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Dietary Soy Protein Isolate Attenuates Metabolic Syndrome in Rats via Effects on PPAR, LXR, and SREBP Signaling[1–3]

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Abstract

To determine the effects of feeding soy or isoflavones on lipid homeostasis in early development, weanling rats were fed AIN-93G diets made with casein, soy protein isolate (SPI+), isoflavone-reduced SPI+ (SPI−), or casein supplemented with genistein or daidzein for 14 d. PPARα-regulated genes and proteins involved in fatty acid degradation were upregulated by SPI+ ($P < 0.05$) accompanied by increased promoter binding and expression of PPARα mRNA ($P < 0.05$). Feeding SPI− or pure isoflavones did not alter PPARα-regulated pathways. SPI+ feeding had similar effects on PPARγ signaling. SPI+, SPI−, and casein plus isoflavones all increased liver X-receptor (LXR)α-regulated genes and enzymes involved in cholesterol homeostasis. Feeding SPI+ increased promoter binding of LXRα, expression of the transcription factor mRNA, and protein ($P < 0.05$). In a second experiment, male Sprague-Dawley rats were fed casein diets from postnatal d (PND) 24 to PND64 or were fed high-fat Western diets containing 5 g·kg^{-1} cholesterol made with either casein or SPI+. Insulin resistance, steatosis, and hypercholesterolemia in the Western diet-fed rats were partially prevented by SPI+ ($P < 0.05$). Nuclear sterol receptor element binding protein (SREBP)-1c protein and mRNA and protein expression of enzymes involved in fatty acid synthesis were increased by feeding Western diets containing casein but not SPI+ ($P < 0.05$). These data suggest that activation of PPAR and LXR signaling and inhibition of SREBP-1c signaling may contribute to insulin sensitization and improved lipid homeostasis in SPI+-fed rats after consumption of diets high in fat and cholesterol. J. Nutr. 139: 1431–1438, 2009.

Introduction

There has been a surge of interest in recent years in the potential health beneficial effects of adding soy foods to the diet (1–4). One of the strongest health claims involves protection against coronary heart disease, based upon reductions in plasma cholesterol and triglycerides, and protection against atherosclerosis in animal models (4–7). Additional health benefits have been suggested to include antidiabetic effects, reduced weight gain, and improved body composition (8–11). However, the potential health effects of soy foods in humans remains highly controversial (2,9,12,13). It has been suggested that many of the health effects of soy may be related to activation of estrogen receptor (ER)[7] -α and ERβ, mediated by soy-associated phytoestrogens, especially the isoflavones genistein and daidzein (1–4). Recent studies on adipose tissue deposition in ovariectomized mice fed genistein suggested that decreases in fat mass were associated with reduced adipose tissue lipoprotein lipase activity mediated via ERα signaling (14). Moreover, improvement in lipid and glucose signaling after soy feeding in mice has recently been linked to estrogenic activation of AMP-activated protein kinase in adipose tissue and skeletal muscle (15). Alternatively, effects of soy feeding on insulin sensitivity and lipid homeostasis have been suggested to involve altered signaling in the liver through promiscuous nuclear receptors, including the PPARα and PPARγ and liver X-receptor (LXR)-α (9–11,16,17), or through other transcription factors, such as the sterol regulatory element binding protein (SREBP) (10, 18–21). The identity of the component or components of soy responsible for nuclear receptor regulation has remained the subject of debate among researchers (9–11,16–21).

It is increasingly clear that the effects of soy and soy-associated isoflavones during early development may differ from those in adults as a result of potential long-term programming effects (1,17,18) and the differing effects of soy-associated phytoestrogens

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[3] Supplemental Tables 1 and 2 and Supplemental Figures 1 and 2 are available with the online posting of this paper at jn.nutrition.org.
[7] Abbreviations used: ACO, acyl Co-A oxidase; CAS, casein diet; C+D, casein + daidzein diet; C+G, casein + genistein diet; ChIP, chromatin immunoprecipitation; CPT, carnitine palmitoyl transferase 1A; CYP, cytochrome P450; CYP7A1, cholesterol 7α-hydroxylase; ER, estrogen receptor; FASN, fatty acid synthase; GAPDH; glucose 6-phosphate dehydrogenase; HADHA, mitochondrial trifunctional enzyme subunit A; IGF-1, insulin-like growth factor-1; LXR, liver X-receptor; OGTT, oral glucose tolerance test; PND, postnatal day; PPRE, peroxisomal proliferator receptor response element; SREBP, sterol regulatory element binding protein; SPI+, soy protein isolate; SPI−, soy protein isolate stripped of phytochemicals by successive ethanol washes; Western casein, Western diet made with casein; Western SPI+, Western diet made with SPI+.
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in the face of different levels of endogenous sex steroids (1,19–21). Few studies have examined the health effects of soy feeding prior to puberty despite the fact that in the US, a large proportion of soy consumption occurs in neonates fed soy formula (1).

We studied the effects of feeding soy protein isolate (SPI+), the sole protein source in soy infant formula. We also compared the effects of SPI+ with those of SPI− (SPI processed to have negligible levels of phytochemicals, including isoflavones) or casein diets supplemented with the purified isoflavones, genistein or daidzein. Expression of hepatic genes involved in fatty acid, cholesterol, and glucose homeostasis and the expression and activation of 3 nuclear receptors (PPARα, PPARγ, and LXRα) involved in the regulation of these genes were all studied. In addition, we examined the effects of SPI+ early in life on the physiological, endocrine, and metabolic responses associated with consumption of a high-fat, high-cholesterol Western diet.

Materials and Methods

Materials. All the chemicals, unless otherwise noted, were purchased from Sigma Aldrich. For Western blotting, goat polyclonal antibodies against rat cytochrome P450 (CYP)7A1 (sc-14423), goat polyclonal antibodies against rat SKl-1/S1P (sc-9785), and mouse monoclonal antibodies against human SREBP-1 (sc-13551) were purchased from Santa Cruz Biotechnology. Goat polyclonal antibodies specific for rat liver carnitine palmitoyl transferase (CPT)-1A were generated as previously described (22) and were the kind gift of Dr. Victoria Esser, University of Texas Southwestern Medical Center, Dallas, TX. Rabbit polyclonal antibodies against PPARγ and mouse monoclonal antibodies against LXRα were from Novus Biological and R&D Systems, respectively. Mouse monoclonal antibodies to rat CYP2C11 were the gift of Dr. Paul Thomas, Rutgers University, NJ. A polyclonal antibody was raised against a peptide coding for a unique region of rat LXRα: NH_2-PRVSSPPQVLPQLSP-OH (Bio synthesis). This antibody recognized a nuclear protein of the same mass as LXRα (50 kDa). Expression of this protein was elevated in Western blots and binding to an LXR response element on the CYP7A1 promoter was increased in chromatin immunoprecipitation (ChIP) assays after SPI+ feeding coincident with increased expression of LXR-target genes. SPI+ and SPI− proteins were supplied by the Solae Company. SPI− contained <3 mg/kg of any isoflavone. Genistein and daidzein were purchased from Indofine.

Rats and diets. The experiments received prior approval from the Institutional Animal Care and Use Committee at University of Arkansas for Medical Sciences. Weanling and time-impregnated Sprague-Dawley rats were obtained from Charles River and were housed in polycarbonate cages in an Association of Laboratory Animal Care-approved animal facility in an environmentally controlled room at 22°C with a 12-h-light/-dark cycle.

Expt. 1. Time-impregnated female rats consumed ad libitum water and diets formulated with casein as the sole protein source (CAS) (23). At birth, litters were culled to 5 male and 5 female pups per litter. Dams continued to be fed the CAS diet throughout lactation. Beginning at postnatal d (PND) 15, CAS litters were given continued access to the CAS diet. Additional litters were switched to diets in which casein was replaced by SPI+ (IB1.2 UN 30CA, lot no. 200–1, Solae) (SPI+). The diets were formulated according to the AIN-93G formulation, except that SPI+ replaced CAS and essential amino acids were added to match CAS levels and corn oil replaced soy oil (23). The SPI+ diet contained 286 $mg \cdot kg^{-1}$ genistein and 226 $mg \cdot kg^{-1}$ daidzein. Rat pups fed the SPI+ diet were calculated to consume a mean of 80 mg of total isoflavones $\cdot kg^{-1} \cdot d^{-1}$ from weaning until killing on PND33. Additional litters were fed AIN-93G diets in which CAS was replaced by SPI− (FXP H0140, lot no. M320000318, Solae) in the same manner as SPI+. Other litters were fed AIN-93G diets containing casein supplemented with 250 $mg \cdot kg^{-1}$ genistein (C+G) or casein supplemented with 250 $mg \cdot kg^{-1}$ daidzein (C+D). Pups were killed on PND33. Serum and liver were collected and stored at −70°C until use.

Expt. 2. Male rats (n = 10) consumed ad libitum water and AIN-93G diet formulated with casein as the sole protein source (casein), as previously described (23), from PND24 to 64. Additional groups of rats (n = 10) were pair-fed a high-fat/high-cholesterol Western diet (Harlan Teklad TD.88137; modified to contain 18.84 $MJ \cdot g^{-1}$ energy, 195 $g \cdot kg^{-1}$ protein, 483 $g \cdot kg^{-1}$ carbohydrate, 210 $g \cdot kg^{-1}$ anhydrous milk fat, 5 $g \cdot kg^{-1}$ cholesterol, and 50 $g \cdot kg^{-1}$ cellulose fiber) made with either casein (Western casein) or with SPI+ (Western SPI+) as the sole protein source. After the rats were killed, serum, liver, and retroperitoneal fat pads were collected and stored at −70°C until use. In addition, liver samples were fixed in optimal cutting temperature compound for Oil red O analysis of fat droplets.

Body composition analysis. Body composition was assessed via whole animal body composition by NMR (Echo Medical Systems) performed in conscious unanesthetized rats (24) and postmortem dissected weights of retroperitoneal fat pads.

Endocrine and biochemical analyses. Serum from Expt. 1 was assayed for insulin-like growth factor-1 (IGF-1) by RIA using kit DSL-2900 from Diagnostic Systems Laboratories. Seven days prior to killing, 5 rats from each group in Expt. 2 were given an oral glucose tolerance test (OGTT) as described previously in our laboratory (24). Serum insulin concentrations were measured using an ELISA (EZRMI-13K) for rat insulin (Linco Research). At killing, serum insulin was also assayed and serum glucose measured using the glucose oxidase method (IR070, Synermed). We measured serum triglycerides and total cholesterol using commercially available reagents (IR140-R, IR060-R, Synermed). Liver sections were stained for lipid droplets using 60% Oil red O stock solution (14). Lipids were extracted from liver homogenates with chloroform/methanol (2:1, v:v) and triglycerides and cholesterol concentrations assayed as described above for serum. 16α-Hydroxylation of testosterone was measured in hepatic microsomes using [^{14}C]testosterone and the TLC method of Ronis et al. (25).

Western immunoblot analysis. Hepatic microsomes were prepared by differential ultracentrifugation (26). Nuclear extracts were prepared using a Cell Lytic kit (N-XTRACT, Sigma). Mitochondria were isolated using a kit from Pierce (PI89801). Microsomes were analyzed for expression of CYP2C11 apoprotein and CYP7A1 apoprotein. Mitochondrial fractions were analyzed for CPT-1A protein. PPARγ, LXRα, and SREBP-1c protein expression was assessed in nuclear extracts. Fatty acid synthase (FASN) and SKl-1/S1P proteins were immunoquantitated from whole liver homogenates. Even loading of microsomal protein onto gels was confirmed by Coomassie Blue protein staining of duplicate gels. Loading variation of proteins across gels was ± 3%. Nuclear SREBP-1c was normalized to laminin and cytosolic proteins to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or α-tubulin depending on molecular weight. Mitochondrial CPT1c expression was normalized to actin.

Real-time RT-PCR analysis of mRNA expression. Real time RT-PCR was conducted as described previously (26) using gene-specific probes (**Supplemental Table 1**). GAPDH or cyclophilin amplicons were used to normalize the values. The groups did not differ in expression of these housekeeping genes.

ChIP analysis. Liver pooled from 3 rats per sample was used for ChIP analysis of PPARγ binding to the peroxisomal proliferator receptor response element (PPRE) element in the promoter of the glucokinase gene and of LXRα binding to the LXR-response element in the promoter of the CYP7A1 gene. ChIP was carried out using a ChIP assay kit (ChIP-IT, Active Motif) according to the manufacturer's instructions. Chromatin was immunoprecipitated with antibodies against PPARγ (Novus) or raised against rat LXRα (Biosynthesis). DNA extracted from the PPARγ immune complex was amplified by PCR with a 5′-primer (CAA GGA CTT CCG CAC TAA CG) −276 to −157 and a 3′-primer (ACC TCT CCA CAG CAA GTC CA) −93 to −74 specific for the PPRE site on the glucokinase promoter. DNA extracted from the LXRα immune complex was amplified by PCR with a 5′-primer (ATG TGC ACA GGA CCA TGA TC) −3613 to −3594 and a 3′ primer (TGG ACT TAG TTC

AAG GCC GG) −3496 to −3477, specific for an identified LXR binding sequence in the CYP7A1 promoter. PCR amplifications were performed (32 cycles for the input, 35 and 38 cycles for the PPARγ and LXRα immunoprecipitates, respectively, and the no-antibody precipitation negative control).

Statistical analysis. Data are presented as means ± SEM. All data were analyzed using Sigma Stat for Windows (version 3.5 Systat Software). In Expt. 1, data were analyzed by 2-way ANOVA (diet × gender) and in Expt. 2 by 1-way ANOVA. When variances were heterogeneous, such as in the case of FASN expression, data were analyzed by ANOVA on ranks. Differences among means were determined by the Student-Newman-Keuls test and were considered significant at $P < 0.05$.

Results

Serum genistein. The total genistein concentration (aglycone + conjugates) in serum of male rats at PND50 after developmental feeding was 808 ± 119 nmol·L^{-1} after SPI+ feeding and 2700 ± 100 nmol·L^{-1} after feeding C+G. However, most of the circulating isoflavones (92–96%) are found as inactive conjugates as a result of first pass glucuronidation and sulfation in the intestine and liver (2,27,28).

Growth, growth hormone, and body composition. In Expt. 1, body weight at PND33 was lower in male rats fed SPI+ or SPI− diets compared with those fed CAS ($P < 0.05$) (**Supplemental Table 2**). Body weight in female rats fed SPI− was less than in those fed CAS ($P < 0.05$). Reduced weight gain coincided with disruption of GH signaling after soy consumption, because serum IGF-1 concentrations were lower ($P < 0.05$) in males fed either SPI+ or SPI− than in male rats fed CAS. Furthermore, expression and activity of male hepatic CYP2C11, which is dependent on male patterns of GH-secretion (29), were lower ($P < 0.05$) relative to the CAS group after both SPI+ and SPI− feeding (Supplemental Table 2; **Supplemental Fig. 1**).

PPARα-regulated pathways. Expression of mRNA-encoding hepatic genes involved in peroxisomal and mitochondrial fatty acid β-oxidation [acyl Co-A oxidase (ACO) and the mitochondrial trifunctional protein α subunit (HADHA)] and fatty acid transport into the mitochondria CPT-1A was greater in SPI+-fed male and female rats than in rats fed CAS ($P < 0.05$) (**Table 1**). In contrast, feeding SPI−, C+G, or C+D resulted in no induction except for HADHA mRNA, which was greater than in the CAS group only in female pups ($P < 0.05$). All 3 genes are positively regulated by the transcription factor PPARα (30). Greater mitochondrial CPT-1A protein expression in SPI+-fed ($P < 0.05$) but not SPI−, C+G, or C+D-fed groups compared with CAS feeding was confirmed by Western immunoblot in livers of female rats (**Fig. 1**). We have previously reported that PPARα binding was 2- to 3-fold greater in both males and females fed SPI+ compared with those fed CAS in this experiment using electrophoretic mobility shifty assay analysis of protein binding to the PPRE element in the ACO promoter ($P < 0.05$) (data not shown) (11). Expression of the mRNA encoding for PPARα itself was greater in both male and female rats fed SPI+ compared with those fed CAS ($P < 0.05$) (Table 1).

PPARγ-regulated pathways. Glucokinase, a key enzyme involved in regulation glucose metabolism, and CD36, a scavenger receptor involved in hepatic fatty acid uptake, are 2 hepatic genes regulated by PPARγ (31,32). Both genes were expressed more highly in female than in male liver ($P < 0.05$) (Table 1).

TABLE 1 PPARα- and PPARγ-regulated pathways in livers of rats fed CAS, SPI+, SPI−, C+G, or C+D (Expt. 1)[1]

Gene name and function	CAS	SPI+	SPI−	C+G	C+D
ACO, Peroxisomal β-oxidation (PPARα-target)			*Fold of male CAS*[2]		
Male	1.00 ± 0.06[a]	1.86 ± 0.08[b]	1.12 ± 0.09[a]	0.69 ± 0.19[a]	0.65 ± 0.07[a]
Female	1.03 ± 0.13[a]	2.93 ± 0.12[b]*	1.16 ± 0.18[a]	0.90 ± 0.09[a]	0.75 ± 0.02[a]
CPT-1A, Fatty acid transport into mitochondria (PPARα-target)					
Male	1.00 ± 0.15[b]	1.74 ± 0.19[c]	0.99 ± 0.15[b]	0.47 ± 0.13[a]	0.32 ± 0.03[a]
Female	0.42 ± 0.05[a,*]	3.55 ± 0.3[c]*	1.28 ± 0.37[a,b]	0.87 ± 0.15[a,b]*	0.51 ± 0.09[a]
HADHA, Mitochondrial β-oxidation (PPARα-target)					
Male	1.00 ± 0.12[b]	1.61 ± 0.19[c]	1.10 ± 0.07[b]	0.77 ± 0.16[a,b]	0.55 ± 0.05[a]
Female	0.81 ± 0.04[a]	1.46 ± 0.11[b]	2.10 ± 0.40[b]	1.43 ± 0.06[b]*	1.39 ± 0.05[b]*
PPARα					
Male	1.00 ± 0.08[a]	1.50 ± 0.06[b]	n.d[3]	n.d.	n.d.
Female	0.66 ± 0.05[a]*	1.18 ± 0.05[b]*	n.d.	n.d.	n.d.
Glucokinase, Glucose sensor, regulator of hepatic glucose (PPARγ-target)					
Male	1.00 ± 0.24[a]	6.10 ± 1.90[b]	0.94 ± 0.12[a]	1.61 ± 0.27[a]	1.22 ± 1.44[a]
Female	5.43 ± 0.38*	8.00 ± 2.8	6.90 ± 1.75*	4.15 ± 1.03*	5.33 ± 1.02*
CD36, Scavenger receptor involved in fatty acid transport (PPARγ-target)					
Male	1.00 ± 0.25[a]	1.91 ± 0.37[b]	2.50 ± 0.32[b]	1.04 ± 0.12[a]	1.54 ± 0.13[a]
Female	3.53 ± 0.28[b]*	5.45 ± 0.92[b]*	5.97 ± 0.71[b]*	3.84 ± 0.83[b]*	1.81 ± 0.21[a]
PPARγ					
Male	1.00 ± 0.06[a]	2.20 ± 0.28[b]	n.d.	n.d	n.d.
Female	1.04 ± 0.08[a]	1.87 ± 0.13[b]	n.d.	n.d.	n.d

[1] Data are mean ± SEM, $n = 3$ mRNA pools each from the livers of 3 rats. Means in a row without a common letter differ, $P < 0.05$.
*Different from corresponding value in males, $P < 0.05$.
[2] Normalized to GAPDH (housekeeping gene) and expressed relative to male CAS, which was set at 1.0.
[3] n.d., Assay not done.

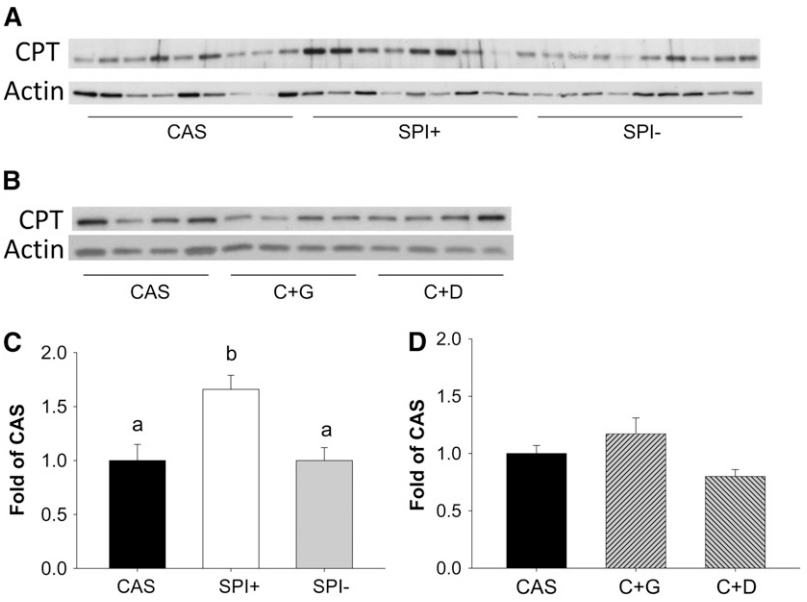

FIGURE 1 Western immunoblot of CPT-1A protein expression in Expt. 1 in mitochondria from the liver of PND33 male rats previously weaned from CAS-fed dams onto CAS, SPI+, or SPI− diets (*A*). Each lane represents samples from 1 rat per treatment. Western immunoblot of CPT-1A protein expression in mitochondria from the liver of PND33 male rats previously weaned from CAS-fed dams onto CAS, C+G, or C+D (*B*). Quantitation of Western immunoblots in *A* and *B* are shown in C and D, respectively. Densitometric data for CPT-1A were normalized to actin (loading control) and expressed relative to CAS, which was set at 1.0. Data are mean ± SEM, $n = 9$ (*C*) or 4 (*D*). Means without a common letter differ, $P < 0.05$.

Expression of both genes was greater after SPI+ consumption in male rats compared with those fed CAS ($P < 0.05$), whereas other diets had no effects, with the exception of greater CD36 mRNA expression after feeding SPI− in males ($P < 0.05$). Expression of these genes did not differ among the female groups. ChIP analysis had greater PPARγ binding to its response element on the glucokinase promoter after feeding SPI+ in males than in those fed CAS ($P < 0.05$) (**Fig. 2***A*). PPARγ transcription factor expression (protein and mRNA) was also greater ($P < 0.05$) in nuclear extracts from male rats fed SPI+ compared with those fed CAS (Fig. 2*B*; Table 1).

LXRα-regulated pathways. Expression of hepatic genes regulated by LXRα was examined. These included CYP7A1, which catalyzes cholesterol 7α-hydroxylation, the rate-limiting step in the formation of bile acids (33,34), and the sterol transporters ABCG5 and ABCG8 (35). Expression of CYP7A1 and ABCG8 mRNA was greater after feeding SPI+ than after feeding CAS in both sexes ($P < 0.05$), with a smaller effect on ABCG5 mRNA, which was elevated significantly in only SPI+ fed females (**Table 2**). Greater expression of CYP7A1 after feeding SPI+ compared with CAS was confirmed at the apoprotein level by Western immunoblot (**Fig. 3***A*). In male but not female rats, there was greater expression of CYP7A1 and ABCG8 after SPI− feeding to the same degree as after feeding SPI+ (Table 2; Fig. 3*A*). Expression of all 3 mRNA and of CYP7A1 apoprotein was greater in the C+G and C+D groups compared with the CAS-fed groups of both sexes ($P < 0.05$) (Table 2; Fig. 3*B*). ChIP analysis of LXRα binding to a response element identified in the CYP7A1 promoter had greater LXRα binding compared with rats fed CAS ($P < 0.05$), but feeding SPI− did not affect LXRα binding (Fig. 3*C*). LXRα mRNA and protein expression was greater ($P < 0.05$) in nuclear extracts of SPI+-fed than in CAS-fed male rats (Table 2; Fig. 3*D*).

Insulin sensitivity and lipid homeostasis. Feeding the Western casein diet in Expt. 2 from PND24 to 64 resulted in greater weight gain, percent body fat, and percent liver weight compared with the casein-fed control group ($P < 0.05$) (**Table 3**). In contrast, feeding a Western SPI+ diet protected against the effects of fat and cholesterol on body composition ($P < 0.05$). Analysis of OGTT after 33 d of feeding the Western casein diet resulted in a greater serum insulin concentration at 60 and 90 min post-glucose challenge compared with either the casein

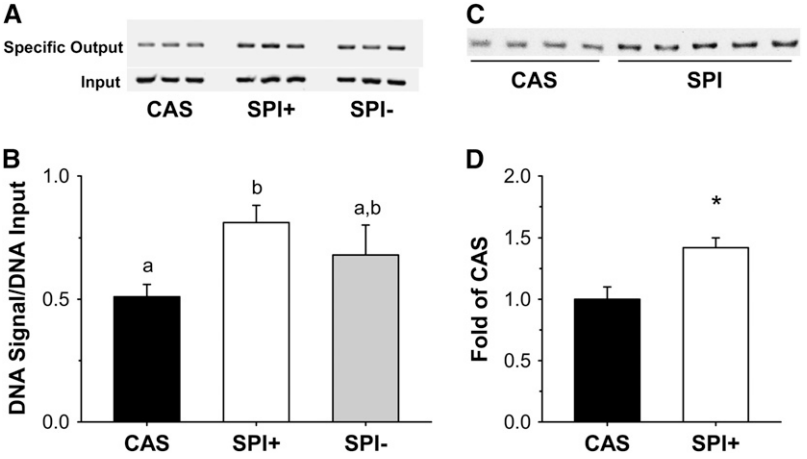

FIGURE 2 ChIP analysis in Expt. 1 of PPARγ binding to the PPRE element in the glucokinase promoter in nuclear extracts from the liver of PND33 male rats previously weaned from CAS-fed dams onto CAS or SPI+ (*A*). Each lane contains samples from 3 male livers pooled from a litter from each treatment group. Data are mean ± SEM, $n = 3$. Means without a common letter differ, $P < 0.05$ (*B*). Western immunoblot of PPARγ protein in nuclear extracts from the liver of PND33 male rats previously weaned from CAS-fed dams onto CAS or SPI+ (*C*). Each lane contains nuclear extracts from 1 rat from each treatment group. Data are mean ± SEM, $n = 4$–5. *Differs from CAS, $P < 0.05$ (*D*).

TABLE 2 LXRα-regulated pathways in rat liver after feeding CAS, SPI+, SPI−, C+G, or C+D (Expt. 1)[1]

Gene name and function	CAS	SPI+	SPI−	C+G	C+D
			Fold of male CAS[2]		
Cholesterol 7α-hydroxylase (CYP7A1), Bile acid formation rate-limiting step					
Male	1.00 ± 0.19[a]	3.12 ± 0.47[a,b]	4.60 ± 0.06[b]	4.12 ± 0.50[b]	3.37 ± 0.41[b]
Female	1.45 ± 0.28[a]	3.84 ± 0.29[b]	2.58 ± 0.56[a]	5.44 ± 0.66[b]	5.90 ± 1.44[a,b]
ABCG5, Cholesterol transport into bile					
Male	1.00 ± 0.19[b]	1.45 ± 0.06[b]	0.63 ± 0.13[a]	1.23 ± 0.25[b]	0.78 ± 0.13[a,b]
Female	0.41 ± 0.06[a]*	2.17 ± 0.28[b]	0.80 ± 0.07[a]	1.71 ± 0.31[b]	1.02 ± 0.12[b]
ABCG8, Cholesterol transport into bile					
Male	1.00 ± 0.17[a]	2.85 ± 0.42[b]	3.34 ± 0.26[b]	13.45 ± 3.38[c]	13.15 ± 1.53[c]
Female	1.25 ± 0.17[a]	4.78 ± 0.86[b]	1.90 ± 0.30[a]	3.48 ± 0.93[b]*	1.90 ± 0.33[a]*
LXRα					
Male	1.00 ± 0.04[a]	1.38 ± 0.06[b]	n.d.[3]	n.d.	n.d.
Female	1.42 ± 0.06[a]	1.48 ± 0.06[b]	n.d.	n.d.	n.d.

[1] Data are mean ± SEM, n = 3 mRNA pools each from the livers of 3 rats. Means in a row without a common letter differ, $P < 0.05$. *Different from corresponding value in males, $P < 0.05$.
[2] Gene expression normalized to GAPDH (housekeeping gene) and expressed relative to male CAS, which was set at 1.0.
[3] n.d., Assay not done.

control or the Western SPI+ diet group ($P < 0.05$) (**Fig. 4**). Area under the insulin vs. time curve was 11.5 ± 7.2 nmol·L^{-1}·150 min (casein control) vs. 23.2 ± 2.1 nmol·L^{-1}(150 min) (Western casein) (P = 0.18) and 4.3 ± 3.2 nmol·L^{-1}(150 min) in the Western SPI+ group (P = 0.003; compared with the Western casein group). Insulin sensitivity appeared to be greater in the Western SPI+ diet group and was accompanied by a reversal of the greater serum glucose concentration in the Western casein group compared with the casein control group (Table 3). Feeding the Western casein diet resulted in greater serum and hepatic triglyceride and cholesterol concentrations and in hepatic steatosis compared with casein-fed controls ($P < 0.05$) (Table 3; **Fig. 5***A–C*). Feeding the Western SPI+ diet resulted in reduced serum cholesterol and hepatic triglyceride concentrations compared with feeding the Western casein diet and reduced steatosis ($P < 0.05$) (Table 3; Fig. 5*A–C*; **Supplemental Fig. 2**).

Fatty acid synthesis and SREBP-1c activation. Because decreases in steatosis produced by soy feeding have been suggested to involve reduced fatty acid synthesis in addition to increased fatty acid degradation, we examined the effects of SPI+ feeding on expression and activation of SREBP-1c, a transcription factor that regulates expression of rate-limiting genes in fatty acid synthesis and desaturation. Real-time RT-PCR analysis demonstrated no effects on SREBP-1c mRNA (**Table 4**). However, cleaved SREBP-1c protein was greater ($P < 0.05$) in the Western casein group than in casein-fed controls. Cleaved SREBP-1c was lower in nuclear extracts from the Western SPI+ group than in extracts from the Western casein group ($P < 0.05$) (Table 4; Fig. 5*D*). Hepatic expression of the serine protease SKI-1/S1P was greater in the Western casein group than in the casein controls ($P < 0.05$). However, expression of this protein did not differ between the Western SPI+ and the Western casein groups (Table 4; Fig. 5*E*). mRNA for downstream gene targets of

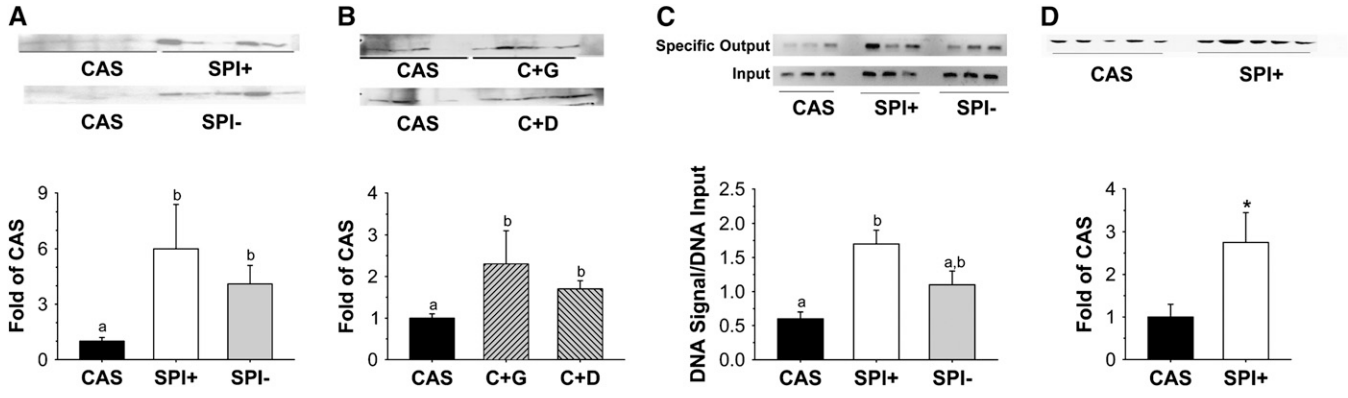

FIGURE 3 Western immunoblot in Expt. 1 of CYP7A1 apoprotein expression in microsomes from the liver of PND33 male rats previously weaned from CAS-fed dams onto CAS, SPI+, SPI−, C+G, or C+D (*A* and *B*). Each lane represents samples from 1 rat per treatment. Data are mean ± SEM, n = 9–10. Means without a common letter differ, $P < 0.05$. ChIP analysis of LXRα binding to an LXR response element in the CYP7A1 promoter in PND33 liver from male rats weaned from CAS-fed dams onto CAS; SPI+ or SPI− (*C*). Each lane contains samples from 3 male livers pooled from each treatment group. Data are mean ± SEM, n = 3. Means without a common letter differ, $P < 0.05$. Western immunoblot of LXRα protein in nuclear extracts from the PND33 liver of male rats weaned from CAS-fed dams onto CAS or SPI+ (*D*). Each lane contains nuclear extracts from 1 rat per treatment. Data are mean ± SEM, n = 5. *Differs from CAS, $P < 0.05$ (*D*).

TABLE 3 Effects of CAS or SPI+ on physiological and metabolic responses of male rats to Western diets (Expt. 2)[1]

Diet group	Casein	Western casein	Western SPI+
Final body weight, *g*	388.00 ± 4.10[a]	439.00 + 4.80[b]	402.00 ± 7.80[a]
Body weight gain, *g·d*$^{-1}$	8.10 ± 0.10[a]	9.20 ± 0.10[b]	8.30 ± 0.20[a]
Lean body mass,[2%]	76.40 ± 0.90[b]	72.00 ± 0.30[a]	75.60 ± 1.10[b]
Body fat mass, *%*	14.80 ± 0.80[a]	19.00 ± 0.30[b]	16.00 ± 1.20[a]
Perirenal adipose tissue, *g·100 g body weight*$^{-1}$	0.91 ± 0.08[a]	1.60 ± 0.14[b]	1.29 ± 0.140[b]
Liver			
Relative weight, *g·100 g body weight*$^{-1}$	4.10 ± 0.12[a]	4.70 ± 0.17[b]	4.20 ± 0.14[a]
Triglycerides, *μmol·g wet tissue*$^{-1}$	44.70 ± 12.70[a]	100.60 ± 14.40[b]	51.20 ± 11.30[a]
Total cholesterol, *μmol·g wet tissue*$^{-1}$	51.00 ± 10.00	80.00 ± 22.00	55.00 ± 15.00
Serum			
Triglyceride, *mmol·L*$^{-1}$	3.40 ± 0.20	4.80 ± 0.24	5.20 ± 0.20
Total cholesterol, *mmol·L*$^{-1}$	2.10 ± 0.33[a]	4.40 ± 1.00[b]	2.60 ± 0.36[a]
Glucose, *mmol·L*$^{-1}$	4.50 ± 0.13[a]	5.10 ± 0.17[b]	4.50 ± 0.21[a]
Insulin, *nmol·L*$^{-1}$	1.60 ± 0.40	1.70 ± 0.20	1.30 ± 0.20

[1] Values are means ± SEM, n = 7–10. Means in a row with superscripts without a common letter differ, $P < 0.05$.

SREBP-1c controlling fatty acid synthesis and desaturation, FASN, and SCD1 was greater after feeding the Western casein diet than the casein diet ($P < 0.05$). Both these genes and another SREBP-1c target gene, ACC1, were lower in the Western SPI+ group than in the Western casein group ($P < 0.05$) (Table 4).

Discussion

Previous studies have suggested that dietary soy can improve body composition. However, disagreement exists regarding both the potential of soy to reduce obesity and the potential mechanism (8,11,14,15,19,36,37). Although consumption of SPI+ produced decreases in somatic growth compared with casein feeding in Expt. 1, this coincided with reduced IGF-1 concentrations and was not accompanied by changes in adiposity. Adiposity significantly decreased in male Western diet-fed rats where SPI+ was the protein source compared with rats fed the same diet made with casein. These data are in agreement with another recent high-fat feeding study (19). Preliminary findings suggest reduced percent body fat in male soy-fed infants in a large longitudinal study currently in progress (38). SPI is a complex mixture of proteins and peptides associated with at least 139 phytochemicals, including saponins, phytosterols, isoflavones, and fatty acids (39). However, the majority of mechanistic studies have focused on the isoflavone genistein. Genistein has been suggested to reduce adult obesity as a result of estrogenic actions on genes regulating lipogenesis, lipolysis, and adipocyte differentiation via ERβ (14,40), epigenetic effects when exposure occurs in utero (37), and ERα-mediated effects on AMPK signaling in muscle and adipose tissue (15). However, isoflavone actions on adipose tissue appear to be dose and sex dependent (40). Effects of soy during early development may differ from those in adults as a result of different expression of ER, different levels of endogenous estrogens, and via estrogen-independent actions of isoflavones. Furthermore, other soy components, such as equol generated by gut bacteria or soy proteins and bioactive peptides generated during digestion such as β-conglycinin, may account for differential effects (2,27,36). In regard to the latter, processing to remove phytochemicals from SPI+ may also alter the protein/peptide composition of SPI− and result in different properties than SPI+.

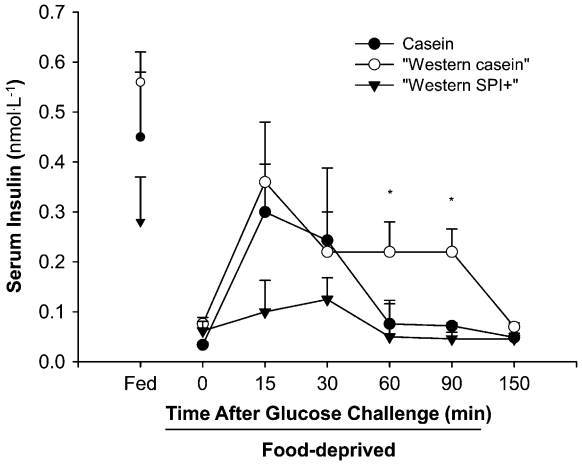

FIGURE 4 Serum insulin concentrations in Expt. 2 after an OGTT in PND64 male rats fed casein, Western casein, or Western SPI+ diets from PND24. Data are mean ± SEM, n = 10 rats killed at 0800 with or without overnight food deprivation and sampled over a 150-min period following an oral glucose challenge with 2.5 g.kg^{-1}. *Differs from casein and Western SPI+ groups, $P < 0.05$.

Our data are consistent with studies in yellow A^{vy}/a mice demonstrating that soy feeding can activate hepatic PPARα signaling in vivo (11). This might contribute to a reduction of hepatic triglycerides and steatosis. We have previously demonstrated increased protein binding to the PPRE on the ACO gene in SPI+-fed rats (11). Previous studies have also reported increases in PPARα-regulated gene expression and a lack of effects of SPI+ feeding on these genes in PPARα knockout mice (17). Our data do not support previous in vitro data that PPARα activation in vivo following soy consumption are the result of actions of isoflavone components in SPI+ (9,16). Because feeding SPI− was also ineffective, it is likely that nonisoflavone phytochemicals or their metabolites were responsible for the effect of feeding SPI+.

Other investigators have suggested that reduced SREPB-1c signaling could lead to lower fatty acid synthesis after soy consumption (10,18–21). SREBP-1c mRNA expression has been shown to decrease in liver of obese Zucker *fa/fa* rats fed SPI+ and in liver of rats fed SPI+ with high-fat diets (10,18,19). This has been attributed to reduced plasma insulin levels or to decreased LXR-mediated signaling. However, in the current study, SPI+ feeding did not affect SREBP-1c mRNA in rats fed Western diets. Moreover, LXR signaling was increased rather than decreased. Several in vitro studies have also observed no effects of isoflavones on SREBP-1c expression (18,20). However, a recent report using HepG2 cells suggested that genistein reduces levels of mature nuclear SREBP-1c protein as the result of lower proteolytic cleavage by the enzyme SKl-1/S1P (21). We observed increased nuclear SREBP-1c protein following consumption of Western diets. However, despite reductions in nuclear SREBP-1c protein and downstream pathways in Western diet-fed rats containing SPI+, SPI+ feeding did not affect S1P protein expression.

To our knowledge, this is the first report to demonstrate that feeding SPI+ results in activation of known PPARγ-regulated genes in the liver in vivo. This could partially explain previously

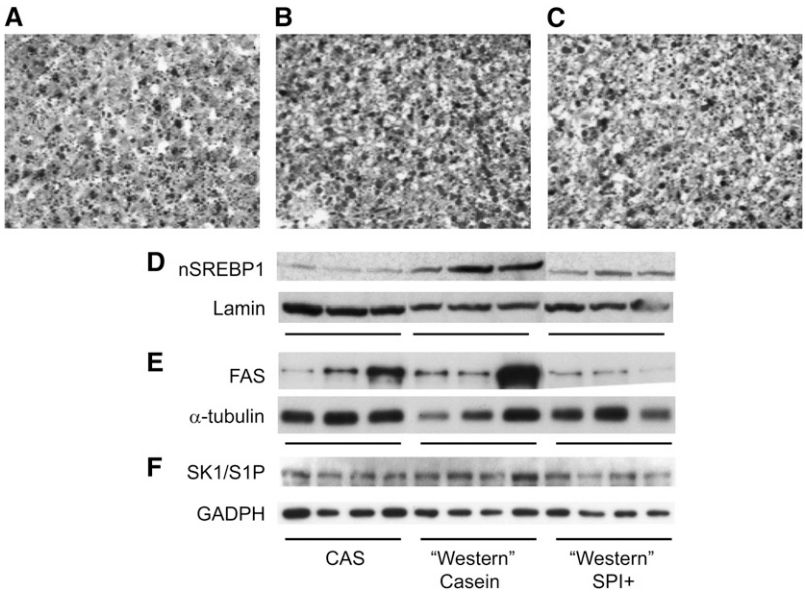

FIGURE 5 Oil red O staining in Expt. 2 of fat droplets in 10× liver sections from PND64 male rats fed casein (*A*), Western casein (*B*), or Western SPI+ (*C*) diets. Representative Western blots of mature SREBP-1 protein expression in nuclear extracts from the liver of rats from Expt. 2 (*D*). Each lane contains nuclear extracts from 1 rat per treatment. Representative Western blots of FASN protein expression in whole homogenates from the liver of rats from Expt. 2 (*E*). Representative Western blots of SKI/S1P protein expression in homogenates from the liver of rats from Expt. 2 (*F*). Each lane contains homogenate from 1 rat per treatment.

described antidiabetic effects and increased whole-body insulin sensitivity in male rats fed SPI+ (8,9,15,41). The current data suggest that PPARγ-regulated genes are more highly expressed in female than in male rat liver and that SPI+ has greater effects on PPARγ-regulated genes in males. This is consistent with a recent report demonstrating female-predominant expression of PPARγ-regulated CD36 in rat liver and regulation by estrogens (42).

Although previous studies have demonstrated in vitro activation of PPARγ by isoflavones (9,16), in the current in vivo study, purified isoflavones were ineffective. Increased PPARγ signaling was accompanied by increased receptor expression. Similar increases in PPARγ mRNA expression have been reported in adipose tissue from Zucker rats fed SPI+ (10). In contrast, recent studies have demonstrated reduced PPARγ mRNA in the pancreas (41), suggesting that the effects of soy on PPARγ may be tissue specific.

Many previous studies have reported reductions in plasma and liver cholesterol concentrations in both animal models and humans fed soy (3–7). However, the molecular mechanisms have remained the subject of dispute (3,10,18,20,42–44). Our data are consistent with previous reports suggesting soy feeding increases bile acid excretion in both animals and infants fed soy formula (43,45) and link this to activation of LXRα-regulated gene expression. Like the PPAR, activation of LXRα pathways was accompanied by significant elevation in receptor expression. However, in this case, both soy protein and isoflavone components of the diet appear to be involved. Effects of genistein and daidzein feeding in vivo on LXR activation appear to be considerably greater than the marginal effects observed with these isoflavones in vitro (16). It is possible that genistein and daidzein are metabolized to more potent bioactive metabolites such as equol by gut bacteria or the rat in vivo. Feeding genistein has also been shown to increase LXR mRNA expression in adipose tissue of C57BL/6 mice in vivo (40). However, our data differ from a previous report that SPI+ results in suppressed hepatic expression of LXR-regulated CYP7A1 and ABCA1 in hyperinsulinemic obese Zucker rats (10). This may be the result of differences in responses to SPI+ between rat strains or the result of altered leptin signaling in these animals.

TABLE 4 Differences in SREBP-1c signaling in liver of male rats fed Western diets with CAS or SPI+ as the protein source (Expt.)[1]

Diet group	Casein	Western casein	Western SPI+
SREBP processing		*Fold of casein*[1]	
SKI-1/S1P mRNA[2]	1.00 ± 0.11	1.44 ± 0.22	1.31 ± 0.13
SK1/S1P protein[3]	1.00 ± 0.11[a]	1.39 ± 0.10[b]	1.34 ± 0.04[b]
SREBP-1c mRNA[2]	1.00 ± 0.03	1.30 ± 0.23	1.36 ± 0.23
SREBP-1c protein (nucleus)[3]	1.00 ± 0.03[a]	2.88 ± 0.40[b]	1.60 ± 0.16[a]
Fatty acid synthesis			
FASN mRNA[2]	1.00 ± 0.31[a]	4.60 ± 2.14[b]	0.56 ± 0.19[a]
FASN protein[3]	1.00 ± 0.24	2.90 ± 1.07	1.39 ± 0.34
ACC1 mRNA[2]	1.00 ± 0.13[b]	1.22 ± 0.16[b]	0.54 ± 0.07[a]
SCD1 mRNA[2]	1.00 ± 0.25[a]	2.68 ± 0.49[b]	1.62 ± 0.34[a]

[1] Values are means ± SEM, n = 7–10. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] mRNA normalized to cyclophilin (housekeeping gene) and expressed relative to casein, which was set at 1.0.
[3] Protein immunoquantitation of Western blots by densitometric scanning expressed relative to casein, which was set at 1.0.

In conclusion, we have demonstrated that feeding SPI+-containing diets to prepubertal rats results in increased expression of hepatic genes regulated by the promiscuous nuclear receptors PPARα, PPARγ, and LXRα decreased expression of genes regulated by SREBP-1c. These effects may partially explain the antisteatotic, cholesterol-lowering, and insulin-sensitizing effects of soy. Moreover, feeding SPI+ or isoflavones in the diet had different effects than those of purified isoflavones in in vitro cell culture models. These data suggest that study of purified soy components in vitro may produce misleading results and need to be interpreted with caution relative to results of feeding whole diets.

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