Soy Protein Isolate Induces CYP3A1 and CYP3A2 in Prepubertal Rats

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Feeding soy diets has been shown to induce cytochrome P450s in gene family CYP3A in Sprague-Dawley rat liver. We compared expression of CYP3A enzymes on postnatal Day 33 (PND33) rats fed casein or soy protein isolate (SPI+)-based AIN-93G diets continuously from gestational Day 4 through PND33 or the diets were switched on PND15 (n = 3–6 litters) to examine the potential imprinting effects of soy on drug metabolism. In addition rats were fed casein, SPI+, SPI+ stripped of phytochemicals (SPI–), or casein diets supplemented with the soy-associated isoflavones genistein or daidzein from weaning through PND33 to examine the hypothesis that the isoflavones are responsible for CYP3A induction by soy feeding. Feeding SPI either continuously or from weaning induced hepatic CYP3A1 and CYP3A2 mRNA, apoprotein, and CYP3A-dependent testosterone 6β-hydroxylase activity in liver microsomes 2- to 5-fold ($P < 0.05$). CYP3A mRNA expression was also elevated 2- to 3-fold in the jejunum of SPI-fed rats ($P < 0.05$). CYP3A was not induced in livers of rats switched to casein from soy at weaning. Induction of CYP3A1 also did not occur in rats fed SPI–, but CYP3A2 mRNA and apoprotein were induced ($P < 0.05$) in females fed SPI–. Offspring weaned onto genistein-supplemented diets had no elevation of CYP3A mRNAs or apoproteins. Weaning onto daidzein diets increased CYP3A2 mRNA and apoprotein expression in male rats ($P < 0.05$). These data suggest that early soy consumption may increase the metabolism of a wide variety of CYP3A substrates, but that soy does not imprint the expression of CYP3A enzymes. Effects on CYP3A1 expression appear to be primarily due to phytochemical components of SPI other than isoflavones. In contrast, consumption of soy protein and daidzein appear to be associated with the induction of CYP3A2. Exp Biol Med 231:60–69, 2006

Key words: soy protein isolate; CYP3A; isoflavones; rat; weaning

Introduction

Drug-metabolizing enzymes, such as the hepatic cytochrome P450-dependent microsomal monooxygenases, display a large degree of interindividual variability of expression in human liver (1). At least part of this variability may be explained by dietary differences. Diet has been shown to alter cytochrome P450 expression and activity in both humans and animal models (2, 3). The CYP3A enzymes are the major P450 forms expressed in human fetal and neonatal liver (4). They catalyze the rate-limiting step in the metabolism and clearance of a large proportion of clinical medications, including many pediatric drugs, and have been implicated in the bioactivation of procarcinogens, such as aflatoxin B1 (5–8). In addition, CYP3As are important in the metabolism of endogenous compounds, such as bile acids and estrogens (9, 10).

We have previously demonstrated that lifetime feeding of an AIN-93G diet made with soy protein isolate (SPI+) results in alterations in the expression of a number of different cytochrome P450 enzymes in rat liver and mammary gland. These include suppression of CYP1A1 and CYP1B1 expression, inducibility by polycyclic aromatic hydrocarbons, and suppression of constitutively expressed CYP4A1 and CYP2C11, but there is relatively little effect on the expression and inducibility of CYP2B1 (11–14). In addition, we have reported a modest, but significant, elevation of constitutive CYP3A apoprotein expression, glucocorticoid inducibility, and CYP3A-dependent activities such as erythromycin, ethylmorphine N-demethylase, and testosterone 6β-hydroxylase (14) in adult male rat liver relative to rats fed AIN-93G casein-based diets. Subsequently, we reported that soy consumption in rats during early development (exposure *in utero* through

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postnatal Day 25 [PND25]) resulted in a much greater increase in constitutive expression of two rat CYP3A enzymes (i.e., CYP3A1, CYP3A2) and their activities than observed during adulthood, while the two other rat CYP3A enzymes (i.e., CYP3A9, CYP3A18) appeared relatively unaffected (15). CYP3A1 and a closely related variant, CYP3A23, which are rat orthologues of the major human enzyme CYP3A4, are expressed at low levels constitutively in rat liver and are highly inducible by a wide variety of xenobiotics including glucocorticoids (i.e., dexamethasone), antiglucocorticoids (i.e., pregnenolone 16α-carbonitrile), macrolide antibiotics, and imidazole fungicides (16–19). In contrast, CYP3A2 is constitutively expressed in a male-specific fashion after puberty in rat liver and is not inducible to the same extent as CYP3A1 (20–22).

Because SPI+ is the protein source used in soy infant formula, which is fed to 20%–25% of all formula-fed infants in the United States (23), our previous data raise questions regarding the effects of soy-formula feeding on the clearance and efficacy of pediatric medications that are CYP3A substrates. However, it was unclear from these previous studies if the effects of soy on CYP3A expression were the results of metabolic imprinting during fetal or neonatal development or the result of a direct effect of the diet. In the current study, direct and imprinting effects of feeding the SPI+ diet were assessed in crossover studies by weaning rat pups fed casein-based diets during *in utero* and postnatal periods onto SPI+ -containing diets from PND15 to PND33 or weaning rat pups fed SPI+ diets onto casein and measuring hepatic CYP3A1 and CYP3A2 apoprotein and mRNA expression. We also examined the effects of feeding SPI+ on CYP3A expression in the small intestine to determine if similar induction of CYP3As by soy feeding occurs at the major extrahepatic site of CYP3A expression.

Finally, metabolic effects of soy feeding have been variously ascribed to the soy protein itself or to phytochemicals associated with the soy (24, 25). Potentially bioactive phytochemical components of soy include the isoflavone phytoestrogens genistein and daidzein, saponins, phytosterols, phytic acid, phenolic acids, and coumarins (26). Whether the effects of SPI+ on CYP3A expression are due to the soy protein itself or due to phytochemicals such as the isoflavones is unknown. Previous *in vivo* studies with purified soy isoflavones or isoflavone mixtures have reported either no effects or inhibition of cytochrome P450 expression (27–31). A limited amount of data are available on the effects of other soy-associated phytochemicals on cytochrome P450 expression. However, saponins from other plant species have been described to have both suppressive and inductive effects on hepatic P450 enzymes (32, 33). Therefore, in a second experiment, in addition to weaning onto SPI+ diets, groups of casein-fed rat pups were weaned onto diets containing SPI stripped of bound phytochemicals (SPI–) or onto casein diets supplemented with the isoflavones genistein (C+G) or daidzein (C+D) at a level of 250 mg/kg, similar to that found in SPI+.

Materials and Methods

Chemicals and Reagents. Supersomes expressing recombinant rat CYP3A1 or CYP3A2 apoproteins were obtained from Gentest Inc. (Woburn, MA). Rabbit polyclonal antibodies against purified rat CYP3A1 were the gift of Dr. Magnus Ingelman-Sundberg, Karolinska Institute, Stockholm (34). [^{125}I]goat anti-rabbit IgG was purchased from ICN Biomedicals (Costa Mesa, CA).

Generation of Peptide-Specific Rabbit-Polyclonal Antibodies Against Rat CYP3A1 and CYP3A2 Apoproteins. Specific rabbit anti-rat CYP3A1 and CYP3A2 polyclonal antibodies were generated based on unique amino acid sequences from rat CYP3A1 and CYP3A2. Rat CYP3A1 amino acids 378–389 (CKKDVEINGVFM) and rat CYP3A2 amino acids 378–389 (CKKDIELDGLFI) were selected based on searches of the National Center for Biotechnology Information protein sequence database and hydropathic profile analysis. Peptides were synthesized and conjugated to keyhole-limpet hemocyanin, and rabbit polyclonal antisera were raised to the conjugated peptides by Biosynthesis Inc. (Lewisville, TX).

Primers for Real-Time Reverse Transcription-Polymerase Chain Reaction Analysis. Rat CYP3A1-specific primer set: forward, GCCATCACGGACACA-GAAATA starting at 328 base pairs; reverse, GAACGTGGGTGACAGTAAGGCT ending at 477 base pairs. Rat CYP3A2-specific primer set: forward, TCTCTACCGATTGGAACCCATAG starting at 131 base pairs; reverse, TTGTAGTAATTCAGCACAGTGCCTAA ending at 232 base pairs.

Animals and Diets. Time-impregnated female Sprague-Dawley rats were purchased from Harlan Industries (Indianapolis, IN) on gestational Day 4 (GED4) and individually housed in an Association for Assessment and Accreditation of Laboratory Animal Care-approved animal facility at 22°C with constant humidity and lights on from 0600–1800 hrs. In Experiment 1 (Fig. 1A), the dams had *ad libitum* access to water and a pelleted AIN-93G diet formulated with casein or SPI+ (The Solae Company, St. Louis, MO) from GED4 as previously described (35) except that soybean oil was replaced with corn oil. At birth, litters were culled to 5 male and 5 female pups per litter. Dams continued to be fed the casein or SPI+ diets throughout lactation. Beginning at PND15, 6 casein litters were given continued *ad libitum* access to the casein diets and three SPI+ litters were continued on SPI+. An additional five litters were switched from casein to SPI+ diets at PND15, while four litters were switched from SPI+ to casein diets. Crib feeding was accomplished by adding the respective food pellets to the bottom of the cages. At PND21, rat pups were removed from the dams and weaned to their respective diets. On PND33, the pups were sacrificed by overdose of Nembutal and livers were collected. In Experiment 2 (Fig. 1B), dams were fed casein diets as in Experiment 1 from GED4 until

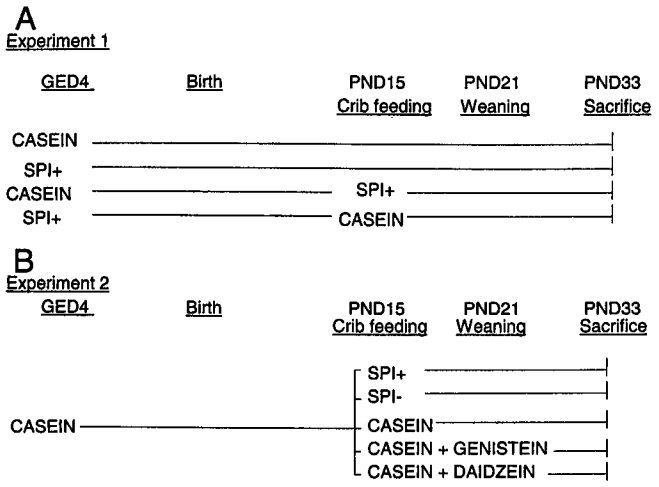

Figure 1. Experimental design. (A) Experiment 1: Diet switchover study. (B) Experiment 2: Weaning from casein to diets containing SPI+ or soy components (SPI–, genistein, or daidzein).

postnatal Day (PND) 15. At that time, litters of pups were either crib fed the casein diet and, subsequently, weaned onto it at PND21 ($n=6$) or were crib fed and weaned to SPI+ ($n=3$). The SPI+ diet contained 276 mg/kg genistein and 132 mg/kg daidzein, and rat pups fed SPI+ were calculated to consume an average of 65 mg of total isoflavones/kg/day from weaning until sacrifice. Additional casein-fed litters were crib fed and weaned to one of three other diets consisting of AIN-93G formulation made with: 1) low-isoflavone SPI– that had been processed by successive ethanol washes to be essentially devoid of phytochemicals (i.e., <5% of the isoflavones found in SPI+; $n=3$; The Solae Company), 2) casein supplemented with 250 mg/kg genistein (C+G; $n = 3$), or 3) casein supplemented with 250 mg/kg daidzein (C+D; $n = 3$). Genistein and daidzein were purchased from Indofine (Hillsborough, NJ). Pups were sacrificed on PND33. Liver and jejunum were collected, snap frozen in liquid nitrogen, and stored at –70°C until use. Hepatic microsomes were prepared by differential ultracentrifugation (36).

Testosterone 6β-Hydroxylase. Testosterone hydroxylation at position 6β that is catalyzed by CYP3A enzymes and, in particular, CYP3A2, was measured in microsomes from male rats by high-performance thin-layer chromatography using [^{14}C]testosterone as previously described (14, 18) using 0.1 mg of microsomal protein and a substrate concentration of 0.04 μ*M*.

Western Immunoblot Analysis. Western blotting of CYP3A enzymes is complicated by the high degree of homogeneity of rat CYP3A forms (80%) and their virtually identical molecular weights (15). To circumvent this problem, Western immunoblot analysis of CYP3A1 and CYP3A2 apoproteins was conducted on liver microsomes using rabbit polyclonal antibodies directed against specific peptides in the CYP3A1 and CYP3A2 amino acid sequences and a polyclonal antibody raised to purified CYP3A1. The peptide-specific antisera were used at a dilution of 1:250 (CYP3A1) or 1:500 (CYP3A2) with [^{125}I]-labeled goat anti-rabbit IgG at a dilution of 1:1000 as the secondary antibody. Cross-reactivity with the other CYP3A apoprotein was compared in assays using 1 μg of protein from supersomes expressing recombinant rat CYP3A1 or CYP3A2 (Gentest Inc., Woburn, MA). Rat liver microsomes were loaded at 50 μg/well and probed under the same conditions as the supersomes. In addition, rat microsomes were Western blotted with a rabbit polyclonal antibody raised against purified rat CYP3A1 as previously described (14). Protein concentrations were determined using bicinchoninic acid reagent (Pierce Biotechnology, Rockford, IL) and even loading of protein onto gels confirmed by Coomassie blue protein staining of duplicate gels. Loading variation of total protein across gels was + 3%. Autoradiographs were quantitated by densitometric scanning using a GS525 molecular imager (Bio-Rad Laboratories, Hercules, CA). Densities of the immunoreactive bands were normalized against a single microsomal sample from a male SPI+-fed rat that was run on every gel, and the data were expressed relative to the mean value for casein-fed male rats = 1.

Real-Time Reverse-Transcription Analysis. Total mRNA was isolated from liver and jejunum using the TRI Reagent according to the manufacturer's protocol (Molecular Research Center, Cincinnati, OH). RNA was treated with RNase-free DNase (Ambion Inc., Austin, TX) to remove contaminating genomic DNA and was further purified with an RNeasy mini kit (QIAGEN Inc., Valencia, CA). cDNA was synthesized from 1 μg of total RNA in 20 μl using random hexamers and avian myeloblastosis virus-reverse transcriptase (Promega Corp., Madison, WI). One twentieth of the reverse transcription mixture was mixed with 2 × SYBR Green PCR master mixture and gene-specific primers in a final volume of 50 μL. Real-time RT-PCR was performed in an ABI Prism Model 7000 (Foster City, CA) sequence detection system. Probe specificity was verified by assessment of a single PCR product on agarose gels and a single temperature dissociation peak, and real-time RT-PCR was quantitated within the linear range of product amplification. Ribosomal 18S rRNA and GAPDH mRNA amplicons were used as endogenous internal standards to normalize the values. The relative quantification for any given gene was expressed as signal relative to the average signal value for the internal standard.

Statistical Analysis. Data are means ± SEM. All data were analyzed using the SigmaStat for Windows program (Jandal Scientific Software, San Rafael, CA) by one-way ANOVA followed by a Student-Newman-Keuls test for all pair-wise mean comparisons to detect differences among groups, except for the testosterone 6β-hydroxylase data which was not normally distributed. This was analyzed by one-way ANOVA of Ranks followed by Mann-Whitney Rank Sum tests to compare median activities in rats fed one diet versus another. An α-level of 0.05 was set to determine significance.

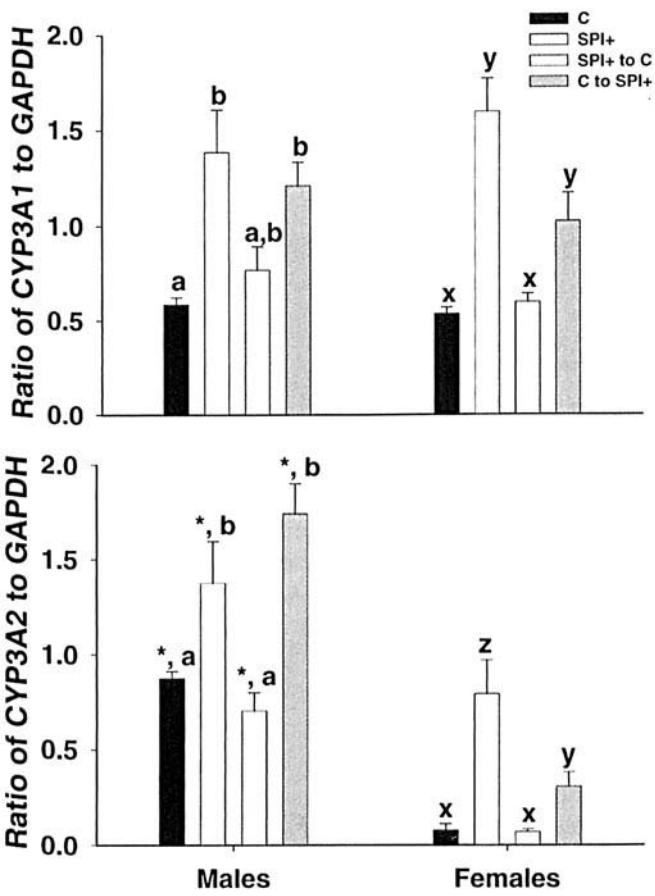

Figure 2. Real-time RT-PCR quantitation of CYP3A1 and CYP3A2 mRNA expression in the liver from rats in Experiment 1. Groups of rat pups were continuously fed casein ($n = 15$) or SPI+ ($n = 5$) throughout development (GED4–PND33) or weaned from casein to SPI+ ($n = 10$) or SPI+ to casein ($n = 15$) on PND15 and sacrificed on PND33. Data are presented as means ± SEM. Means not sharing a common letter differ significantly ($P < 0.05$; a < b; x < y < z). *, Males > females within each diet group ($P < 0.05$).

Results

Hepatic CYP3A mRNA Is Not Induced in Prepubertal Rats by *In Utero* and Neonatal Exposure to Soy Followed by Switching to Casein Diets at Weaning. Results from Experiment 1 are shown in Figure 2. This depicts the effects of SPI+ diets on hepatic CYP3A mRNA expression when fed during three developmental windows: from weaning onward, just during gestation and lactation, or continuously throughout development (gestational Day (GED) 4–PND33). CYP3A1 mRNA and CYP3A2 mRNA were induced ($P < 0.05$) when SPI+ was continuously fed or for a short time beginning at weaning. In contrast, no induction of hepatic CYP3A mRNAs was observed in pups of either gender when rats were fed SPI+ during gestation and lactation and subsequently switched to casein when compared with rats continuously fed casein.

Effects of Weaning Rats to Diets Containing SPI or Isoflavones on CYP3A Apoprotein Expression. Cross-reactivity studies with our peptide specific antisera demonstrated 8-fold greater recognition of recombinant CYP3A1 and 9-fold greater recognition of recombinant CYP3A2 by the corresponding antiserum in blots where the proteins were compared at the same concentration (Figs. 3 and 4). As demonstrated in representative Western blots using pooled samples, CYP3A1 apoprotein was detectable at low levels in hepatic microsomes from both male and female casein-fed rats at PND33 (Fig. 3). In contrast, CYP3A2 apoprotein was found at significant levels in male liver microsomes, but was barely detectible in females at this age (Fig. 4). Figure 5 depicts relative CYP3A1 and CYP3A2 apoprotein expression based on Western immunoblots of individual liver microsomes from 10 pups of each gender, from each diet group, in Experiment 2. In rat pups of both genders, weaning onto diets containing SPI+ from casein resulted in increases ($P < 0.05$) in the expression of CYP3A1 apoprotein (Figs. 3 and 5). These effects were confirmed using a second polyclonal antibody against rat CYP3A1 (data not shown). CYP3A2 apoprotein expression was also increased by weaning from casein to SPI+ in female pups ($P < 0.05$), but not in males (Figs. 4 and 5). In contrast, weaning to diets containing SPI– resulted in no significant change in expression of CYP3A1 in females, while CYP3A2 apoprotein expression was increased in hepatic microsomes from only female pups ($P < 0.05$). Weaning to casein diets supplemented with genistein at levels similar to those found in SPI+ had no statistically significant effect on CYP3A1 or CYP3A2 apoprotein expression in males and actually suppressed expression in females. Weaning to casein diets supplemented with daidzein resulted in no significant effects on CYP3A1 apoprotein expression in either gender, but it increased CYP3A2 apoprotein expression ($P < 0.05$) in liver microsomes from the male rats when compared with casein controls.

Effects of Weaning Rats to Diets Containing SPI or Isoflavones on Expression of CYP3A mRNAs. Weaning to SPI+ -containing diets from casein resulted in increased ($P < 0.05$) hepatic levels of both CYP3A1 and CYP3A2 mRNAs at PND33 (Fig. 6). As was observed with apoprotein expression, hepatic CYP3A2 mRNA levels were elevated more in rats fed SPI– diets than in those fed SPI+ diets in female pups. Weaning to diets containing casein supplemented with genistein had no effect on the levels of either hepatic CYP3A1 or CYP3A2 mRNA. Weaning to casein diets supplemented with daidzein increased mean expression of hepatic CYP3A2 mRNA ($P < 0.05$) to levels statistically indistinguishable with those observed following feeding SPI+ diets. In addition to effects on hepatic CYP3A expression, weaning onto SPI+ diets also induced CYP3A1 expression in the jejunal region of the small intestine in pups of both genders ($P < 0.05$; Fig. 7), but CYP3A1 mRNA was expressed at overall higher levels in jejunum from female rats compared to males. CYP3A2 mRNA was barely detectable in jejunum, and expression was not sexually dimorphic. Feeding SPI+

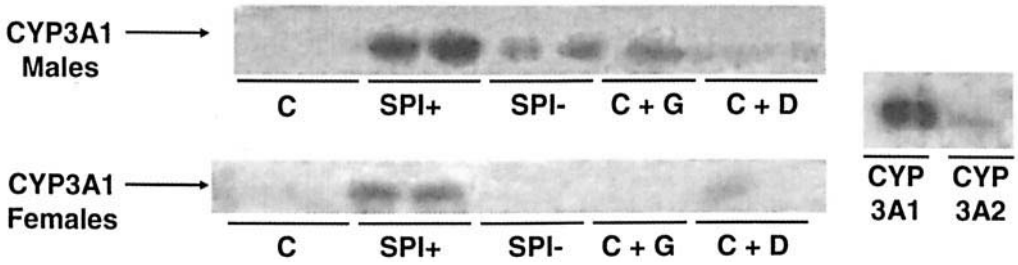

Figure 3. Representative Western blots showing expression of CYP3A1 in hepatic microsomes from rats weaned to diets containing soy or soy components at PND15 and sacrificed on PND33. Top panel: Duplicate sample pools from male rats containing 50 μg of pooled hepatic microsomal protein (5 μg from each of 10 male rats in each diet group). Rats were weaned to casein, SPI+, SPI–, C+G, or C+D. Liver microsomes are compared to samples containing 1 μg protein from supersomes expressing recombinant rat CYP3A1 or rat CYP3A2 (inset to right). Bottom panel: Duplicate sample pools from female rats.

variably increased jejunal CYP3A2 mRNA expression, but only in the male pups.

Effects of SPI and Isoflavone Feeding on CYP3A-Dependent Testosterone 6β-Hydroxylase Activity in Rat Liver. CYP3A-dependent testosterone 6β-hydroxylase activity in liver microsomes from male rat pups fed SPI+, SPI–, and pure isoflavones is shown in Figure 8. Testosterone 6β-hydroxylase activity is preferentially catalyzed by CYP3A2, but can also be catalyzed by CYP3A1 (37). Median activities were induced by feeding SPI+, SPI–, and daidzein ($P < 0.05$) compared to casein, but were unaffected by feeding genistein.

Discussion

We have previously demonstrated increased expression of CYP3A-dependent enzyme activities, apoprotein, and CYP3A1 and CYP3A2 mRNAs in the livers of weanling and adult rats fed SPI+ from GED4 throughout life (14, 15). Those studies suggested that soy-fed infants might have altered clearance and efficacy of pediatric medications and the potential for increased activation of carcinogens relative to breast-fed or dairy formula–fed babies. However, additional questions were raised. Are the effects of SPI+ direct effects of the diet or the result of metabolic programming or imprinting during fetal or neonatal development? What component of SPI+, protein, or phytochemicals bound to the protein is responsible for the effect? The current studies were designed to address these issues.

The data presented in Figure 2 demonstrate that feeding SPI+ to rats for a short period postweaning produced similar increases in the expression of CYP3A1 and CYP3A2 mRNA and apoprotein as those observed in rats fed SPI+ diets starting in early gestation. No induction was observed in rats fed SPI+ during gestation and lactation and then subsequently switched to casein. We conclude that the soy effect on CYP3A can occur *via* a direct effect of the diet and that gestational/neonatal soy consumption does not result in metabolic imprinting, at least of this enzyme family.

It is difficult to determine which component of SPI+ is responsible for the CYP3A induction. In Experiment 2 of the current study, we used two sources of soy (i.e., SPI+, SPI–) in an attempt to ascertain the effects of soy protein devoid of phytochemicals on CYP3A expression. For CYP3A1, it is clear that increased expression of both mRNA and apoprotein occurs when rats are fed SPI+, but does not occur to the same extent on feeding SPI– (Figs. 5 and 6). These data could suggest that phytochemicals bound to SPI+ are responsible for CYP3A1 induction, or they could indicate that processing significantly altered the properties of the SPI– protein, which results in loss of bioactivity. In this regard, it has been reported that processing SPI+ to SPI– alters the pattern of soy proteins as determined by two-dimensional gel electrophoresis (38).

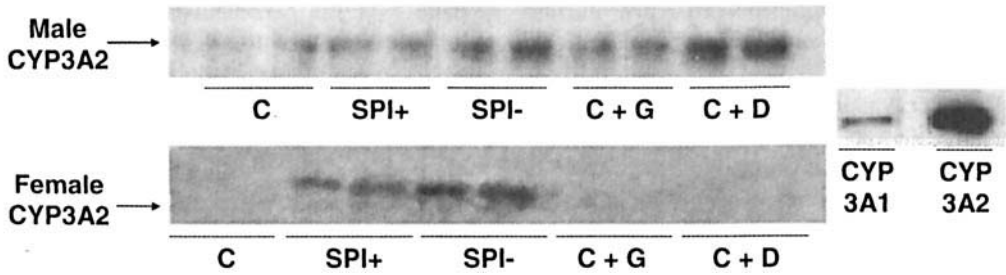

Figure 4. Representative Western blots showing expression of CYP3A2 in hepatic microsomes from rats weaned to diets containing soy or soy components at PND15 and sacrificed on PND33. Top panel: Duplicate sample pools from male rats containing 50 μg of pooled hepatic microsomal protein (5 μg from each of 10 male rats in each diet group). Rats were weaned to casein, SPI+, SPI–, C+G, or C+D. Liver microsomes are compared to samples containing 1 μg protein from supersomes expressing recombinant rat CYP3A2 or rat CYP3A1 (inset to right). Bottom panel: Duplicate sample pools from female rats.

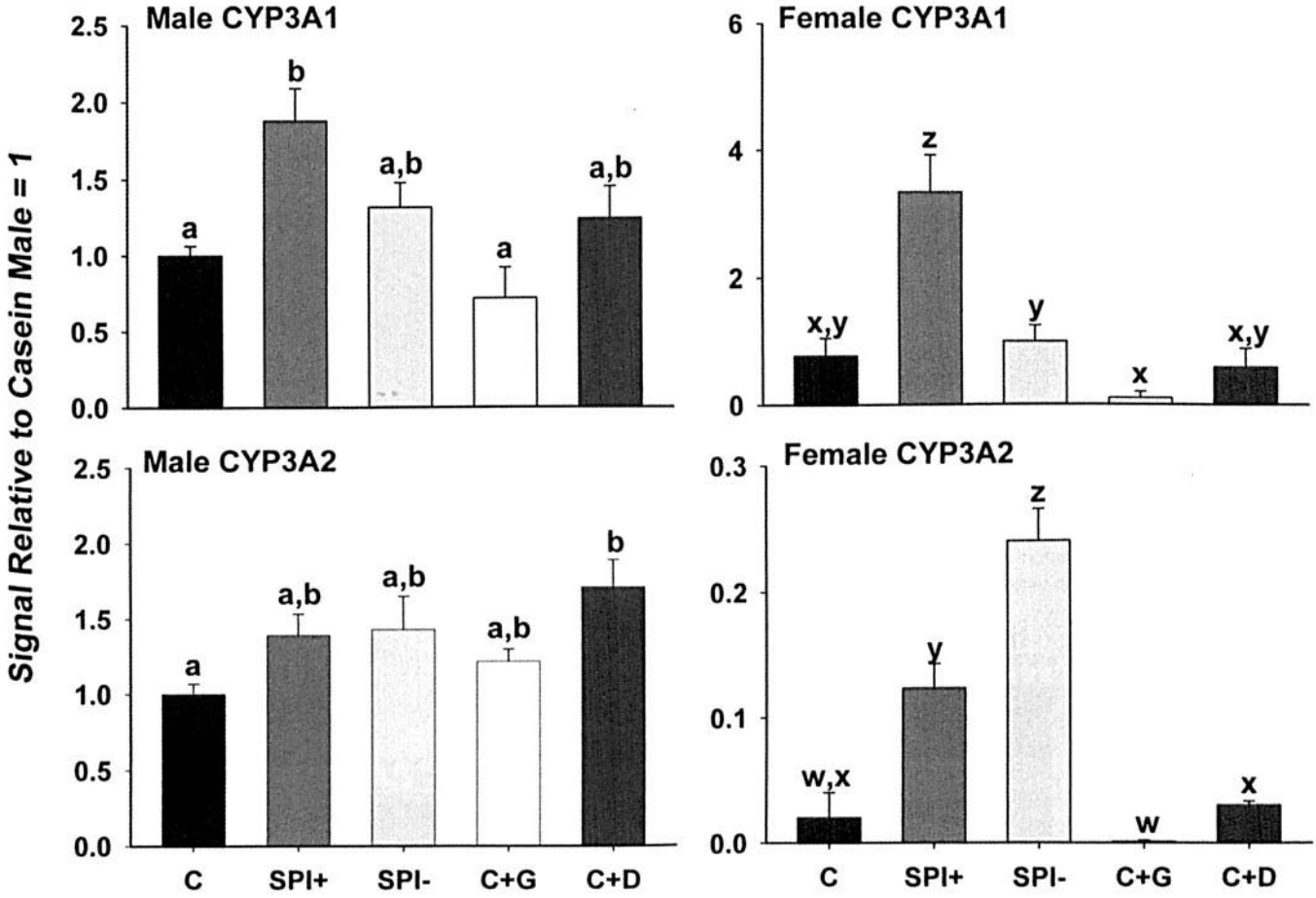

Figure 5. Immunoquantitation of CYP3A1 and CYP3A2 apoprotein expression in hepatic microsomes from individual rat pups ($n = 10$) weaned to casein, SPI+, SPI–, C+G, or C+D beginning on PND15 in Experiment 2 and sacrificed at PND33. Data are presented as means ± SEM. Means not sharing a common letter differ significantly ($P < 0.05$; a < b; w < x < y < z).

Although feeding SPI+ significantly increased CYP3A2 mRNA levels in both genders and CYP3A2 apoprotein in females, a similar or even larger increase was observed following the feeding of SPI–. This suggests that soy proteins or peptides resulting from the digestion of these proteins may be involved in CYP3A2 regulation following SPI+ consumption.

In an attempt to determine if the isoflavones bound to SPI+ were involved in the CYP3A induction response, we fed casein diets supplemented with genistein or daidzein at concentrations similar to those found in SPI+. The results were mixed. Genistein did not appear to significantly induce CYP3A1 or CYP3A2 expression. CYP3A1 expression was also unaffected in rats fed either SPI– or daidzein. The lack of effects of isoflavones on CYP3A1 expression are consistent with previous reports in which diets containing the pure isoflavones or isoflavone extracts from soy given to rats had little effect on P450 expression (29–31) and agree with the conclusions of Kishida *et al.* (31) that induction of some P450s following consumption of soy diets appears to depend on chemicals other than isoflavones. However, daidzein-fed rats had significant induction of CYP3A2 mRNA and apoprotein and CYP3A-dependent testosterone 6β-hydroxylation in males (Figs. 5, 6, 8). In contrast, feeding daidzein had no significant effect on CYP3A2 expression in female pups.

Li and Shay (39) recently demonstrated induction of the human CYP3A1 orthologue CYP3A4 in HepG2 hepatoma cells transfected with a CYP3A4 reporter construct and induction in primary human hepatocytes *in vitro* after treatment with isoflavone extracts and purified isoflavones, including genistein and equol. However, these effects on CYP3A4 transcription were reported at isoflavone concentrations above 10 μ*M*, much higher than can be attained *in vivo* following dietary consumption of SPI+. Isoflavones are found as glucosyl-conjugates (genistin and daidzin) bound to soy and degraded in the gut to aglycones genistein and daidzein, and a proportion of daidzein is further metabolized by gut bacteria to equol (40, 41). The majority (>90%) of these isoflavone aglycones are conjugated by uridine diphosphate-glucoronyltransferases and sulfatases as they are absorbed from the gastrointestinal tract following consumption of SPI+ (42, 43). We have found <1 μ*M* of the biologically active genistein aglycone in rat plasma and

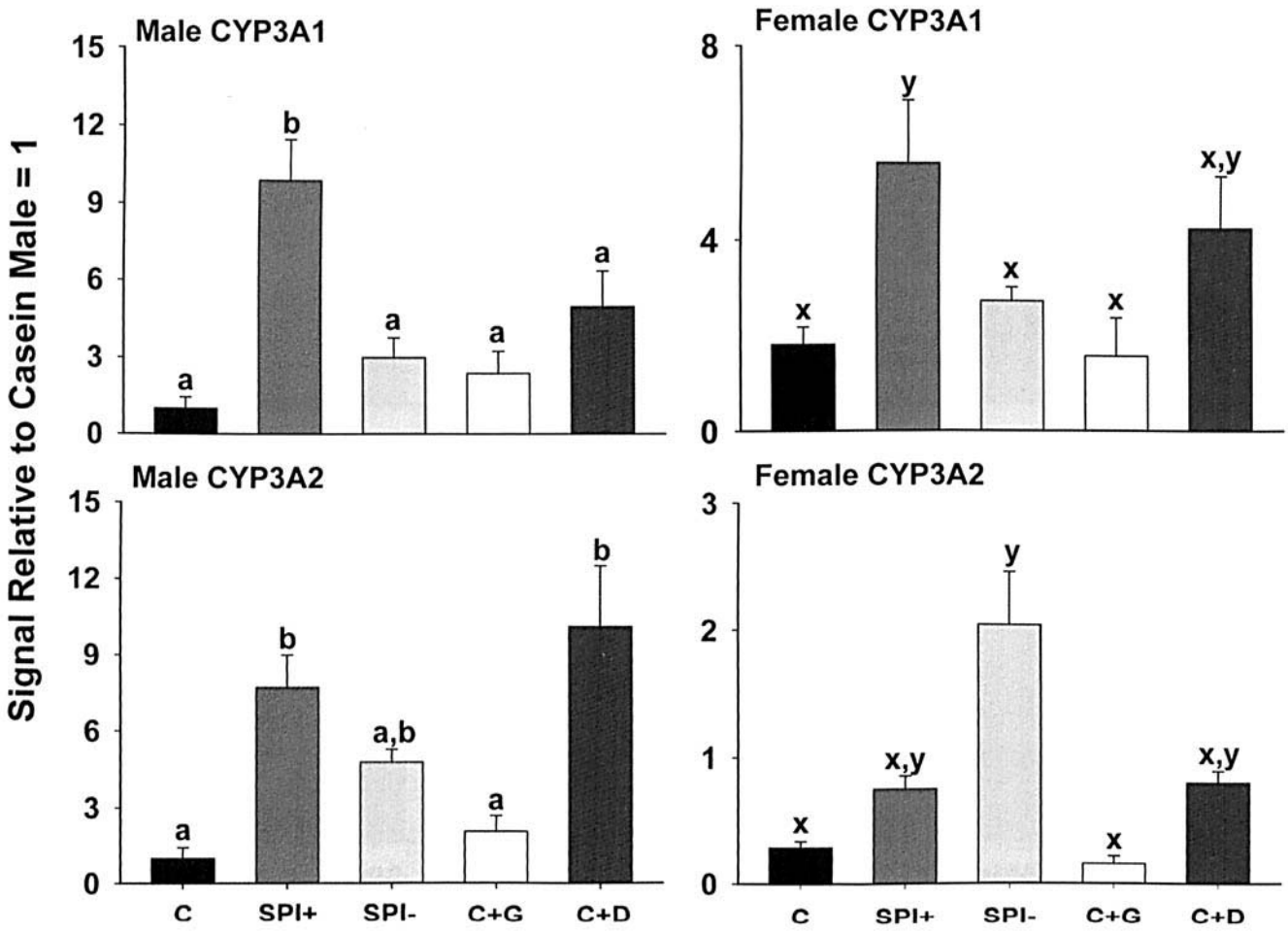

Figure 6. Real-time RT-PCR quantitation of CYP3A1 and CYP3A2 mRNA in livers from individual rat pups ($n = 10$) weaned to casein, SPI+, SPI–, C+G, or C+D beginning on PND15 in Experiment 2 and sacrificed at PND33. Data are presented as means ± SEM. Means not sharing a common letter differ significantly ($P < 0.05$; a < b; w < x < y < z).

liver following consumption of SPI+ (43, 44) and, based on the current data showing a lack of effects of feeding pure genistein at levels comparable to those found in SPI+ on CYP3A1 apoprotein expression *in vivo*, it is unclear if the *in vitro* results of isoflavone actions on CYP3A4 gene transcription in hepatocyte cultures have any relevance to the effects we have observed on hepatic CYP3A1 apoprotein expression and activity following consumption of dietary SPI+ in the current and previous studies (14, 15). Moreover, we have reported that the effects on hepatic CYP3A1 expression following the soy consumption *in vivo* appear to involve post-transcriptional effects rather than increased gene transcription because mRNA levels were found to increase with no significant change in levels of the primary transcript measured in heterologous nuclear RNA (15). It should be noted that SPI+ contains conjugated isoflavone glucosides (i.e., genistein, daidzin), whereas we fed the aglycones (i.e., genistein, daidzein). Unpublished data from our laboratory (Gu, Ronis, Badger) indicate that the pharmacokinetics of isoflavones fed as aglycones or as SPI+, as well as the tissue levels attained, are significantly different. This is a limitation of the present study and of virtually all published studies that employ aglycones to investigate the contribution of isoflavones to the effects of feeding soy.

CYP3A2 is known to be regulated differently than CYP3A1. CYP3A2 exhibits sexual dimorphism in rats with male predominant expression related to suppression by female patterns of growth-hormone secretion (20, 37). CYP3A1 and CYP3A2 are differentially regulated during development (20, 45), and the CYP3A2 promoter is known to bind a different set of transcription factors than that of CYP3A1 (46). Therefore, it is not surprising that CYP3A2 should be regulated by SPI+ and isoflavones in a different manner than CYP3A1. Rats are known to produce large quantities of equol from daidzein after soy consumption (47), and equol may be partly responsible for the effects of daidzein on CYP3A2 induction.

In addition to hepatic expression, CYP3A enzymes, in particular CYP3A1 and its orthologues such as CYP3A4 in humans, are expressed in the small intestine and are known to play an important role in the first-pass metabolism of

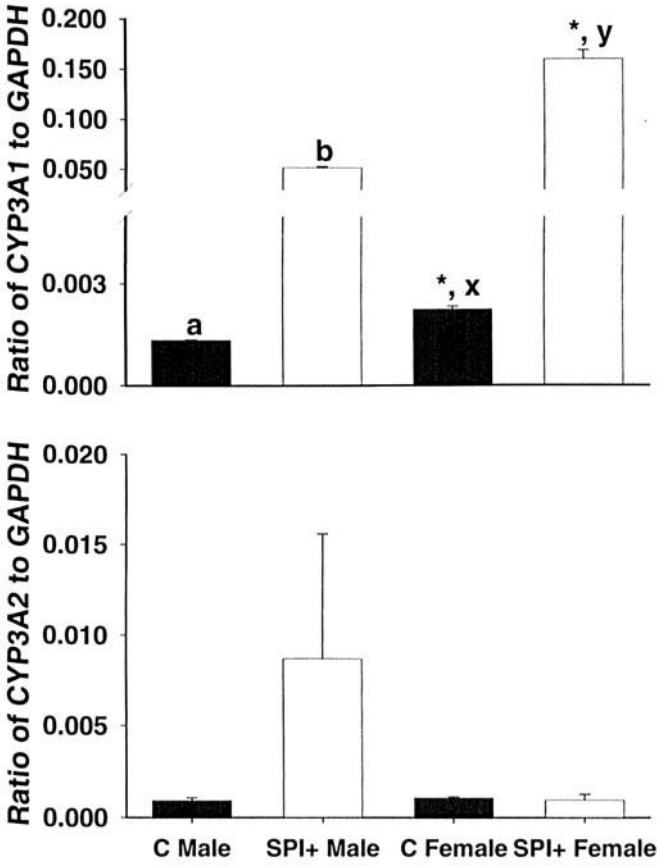

Figure 7. Real-time RT-PCR quantitation of CYP3A1 and CYP3A2 mRNA expression in the jejunum from individual rat pups ($n = 10$) weaned from casein to casein or to SPI beginning on PND15 in Experiment 2 and sacrificed on PND33. Data are presented as means ± SEM. Means not sharing a common letter differ significantly ($P < 0.05$). *, Females > males within each diet group ($P < 0.05$; a < b; x < y).

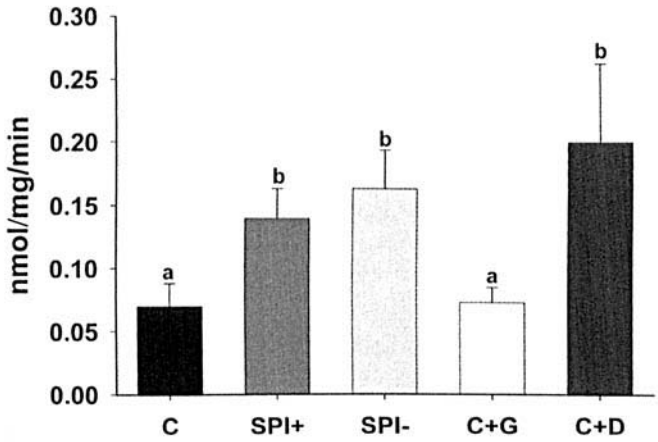

Figure 8. Testosterone 6β-hydroxylase activity in liver microsomes prepared from male rat pups ($N = 10$). Rats were weaned onto different diets (i.e., casein, SPI+, SPI–, C+G, C+D) from casein beginning on PND15 in Experiment 2 beginning at PND15 and sacrificed on PND33. Data are presented as means ± SEM. Medians differ significantly by one-way ANOVA of Ranks ($P < 0.05$).

many orally administered medications (48–50). In the current study, we report for the first time that SPI+ consumption also significantly up-regulates CYP3A1 mRNA expression in the rat jejunum (Fig. 7). This suggests that soy consumption may also increase first pass as well as hepatic metabolism of CYP3A substrates. There are variable reports regarding the presence of CYP3A2 in rat small intestine (49–52). We observed CYP3A2 mRNA to be present in the jejunum, but at levels too low to accurately determine effects of soy feeding.

Thus, we have demonstrated that the increase in hepatic CYP3A expression we previously observed in rats fed SPI+ during early development appears to occur as a direct effect of consumption of soy rather than as the result of metabolic imprinting, and that CYP3A1 expression is also upregulated by soy feeding in the small intestine. The induction effects appear not to be associated with genistein, but may in part be mediated by daidzein or its metabolites; by other nonisoflavone, phytochemical components of soy such as saponins; and by the soy protein itself. It remains unknown if infants fed soy-based formula have increased CYP3A expression and activity relative to breast-fed or dairy formula–fed infants, but a recent *in vitro* study reported that the addition of soy infant formula to culture medium increased CYP3A4 expression in HepG2 cells, while addition of breast milk had no effect (53). If CYP3A induction does occur in soy-fed infants, there may be significant effects on clearance and efficacy of many pediatric medications. Noninvasive studies of CYP3A activity in formula-fed and breast-fed infants are in progress.

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