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Effects of Soy Containing Diet and Isoflavones on Cytochrome P450 Enzyme Expression and Activity

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Abstract

Cytochrome P450s (CYP) plays an important role in metabolism and clearance of most clinically utilized drugs and other xenobiotics. They are important in metabolism of endogenous compounds including fatty acids, sterols, steroids and lipid soluble vitamins. Dietary factors such as phytochemicals are capable of affecting CYP expression and activity which may be important in diet-drug interactions and in development of fatty liver disease, cardiovascular disease and cancer. One important diet-CYP interaction is with diets containing plant proteins, particularly soy protein. Soy diets are traditionally consumed in Asian countries and are linked to lower incidence of several cancers and of cardiovascular disease in Asian populations. Soy is also an important protein source in vegetarian and vegan diets and the sole protein source in soy infant formulas. Recent studies suggest that consumption of soy can inhibit induction of CY1 enzymes by polycyclic aromatic hydrocarbons which may contribute to cancer prevention. In addition, there is data to suggest that soy components promiscuously activate several nuclear receptors including PXR, PPAR and LXR resulting in increased expression of CYP3As, CYP4As and CYPs involved in metabolism of cholesterol to bile acids. Such soy-CYP interactions may alter drug pharmacokinetics and therapeutic efficacy and be associated with improved lipid homeostasis and reduced risk of cardiovascular disease. The current review summarizes results from *in vitro*; *in vivo* and clinical studies of soy-CYP interactions and examines the evidence linking the effects of soy diets on CYP expression to isoflavone phytoestrogens, particularly genistein and daidzein which are associated with soy protein.

Keywords

Cytochrome P450; soy; isoflavone; genistein; daidzein; nuclear receptor

Introduction

The cytochrome P450 (CYP)-dependent monooxygenase system is highly versatile in its ability to catalyze the oxidative metabolism of a vast range of xenobiotics and endogenous compounds. In the majority of cases this results in increased clearance and detoxication. However, in some instances oxidative metabolism can result in formation of reactive

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intermediates capable of producing cellular injury or acting as mutagens. As a result, changes in patterns of expression and activity of multiple CYP enzymes can affect drug metabolism, clearance and therapeutic efficacy, and the risk of development of many chronic diseases including cardiovascular disease and cancer. Over the past 50 years, studies have demonstrated that dietary factors and diet composition have a profound influence on CYP regulation with consequences for drug metabolism, toxicity and disease prevalence (Ioannides, 1999). This was amply demonstrated in an early study in rabbits by Pineau et al. (1991) which revealed large increases in CYP3A6 expression and activity associated with switch of diet from breast milk to solid diets at weaning. Subsequently, many investigators have focused on the CYP-regulatory effects of specific dietary components including dietary fats (reviewed by Hardwick et al., 2009); ethanol (reviewed by Ingelman-Sundberg et al., 1994; Ronis et al., 1996; Li and Cederbaum 2008) and phytochemicals in fruits and vegetables (reviewed by Ioannides, 1999; Lamps and Petersen, 2002; Murray 2006; Rodriguez-Fragoso et al., 2011; An et al. 2015). In addition, a significant amount of work has examined the effects of dietary protein composition, in particular soy protein and its associated phytochemicals, on CYP expression and activity. Such effects are potentially important since soy products are an important source of calories in Asians eating traditional diets; vegetarians and vegans and are the sole protein source in the million children each year who consume soy infant formula (Badger et al., 2002). In addition, soy constitutes a significant portion of rodent chow diets and may influence the outcome of pre-clinical studies of new therapeutic agents. This is the topic of the current review.

Soy, Aryl Hydrocarbon Receptor (AhR) Signaling and CYP1 Enzymes

Epidemiological data have linked consumption of traditional Asian diets rich in soybean products with a lower incidence of several cancers including breast cancer (Wu et al., 2008; Korde et al. 2009, Dong and Qin, 2011, Ollberg et al. 2012; Mahmoud et al. 2014). In addition, we and others have reported that consumption of soy protein isolate (SPI) results in significant protection against chemically-induced breast cancer in several animal models, including those involving treatment with the polycyclic aromatic hydrocarbon (PAH) dimethylbenz(a)anthracene (DMBA) (Hakkak et al. 2000; Constantinou et al. 2001). One potential protective mechanism related to chemical carcinogenesis would be inhibition of procarcinogen bioactivation to reduce cancer initiation (Badger et al. 2005). In this regard cytochrome P450 enzymes in the CYP1 family under the regulation of the Ah receptor play an important role in bioactivation of PAHs to mutagenic metabolites (Shimada and Fujii-Kuriyama, 2004). There is now extensive evidence that consumption of SPI results in downregulation of CYP1 family member inducibility by PAHs in the liver and mammary gland of rats. Ronis et al. (2001) examined the inducibility of CYP1A1 and CYP1A2 in the liver of male rats fed lifetime diets containing casein, SPI or whey as the sole protein source by the PAH and AhR ligand 3-methylcholanthrene (3MC). SPI feeding significantly reduced CYP1A1 mRNA and apoprotein induction relative to casein and whey fed groups. This correlated with reduced expression of the hepatic AhR after SPI feeding. In contrast, both SPI and whey feeding reduced 3MC induction of CYP1A2 mRNA. In contrast, CYP1A1 mRNA and apoprotein induction by an AhR-independent mechanism after treatment with isosafrole was significantly enhanced in the SPI-fed compared to casein or whey fed groups.

A similar study was performed in female rats. CYP1 enzymes were induced by DMBA in animals fed casein, SPI or whey. Rowlands et al. (2001) reported that DMBA-induction of ethoxyresorufin O-deethylase, CY1A1 apoprotein and mRNA in liver was reduced significantly by feeding SPI compared to casein. Similarly, DMBA induction of hepatic methoxyresorufin O-demethylase activity, CYP1A2 mRNA and CYP1B1 mRNA were lower in SPI-fed compared to casein-fed female rats. Moreover, feeding SPI significantly suppressed both constitutive and DMBA-induced apoprotein expression of all three CYP1 enzymes, CYP1A1, CYP1A2 and CYP1B1 in the mammary gland of female rats. This was accompanied by reduced expression of AhR protein and its heterodimerization partner ARNT (Rowlands et al. 2001). In contrast, no differences were observed in inducibility of CYP1A1 and CYP1B1 in the uterus of female rats fed casein or SPI and treated with DMBA (Ronis et al. 2016). Also in contrast to data in rats, no significant change in expression of CYP1A1 or CP1B1 was observed in the mammary gland of ovariectomized female cynomolgus macaques fed soy diets with or without bound phytochemicals for 36 months (Scott et al. 2008).

In subsequent mechanistic studies, suppression of DMBA induction of CYP1A1, and of binding of AhR/ARNT heterodimers to the XRE element on the CYP1A1 promoter in rat liver by feeding SPI, was found to require the presence of SPI bound phytochemicals. These effects did not occur after feeding SPI protein stripped of phytochemicals by successive ethanol washes (Singhal et al., 2007). However, the phytochemical component involved in this response appeared not to be isoflavones. Diets supplemented with the same levels of pure genistein or diadzein found in SPI failed to suppress hepatic AhR expression or CYP1A1 induction (Singhal et al., 2008). Studies using FGC-4 rat hepatoma cells exposed to DMBA and serum from casein and SPI-fed rats demonstrated that factors from the soy diet triggered ubiquitination and proteasomal degradation of AhR (Singhal et al. 2007). Treatment with serum from SPI-fed rats resulted in reduced association of AhR with the cytosolic chaperone protein immunophillin-like X-associated protein (XAP2) and translocation of AhR from the cytosol into the nucleus. However, the translocated receptor appeared unable to heterodimerize with ARNT or bind to xenobiotic response elements (XREs) on the CYP1A1 promoter, but was rather targeted for degradation (Singhal et al. 2007). The suppressive effect of SPI on DMBA induction of CYP1A1 appears to be dependent on the presence of estrogens. The effect was lost after ovariectomy of female rats and restored after replacement with estradiol. This was associated with reduced recruitment of AhR and ERα to XRE elements on the CYP1A1 promoter (Singhal et al. 2009).

Isoflavones, AhR Signaling and CYP1 Enzymes

Although there are over 100 phytochemicals associated with soy protein products (Fang et al., 2004, Kang et al. 2010), the major phytochemical constituents are the isoflavones genistein and daidzein. These compounds have structures similar to 17β-estradiol and are weakly estrogenic with a higher affinity for estrogen receptor (ER)β than ERα (Badger et al. 2002; Szkudelska and Nogowski 2007; Dinsdale and Ward, 2010). In addition, breakdown of daidzein by gut bacteria results in the production of the more etrogenic metabolite equol in ~20% of soy consumers (Shor et al. 2012). It is commonly assumed that the biological effects of soy result from the actions of these phytoestrogen components

acting via ER signaling (McCarver et al. 2011). The majority of studies examining the effects of pure isoflavones on CYP1 expression and activity have been performed *in vitro* and appear to vary depending on the isoflavone and cell line examined. Genistein was reported to inhibit metabolism of ethoxyresorufin in mouse liver microsomes and to be a non-competitive inhibitor of recombinant human CYP1A2 at mM concentrations (Helsby et al., 1998). Genistein was also reported to inhibit recombinant human CYP1A1 and CYP1B1 activity at lower Ki of 15 and 0.7 μM respectively wheras daidzein was ineffective (Chan and Leung, 2003). In contrast, genistein, daidzein and the daidzein metabolite equol were all shown to be dose-dependent CYP1A1 and CYP1B1 inhibitors in transfected V79 cells at concentrations from 3–30 μM (Scott et al., 2008). In H411E rat hepatoma cell cultures genistein and daidzein were both reported to inhibit induction of CYP1A1 by omeprazole which activates the AhR/ARNT complex without AhR binding. However, neither were able to block induction of CYP1A1 by the AhR ligands PAH benzo(a)pyrene or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Backlund et al., 1997). In contrast, in MCF-7 human mammary carcinoma cells, human ketatinocytes and human alveolar type II A549 lung adenocarcinomas, genistein and daidzein have been reported to inhibit dioxin and DMBA-induced CYP1A1 expression (Gradin et al., 1994; Hukkanen et al. 2000; Chan and Leung, 2003, Wagner et al. 2008). In keratinocytes, genistein inhibition was shown to target the ligand binding domain of AhR and interactions with the chaperone protein HSP-90 but did not result in AhR degradation (Gradin et al., 1994). In contrast to CYP1A1, CYP1B1 expression and induction by TCDD has been reported to be unaffected by genistein in both MCF-7 and lung adenocarcinoma cells (Hukkanen et al., 2000; Wagner et al. 2008). Moreover, Wei et al. (2015) reported synergistic induction of CYP1B1 in MCF-7 cells by genistein and DMBA. Only one human clinical study has examined the effects of genistein on CYP1 activity. Daily ingestion of 1 g genisten/day for 14 days by healthy human volunteers resulted in a 41% decrease in caffeine metabolite ratios used to assess CYP1A2 activity (Chen et al. 2011).

Soy and CYP2 Enzymes

In contrast to effects on CYP1 enzymes, the effects of soy feeding on expression of CYP family 2 members appear more limited. Mezei et al. (2002) reported 3-fold upregulation of a member of the CYP2A subfamily in the liver of gerbils fed SPI for 28 days relative to gerbils fed a casein-based diet. In this study, SPI stripped of phytochemicals by ethanol washing was almost as effective in CYP2A induction as intact SPI, wheras extracted phytochemical extracts also significantly increased CYP expression. Induction of pentoxyresorufin O-depentylase, a marker of CYP2B activity was shown to be significantly increased in rats fed SPI and treated with the glucocorticoid dexamethasone relative to dexamethasone-treated rats fed casein (Ronis et al. 1999). However, no similar increase was observed in CYP2B1 apoprotein. In addition, array analysis of hepatic gene expression profiles of ovariectomized female rats fed SPI for 14 days revealed a significant 3-fold increase in expression of CYP2C13 mRNA (Singhal et al. 2009a). Interestingly, a similar increase in CYP2C13 expression was observed when these rats where treated with estradiol to restore physiological hormone levels. Array analysis of hepatic gene expression in neonatal pigs fed cow's milk or soy-based infant formula indicated a significantly greater

expression of CYP2D6 mRNA in both male and female piglets fed soy formula (Ronis et al. 2011a).

Isoflavones and CYP2 Enzymes

Information on the effects of isoflavones on CYP2 enzymes is also limited. Genistein, daidzein and glycitein have been shown to non-competitively inhibit nicotine C-oxidation catalyzed by recombinant human CYP2A6 at Ki concentrations of 1.3, 0.7 and 5.2 μM respectively. In addition, isoflavone supplement consumption for 5 days was shown to significantly decrease nicotine metabolism in a Japanese clinical study (Nakajima et al. 2006). In rats, consumption of an isoflavone extract at levels of up to 300 mg/kg diet was reported to have no effect on hepatic expression of CYP2B1/2, CYP2C11 or CYP2E1 mRNAs (Kishida et al. 2004). At higher dietary concentrations of 1250 mg/kg, genistein was found to decrease expression of CYP2C11 apoprotein and testosterone 2α- and 16α-hydroxylation (Laurenzana et al. 2002). Genistein and equol were also shown to be non-competitive inhibitors of CYP2E1 activity at Ki concentrations of 10 mM and 560 μM (Helsby et al. 1998). In an *in vivo* pharmacokinetic study in rats, genistein was also found to inhibit the clearance of the anticancer drug paclitaxel whether administered orally or by i.v. (Li and Choi 2007). However, it is unclear if this is associated with inhibition of CYP2C or CYP3A activity or effects on ABC transporter expression.

Soy, PXR-Signaling and CYP3A Enzymes

The effects of soy on CYP3A enzymes has received a significant amount of attention given the widespread consumption of soy products and the importance of CYP3As in the metabolism of a majority of clinically utilized drugs in both adults and pediatric patients (Ronis and Ingelman-Sundberg, 1998,de Wildt et al. 1999). Ronis et al. (1999) reported that lifetime SPI feeding of male rats resulted in significantly higher hepatic CYP3A apoprotein expression and dexamethasone inducibility compared to rats fed diets using casein as the sole protein source in adults. Feeding SPI also resulted in significantly increased microsomal 6β-hydroxylation of the CYP3A substrates testosterone and corticosterone and increased CYP3A-dependent N-demethylation of erythromycin and ethylmorphine after dexamethasone treatment relative to casein-fed rats (Ronis et al. 1999). In subsequent studies in weanling rats fed casein or SPI, Ronis et al. (2004) demonstrated that SPI consumption resulted in expression of hepatic CYP3A1 (CYP3A23) and CYP3A2 mRNA in rats as young as age 25 days. These enzymes were undetectable in rats fed casein. SPI consumption increased metabolism of the CYP3A substrate midazolam 2-fold. Feeding SPI also significantly increased induction of CYP3A2 mRNA and CYP3A apoprotein by dexamethasone and clotrimazole at this age relative to feeding casein. Using peptide-specific antibodies against CYP3A1 and CYP3A2, Ronis et al. (2006b) demonstrated that the effects of SPI feeding on hepatic CYP3A expression was a direct effect of the diet as opposed to developmental programming associated with early soy consumption. In addition, CYP3A1 was observed to be expressed at high levels in both liver and the intestinal jejunum of SPI-fed rats at age 33 days relative to casein-fed rats. More recent studies by Ronis et al. (2011b) examining quantitation of newly synthesized hepatic CYP3A1 transcripts by nuclear run-on analysis demonstrated a higher rate of basal transcription of the *CYP3A1* gene in SPI-fed

compared to casein fed rats accompanied by increased recruitment of the pregnane X-receptor to a response element on the *CYP3A1* promoter. The rat is not the only species in which increased expression of CYP3A enzymes has been reported in response to feeding soy products. Li et al. (2009) reported a 5.7-fold increase in expression of CYP3A11 in mice fed SPI diets containing high levels of isoflavones relative to mice fed diets containing SPI stripped of phytochemicals by successive ethanol washes. Ronis et al. (2011a) reported significantly increased expression of hepatic CYP3A29 and CYP3A46 mRNA, increased expression of CYP3A apoprotein and increased CYP3A-dependent microsomal metabolism of erythromycin and 6β-hydroxylation of testosterone in neonatal male and female piglets fed soy-based infant formula relative to piglets which were fed cow's milk formula or breastfed sows milk. Collectively these data suggest that consumption of soy products by Asians eating traditional diets, vegetarians, vegans and soy formula consumption by infants may alter the clearance and therapeutic efficacy of a wide variety of medications and require dosage adjustment.

Isoflavones, PXR-Signaling and CYP3A Enzymes

In contrast to the consistent reports of increased expression of CYP3A mRNAs, protein and activities observed in animals fed soy products, the literature on the effects of pure isoflavones on CYP3A enzymes is more variable. Significant inhibition of activity of human CYP3A4 stably transfected into V79 cells by daidzein at concentrations of 1–30 μM has been reported. In contrast no effect was observed with genistein while equol appeared to increase CYP3A4 activity in a dose-dependent manor by post-transcriptional mechanisms (Scott et al. 2008). In contrast, genistein has been reported to inhibit vitamin D3-induced CYP3A4 expression in human intestinal Caco-2 cells (Sergent et al. 2009). Administration of isoflavone containing soy extracts to rats at doses up to 300 mg/kg diet was reported to have no effect on expression of hepatic CYP3A1 or CYP3A2 mRNAs (Kishida et al. 2004). A higher dietary dose of 1250 mg/kg genistein given throughout development was reported to decrease hepatic CYP3A apoprotein expression in male rats (Laurenzana et al. 2002). In contrast, genistein, daidzein and equol have been reported to upregulate human CYP3A4 promoter directed luciferase expression in HepG2 cells at concentrations of 5–10 μM and to increase CYP3A11 expression in primary mouse hepatocytes from wild type but not PXR −/− mice (Li et al. 2009). It was reported that the isoflavones differed in their affinity for mouse and human PXR with genistein being the most potent mouse ligand while equol was the most potent human ligand. Additional evidence for activation of PXR by isoflavones come from studies in HepG2 cells transfected with Gal4 luciferase reporter and a chimeric plasmid in which the Gal4 DNA-binding domain is fused to PXR (Ricketts et al. 2005). However, the concentration of isoflavones required to activate PXR and induce CYP3A in these *in vitro* assays was super physiological. Total isoflavone concentrations following consumption of SPI range from 1–2 μM depending on the species, age and sex (Gu et al., 2006, Singhal et al. 2009b). Moreover, almost all of the isoflavones are conjugated with glucuronide or sulfate as they are absorbed from the small intestine and plasma concentrations of biologically active isoflavone aglycones only range from 0.5–7% of the total (Gu et al., 2006, Ronis et al. 2006a). Thus total plasma isoflavone aglycone concentrations after consumption of SPI are in the range of 10–140 nM. It is therefore

unclear to what degree isoflavones are responsible for the increase in CYP3A expression observed after consumption of SPI. Ronis et al. (2006b) reported a lack of effect of casein diets containing 250 mg/kg pure genistein (a level comparable to that found in SPI) on expression of hepatic CYP3A1 or CYP3A2 mRNA or protein in weanling rats. A similar lack of effect on expression of hepatic CYP3A1 was observed in rats fed casein diets containing pure daidzein at 250 mg/kg or fed diets containing SPI stripped of phytochemicals. These data suggest that soy phytochemicals other than isoflavones are responsible for PXR activation and CYP3A1 upregulation. In contrast, although genistein diets also had no effect on expression of rat hepatic CYP3A2, both dietary daidzein and phytochemical stripped soy protein diets significantly increased expression of CYP3A2 mRNA, CYP3A2 protein and testosterone 6β-hydroxylase activity (Ronis et al. 2006b). A recent clinical study in healthy Chinese volunteers receiving genistein for 14 days reported significant increases in clearance of CYP3A substrates midazolam and talinolol suggesting that consumption of genistein supplements may result in a modest increase in CYP3A in humans (Xiao et al. 2012).

Soy, PPARα-Signaling and CYP4 Enzymes

Consumption of SPI has been shown to inhibit the development of hepatic triglyceride accumulation and steatosis in several different rodent models including rats fed high fat diets, rats overfed with high fat diets by total enteral nutrition; high fat fed 129/SV mice; in obese yellow A^{vy}/a mice and in obese Zucker rats (Mezei et al. 2003; 2006; Badger et al. 2008, Ronis et al. 2009). The anti-steatotic effects of SPI appear due to activation of signaling through both the peroxisome proliferator activated receptor (PPARα) pathway to increase fatty acid degradation. A variety of downstream PPARα-regulated genes including, acyl CoA oxidase, carnitine palmitoyl transferase and several CYP4As have been reported to be elevated after feeding diets containing SPI relative to feeding diets made with casein. CYP4A14 mRNA was induced 4-fold in SPI-fed yellow A^{vy}/a mice relative to mice fed casein (Badger et al. 2008). Ronis et al. (2011) reported a 2–6-fold increase in expression of hepatic CYP4A21 in male and female neonatal piglets fed soy-based infant formula relative to piglets which were sow fed or fed cow's milk based formula. However, not all CYP4A enzymes were reported to be elevated after SPI feeding. Simmen et al. (2010) reported a reduction in rat hepatic CYP4A10 after lifetime feeding of SPI compared to casein. Ronis et al. (2011a) reported that unlike CYP4A21, pig hepatic CYP4A24 was unresponsive to feeding of soy infant formula. Mezei et al. 2006 reported that SPI improved steatosis in high fat-fed mice relative to mice fed SPI stripped of phytochemicals and that this effect was blocked in PPARα −/− mice. Consistent with an effect via PPARα activation Badger et al. (2008) reported increased binding of PPARα to the PPRE element in the acyl COA oxidase promoter in SPI-fed yellow A^{vy}/a mice relative to mice fed casein using electrophoretic mobility shift assays. In addition, feeding SPI has been reported to increase hepatic PPARα mRNA expression (Ronis et al. 2009).

Isoflavones, PPAR-signaling and CYP4 Enzymes

A number of studies have examined the effects of isoflavones on PPARα activation. In vitro studies by Mezei et al. 2002; 2006; Shay and Banz 2005 and Ricketts et al. 2005

demonstrate activation of PPARα and PPARγ by isoflavone extracts and purified isoflavones. However, the same issues apply to PPAR activation as to PXR activation by isoflavones in comparison to bioactivity associated with other phytochemical and protein/peptide components of SPI. The in vitro studies on PPAR activation also use super-physiological levels of isoflavones not attainable in vivo after feeding SPI. Only one study has directly examined the effects of pure isoflavones on CYP4 expression. Comparison of the effects of diets containing intact SPI, phytochemical stripped SPI and casein diets + genistein or daidzein at concentrations found in SPI on PPAR expression and expression of downstream PPAR-regulated genes was reported in rats by Ronis et al. (2009). Significant increases in these pathways were observed in rats fed either intact SPI or stripped of phytochemicals compared to casein. In contrast, neither dietary genistein nor daidzein at levels found in SPI had effects on these pathways. Thus it appears that while high levels of isoflavones can have effects on lipid metabolism via PPAR activation which may be of relevance to consumers of isoflavone supplements, protein/peptide components of SPI appear to be responsible for the effects of soy on PPAR-mediated pathways. Only one other paper has been published on the effects of pure isoflavones on CYP4 enzymes. Hsu et al. (2011) reported that AMPK activation and genistein upregulated the transcription of CYP4F2 mRNA in human HepG2 hepatoma cells. The effects of genistein on CYP4F2 were blocked by inhibitors of SIRT1 and calmodulin kinase kinase and it was suggested that these pathways were upstream of activation of AMPK.

Effects of Soy on CYP-dependent metabolism of cholesterol

There is convincing evidence that soy consumption is linked to protection against cardiovascular disease as the result of improved serum lipoprotein profile (Clarkson, 1998; Gil-Izquierdo et al., 2012). Meta analyses suggest a 5% decrease in serum LDL cholesterol associated with soy protein intake (Zhan and Ho, 2005; Anderson and Bush, 2011). Similar significant decreases in plasma and hepatic cholesterol have been reported in obese Zucker rats fed SPI (Mezei et al., 2002); in rats fed high fat/0.5% cholesterol diets (Ronis et al. 2009) and in piglets fed soy-based infant formula relative to sow-fed piglets (Ronis et al. 2011a). This effect has been linked to increased CYP-dependent conversion of cholesterol to bile acids by CYP7A1 (cholesterol 7α hydroxylase). CYP7A1 mRNA and apoprotein were reported to be significantly upregulated by feeding high fat/0.5% cholesterol diets containing SPI with or without phytochemical stripping to rats for 40 days from weaning relative to rats fed the same diet made with casein (Ronis et al 2009). Increased CYP7A1 expression was accompanied by evidence of increased expression and activation of the nuclear receptor LXR in the liver of SPI fed rats. In addition, feeding of soy infant formula to neonatal piglets also resulted in significantly increased expression of hepatic CYP7A1 relative to piglets fed sow milk or cow's milk formula (Ronis et al. 2011a). Additional *in* vivo studies in mice revealed increased hepatic expression of CYP8B1 (sterol 12α hydroxylase) another important CYP enzyme involved in bile acid formation in animals fed intact SPI compared to SPI stripped of phytochemicals (Li et al. 2007).

Effects of isoflavones on CYP-dependent metabolism of cholesterol

In contrast to lack of effects of diets supplemented with pure isoflavones at physiologically relevant levels on expression of hepatic CYP3A and CYP4A enzymes, casein diets supplemented with 250 mg/kg genistein or daidzein were reported to increase expression of CYP7A1 to a similar degree as intact SPI (Ronis et al. 2009). In addition, there is evidence from in vitro studies of isoflavones using a Gal4 fusion reporter system that isoflavones are capable of activating LXR (Ricketts et al. 2005). Additional *in vitro* studies with isoflavones in primary human hepatocytes and in HepG2 cells suggest that isoflavones increase expression of CYP8B1 via activation of hepatocyte nuclear factor 4α (Li et al. 2007).

Effects of Soy and Isoflavones on CYP-dependent Steroid Metabolism

Human clinical studies provide little evidence that consumption of soy products or isoflavones dramatically affect serum sex hormone levels (Hooper et al. 2009; Hamilton-Reeves et al. 2010). However, many *in vitro* and animal studies have suggested that at pharmacological concentrations genistein and daidzein can affect expression and activity of many CYP enzymes involved in synthesis and metabolism of sex steroids, glucocorticoids and vitamin D3 metabolites. In studies in cultured pig granulosa cells genistein was reported to inhibit CYP11A1 activity (cholesterol side chain cleavage enzyme) which catalyzes the conversion of cholesterol to pregnanolone and reduced progesterone production (Piasecka-Srader et al. 2014). Interestingly in this regard, Miousee et al. (2013) reported that feeding SPI compared to casein diets reduced serum progesterone in weanling female but not male rats. In contrast, the reported effects of genistein on CYP19 (aromatase) expression and activity are inconsistent. Rice et. al. (2006) reported that 10μM genistein reduced CYP19 mRNA in human granulosa-luteal cells and Brooks and Thompson (2005) reported a similar dose of genistein inhibited aromatase activity in human MCF-7 mammary cancer cells. On the other hand, Piasecka-Srader et al. (2014) reported no effects of genistein on aromatase expression or activity in pig granulosa cells, Myllymaki et al. (2005) report that genistein stimulated aromatase activity in cultured isolated immature rat ovarian follicles and van Dursen et al. (2011) reported that genistein induced aromatase expression in MCF-7 cells. Mesiandro et al. (1999). Ohno et al. (2002) reported that genistein and daidzein were competitive inhibitors of adrenal CYP21 (steroid 21-hydroxylase). Isoflavones were also able to inhibit basal and ACTH-stimulated glucocorticoid synthesis in pig adrenal glands ex –vivo (Kaminska et al. 2014) and high doses of pure genistein were able to suppress serum corticosterone by 50% in weanling rats *in vivo* (Ohno et al. 2003). It has been demonstrated that genistein can inhibit expression and activity of both 25-hydroxyvitamn D3 1α hydroxylase (CYP27B1) and 24-hydroxylase (CYP24) in prostate cancer cells *in vitro* (Farhan and Cross 2002; Farhan et al. 2003; Swami et al. 2005). In contrast, genistein but not equol was reported to inhibit CYP24 expression and activity but induce CYP27B1 in human colon and breast cancer cells (Lechner et al. 2006).

Conclusions

There is conclusive data to suggest that consumption of dietary SPI and other soy products significantly alter the expression and activity of CYP enzymes in a species, organ and cell

type specific manner. Inhibition of AhR-mediated CYP1 induction may play a role in the reduced cancer risk observed in many Asian populations consuming traditional soy-rich diets. In addition, increased expression of CYP2 and CYP3A enzymes may increase the clearance, reduce the half-life and change the therapeutic efficacy of many clinically utilized drugs in soy-formula-fed infants and adult vegans and vegetarians. Also, increased expression of CYPs involved in fatty acid and sterol metabolism after soy consumption may play a role in improved lipid homeostasis and reduced cardiovascular risk. From the perspective of pre-clinical drug development and toxicity testing it is also important to remember that most rodent chows contain a high level of soy. This will undoubtedly significantly affect both therapeutic and toxic endpoints relative to animals fed soy free diets such as the 2020X Teklad Global diet or semi-purified diets such as AIN-93G (Hakkak et al. 1993). The role of isoflavones in the effects of soy on CYP expression is unclear. Bioavailability of isoflavones following soy consumption is limited by the food matrix in whole diets and by the nature of isoflavones in soy, where they are found mainly as glycoside conjugates (genistin, daidzin) as opposed to the bioactive aglycones genistein and daidzein (Badger et al. 2002). In addition, extensive first pass conjugation of isoflavone aglycones to inactive glucuronides and sulfates occurs during absorption from the gut (Gu et al., 2006; Ronis et al., 2006a) resulting in very low plasma concentrations of aglycones of less than 1 μM. In vitro studies in cell lines have often utilized supra-physiological concentrations of isoflavones only attainable as the result of supplement consumption as opposed to eating of soy products. Some similar effects on CYP3A CYP4, and CYP7A1 expression associated with activation of nuclear receptors such as PXR, PPARs and LXR occur in *in vitro* systems at isoflavone concentrations considerably higher than observed *in vivo* after feeding SPI. Comparisons of CYP expression in rodents fed casein, SPI with or without phytochemical striping or fed casein with pure isoflavone supplementation suggest that other protein/peptide and phytochemical components on SPI may also play a role in CYP regulation. It is clear that reductionist approaches to establishing molecular mechanisms underlying the biological effects of whole diets can be highly misleading. This is certainly the case when it comes to soy and isoflavones. Many investigators and regulatory agencies have equated the biological effects of soy with those of pure genistein, particularly in relation to estrogen receptor-mediated effects (Hsieh et al. 1998; McCarver et al. 2011, Jefferson et al. 2009). However, we have comprehensively demonstrated that unlike genistein, SPI consumption as the sole protein source does not have estrogenic actions on gene expression profiles either in the liver (Singhal et. al. 2009, Ronis et al., 2011a) or in estrogen-sensitive reproductive tissues such as the mammary, uterus or testis (Ronis et al., 2012; Miousse et al., 2013; Ronis et al., 2014; Ronis et al., 2016). These data suggest that although studies on the effects of pure isoflavones on CYP expression and activity may be of importance in consumers of isoflavone supplements, they probably have limited relevance to soy consumers.

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References

An G, Mukker JK, Derendorf H, Frye RF. Enzyme and transporter-mediated beverage-drug interactions: An update on fruit juices and green tea. J Clin Pharmacol. 2015; 55:1313–1331. [PubMed: 26095990]

Anderson JW, Bush HM. Soy protein effects of serum lipoproteins: a quality assessment and meta-analysis of randomized, controlled studies. J Am Coll Nutr. 2011; 30:79–91. [PubMed: 21730216]

Backlund M, Johansson I, Mkrchian S, Ingelman-Sundberg M. Signal-transduction-mediated activation of the aryl hydrocarbon receptor in rat hepatoma H4IIE cells. J Biol Chem. 1997; 272:31755–31763. [PubMed: 9395520]

Badger TM, Ronis MJJ, Hakkak R, Korourian S. The health consequences of early soy consumption. J Nutr. 2002; 132:559S–565S. [PubMed: 11880593]

Badger TM, Ronis MJJ, Simmen R, Simmen F. Soy protein isolate and protection against cancer. J Am Coll Nutr. 2005; 24:146S–149S. [PubMed: 15798082]

Badger TM, Ronis MJJ, Wolff G, Stanley S, Ferguson M, Shankar K. Consumption of Soy Protein Isolate Reduces Hepatosteatosis in Obese Yellow Agouti (Avy) Mice, but Does Not Alter the Coat Color Phenotype. Exp Biol Med. 2008; 233:1242–1254.

Brooks JD, Thompson LU. Mammalian lignans and geniostein decrease the activities of aromatase and 17β-hydroxysteroid dehydrogenase in MCF-7 cells. J Steroid Biochem Mol Biol. 2005; 94:461–467. [PubMed: 15876411]

Chan HY, Leung LK. A potential protective mechanism of soya isoflavones against 7,12-dimethylbenz[a]anthracene tumor initiation. Br J Nutr. 2003; 90:457–465. [PubMed: 12908908]

Chen Y, Xiao CQ, He YJ, Chen BL, Wang G, Zhou G, et al. Genistein alters caffeine exposure in healthy female volunteers. Eur J Clin Pharmacol. 2011; 67:247–353.

Clarkson TB, Anthony MS. Phytoestrogens and coronary heart disease. Baillieres Clin Endocrinol Metab. 1998; 12:589–604. [PubMed: 10384815]

Constintinou AL, Lantvit D, Hawthorne M, Xu X, van Breemen RB, Pezzuto JM. Chemopreventive effects of soy protein and purified soy isoflavones oon DMBA-induced mammary tumors in female Sprague-Dawley rats. Nutr Cancer. 2001; 41:75–81. [PubMed: 12094632]

De Wildt SN, Kearn GL, Leeder JS, van den Anker JN. Chtochrome P450 3A: ontogeny and drug disposition. Clin Pharmacokinet. 1999; 37:485–505. [PubMed: 10628899]

Dinsdale EC, Ward WE. Early exposure to soy isoflavones and effects on reproductive health: a review of human and animal studies. Nutrients. 2010; 2:1156–1187. [PubMed: 22254003]

Dong JY, Qin LQ. Soy isoflavones consumption and risk of breast cancer incidence and recurrence. Breast Cancer Res Treat. 2011; 125:315–323. [PubMed: 21113655]

Fang N, Yu S, Badger TM. Comprehensive phytochemical profile of soy protein isolate. J Agric Food Chem. 2004; 52:4012–4020. [PubMed: 15186131]

Farhan H, Cross HS. Transcriptional inhibition of CYP24 by genistein. Ann NY Acad Sci. 2002; 973:459–462. [PubMed: 12485911]

Farhan H, Wahala K, Cross HS. Genistein inhibs vitamin D hydroxylases CYP24 and CYP27B1 expression in prostate cells. J Steroid Biochem Mol Biol. 2003; 84:423–429. [PubMed: 12732287]

Gradin K, Whitelaw ML, Toftgard R, Poellinger L, Bergard A. A tyrosine kinase-dependent pathway regulates ligand-dependent activation of the dioxin receptor in human keratinocytes. J Biol Chem. 1994; 269:23800–23807. [PubMed: 8089152]

Gil-Izquierdo A, Penalvo JL, Gil JI, Medina S, Horcajada MN, Lafay S, et al. Soy isoflavones and cardiovascular disease epidemiological, clinical and –omics perspectives. Curr Pharm Biotechnol. 2012; 13:624–631. [PubMed: 22122477]

Gu L, Prior RL, Fang N, Ronis MJJ, Clarkson TM, Badger TM. Metabolic phenotypes of isoflavones are different in female rats, pigs, monkeys and humans. J Nutr. 2006; 136:1215–1226. [PubMed: 16614407]

Hakkak R, Ronis MJJ, Badger TM. Effects of enteral nutrition and ethanol on cytochrome P450 distribution in small intestine of male rats. Gastroenterology. 1993; 104:1611–1618. [PubMed: 8500717]

Hakkak R, Korourian S, Shelnutt SR, Lensing S, Ronis MJJ, Badger TM. Diets Containing Whey Protein or Soy Protein Isolate Protect Against DMBA- Induced Mammary Tumors in Female Rats, Cancer Epidemiol. Biomarkers Prev. 2000; 9:113–117.

Hamilton-Reeves JM, Vazquez G, Duval SJ, Phipps WR, Kurzer MS, Messina MJ. Clinical studies show no effects of soy protein or isoflavones on reproductive hormones in men: results of a meta-analysis. Fertil Steril. 2010; 94:997–1007. [PubMed: 19524224]

Hardwick JP, Osei-Hyiaman D, Wiland H, Abdelmegeed MA, Song BJ. PPAR/RXR Regulation of Fatty Acid Metabolism and Fatty Acid omega-Hydroxylase (CYP4) Isozymes: Implications for Prevention of Lipotoxicity in Fatty Liver Disease. PPAR Res. 2009; 2009:952734. [PubMed: 20300478]

Helsby NA, Chipman JK, Gescher A, Kerr D. Inhibition of mouse and human CYP1A- and 2e1-dependent substrate metabolism by the isoflavinoids genistein and equol. Food Chem Toxicol. 1998; 36:375–382. [PubMed: 9662412]

Hooper L, Ryder JJ, Kurzer MS, Lampe JW, Messina MJ, Phipps WR, et al. Effects of soy protein and isoflavones on circulating hormone concentrations in pre-m and post-menopausal women: a systemic review and meta-analysis. Hum Reprod Update. 2009; 15:423–440. [PubMed: 19299447]

Hsieh CY, Santell RC, Haslam SZ, Helferich WG. Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells in vitro and in vivo. Cancer Res. 1998:3833–3838. [PubMed: 9731492]

Hsu MH, Savas U, Lasker JM, Johnson EF. Genistein, resveratrol, and 5-aminoimidazole-4-carboxamide-1β-D-ribofuranoside induce cytochrome P450 4F2 expression through an AMP-activated protein kinase-dependent pathway. J Pharmacol Exp Ther. 2011; 337:125–136. [PubMed: 21205922]

Hukkanen J, Lassila A, Paivarinta K, Valanne S, Sarpo S, Hakkola J, et al. Induction and regulation of xenobiotic-metabolizing cytochrome P450s in the human A549 lung adenocarcinoma cell line. Am J Respir Cell Mol Biol. 2000; 22:360–366. [PubMed: 10696073]

Ingelman-Sundberg M, Ronis MJ, Lindros KO, Eliasson E, Zhukov A. Ethanol-inducible CYP2E1: regulation, enzymology and molecular biology. Alcohol Alcohol Suppl. 1994; 2:131–139. [PubMed: 8974327]

Ioannides C. Effect of diet and nutrition on the expression of cytochromes P450. Xenobiotica. 1999; 29:109–154. [PubMed: 10199591]

Jefferson WN, Doerge D, Padilla-Banks E, Woodling KA, Kissling GE, Newbold R. Oral exposure to genistein during neonatal life adversely affects the female reproductive system. Environ. Health Perspect. 2009; 117:1883–1889.

Kaminska B, Czerwinska J, Wojciechowicz B, Nynca A, Ciereszko R. Genistein and daidzein affect in vitro steroidogenesis but not gene expression of steroidogenic enzymes in adrenals of pigs. J Physiol Pharmacol. 2014; 65:127–133. [PubMed: 24622837]

Kang J, Badger TM, Ronis MJJ, Badger TM. Non-isoflavone phytochemicals in soy and their health effects. J Agric Food Chem. 2010; 58:8119–8133. [PubMed: 20578706]

Kishida T, Nagamoto M, Ohtsu Y, Watakabe M, Ohshima D, Nashiki K, et al. Lack of an inducible effect of dietary soy isoflavones on the mRNA abundance of hepatic cytochrome P-450 isozymes in rats. Biosci Biotechnol Biochem. 2004; 68:508–515. [PubMed: 15056880]

Korde LA, Wu AH, Fears T, Nomura AM, West DW, Kolonel LN, et al. Childhood soy intake and breast cancer risk in Asian American women. Cancer Epidemiol Biomarkers Prev. 2009; 18:1050–1059. [PubMed: 19318430]

Lamps JW, Petersen S. *Brassica*, biotransformation and cancer risk: Genetic polymorphisms alter the preventive effects of cruciferous vegetables. J Nutr. 2002; 132:2991–2994. [PubMed: 12368383]

Laurenzana EM, Weis CC, Bryant CW, Newbold R, Delclos KB. Effect of dietary administration of genistein, nonylphenol or ethinyl estradiol on hepatic testosterone metabolism, cytochrome P450 enzymes and estrogen receptor alpha expression. Food Chem Toxicol. 2002; 40:53–63. [PubMed: 11731036]

Lechner D, Bajna E, Adlercreutz H, Cross HS. Genistein and 17β-estadiol, regulate vitamin D synthesis in human colon and breast cancer cells. Anticancer Res. 2006; 26:2597–2603. [PubMed: 16886669]

Li X, Choi JS. Effect of genistein on the pharmacokinetics of paclitaxel administered orally or intravenously in rats. Int J Pharm. 2007; 337:188–193. [PubMed: 17267149]

Li Y, Mezei O, Shay NF. Human and murine hepatic sterol-12α-hydroxylase and other xenobiotic metabolism mRNA are upregulated by soy isoflavones. J Nutr. 2007; 137:1705–1712. [PubMed: 17585019]

Li Y, Cederbaum AI. CYP2E1 and oxidative liver injury by alcohol. Free Radic Biol Med. 2008; 44:723–738. [PubMed: 18078827]

Li Y, Ross-Viola J, Shay NF, Moore DD, Ricketts M-L. Human CYP3A4 and murine CYP3A11 are regulated by equol and genistein via the pregnane-X-receptor in a species-specific manner. J Nutr. 2009; 139:898–904. [PubMed: 19297428]

Mahmoud AM, Yang W, Bosland MC. Soy isoflavones and prostate cancer: a review of molecular mechanisms. J Steroid Biochem Mol Biol. 2014; 140:116–132. [PubMed: 24373791]

McCarver G, Bhatia J, Chambers C, Clarke R, Etzel R, Foster W, et al. NTP-CERHR expert panel report on the developmental toxicity of soy infant formula. Birth Defects Res B Dev Reprod Toxicol. 2011; 92:421–468. [PubMed: 21948615]

Mesiano S, Katz SL, Lee JY, Jaffe RB. Phytoestrogens alter adrenocortical function: genistein and daidzein suppress glucocorticoid and stimulate androgen productin by cultured adrenal cortical cells. J Clin Endocrinol Metab. 1999; 84:2443–2448. [PubMed: 10404819]

Mezei O, Chou CN, Kennedy KJ, Tovar-Palacio C, Shay NF. Hepatic cytochrome P450-2A and phosphoribosylpyrophosphate synthase-associated protein mRNA are induced in gerbils after consumption of isoflavone-containing protein. J Nutr. 2002; 13:2358–2544.

Mezei O, Banz WJ, Steger RW, Peluso MR, Winters TA, Shay N. Soy isoflavones exert antidiabetic and hyperlipidemic effects through the PPAR pathways of obese Zucker rats and murine RAW 264.7 cells. J Nutr. 2003; 133:1238–1243. [PubMed: 12730403]

Mezei O, Li Y, Mullen E, Ross-Viola JS, Shay N. Dietary isoflavone supplementation modulates lipid metabolism via PPARα-dependent and independent mechanisms. Physiol Genomics. 2006; 26:8–14. [PubMed: 16507786]

Miousse IR, Sharma N, Blackburn M, Vantrese J, Gomez-Acevedo H, Hennings L, et al. Feeding soy protein isolate and treatment with estradiol have different effects on mammary gland morphology and gene expression in weanling male and female rats. Physiol Genomics. 2013; 45:1072–1083. [PubMed: 24046281]

Murray M. Altered CYP expression and function in response to dietary factors: Potential roles in disease pathogenesis. Curr Prog Drug Met. 2006; 7:67–81.

Myllymaki S, Haavisto T, Vainio M, Topparu J, Paranko J. In vitro effects of diethylstilbestrol, genistein 4-tert-butylphenol and 4-tert-octylphenol on steroidogenic activity iof isolated immature rat ovarian follicles. Toxicol Appl Pharmacol. 2005; 204:69–80. [PubMed: 15781295]

Nakajima M, Itoh M, Yamanaka H, Fukami T, Tokudome S, Yamamoto Y, et al. Isoflavones inhibit nicotine C-oxidation catalyzed by human CYP2A6. J Clin Pharmcol. 2006; 46:337–344.

Ohno S, Shinoda S, Toyoshima S, Nakazawa H, Makino T, Nakajin S. Effects of flavonoid phytochemicals on cortisol production and on activities of steroidogenic enzymes in human adrenocortical H295R cells. J Steroid Biochem Mol Biol. 2002; 80:355–363. [PubMed: 11948020]

Ohno S, Nakajima Y, Inoue K, Nakazawa H, Nakajin S. Genistein administration decreases serum corticosterone and testosterone levels in rats. Life Sci. 2003; 74:733–742. [PubMed: 14654166]

Ollberding NJ, Lim U, Wilkens LR, Setiawan VW, Shvetsov YB, Henderson BE, et al. Lergume, soy, tofu and isoflavone intake and endometrial cancer risk in postmenopausal women in the multiethnic cohort study. J natl Cancer Inst. 2012; 104:67–76. [PubMed: 22158125]

Plasecka-Srader J, Kolomycka A, Nynca A, Ciereszko RE. Effects of 2,3,7,8-tetrachlorodibenz0-p-dioxin and phytoestrogen genistein on the activity and the presence of steroidogenic enzymes proteins in cultured granulosa cells of pigs. Anim Reprod Sci. 2014; 148:171–181. [PubMed: 25022331]

Pineau T, Daujat M, Pichard L, Girard F, Angevain J, Bonfils C, et al. Developmental expression of rabbit cytochrome P450 CYP1A1, CYP1A2 and CYP3A6 genes. Eur J Biochem. 1991; 197:145–153. [PubMed: 2015817]

Rice S, Mason HD, Whitehead SA. Phytoestrogens and their low dose combinations inhibit mRNA expression and activity of aromatase in human granulosa-luteal cells. J Steroid Biochem Mol Biol. 2006; 101:216–225. [PubMed: 16965912]

Ricketts M-L, Moore DD, Banz WJ, Mezei O, Shay NF. Molecular mechanisms of action of the soy isoflavones includes activation of promiscuous nuclear receptors. A review. J Nutr Biochem. 2005; 16:321–330. [PubMed: 15936643]

Rodriguez-Fragoso L, Martinez-Arismendi JL, Orozco-Bustos D, Reyes-Esparza J, Torres E, Burchiel SW. Potential risks resulting from fruit/vegetable-drug interactions: Effects on drug metabolizing enzymes and transporters. J Food Sci. 2011; 76:R112–R124. [PubMed: 22417366]

Ronis, MJJ., Lindros, K., Ingelman-Sundberg, M. The CYP 2E1 Family. In: Ioneddes, C., editor. Cytochrome P450: Biochemical and Toxicological Aspects. CRC Press; 1996. p. 211-239.

Ronis, MJJ., Ingelman-Sundberg, M. Induction of Human P450 Enzymes: Mechanisms and Implications. In: Woolf, T., editor. A Handbook of Human Toxicology. Marcel Decker; 1998. p. 239-262.Chapter 10

Ronis MJJ, Rowlands JC, Hakkak R, Badger TM. Expression and Glucocorticoid-Inducibility of CP3A and CYP2B Enzymes in Male Rat Liver: Effects of Diets Containing Soy Protein Isolate. J Nutr. 1999; 129:1958–1965. [PubMed: 10539769]

Ronis MJJ, Rowlands JC, Hakkak R, Badger TM. Altered inducibility of hepatic CYP1A enzymes by 3-methylcholanthrene and isosafrole in male rats fed diets containing casein, soy protein isolate or whey. J Nutr. 2001; 131:1180–1188. [PubMed: 11285323]

Ronis MJJ, Chen Y, Jo CH, Simpson P, Badger TM. Increased hepatic CYP3A expression and inducibility in weanling rats fed diets containing soy protein isolate during development. J Nutr. 2004; 134:3270–3276. [PubMed: 15570024]

Ronis MJJ, Little J, Barone GW, Chen G, Radominska-Pandya A, Badger TM. Sulfation of the isoflavones genistein and daidzein by human and rat liver and gastrointestinal tract. J Medicinal Food. 2006a; 9:348–355.

Ronis MJJ, Chen Y, Badeaux J, Laurenzana E, Badger TM. Induction of rat hepatic CYP3A1 and CYP3A2 by soy protein isolate or isoflavones fed during early development. Exp Biol Med. 2006b; 231:60–69.

Ronis MJJ, Badger TM, Chen Y, Badeaux J. Dietary soy protein isolate attenuates metabolic syndrome in rats via effects on PPAR, LXR and SREBP-1c signaling. J Nutr. 2009; 139:1431–1438. [PubMed: 19515742]

Ronis MJJ, Chen Y, Shankar K, Gomez-Acevedo H, Badeaux J, Blackburn ML, et al. Formula feeding and protein source alter hepatic gene expression, iron and cholesterol homeostasis in neonatal piglets. Physiol Genomics. 2011a; 43:1281–1293. [PubMed: 21954453]

Ronis MJJ, Chen Y, Liu X, Shankar K, Fang N, Badger TM. Over expression and enhanced glucocorticoid-inducibility of hepatic CYP3A in rats fed soy protein isolate occurs through increased recruitment of PXR to promoter response elements. J Nutr. 2011b; 141:10–16. [PubMed: 21084653]

Ronis MJJ, Shankar K, Gomez-Acevedo H, Hennings H, Singhal R, Blackburn ML, et al. Mammary Gland Morphology and Gene Expression Differ in Female Rats Treated with 17β-Estradiol or Fed Soy Protein Isolate. Endocrinology. 2012; 153:6021–6032. [PubMed: 23027806]

Ronis M, Hennings L, Gomez-Acevedo H, Badger TM. Different responses to soy and estradiol in the reproductive system of prepubertal male rats and neonatal male pigs. Experimental Biology A. 2014; 818:1.

Ronis MJJ, Gomez-Acevedo H, Blackburn ML, Cleves MA, Singhal R, Badger TM. Uterine responses to feeding soy protein isolate and treatment with 17β-estradiol differ in ovariectomized female rats. Toxicol Appl Pharmacol. 2016; 297:68–80. [PubMed: 26945725]

Rowlands JC, He L, Hakkak R, Ronis MJJ, Badger TM. Soy and whey proteins downregulate DMBA-induced liver and mammary gland CYP1 expression in female rats. J Nutr. 2001; 131:3281–3287. [PubMed: 11739881]

Scott LM, Durant P, Leone-Kabler S, Wood CE, Register TC, Townsend A, et al. Effects of prior oral contraceptive use and soy isoflavonoids on estrogen-metabolizing cytochrome P450 enzymes. J Steroid Biochem Mol Biol. 2008; 112:179–185. [PubMed: 18955142]

Sergent T, Dupont I, Van der Heiden E, Scippo ML, Pussemier L, Larondelle Y, et al. CYP1A1 and CYP3A4 modulation by dietary flavonoids in human intestinal Caco-2 cells. Toxicol Lett. 2009; 15:216–222.

Shay NF, Banz B. Regulation of gene transcription by botanicals: Novel regulatory mechanisms. Ann Rev Nutr. 2005; 25:297–315. [PubMed: 16011469]

Shimada T, Fujii-Kuriyama Y. Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. Cancer Sci. 2004; 95:1–6. [PubMed: 14720319]

Singhal R, Badger TM, Ronis MJJ. Reduction in CYP1A1 induction following soy consumption is mediated via degradation of the aryl hydrocarbon receptor. J Nutr. 2007; 137:1–6. [PubMed: 17182792]

Shor D, Sathyapalan T, Atkin SL, Thatcher NJ. Does equol production determine endocrine effects? Eur J Nutr. 2012; 51:389–398. [PubMed: 22366740]

Simmen FA, Mercado CP, Zavacki AM, Huang SA, Greenway AD, Kang P, et al. Soy protein diet alters expression of hepatic genes regulating fatty acid and thyroid hormone metabolism in the male rat. J Nutr Biochem. 2010; 21:1106–1113. [PubMed: 19962299]

Singhal R, Badger TM, Ronis MJJ. Rats fed soy have impaired hepatic CYP1A1 induction by polycyclic hydrocarbons as a result of interference with Ah receptor signaling. Toxicol Appl Pharmacol. 2008; 227:275–283. [PubMed: 18078967]

Singhal R, Badger TM, Ronis MJJ. Estrogenic status modulates the effects of soy on hepatic responses to 7,12-dimethylbenz(a)anthracene (DMBA). Toxicol Appl Pharmacol. 2009a; 234:89–97. [PubMed: 18992763]

Singhal R, Shankar K, Badger TM, Ronis MJJ. Soy protein isolate mediated hepatic gene expression differs from that of 17β-estradiol in female rats. J Endocrinol. 2009b; 202:143–154.

Swami S, Krishnan AV, Peehl DM, Feldman D. Genistein potentiates the growth inhibitory effects of 1,25-dihydroxyvitamin D3 in DU145 human prostate cancer cells: role of the direct inhibition of CYP24 enzyme activity. Mol Cell Endocrinol. 2005; 28:49–61.

Szkudelska K, Nogowski L. Genistein – a dietary compound inducing hormonal and metabolic changes. J Steroid Biochem Mol Biol. 2007; 105:37–45. [PubMed: 17588743]

Van Duursen MB, Mijmeijer SM, de Morree ES, de Jong PC, van den Berg M. Genistein induces breast cancer-associated aromatase and stimulates estrogen-dependent tumor cell growth in in vitro breast cancer model. Toxicology. 2011; 289:67–73. [PubMed: 21854827]

Wagner JI, Jiang L, Lehmann L. Phytoestrogens modulate the expression of 17alpha-estradiol metabolizing enzymes in cultured MCF-7 cells. Adv Exp Med Biol. 2008; 617:625–32. [PubMed: 18497090]

Wei YK, Gamra I, Davenport A, Lester R, Zhao L, Wei Y. Genistein Induces Cytochrome P450 1B1 Gene Expression and Cell Proliferation in Human Breast Cancer MCF-7 Cells. J Environ Pathol Toxicol Oncol. 2015; 34:153–159. [PubMed: 26081033]

Wu AH, Yu MC, Tseng CC, Pike MC. Epidemiology of soy exposures and breast cancer risk. Br J Cancer. 2008; 98:9–14. [PubMed: 18182974]

Xiao CQ, Chen R, Lin J, Wang G, Chen Y, Tan ZR, Zhou HH. Effect of genistein on the activities of cytochrome P450 3A and p-glycoprotein in Chinese healthy participants. Xenobiotica. 2012; 42:173–178. [PubMed: 21943317]

Zhan S, Ho SC. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Am J Clin Nutr. 2005; 81:397–408. [PubMed: 15699227]

Table 1

Effects of Soy and Isoflavones on CYP1

Agent	Inducer	CYP	Effect	Proposed Mechanism	Reference
Diet in Vivo					
SPI	3-MC	CYP1A1	Suppressed Hepatic Inductiion	Reduced AhR	Ronis et al. (2001)
SPI	DMBA	CYP1A1, CYP1A2 CYP1B1	Suppressed Expression/Induction in Liver and Mammary	Reduced AhR Reduced ARNT	Rowlands et al. (2001)
SPI	DMBA	CYP1A1	Suppressed Hepatic Induction	AhR Degradation	Singhal et al. (2007 Singhal et al. (2009)
SPI	DMBA	CYP1A1	Suppressed Hepatic Induction	Heterodimerization Failure with ARNT	Singhal et al. (2009)
SPI	DMBA	CYP1A1, CYP1B1	1 No effect on Uterine Induction		Ronis et al. (2016)
SPI	Isosafrole	CYP1A1	Increased Hepatic Induction	AhR Independent	Ronis et al. (2001)
In Vitro Studies					
Genistein		CYP1A2	Activity Inhibition Recombinant Enzyme	Non-competitive	Helsby et al. (1998)
Genistein		CYP1A1, CYP1B1	Activity inhibition Recombinant Enzyme		Chan and Leung (2003)
Genistein, Daidzein, Equol		CYP1A1, CYP1B1	Activity Inhibition Transfected V79 cells	Dose-Dependent	Scott wet al. (2008)
Genistin, Daidzein	Omeprozole	CYP1A1	Inhibited Induction Hep411E cells	AhR Independent	Backlund et al. (1997)
Genistein, Daidzein	TCDD, B(a)P	CYP1A1	No effect Hep411 E cells	AhR Dependent	Backlund et al. (1997)
Genistein	TCDD	CYP1A1	Inhibited Induction in keratinocytes	HSP-90 Phosphorylation	Gradin et al. (1994)
Genistein	TCDD	CYP1A1	Inhibited Induction in lung, mammary cells		Hukkanen et al. (2000), Wagner et al. (2008)
Genistein	TCDD	CYP1B1	No Effect on Induction in lung, mammary cells		Hukkanen et al. (2000), Wagner et al. (2008)
Clinical Studies					
Genistein		CYP1A2	Inhibited caffeine metabolism		Chen (2011)

3-MC - 3-methylcholanthrene; DMBA - dimethylbenzanthracene; TCDD - dioxin; B(a)P - benzo(a)pyrene.

Table 2

Effects of Soy and Isoflavones on CYP3A and PXR Signaling

Agent	Inducer	CYP	Effect	Proposed Mechanism	Reference
Diet in Vivo					
SPI	DEX	CYP3A1, CYP3A2	Enhanced induction		Ronis et al. (1999; 2004a) (2006a b) (2011b)
SPI		CYP3A1	Increased hepatic and GI Expression		Ronis et al. (2004)
SPI+ vs SPI-		CYP3A11	Increased Hepatic Expression	PXR activation	Li et al. (2009)
SPI		CYP3A1	Increased transcription	PXR activation	Ronis et al. (2011b)
Soy Formula		CYP3A29, CYP3A46	Increased Hepatic Expression		Ronis (2011a)
Genistein		CYP3A	Reduced Hepatic Expression		Laurenzana et al. (2002)
Isoflavone Extract		CYP3A1, CYP3A2	No Effect		Kishida et al. (2004)
Genistein		CYP3A1, CYP3A2	No Effect		Ronis et al. (2006b)
SPI-; Daidzein		CYP3A2	Increased Hepatic Expression		Ronis et al. (2006b)
In Vitro Studies					
Daidzein		CYP3A4	Activity Inhibition transfected V79 cells		Scott et al. (2008)
Genistein, Equol		CYP3A4	Activity Increased transfected V79 cells	Post-Transcriptional	Scott et al.(2008)
Genistein, Daidzein, Equol		CYP3A4	Increased Transcription HepG2 cells	PXR Activation	Li et al. (2009)
Isoflavones			PXR Activation 2-Hybrid System	Ligand Binding	Rickets et al. (2005)
Clinical Studies					
Genistein		CYP3A4	Increased substrate clearance		Xiao et al. (2012)

DEX - dexamethasone; CYP3A1/2 - rat; CYP3A11 - mouse; CYP3A29/46 - piglet; SPI+ intact soy protein isolate with phytochemicals; SPI- SPI stripped of phytochemicals

Table 3

Effects of Soy and Isoflavones on CYP4 and PPARα Signaling

Agent	CYP	Effect	Proposed Mechanism	Reference
Diet in Vivo				
SPI	CYP4A14	Increased Hepatic Expression	PPARα Activation	Badger et al. (2008)
Soy Formula	CYP4A21	Increased Hepatic Expression		Ronis et al. (2011a)
SPI	CYP4A10	Reduced Hepatic Expression		Simmen et al. (2010)
Soy Formula	CYP4A24	No Effect		Ronis et al. (2011a)
SPI		Reduced Steatosis, Increased	PPARα Activation	Mezei et al. (2003; 2006)
		Downstream Gene Expression		Badger et al. (2008), Ronis et al. (2009)
SPI		Increased PPARα Expression		Ronis et al. (2009)
Genistein, Daidzein		No Effect on PPARα-Dependent Genes		Ronis et al. (2009)
In Vitro Studies				
Genistein, Daidzein		PPARα Activation, Induction of Dependent Genes	Ligand Binding	Mezei et al. (2003; 2006) Shay and Banz (2005)
Genistein, Daidzein, Isoflavones		PPARα Activation, 2 Hybrid System	Ligand Binding	Ricketts et al. (2005)
Genistein	CYP4F2	Induction in HepG2 cells	AMPK Activation	Hsu et al. (2011)

CYP4A14 - mouse; CYP4A21, CYP4A24 - piglet; CYP4A10 - rat; CYP4F2