Plasma genistein was significantly increased after just 2 hours and reached a peak concentration at about 8 hours. Daidzein concentration peaked at approximately the same time but did not achieve the level of genistein (Figure 4).

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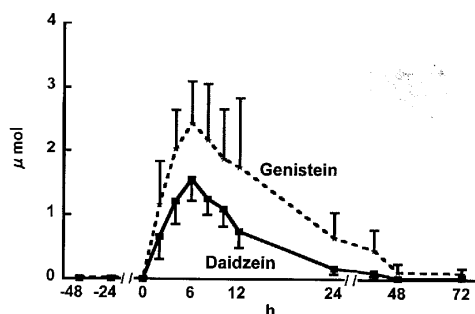
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Figure 4 (From Watanabe, 1998)

FIGURE 1 Plasma genistein and daidzein concentrations in men after ingestion of 60 g kinako (baked soybean powder). Each point represents the mean ± SD, n = 7.

23. Plasma levels of O-DMA and equol peaked after genistein in 2 and 4 of the 7 subjects, respectively (Figure 5). In contrast to plasma, daidzein was the major urinary
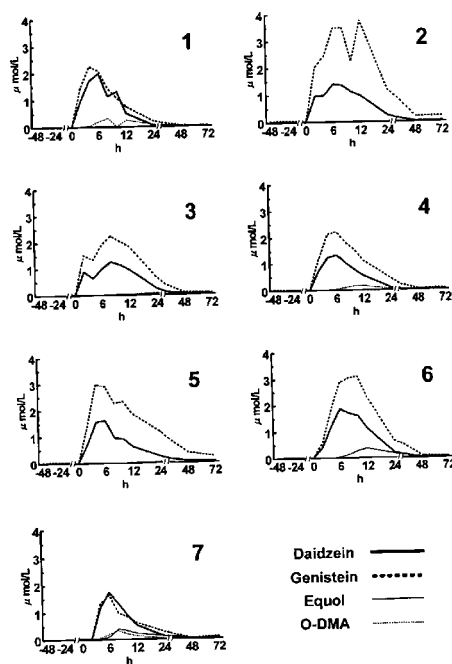
Figure 5 (From Watanabe, 1998)

FIGURE 2 Changes in plasma genistein, daidzein, O-desmethy-langlolensin (O-DMA) and equol concentrations in seven men after ingestion of 60 g kinako (baked soybean powder).

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component (Figure 6 A and B). Respective recoveries of ingested daidzein and genistein in urine were 35.8% and 17.6 % and probably reflected the higher polarity of daidzein conjugates compared to those of genistein.
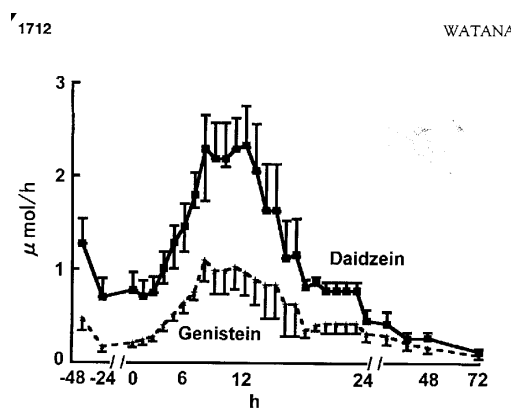
Figure 6 (From Watanabe, 1998)

A

B

FIGURE 3   Urinary daidzein and genistein excretion after ingestion in men of 60 g kinako. Each point the the mean ± SD, n = 7.
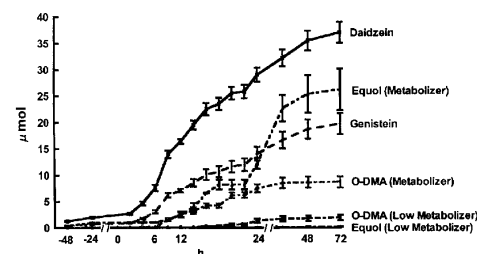
FIGURE 4   Cumulative excretions of daidzein, genistein, O-des-methylanglolensin (O-DMA) and equol in the urine of men after ingestion of 60 g kinako (baked soybean powder). "Metabolizers" excreted metabolites of daidzein in the urine at levels >1% of ingested daidzein. Values are mean ± SD, n = 7.

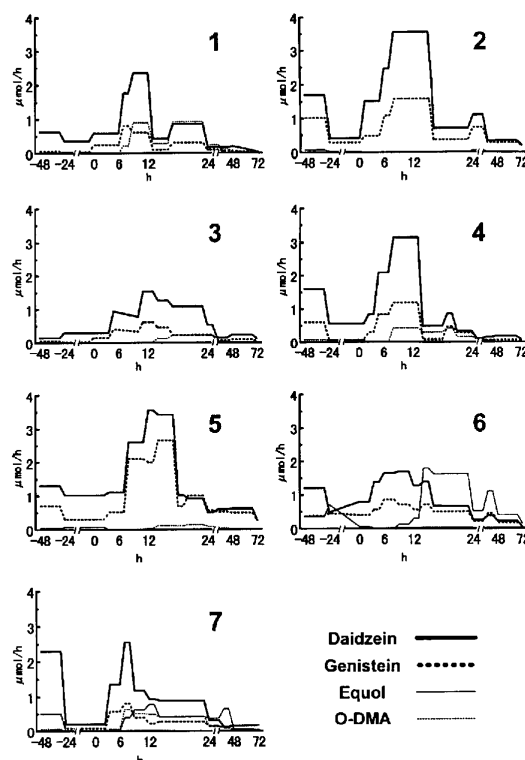## Figure 7 (From Watanabe, 1998)

FIGURE 5   Different patterns of urinary daidzein, genistein, O-des-methylanglolensin (O-DMA) and equol excretion in seven men after ingestion of 60 g kinako (baked soybean powder).

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24.   Equol excretion was observed in only 2 subjects (6 and 7) and followed peak daidzein excretion (Figure 7).

25.   The majority of ingested isoflavones in faeces was recovered in the second and third days after ingestion (Figure 8). Excretion of total diphenolics, including equol, was often higher in the second and third days than in the first 24 hours following ingestion, suggesting that faecal isoflavones and equol represented biliary excretion. Total recovery of daidzein and its metabolites, O-DMA and equol, from urine and faeces was ~55% and~20% of administered genistein was recovered as genistein.

26.   Plasma half-lives for genistein and daidzein were 8.4 and 5.8 hours, respectively. There was significant inter-individual variation of plasma and urinary equol and O-DMA. The concentrations of genistein and/or daidzein peaked twice in some of the subjects, in plasma and particularly in urine, most likely due to enterohepatic circulation.

27.   Husband et al (1999) 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
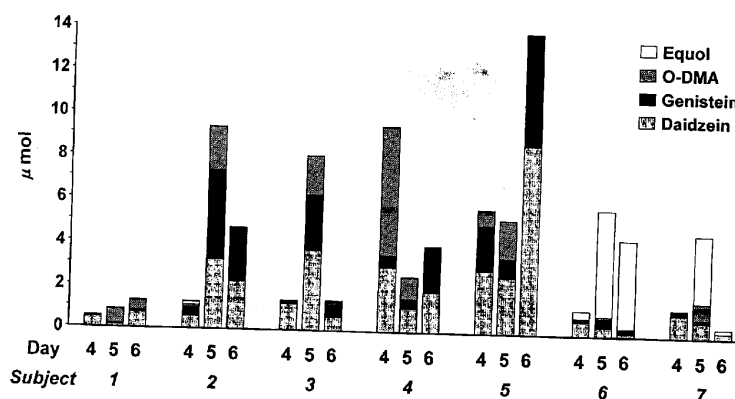
Figure 8 (From Watanabe, 1998)

FIGURE 6 Excretion in the feces of daidzein, genistein, O-desmethylanglo-lensin (O-DMA) and equol isoflavones and equol in seven men after ingestion of 60 g kinako (baked soybean powder). Day 4 is the first day after ingestion of kinako.

Note: In figure 8, days 4, 5 and 6 refer to the day of administration with breakfast (first day) and the second and third days after ingestion, respectively.

Table 1 (from Watanabe et al, 1998)

TABLE 1

Total recovery of isoflavones and isoflavane equol in urine and feces of men for 3 d after ingestion of 60 g kinako (baked soybean powder)

Subject	Daidzein		O-DMA		Equol		Total	%	Genistein	
	Urine	Feces	Urine	Feces	Urine	Feces			Urine	Feces
					μmol			*%*	*μmol*	
1	27.28	1.08	11.70	0.99	0.16	0.08	51.60	24.0	10.12	0.20
2	47.01	5.28	0.97	1.90	0.22	0.21	81.53	37.9	27.24	6.15
3	28.06	4.91	3.55	1.78	0.07	0.10	49.94	23.2	11.02	2.78
4	29.67	5.28	6.16	6.12	0.13	0.05	58.27	27.1	11.92	2.69
5	64.56	12.59	2.38	2.14	0.30	0.11	128.67	59.8	47.91	7.14
6	32.07	1.07	0.59	0.08	33.51	8.21	92.03	42.8	20.10	0.73
7	30.16	1.77	8.22	0.74	18.80	2.93	69.67	32.4	9.68	0.24
Mean	36.97	4.57	4.80	1.97	7.60	1.67	75.96		19.71	2.85
SD	13.90	4.04	4.10	1.97	13.37	3.07	27.96		14.03	2.82
Recovery, %	35.8	4.4	4.01	1.91	7.01[1]	1.61	35.3		17.6	2.5

[1] These values are the percentage of recovery of daidzein ingested.
[2] Percentage of recovery of daidzein, including its metabolites in urine and feces together. O-DMA; O-desmethylangolensin.

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. [3]

28.   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 (King and Bursill, 1998). 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 Watanabe et al (1998). However, a study by Setchell et al (referred to in Setchell, 1998), 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.

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[3] The accumulation of daidzein and genistein observed in this study seems inconsistent with the plasma half-lives for these compounds determined in other studies. This may indicate that the pharmacokinetics of daidzein and genistein are altered on repeated dosing .

29.   In a study in women, reported by Xu et al (1994), plasma concentrations of daidzein and genistein were both significantly increased after 6.5 hours and ~ equal to each other following consumption of a soy milk powder drink containing isoflavones (44% of which was daidzein and 56% genistein). Faecal excretion was very low (1-2%). On the basis that urinary excretion of daidzein (21%) was much higher than that of genistein (9%), the authors concluded that daidzein had the greater bioavailability.

30.   Coward et al (1996) reported that the molar ratios of genistein to daidzein in plasma did not vary significantly from that present in the administered soy beverage, thus suggesting similar bioavailabilities of the two isoflavones. However, when administered to rats as conjugates in the form of a soy extract, daidzein was reported to be more bioavailable than genistein (King, 1998). It was suggested that there may be a difference in susceptibility to hydrolysis and/or that daidzein may be more resistant to metabolism since the excretion of the genistein metabolite, *p*-ethyl-phenol, was much greater than the excretion of the daidzein metabolite, equol. Interestingly, Zhang et al (1999) 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.

Glycosidic versus aglycone forms
31.   Some data have suggested that the availability of isoflavonoids in food may be greater when they are in the form of aglycones, as in fermented soy, than when they are present as glycosides, as in unprocessed soy. Hutchins et al (1995) reported that the recovery of urinary daidzein and genistein was higher when subjects consumed a diet consisting of tempeh (fermented soy) compared to when they consumed a similar daily dose of isoflavones in the form of unfermented soy pieces. Total isoflavone urinary excretion was similar following both diets. Slavin et al (1998) reported that while fermentation of soy decreased the isoflavone content of a product, it increased urinary isoflavonoid recovery, thus also suggesting that fermentation increased the availability of isoflavones in the soy. Animal studies, however, have indicated that, although the initial rates of absorption and excretion for the aglycone forms were greater than for the conjugated forms, the total percentage recoveries in urine (and in faeces) did not differ significantly. Consequently, the extent of absorption and the bioavailabilities of aglycone and glycosidic forms were considered to be similar (King et al, 1996). In further contrast, however, Setchell (1998), refers to a study where the glycosidic conjugates of daidzein and genistein were more bioavailable than their aglycones.

Transfer to breast milk
32.   Franke and co-workers (Franke and Custer, 1996; Franke et al, 1998 *a & b*) 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 (Figure 9). Maximum levels were attained 10-14 hours after ingestion and were followed by a smaller secondary peak. This biphasic pattern had also been observed in plasma and urine and was thought to be a reflection of the process of enterohepatic circulation. A return to baseline levels was complete within 2-4 days. The pattern of metabolites found in the breast milk reflected that observed in plasma.

Figure 9 (from Franke et al, 1998).
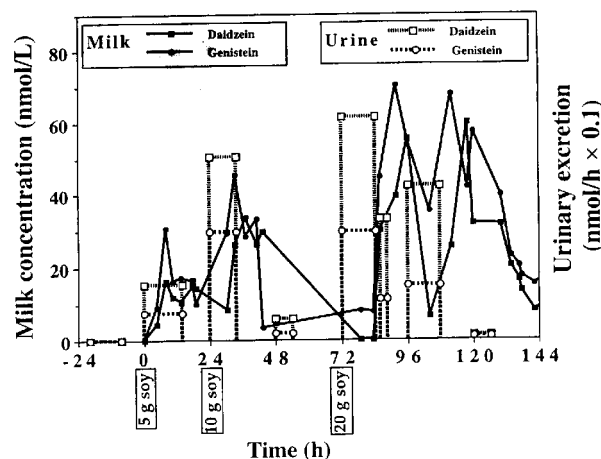
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FIGURE 3. Daidzein and genistein concentrations in human breast milk and urine after a challenge with 5, 10, and 20 g roasted soybeans given at 0, 24, and 72 h. Concentrations were determined by HPLC with diode-array ultraviolet scanning. Milk samples were collected at the time of feeding. Six overnight urine samples and 2 additional samples, collected 12 h before and 87 h after the first soybean intake, were obtained to monitor urinary isoflavone excretion.

Pharmacokinetics of lignan compounds

33.   Nesbitt et al (1999) 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. Significant increases over baseline in plasma lignan levels were observed 9 hours after the first ingestion on day 1. These increases were maintained at 12 and 24 hours[4]. Significantly more lignan was excreted within the 12-24 hour period than within the first 12-hour period. The plasma AUC on day 8 was greater than on day 1, indicating accumulation of the lignans. Urinary excretion was the same for both the 0-12 and 12-24 hour collection periods and was greater than on day 1.

34.   The greater time required for the lignans to achieve peak plasma concentrations, compared to isoflavonoids, was also observed by Morton et al (1994, 1997). 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

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[4] Plasma half-lives for the lignans could not be determined from the given data. However, the data did indicate a slow absorption and/or excretion of these compounds.

not observed until 8.5 hours following consumption. After 24 hours, plasma concentrations of both lignans and isoflavonoids remained above pre-dose levels. The mean 24 hour plasma AUCs on day 1 and day 8 were 1027±96 and 1840±343 nmol.hr/L, respectively. These values differed significantly from each other (P<0.01).
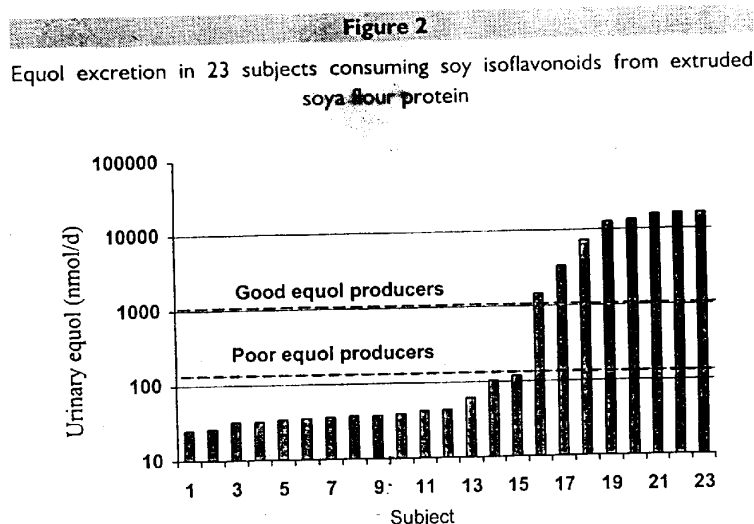
Summary of pharmacokinetic findings

35.   There is a relative paucity of data regarding the pharmacokinetics of phytoestrogens, particularly for the lignans. However, to summarise the isoflavonoid data, only one study has concurrently profiled the appearance and disappearance of soy-derived isoflavones and their major metabolites in plasma, urine and faeces. Few studies have determined metabolite profiles as well as the parent isoflavones. The total recovery of dose is not stated and unlikely to be 100% since the full metabolic fate of the dose is never established. Differences in findings between studies regarding various pharmacokinetic parameters for genistein and daidzein are probably a reflection of the different forms in which the isoflavones were administered (e.g. soy flour based meal versus soy milk powder drink versus fermented soy versus purified isoflavones given as a single bolus), the individuals used as subjects (sex, age, usual dietary habits) and/or study design (e.g. sampling points).

36.   Nonetheless and generally speaking, it would appear that, if present, methylated precursors of genistein and daidzein undergo extensive and rapid demethylation prior to absorption. Following ingestion, plasma levels of genistein and daidzein begin to rise within 2 hours, and maybe as early as 15 min. Peak concentrations of daidzein and genistein are achieved in 5-8 hours. Some individuals show more than one plasma peak, probably reflecting the occurrence of enterohepatic circulation. Plasma half lives for genistein and daidzein have been found to range between 5-8 hours. If a difference has been found between genistein and daidzein in terms of plasma half-life, daidzein has always shown the shorter. Onward from this, there is also conjecture as to whether the bioavailability of genistein is greater or similar to that of daidzein. It is likely that this may depend upon the form in which the isoflavones are presented since in the pure form, the bioavailability of genistein and daidzein were shown to be the same. Similarly, there is conjecture as to whether aglycones, as present in fermented soy products, are more available than the glycosides. This may ultimately be attributed to inter-individual variation and gut microflora (see below). Studies in rats have shown the bioavailabilities of aglycones and glycosides to be similar. The majority of isoflavones are excreted in urine, either as parent compound or metabolites with only a small percentage of absorbed isoflavones ending up in the faeces either as parent compounds or metabolites. The available data clearly indicate considerable inter-individual variation in plasma and excretion profiles for daidzein and genistein and their metabolites, with some (5/7) individuals producing little or no O-DMA and equol (which is consistent with findings of Kelly et al, 1995; Setchell et al, 1984; Morton et al, 1994; Slavin et al 1998; Lampe, 1998; Rowland et al, 1999 - see paragraph 37). Repeat dosing of amounts of isoflavones present in some supplements (e.g. Promensil containing 40 mg of total isoflavones) may lead to accumulation[3].

3

37.   Human metabolism and excretion of isoflavones and lignans are subject to considerable inter-individual variation (Setchell, 1998, references therein). Kelly et al (1993) 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 (Kelly et al, 1995; Setchell et al, 1984; Morton et al, 1994; Slavin et al 1998; Lampe, 1998; Rowland et al, 1999. See Figure 10). 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, 1998, references therein) and equol and *O*- DMA and 6'OH-*O*-DMA (Kelly et al, 1993) excretion. Presumably, low equol excretors are also subject to increased plasma levels of precursor compounds.

Figure 10 (from Rowland et al, 1999)

Figure 2

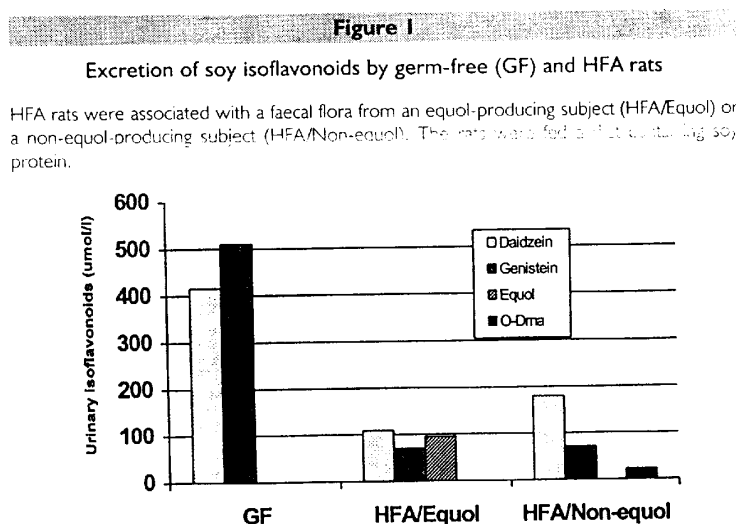Equol excretion in 23 subjects consuming soy isoflavonoids from extruded soya flour protein

38.   Furthermore, Nesbitt et al (1999) showed that 2 of 9 female subjects produced little or no enterolactone following ingestion of lignans from flaxseed and Lampe et al (1994) 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.

Importance of gut microflora
39.   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) showed that incubation of textured vegetable protein with cultured human faecal bacteria resulted

in the formation of equol while Chang and Nair (1995) 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 (Setchell et al, 1981) 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 non-equol- producing human, produced no detectable urinary equol and only trace amounts of *O*-DMA, when fed with soy (Figure 11). 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

Figure 11 (from Rowland et al, 1999)

Figure I

Excretion of soy isoflavonoids by germ-free (GF) and HFA rats

HFA rats were associated with a faecal flora from an equol-producing subject (HFA/Equol) or a non-equol-producing subject (HFA/Non-equol). The rats were fed a diet containing 50% protein.

HFA = human-flora-associated rats
GF = germ-free rats

metabolite was detected in plasma (Rowland et al, 1999; Setchell 1998; Adlercreutz et al, 1987, references therein). 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 (Pettersson et al, 1996).

40.   From the results of a study monitoring the gut microflora metabolism of isoflavones in 14-20 subjects, Hendrich et al (1998) have suggested that there may be

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 mediocre equol producers.

Bacterial activity in the human intestine[5]

41.   Little attempt has been made to identify the types of bacteria specifically involved in the metabolism of soy isoflavones (Setchell et al, 1998) although several groups of bacteria are known to possess β-glucosidase activity, including *Lactobacilli, Bacteroides* and *Bifidobacteria* (Hawkesworth et al, 1971). 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 (Heneghen, 1988). The distal part of the small intestine and the large intestine contain substantial numbers (10^4-10^7 bacteria/g wet wt) of *Lactobacilli, Bacteroides* and *Bifodobacteria* (Gorbach et al, 1967; Mitsuoka 1982) whereas the proximal end of the small intestine contains very few of these bacteria.

42.   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 (Heneghen, 1988). 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 (Xu et al, 1995), thus preventing their reabsorption from the lower bowel. In support of this, strains of *Clostridia,* present in the lower gastro-intestinal 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 (Winter et al, 1989). Consequently, the intestinal microflora profile can have a profound effect both on the magnitude and pattern of isoflavone bioavailability.

Development of the gut microflora in the new-born

43.   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 (Klein, 1998 and references within). Initial colonisation is determined by factors such as the composition of maternal gut flora, the mode of delivery (conventional or caesarian 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 (Kirjavainen and Gibson, 1999). Bacterial enzyme activities increase with age and are greatly influenced by the adoption of an adult diet (Mykkanen et al, 1997). The influence of the diet is greater on gut microflora of previously breast-fed babies than on formula-fed babies.

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[5]The reader is referred to the IEH Report for a detailed treatise of gut floral composition, inter-individual variations and implications for health.

Factors affecting the gut microbial flora

44.　　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 (Kirijavainen and Gibson, 1999; Rowland et al, 1999; IEH document 1997; references therein).

Diet

45.　　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 (Slavin et al, 1998; Lampe et al, 1998). 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 (Bingham et al, 1998; Rowland et al, references therein). 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 (Adlercreutz et al, 1987).

46.　　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 (Tew et al, 1996; Tham et al 1998, references therein). Vegetarians generally have higher faecal weights than omnivores, and a lower faecal bacterial β-glucuronidase activity (Adlercreutz et al, 1987, references therein). 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 non-excretors of equol, in females (Lampe et al, 1998).

47.　　The effect of diet on the metabolism of phytoestrogens, both from the influence on gut microflora and hepatic enzyme profile, may also explain, in part, any ethnic differences in metabolic (and perhaps biological) response to phytoestrogens.

Sex

48.　　Claims have been made that there are sex differences in the urinary excretion of isoflavones. Data reported by Lu and Anderson (1998) suggested that recovery of isoflavone conjugates in urine was greater in women than in men, following soy challenge (Lu and Anderson, 1998). Furthermore, Lu et al (1995) suggested that chronic soy consumption could differentially modulate the metabolism and disposition of isoflavones in men and women. Zhang et al (1999) 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 (Setchell 1998 and references therein). Kirkman et al (1995) 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.

Age
49.   With the exception of the data from neonates and infants, there have been no reports of age related differences, however studies have been limited (Adlercreutz et al, 1993).

Chemical structure and resistance to degradation
50.   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 (Griffiths and Smith, 1972). 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 shown (Winter et al, 1989 – also see paragraph 42). 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.

OTHER FACTORS DETERMINING AVAILABILITY AND BIOAVAILABILITY OF DIETARY PHYTOESTROGENS

Food matrix and transit time
51.   The effects of the food matrix and intestinal transit time have yet to be fully investigated.

Plasma protein binding
52.   In the plasma, the extent of free phytoestrogen available for biological interaction is determined by the extent of plasma protein binding (please refer to previous paper on estrogenic potency).

Hepatic metabolism
53.   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 (for general review, see Pelkonen et al, 1998).

BIOAVAILABILITY AND METABOLISM OF PHYTOESTROGENS IN THE NEW-BORN AND INFANT

54.   On a body weight basis, some of the highest intakes of isoflavones occur in very young babies fed soy-based infant formulas and it has been suggested that the plasma levels achieved may be high enough to elicit biological effects (Setchell et al, 1997). However, several factors may influence age-related differences in the pharmacokinetic and metabolic handling of phytoestrogens in adults and babies. These factors include the composition of the gut microflora, which has a strong influence over the absorption, metabolism and reabsorption of isoflavones and, to a

limited extent, body water content, which is higher in neonates and could result in an altered distribution of hydrophilic compounds. Furthermore, plasma protein binding in infants is lower, resulting in a potentially increased amount of free chemical available for interaction, hepatic enzymes are underdeveloped and elimination capacity is reduced due to lower levels of conjugation and a lower glomerular filtration rate (Tritscher, 1999).

55.   It is uncertain as to precisely when an infant develops a microflora necessary for the metabolism and absorption of isoflavones. Setchell et al (1997, 1998) reported that infants of 4 mo, fed soy-based infant formulas, can absorb significant levels of isoflavones although the efficiency of absorption (dependent on the transit time which is decreased in infants) was poor. Furthermore, the gut microflora was not fully metabolically competent beyond initial hydrolysis of the glycosidic moiety since plasma levels of the isoflavone metabolite, equol, were found to be very low. These findings were consistent with those of Cruz et al (1995). However, an ongoing study, (so far) involving only 4 babies, reported by Irvine (1998), suggested that infants can absorb and excrete daidzein and genistein from soy-based infant formulas as efficiently as adults consuming soy products from an age of 4 weeks. It was suggested that a differences in findings between studies might be attributed to the method of urine collection, spot collections as opposed to sample recovery from diapers (which was the method employed in the latter study).

Isoflavones from soy-based formulas compared to human breast or cows milk
56.   Soy-based formulas contain isoflavones largely in the form of their glycosides and malonyl derivatives. In comparison, human breast milk and cow's milk contain much lower levels of isoflavones, mainly in the form of glucuronide (and some sulphate) conjugates. Human and cow's milk also contain equol, which is not present in soy formula. The levels present are determined by diet (Tritscher, 1999). As yet, it is not known if these compositional differences influence bioavailability.

CONCLUDING REMARKS

57.   It is clear that the gut microflora greatly influence the exposure to ingested phytoestrogens and their metabolites by determining the extent to which they are absorbed, metabolised, reabsorbed and degraded. It is also clear that there is considerable inter-individual variation in the pharmacokinetic and metabolic handling of ingested phytoestrogens which is attributed, at least in part, to an individual's unique gut microflora which, in turn, is influenced by factors including diet, antibiotic use, disease and stress. It is likely that pharmacokinetic handling of phytoestrogens in infants differs considerably from that of the adult.

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