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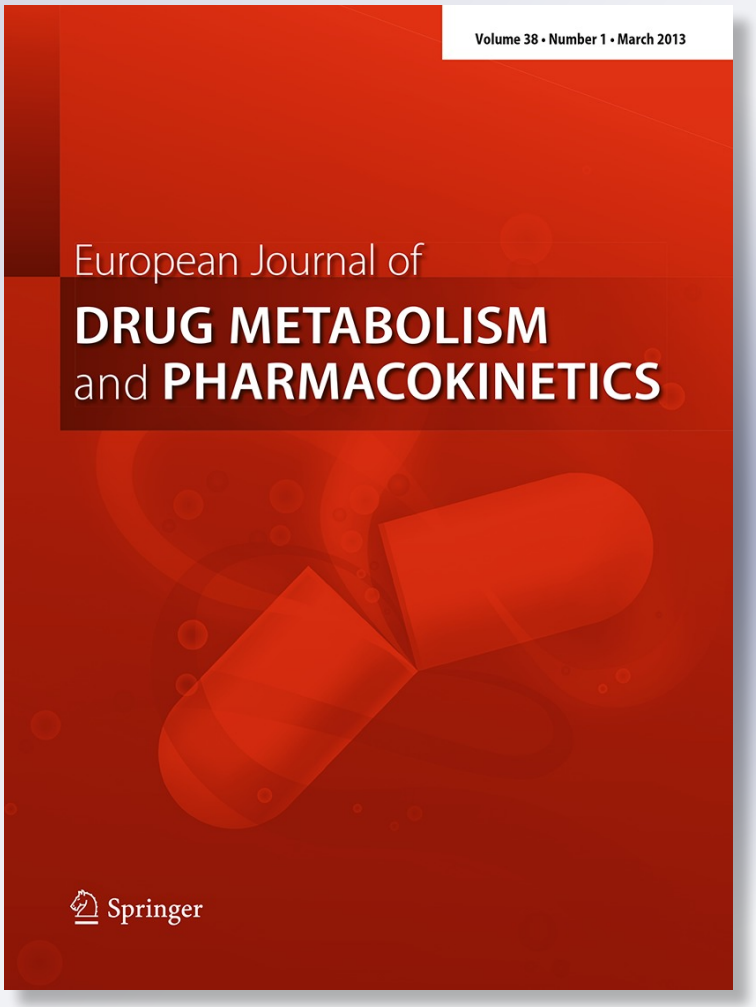

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REVIEW

Isoflavones: estrogenic activity, biological effect and bioavailability

Daniela Cristina Vitale · Cateno Piazza · Barbara Melilli · Filippo Drago · Salvatore Salomone

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Abstract Isoflavones are phytoestrogens with potent estrogenic activity; genistein, daidzein and glycitein are the most active isoflavones found in soy beans. Phytoestrogens have similarity in structure with the human female hormone 17-β-estradiol, which can bind to both alpha and beta estrogen receptors, and mimic the action of estrogens on target organs, thereby exerting many health benefits when used in some hormone-dependent diseases. Numerous clinical studies claim benefits of genistein and daidzein in chemoprevention of breast and prostate cancer, cardiovascular disease and osteoporosis as well as in relieving postmenopausal symptoms. The ability of isoflavones to prevent cancer and other chronic diseases largely depends on pharmacokinetic properties of these compounds, in particular absorption and distribution to the target tissue. The chemical form in which isoflavones occur is important because it influences their bioavailability and, therefore, their biological activity. Glucose-conjugated isoflavones are highly polar, water-soluble compounds. They are hardly absorbed by the intestinal epithelium and have weaker biological activities than the corresponding aglycone. Different microbial families of colon can transform glycosylated isoflavones into aglycones. Clinical studies show important differences between the aglycone and conjugated forms of genistein and daidzein. The evaluation of isoflavone metabolism and bioavailability is crucial to understanding their biological effects. Lipid-based formulations such as drug incorporation into oils, emulsions and self-microemulsifying formulations have been introduced to increase bioavailability. Complexation with cyclodextrin also represent a valid method to improve the physico-chemical characteristics of these substances in order to be absorbed and distributed to target tissues. We review and discuss pharmacokinetic issues that critically influence the biological activity of isoflavones.

Keywords Isoflavones · Phytoestrogens · Estrogenic activity · Biological effect · Bioavailability · Genistein · Daidzein

Abbreviations

ER	Estrogen receptors
HRT	Hormone replacement therapy
AUC	Area under the plasma concentration curve
T_{max}	Time to maximum concentration
IFG	Isoflavone glucoside
TVP	Textured vegetable protein
C_{max}	Maximum plasma concentration
$t_{1/2}$	Plasma concentration half-life
AUC_{0-t}	Area under the plasma concentration curve administration to last observed concentration at time t
SMEDDS	Self-microemulsifying drug delivery system
IFE	Isoflavone extract
CD	Cyclodextrins

1 Introduction

Isoflavones are naturally occurring plant chemicals belonging to the "phytoestrogen" class (Murkies et al. 1998; Price and Fenwick 1985); they are currently heralded as offering

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potential alternative therapies for a range of hormone-dependent conditions, including cancer, menopausal symptoms, cardiovascular disease and osteoporosis. Phytoestrogens are natural estrogenic compounds found in a variety of plants and in their seeds. These compounds have structures similar to mammalian estrogens (Setchell and Adlercreutz 1988) and display both estrogenic and anti-estrogenic effects. Phytoestrogens are found in high amounts in soybeans, flaxseed, alfalfa and other edible plants; they have been categorized according to their chemical structures as isoflavones, lignans and coumestans (Dixon 2004). The metabolism of these compounds is complex and variable. Isoflavones are the most common form of phytoestrogens and their greatest dietary source is soy. Soybeans contain isoflavones, such as genistein, daidzein and glycitein, at concentrations as high as 1–3 mg/g. Each one of these compounds may be found in four different chemical forms (Fig. 1): unconjugated (aglycone, IFA), sugar-conjugated (isoflavone glucoside, IFG), acetylglucosides and malonylglucosides. Coumestans are less abundant in diet and less well studied. Lignans (enterolactone and enterodiol) are found in woody portions of plants, seeds, coats of seed and bran layers in grains, where they form the building blocks for plant cell walls (Adlercreutz and Mazur 1997; Mazur et al. 1998); lignans and isoflavones are the most common estrogenic compounds. Flaxseed is by far the greatest single vegetable source of lignans, but whole grains, vegetables, and tea are also significant sources, including buckwheat sprout and matrix (Dixon 2004; Setchell 1998; Cornwell et al. 2004).

2 Estrogenic activity, biological and clinical effects

There are over 300 plants identified so far that have sufficient estrogenic activity to initiate estrus in animals (Farnsworth et al. 1975; Whitten and Patisaul 2001). The soy isoflavones, particularly genistein, are of greatest interest because of the widespread human consumption of soy. The soy isoflavones can bind to the estrogen receptor and induce estrogen-like effects in vivo in animals and humans, and in vitro in cell cultures (Whitten and Patisaul 2001). The relative estrogenic potency is dependent on the type of assay used to measure hormonal activity, animal species, dosage, route of administration as well as on the duration and timing of exposure (Whitten and Patisaul 2001). Phytoestrogens have relatively weak activity compared to animal estrogens; however, exposure to high dietary levels may result in biological responses in humans and animals, with favorable or unfavorable consequences (Adlercreutz 1998; Bouker and Hilakivi-Clarke 2000; Setchell and Cassidy 1999). Diel et al. (2000) reported that daidzein exhibits a very low uterotropic activity in rat compared to other xenoestrogens, though it strongly modulates the expression of estrogen-sensitive genes, measured as mRNA levels. The soy isoflavones administered during development can cause several forms of estrogen-related toxicity in experimental animals (Doerge and Sheehan 2002). On the other hand, epidemiologic observations and experimental data are suggestive of beneficial effects of isoflavones on human health (Murkies et al. 1998; Setchell 1998; Cassidy 1996; Knight et al. 1996; Messina et al. 1994;

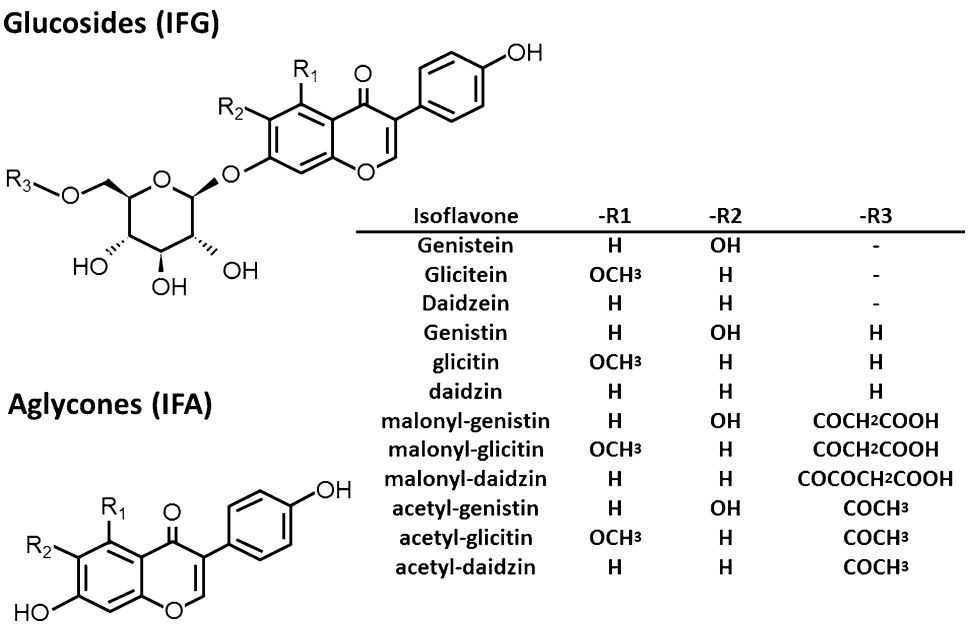

Fig. 1 Chemical structures of isoflavones

Cassidy et al. 1995), but data from large-scale clinical trials are not available. On the basis of their chemical structures, binding of isoflavones to estrogen receptors (ER) can be predicted (Fang et al. 2001); they act, however, in a complex manner, as partial estrogen agonists and antagonists; isoflavones modulate steroid receptors status according to the dose and duration of action, that may be transient and extremely rapid or sustained (Jordan 1990; Kampa et al. 2007). To further complicate this matter, isoflavones have been reported to down-regulate aromatase mRNA in human granulose-luteal cells, a property that may affect in situ steroidogenesis (Rice et al. 2006). Finally, estrogens and phytoestrogens can also exert non-genomic actions that include effects on plasma membrane and on cell signaling pathways (Nilsson et al. 2011; Kim et al. 1998). Estrogens exert their genomic effects mainly via two nuclear receptors, ERα and ERβ (Dahlman-Wright et al. 2006; Kuiper et al. 1996; Paech et al. 1997), that play different roles in gene regulation. Tissue distribution and relative ligand binding affinities of ERα and ERβ differ, which may help to explain the selective action of estrogens in different tissues. Interestingly, ERβ is found in brain, bone, bladder and vascular epithelia (Kuiper et al. 1996, 1997; Hillier et al. 1998), tissues that are responsive to classical hormone replacement therapy (HRT). Analysis of relative molar binding affinities of different estrogenic compounds reveals that phytoestrogens and some environmental xenoestrogens have significantly higher affinities for ERβ and suggests that this receptor subtype may be more relevant to the action of non-steroidal estrogens (Kuiper et al. 1997).

Epidemiologic data and clinical experience indicate that estrogen therapy after the menopause offers protection from cardiovascular disease, reduces the extent of osteoporosis, improves cognitive function and relieves menopausal symptoms associated with acute ovarian estrogen loss (Breckwoldt et al. 1995). Given the poor compliance with conventional HRT that is driven by side effects and fear of increased risk of breast cancer (Breckwoldt et al. 1995; Zumoff 1993), alternative sources of exogenous estrogen are constantly sought. Estrogen deficiency is associated with significant alterations in lipoprotein metabolism and serum levels of cholesterol increase markedly in the post-menopausal years (Cignarella et al. 2010). Preventing or reducing the increase in serum cholesterol is associated with reducing risk for cardiovascular disease (Cignarella et al. 2010). The best documented effect of isoflavones is on plasma lipid and lipoprotein concentrations, with reductions of up to 10 % in LDL cholesterol concentrations (somewhat greater for individuals with high pre-treatment LDL cholesterol concentrations) and small increases in HDL cholesterol concentrations (Clarkson 2002). These effects on plasma lipids are associated with vascular effects, such as improved flow-mediated arterial dilation and systemic arterial compliance (Clarkson 2002). The exact mechanism of the hypocholesterolemic effect of soy remains elusive and is probably multifactorial. Because beside isoflavones many other components are present in soy extract, it is difficult to make definitive conclusions regarding the exact component responsible for the cholesterol lowering effect; furthermore, animal studies where isoflavones were added to casein failed to demonstrate a cholesterol lowering effect similar to that of soy containing comparable levels of isoflavones (Clarkson 2002). In addition to effects on lipids, genistein inhibits the process of coagulation, a key promoter of plaque formation; this effect may be related to inhibition by genistein of growth factors, such as platelet-derived growth factor, with subsequent effects on thrombin formation (Sargeant et al. 1993; Wilcox and Blumenthal 1995). Moreover, genistein is a potent inhibitor of tyrosine kinase (Akiyama et al. 1987), which may affect Syk kinase and thrombin generation (van der Meijden et al. 2012). Another important condition associated to menopausal estrogen decrease is osteoporosis. Lack of estrogen increases bone resorption and decreases the deposition of new bone; HRT slows down the rate of bone turnover, especially bone resorption (Khosla et al. 2011). Soy isoflavones and other rich sources of phenolic compounds, represent an alternative/adjunctive therapy for osteoporosis (Atkinson et al. 2004; Cauley et al. 2003). Their activity in preventing bone loss has been observed in experimental animal models, daidzein being more effective than genistein (Picherit et al. 2000); however, administration of 200 mg soy tablets to menopausal women has been found ineffective in a recently published, randomized, double-blind clinical trial (Levis et al. 2011). Epidemiologic studies suggest that the consumption of soy protein containing isoflavones lowers the risk of some cancers, including prostate, breast (Adlercreutz 1998; Messina 1999) and perhaps colon cancer (Pereira et al. 1994; Messina and Bennink 1998). A case–control study in white subjects who consumed a Western diet reported slight protective effects of genistein consumption against prostate cancer (Strom et al. 1999). Highly purified genistein and daidzein are available; they have already been tested in a small double-blinded, placebo-controlled study for localized prostate cancer (deVere White et al. 2010), though with negative results. Genistein and daidzein inhibit the proliferation of different types of cancer cells (Peterson and Barnes 1991, 1993; Zhou et al. 1999), 3T3 cells (Linassier et al. 1990), and normal colonic mucosa (Majumdar 1990), in tissue culture. The exact mechanism of this antiproliferative effect is unclear. The hypotheses that have been proposed consider that soy isoflavones activate estrogen receptors (Messina 1999), inhibit the activity of growth-promoting steroid hormones by inhibiting the enzymes progesterone 5α-reductase and

17β-hydroxysteroid dehydrogenase (Pereira et al. 1994), have antioxidant activities (Wei et al. 1996; Kapiotis et al. 1997), inhibit protein-tyrosine kinase mediated signal transduction (Akiyama et al. 1987), inhibit DNA topoisomerase (ATP-hydrolyzing) (Yamashita et al. 1990; Constantinou et al. 1995), inhibit transforming growth factor 1-mediated signal transduction (Nilsson et al. 2011), and inhibit angiogenesis (Zhou et al. 1999; Fotsis et al. 1998). Interest in phytoestrogens as natural anticancer agents was stimulated by animal studies using the classical model of chemically induced breast cancer. In this model, soy proteins containing isoflavones were found to significantly reduce tumor formation, in a dose-dependent manner (Adlercreutz 1995). This effect was completely lost when using soy proteins devoid of isoflavones.

3 Pharmacokinetics of isoflavones

Isoflavones might play an important role in human health, but their clinical efficacy largely depends on their pharmacokinetic properties, in particular absorption and distribution to target tissues. Isoflavones exist primarily in soybeans and in most soy foods as a complex mixture of glucoside conjugates that are not bioavailable in this form (Setchell et al. 2002b). After ingestion, the isoflavone glucosides are hydrolyzed by both intestinal mucosal and bacterial β-glucosidases releasing the aglycones (Setchell et al. 2002a; Day et al. 1998; McMahon et al. 1997), that are then either absorbed directly or further metabolized by intestinal microflora in the large intestine into other metabolites (Atkinson et al. 2005; Decroos et al. 2005). The metabolism of isoflavones by gut bacteria plays a key role in the availability and bioactivation of these compounds in the intestine. S-equol [7-hydroxy-3-(49-hydroxyphenyl)-chroman] is by far the most abundant and active intestinal metabolite of daidzein (Setchell et al. 2002a). While daidzein conversion yielding S-equol has been known for some time, the corresponding formation of 5-hydroxy-equol from genistein by a strictly anaerobic bacterium has been also recently reported in mouse (Matthies et al. 2008). In humans, S-equol (Setchell and Clerici 2010), a compound structurally similar to estradiol, may contribute to reduction of some menopausal symptoms. However, the capability of producing S-equol is quite variable among the adult population; Caucasians produce significantly lower amounts of S-equol after eating soy foods containing isoflavones than Asians do (Song et al. 2006). Because S-equol binds to estrogen receptor with higher affinity than daidzein, a greater efficacy of daidzein in equol producers might be expected (Setchell et al. 2002a). Besides S-equol, small amounts of other, still poorly identified, metabolites of isoflavones are produced from gut bacteria (Rafii et al. 2007). Intestinal conjugation and subsequent excretion of phase II metabolites via intestine is a main component of first-pass metabolism of genistein (Chen et al. 2003; Hu et al. 2003); intestinal metabolism of isoflavones and excretion of their conjugates are strongly influenced by their structure (Chen et al. 2005). Excretion of phase II conjugates is dependent on metabolite formation by conjugating enzymes such as UGT and subsequent efflux of phase II metabolites by transporters such as multidrug resistance-associated proteins (Hu et al. 2003; Chen et al. 2005). Recently, daidzein and genistein have been found to increase function and expression of P-glycoprotein in human intestinal epithelial cells (Okura et al. 2010), suggesting that repeated exposure to dietary isoflavones cannot only modify their own bioavailability, but also cause interactions with drugs that bind P-glycoprotein in intestine. Many factors, i.e., age, gender, food matrix, etc., may influence the intestinal metabolism and thereby the bioavailability of isoflavones in humans (Setchell 1998; Adlercreutz et al. 1993). Setchell et al. (2001) fed 50 mg purified genistein, genistin, daidzein, or daidzin to four groups of 3–6 women. This dose was chosen because it was considered, at that time, within the expected range of isoflavone intake by people consuming soy as staple food. The areas under curve (AUC) for each compound, indicating overall absorption, based on plasma concentrations over time, was about 2.94 μg/ml h for daidzein, and about 4.54 μg/ml h for genistein, genistin and daidzin. Mean time to attain peak plasma concentrations (T_{max}) following the administration of the aglycones genistein and daidzein was 5.2 and 6.6 h, respectively, whereas for the corresponding isoflavone glucosides (IFG), the T_{max} was delayed to 9.3 and 9.0 h, respectively, the rate-limiting step for absorption being initial hydrolysis of the sugar moiety in intestine. In a later work, Setchell et al. (2003a) reported that the bioavailability, as apparent from the AUC_{inf}, shows a curvilinear relationship with increasing levels of isoflavones ingested as soy food (single-bolus ingestion of 10, 20 or 40 g of soy nuts). When a compound exhibits nonlinear pharmacokinetics, the AUC_{inf} increases in a manner that is disproportionate to the applied dose. When the increase in AUC_{inf} is higher than that predicted on the basis of a strictly linear relationship with dose, it usually indicates that one or more elimination pathways, such as metabolizing enzymes or transporters (renal or biliary), are saturated. When the AUC_{inf} increase is less than that expected on the basis of a linear relationship, it is indicative of either increased elimination, usually due to induction of metabolizing enzymes, or reduced absorption. In the case of both daidzein and genistein, during the first 24 h [^{13}C]labeled compounds were almost entirely recovered in the urine; however, when the dose for both [^{13}C]isoflavones was doubled, the cumulative percentage

recovery of [^{13}C]daidzein and [^{13}C]genistein in urine did not show a linear increase, suggesting a decreased fractional absorption (Setchell et al. 2003b). The mean $t_{1/2}$ values of daidzein and genistein were 8.0 ± 0.3 and 10.1 ± 0.3 h, respectively, and, as expected, independent of the amount ingested. These values are longer than those reported for isoflavones contained in other soy foods (King and Bursill 1998; Watanabe et al. 1998), possibly because of the effect of certain food matrices on the dynamics of absorption and elimination. Significantly longer half-lives are also found in patients with renal disease (Fanti et al. 1999), and this is because renal clearance is the major route for their elimination from the body. Daidzein is always excreted in greater amounts than genistein in the urine of adults when soy foods are consumed (Hutchins et al. 1995; Franke et al. 1998; Zhang et al. 1999); from this observation, some investigators have incorrectly assumed that daidzein is more bioavailable than genistein (Xu et al. 1994, 2000). However, bioavailability assessed from AUC_{inf} of serum concentrations is higher for genistein than for daidzein (Setchell et al. 2001, 2003a).

3.1 Factors affecting the bioavailability of isoflavones

Human studies on the bioavailability of isoflavones in the aglycone and glucoside forms show contradictory results, that may be accounted for, at least in part, by the different formulations used; some studies used isoflavone extracts (IFEs) in a pharmaceutical formulation, some soy food items, such as soy milk, others pure isoflavones. Some studies found that the apparent bioavailability of genistein and daidzein does not differ when they are consumed as either aglycone or glucoside (Zubik and Meydani 2003; Richelle et al. 2002). In contrast, Izumi et al. (2000) reported that soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides. Setchell et al. (2001) reported that T_{max} values for the aglycone genistein and daidzein are 5.2 and 6.6 h, respectively, whereas for the corresponding β-glucoside (genistin and daidzin) the values are delayed to 9.3 and 9.0 h, respectively. A higher bioavailability of administered pure β-glucosides has been confirmed by Rüfer et al. (2008). Several factors might enhance the absorption of daidzein glucoside: active transport of the glucoside, reduced interaction with food matrix components, higher solubility in the aqueous surface of the intestine, protection of bacterial degradation by the sugar moiety (Rufer et al. 2008; Setchell et al. 2001). Isoflavones undergo significant intestinal metabolism in humans that produces a number of metabolites (Axelson et al. 1982; Bannwart et al. 1984; Joannou et al. 1995; Kelly et al. 1993), but sugar moieties seem to protect them from biotransformation; the idea of attaching protecting groups to molecules is common practice in pharmacology and is generally done to prevent biotransformation of the parent drug, thereby improving its bioavailability. Furthermore, binding of aglycones to diet components, e.g., proteins, is extensive, whereas it may be lower for the more hydrophilic glycoside forms. Quercetin glucoside, for example, shows higher bioavailability than its aglycone, as measured from the appearance of quercetin in plasma (Hollman et al. 1995, 1996). To date, little information is available on bioavailability of isoflavones commonly ingested with soy foods and on age- and gender-related differences. Cassidy et al. (2006) examined the bioavailability of isoflavones, both aglycone and glucoside forms naturally present in food, in a randomized crossover trial including 21 premenopausal women, 17 postmenopausal women and 21 men, receiving three different soy foods: soy milk, textured vegetable protein (TVP) and tempeh. The pharmacokinetic data resulting from this study are in accordance with those available from previous studies. Specifically, C_{max}, T_{max}, $t_{1/2}$, and $AUC_{(0-t)}$ were all within the range of values obtained from other studies using similar dietary intakes of isoflavones (Setchell et al. 2003a; King and Bursill 1998; Watanabe et al. 1998; Xu et al. 2000). After soy milk consumption, the time taken to reach peak plasma isoflavone concentrations was 6.2 and 5.9 h for daidzein and genistein, respectively. Solubility of a substance in the intestine influences the rate of absorption; because isoflavones contained in soy milk are mainly hydrophilic β-glucoside conjugates in solution, faster absorption rates and earlier peak serum concentrations are expected following soy milk ingestion compared to solid soy food matrix (Cassidy et al. 2006). On average, the peak serum concentrations of daidzein and genistein occurred 2 h later for TVP and tempeh than for soy milk. When the two solid food matrices were compared, the difference observed could be explained by the difference in content of chemical form of isoflavone. In particular, C_{max} and $AUC_{(0-t)}$ were significantly higher for tempeh than for TVP, for both daidzein and genistein (Cassidy et al. 2006). Tempeh is a fermented food that contains mainly aglycones of daidzein and genistein, whereas TVP contains mainly the glucoside conjugates. The influence of insoluble dietary fibers, such as fructo-oligosaccharides and inulin, on stimulation of the growth of bacteria and fermentation in the colon has been extensively studied (McFarlane and Cumming 1991). Inulin supplementation may influence plasma concentrations of soybean isoflavones, possibly by increasing their absorption. Considering the relevance of isoflavones on alimentation after menopause, we examined the influence of inulin on plasma concentrations of soybean isoflavones in healthy postmenopausal women, to elucidate whether or not inulin enhanced soybean isoflavone absorption (Piazza et al. 2007). We observed a significant difference in plasma isoflavone concentrations as a result

of the presence of inulin. Twelve healthy postmenopausal women participated in a randomized, double-blind, cross-over study. They consumed 40 mg of a conjugated form of soybean isoflavones (6 mg daidzein and 18 mg genistein as free form) with or without 3.66 g inulin twice daily in two different 21-day experimental phases. After 21 days of supplementation with soybean isoflavones, the AUC_{0-24h} values for daidzein and genistein appeared to be higher than those at baseline (day 1) for either Isoflavone alone (Iso) or Isoflavone + Inulin (Iso + Inu) formulations. However, the AUC_{0-24h} values on day 21 for both isoflavones were higher after the Iso + Inu formulation than after the Iso formulation. In the presence of inulin, the increase of AUC_{0-24h} was 38 % for daidzein and 91 % for genistein (Figs. 2, 3). As shown in Tables 1 and 2, plasma C_{max} values of daidzein appeared to be higher after 21 days of supplementation with both Iso and Iso + Inu formulations; in contrast, plasma C_{max} values of genistein appeared to be higher only after supplementation with Iso + Inu formulation. There was a decrease in T_{max} values for both daidzein and genistein, after 21 days of supplementation with either Iso or Iso + Inu formulations; C_{max} values for plasma isoflavones were found at times ranging from 2 to 8 h after ingestion. Furthermore, the T_{max} values on day 21 after supplementation with Iso formulation did not differ from those with Iso + Inu formulation, for each isoflavone. The mechanism by which isoflavone absorption is facilitated in the presence of inulin is unclear. The chemical form of isoflavone present in food has been considered important to its bioavailability and, therefore, to its biological activity. The glucoside forms are predominant (Kim et al. 2006) and represent the major naturally occurring isoflavones in soybean and soybean-based food products (Murphy et al. 1999), though variation in food-processing techniques may alter the relative content of acyl-glucoside,

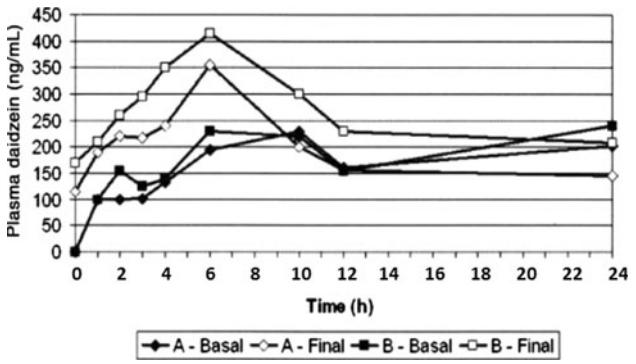

Fig. 2 Mean plasma daidzein concentrations over a 24 h observation period in 12 healthy postmenopausal women. Each of the following 2 formulations was consumed twice daily for 21 days: a formulation containing soybean isoflavones without inulin (**a**) and a formulation containing soybean isoflavones with 3.66 g inulin (**b**). *Basal values* represent the concentration time curve on day 1; *final values* represent the concentration time curve on day 21

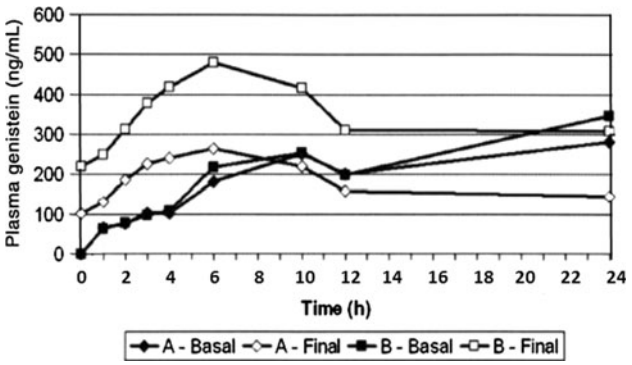

Fig. 3 Mean plasma genistein concentrations over a 24 h observation period in 12 healthy postmenopausal women. Each of the following 2 formulations was consumed twice daily for 21 days: a formulation containing soybean isoflavones without inulin (**a**) and a formulation containing soybean isoflavones with 3.66 g inulin (**b**). *Basal values* represent the concentration time curve on day 1; *final values* represent the concentration time curve on day 21

malonylglucoside, and simple glucosides (Xu et al. 1994). After ingestion, isoflavone glucosides are hydrolyzed to their aglycones by phlorizin lactose hydrolase in the apical membrane of the lumen of the small intestine and by bacterial glucosidases (i.e., from lactobacilli, bacteroides, and bifidobacteria; Xu et al. 1995; Setchell et al. 1984). Conversion of daidzein into its metabolites dihydrodaidzein, *O*-desmethylangolensin, and equol also precedes absorption from the colon (Walsh et al. 2007). After hepatic uptake and excretion into the bile of the β-glycuronides genistein, glycitein, and daidzein and its metabolites, a second round of hydrolysis occurs in the intestine (Sfakianos et al. 1997). Insoluble dietary fibers, such as fructo-oligosaccharides and inulin, may stimulate the growth of bacteria and thereby fermentation by bacterial glucosidases in the colon (McFarlane and Cumming 1991). Thus, we hypothesize that inulin-stimulated bacterial growth induces soybean glucoside breakdown and, hence, facilitates isoflavone absorption. Finally, the influence of menopause on the isoflavone pharmacokinetics deserves attention. We did not directly collect information in premenopausal women; however, bioavailability values in pre-menopause published by others (Cassidy et al. 2006) are higher than those we found in postmenopausal women. Thus, postmenopausal women may show decreased absorption of isoflavones and need higher doses to achieve plasma concentrations similar to those of younger subjects.

3.2 Enhancing oral bioavailability of soy isoflavanones by new drug delivery systems

A variety of formulation strategies have been developed to improve the solubility and bioavailability of isoflavones. Self-microemulsifying formulations improve drug solubility, and enhanced dissolution and absorption, by providing

Table 1 Pharmacokinetic parameters of daidzein

	Iso formulation		Iso + Inu formulation	
	Day 1	Day 21	Day 1	Day 21
AUC_{0-24h} (ng/ml h)	4,083 ± 181	4,614 ± 189	4,372 ± 175	6,386 ± 114*†
C_{max} (ng/ml)	240 ± 10	409 ± 39*	314 ± 19	435 ± 11*
T_{max} (h)	7.9 ± 0.3	5.5 ± 0.2*	10.0 ± 0.7	5.5 ± 0.1*

All values are mean ± standard deviation ($n = 12$) from healthy postmenopausal women

The Iso formulation contained soybean isoflavones without inulin; the Iso + Inu formulation contained soybean isoflavones with inulin. Each of the 2 formulations was consumed for 21 days

AUC_{0-24h}, area under the curve over 24 h; T_{max}, time to reach the maximum concentration; C_{max}, maximum concentration

* $P < 0.05$ versus day 1 value

† $P < 0.05$ versus day 21 value for the Iso formulation. (Wilcoxon matched-pair ranks test, one-way ANOVA)

Table 2 Pharmacokinetic parameters of genistein

	Iso formulation		Iso + Inu formulation	
	Day 1	Day 21	Day 1	Day 21
AUC_{0-24h} (ng/ml h)	4,773 ± 207	4,397 ± 237	5,218 ± 110	8,393 ± 420*†
C_{max} (ng/ml)	295 ± 12	288 ± 18	341 ± 6	483 ± 23*†
T_{max} (h)	20.2 ± 0.7	8.0 ± 0.6*	22.5 ± 0.5	6.3 ± 0.2*

All values are mean ± standard deviation ($n = 12$) from healthy postmenopausal women

The Iso formulation contained soybean isoflavones without inulin; the Iso + Inu formulation contained soybean isoflavones with inulin. Each of the 2 formulations was consumed for 21 days

AUC_{0-24h}, area under the curve over 24 h; T_{max}, time to reach the maximum concentration; C_{max}, maximum concentration

* $P < 0.05$ versus day 1 value

† $P < 0.05$ versus day 21 value for the Iso formulation. (Wilcoxon matched-pair ranks test, one-way ANOVA.)

a large interfacial surface area (Shah et al. 1994; Kang et al. 2004; Gershanik and Benita 2000). An in vivo absorption study was undertaken to determine whether or not the enhanced solubility and in vitro dissolution of daidzein in a self-microemulsifying drug delivery system (SMEDDS) could increase the gastro-intestinal absorption following oral administration (Shen et al. 2010). The plasma profiles of daidzein in rats following oral administration of the control group and SMEDDS forms were compared. Plasma concentration profiles of daidzein in SMEDDS reflected significantly greater absorption compared to control. Pharmacokinetic parameters of daidzein, following oral administration were: C_{max} 170 ± 15.91 ng/ml for control group and 444 ± 49.48 ng/ml for daidzein-SMEDDS; T_{max} 0.38 ± 0.14 h for control group and 0.27 ± 0.17 h for daidzein-SMEDDS; AUC_{0-12h} was 380.98 ± 67.59 ng/ml min for control group and 954.32 ± 158.30 ng/ml min for daidzein-SMEDDS. Compared to the control, SMEDDS enhanced the values of AUC_{0-12h} and C_{max} of daidzein. Complexation with cyclodextrins (CD) represents another valid method to improve the physico-chemical characteristics and reduce the limitations of these substances. In order to improve the solubility and bioavailability of a soy IFE, Lee et al. (2007) developed inclusion complexes (IFE-β-CD) of the IFE with β-cyclodextrin (β-CD). Their results show that the aqueous solubility of the complexes of IFE with β-CD (2.0 mg/ml) was about 26 times higher than that of IFE itself (0.076 mg/ml). The same dosages of IFE and IFE-β-CD were orally administered to rats on an isoflavone glucoside (IFG) basis (daidzin, genistin and glycitin), and the plasma concentrations of daidzein, genistein and glycitein were measured over time to estimate the average AUC of the isoflavones. After the oral administration, the AUC values for daidzein, genistein and glycitein were 340, 11 and 28 μg/ml min, respectively. In contrast, the respective AUC values after the administration of IFE-β-CD were 430, 20 and 48 μg/ml min. The bioavailability of daidzein in IFE-β-CD was increased to 126 % by the formation of inclusion complexes with β-CD, compared with that in IFE. Furthermore, the bioavailability of genistein and glycitein in IFE-β-CD formulation was significantly higher by up to 180 and 170 %, respectively (Lee et al. 2007). These results indicate that the absorption of isoflavone could also be improved by complexation of IFE with β-CD (IFE-β-CD).

4 Conclusions

The beneficial effects of isoflavones on health largely depend on pharmacokinetic properties of these compounds, in particular absorption and distribution to target tissues. The bioavailability of isoflavones depends on the chemical form (aglycone or glucoside) that influences the solubility in the intestinal lumen and the absorption as well as on the metabolism by intestinal and microbial enzymes. Furthermore, diet proteins, such as inulin, might have a significant impact on isoflavone bioavailability, because they bind to aglycones and stimulate the growth of intestinal flora, increasing fermentation activity and isoflavone biotransformation. Recently, new formulations, based on lipid-incorporation in self-microemulsifying formulations or complexation with cyclodextrins, have been developed and tested. These formulations produce more reliable plasma levels following oral administration of isoflavones and increase isoflavone bioavailability.

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