Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 21 (2010) 1106–1113
Journal of Nutritional Biochemistry

Soy protein diet alters expression of hepatic genes regulating fatty acid and thyroid hormone metabolism in the male rat☆

Frank A. Simmen[a,b,*], Charles P. Mercado[a,b], Ann Marie Zavacki[c], Stephen A. Huang[d], Amy D. Greenway[a], Ping Kang[a], Margaret T. Bowman[a], Ronald L. Prior[a]

[a]Arkansas Children's Nutrition Center, Little Rock, AR 72202, USA
[b]Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA
[c]Division of Endocrinology, Diabetes and Hypertension, Brigham and Women's Hospital, Boston, MA 02115, USA
[d]Division of Endocrinology, Children's Hospital Boston, Boston, MA 02115, USA

Received 5 May 2009; received in revised form 16 September 2009; accepted 21 September 2009

Abstract

We hypothesized that consumption of soy protein isolate (SPI) or the soy isoflavone genistein (GEN) would modulate mRNA expression of genes underlying lipid and thyroid hormone metabolism in livers and small intestines of young adult male Sprague–Dawley rats. Early pregnant rat dams were placed on AIN-93G diets containing casein (CAS, control protein), SPI, or CAS+GEN. Litters were weaned to the same diet as their dam. SPI-fed (but not GEN-fed) male rats of 48 days of age had significant reductions in body weight, abdominal fat pad weight and hepatic content of lipid droplets and triglycerides. Hepatic peroxisome proliferator-activated receptor α (*Ppara*) transcripts were elevated with SPI but not GEN diet. Hepatic pyruvate dehydrogenase kinase-4 (*Pdk4*) and cytochrome P450 4A10 (*Cyp4a10*) mRNA abundance was reduced with SPI; the SPI effect on *Cyp4a10* was recapitulated by GEN diet. SPI (but not GEN) suppressed *Pdk4* and 3-hydroxy-3-methylglutaryl-CoA synthase 2 (*Hmgcs2*) mRNA abundance in duodenum. Liver iodothyronine deiodinase types 1 and 2 (*Dio1* and *Dio2*) mRNA levels were increased with SPI diet; the effect on *Dio2*, but not *Dio1* mRNAs, also was observed with GEN. SPI and GEN increased hepatic types 1 and 2 iodothyronine deiodinase (D1 and D2) activities. Effects of SPI and GEN on the above gene expression may contribute to the observed reductions in body and adipose tissue weight and liver lipid content in this model. Identification of the regulation, by genistein and soy protein, of iodothyronine deiodinase synthesis has potential applications for treatment and prevention of fatty liver disease and obesity.

Keywords: Soy protein; Genistein; Deiodinase; Thyroid hormone receptor; Rat

1. Introduction

There is a growing body of evidence for the anti-obesigenic and anti-steatotic actions of dietary soy proteins and soy isoflavones [1,2]. Work with rodent models has demonstrated effects of soy protein isolates to reduce: hepatosteatosis, serum cholesterol and/or triglycerides, hepatic lipogenic enzyme gene expression and insulin resistance [3–12]. While generally agreeing with respect to effects of soy protein on the above in rat models, a consensus from such studies has not emerged regarding the identity of the soy bio-active component(s) and respective mechanism of action. While data supporting the role of soy isoflavones (in particular genistein) in metabolic disease prevention are robust [2,4,5,7,11,13], other data support positive benefits of dietary soy protein isolates containing physiologically insignificant amounts of endogenous isoflavones [4,7,8,14,15]. In addition, genistein (and perhaps other soy isoflavones as well) may exhibit complex dose-dependent inhibitory or stimulatory actions with respect to adipose tissue deposition [2,16]. Moreover, relatively pure β-conglycinin, a major protein component of soy protein isolates, lowered serum triglycerides and hepatic fatty acid synthase mRNA expression when fed to mice, thus recapitulating at least some of the effects of soy protein isolate [17].

Thyroid hormone and its nuclear receptors (thyroid receptors, TRs) are important regulators of liver lipids and adipose tissue deposition [18,19]. T_3 suppresses hepatic lipid content in part, by inducing energy expenditure and fatty acid β oxidation [19–21]. For example, a mouse line with a dominant negative mutant TRβ receptor had increased liver lipids [22]. By contrast, mice expressing a mutant TRα1 receptor lacking T_3 binding were hypermetabolic, resistant to diet-induced obesity, and had increased liver fatty acid catabolism, while mice with a different mutation in TRα1 had increased visceral

☆ This research was supported by the US Department of Agriculture (Grant CRIS 6251-51000-005-03S). Research support also was provided, in part, by Arkansas Children's Hospital Research Institute, the Arkansas Biosciences Institute, and by grant DK076117 from the NIH (AMZ).
* Corresponding author. Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA. Tel.: +1 501 686 8128; fax: +1 501 686 8167.
E-mail address: simmenfranka@uams.edu (F.A. Simmen).

0955-2863/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2009.09.008

obesity and liver lipid contents [23,24]. Soy proteins and soy isoflavones may, at some dietary inputs, affect serum T_4 and/or T_3 levels in animals [25–27], primates [28] and humans [29,30], although not all studies have consistently reported such findings [31]. Interestingly, soy protein isolate, but not soy isoflavones, elevated liver abundance of TRβ1 in male and female Sprague–Dawley rats [26,27]. Thus, increased liver TRβ1 expression in soy protein-fed rats may contribute to reductions in liver lipid content and body weight.

The small intestine has an important role in the digestion and absorption of lipids and cholesterol from the diet and has lipogenic, cholesterogenic and fatty acid oxidative capabilities [32–36]. Considering its size and initial exposure to dietary constituents, it is reasonable that the small intestine may have a significant modulatory role in postnatal growth and body composition. Indeed, fatty acid metabolism–related genes were shown to be more highly expressed in small intestines from obesity-resistant than obesity-prone mice, in response to high fat diet, suggesting physiological linkages of intestinal fatty acid oxidation with lean body phenotype [33,36]. Potential effects of soy protein isolates and soy isoflavones on intestinal lipid and thyroid hormone metabolism have not been significantly explored.

In previous work, we reported that a diet containing soy protein isolate lowered body fat content of mature male Sprague–Dawley rats that had undergone a colon carcinogenesis protocol [37] and suppressed colon fatty acid synthase gene and protein expression in young adult male Sprague–Dawley rats [38]. Here, we have used this same animal model to determine the effects of soy protein isolate and in parallel, the major soy isoflavone genistein on hepatic and intestinal expression of key lipid catabolic and thyroid hormone system genes in the young adult male rat. Results demonstrated significant effects of soy protein isolate on select liver lipid metabolic and thyroid hormone system components (not all of which were mimicked by GEN feeding) and which were correlated with suppressions of liver lipid droplet and triglyceride contents and body fat.

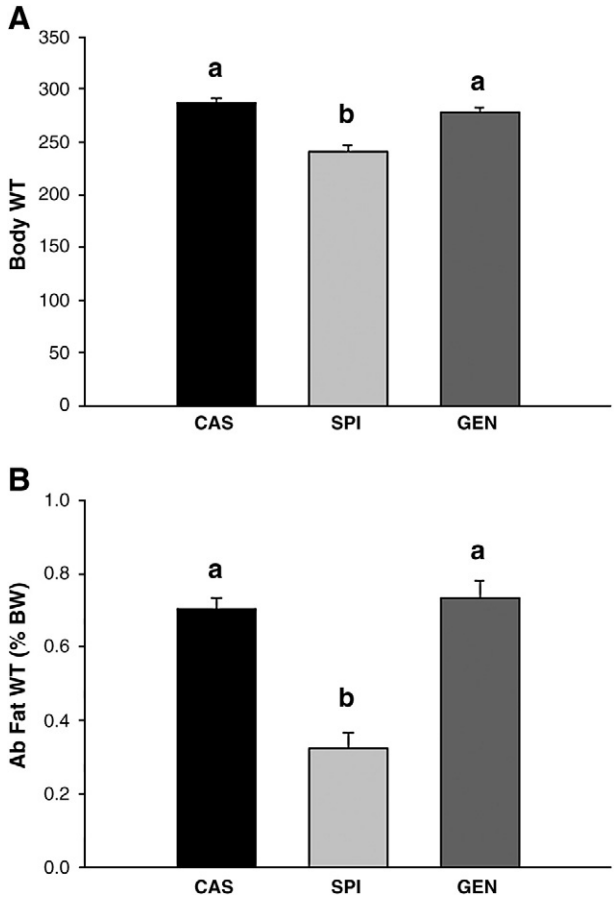

Fig. 1. Reductions in PND 48 body weight (top) and abdominal fat weight (bottom) with SPI but not GEN diet. Bar graphs are means±S.E.M. (n=5–6 rats per diet group). Letters a and b designate differences between group means (P<.05).

2. Materials and methods

2.1. Diets

Isocaloric and isonitrogenous diets contained as sole protein source, either casein (CAS; 20% w/w; Fonterra USA, Rosemont, IL, USA) or soy protein isolate (SPI; 20% w/w; Solae, St. Louis, MO, USA) and were formulated (Harlan Laboratories, Madison, WI, USA) according to the AIN-93G diet formula, except that corn oil replaced soybean oil. The soy protein isolate contained 2.15 mg/g protein of genistein-containing compounds, and 1.24 mg/g protein of the aglycone form of genistein. Soy protein isolate contained 1.41 mg/g protein of daidzein-containing compounds, 0.8 mg/g protein of the aglycone form of daidzein, 0.15 mg/g protein of glycitein-containing compounds and 0.09 mg/g protein of the aglycone form of glycitein. A third diet group contained CAS (20% w/w) to which the aglycone genistein (GEN; Sigma-Aldrich, St. Louis, MO, USA) was added (250 mg genistein/kg diet). These SPI and GEN diets result in similar levels of genistein in rat serum (.4–1.5 μM) and tissues [39]. These serum GEN concentrations are within the range of those found for humans regularly consuming soy foods [40].

Table 1
Sequences of oligonucleotide primers used in qRT-PCR

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
Acot1	GGGTGCTAACATCACCTTTGGA	CACCCAACTGTTTGTGGAAGAA
Acot2	CCCAAGACCATGGAAACCAT	AATCCCAAGCAGCCCAATTC
Cycophilin A (Ppia)	CCCACCGTGTTCTTCGACAT	TCTCCCCAGTGCTCAGAGCA
Cpt1a	CCACAAGGCTACAATGGGACAT	GAAGGAATGCAGGTCCACATC
Cyp4a10	CTGGGATCACCTGGACCAGAT	ATGTGCTGAGTTCTCTGACAATGC
Dio1	TTGACCAGTTCAAGAGACTCGTAGA	GTTCTTAAAAGCCCATCCATCTGT
Dio2	CTTTGAACGTGTGTGCATCGT	TCTCCAGCCAACTTCGGACTT
Dio3	GCTCGAACTGGCAACTTTGTC	GTGAGATGCTGGCGACTTATTG
Hmgcs2	CAGCTTACCGCAGGAAAATCC	CAAAAGGGTGTGTGGAAGATCA
Pdk4	TTGGGAGTATCGACCCCAACT	TTCTGGCGACGTTAGATAATACTGA
Ppara	GGATGACAGTGACATTTCCCTTTT	TCCCCTCCTGCAACTTCTCA
Rpl13a	AGTACCAGGCAGTGACAGCTACTCT	TGCCTGTTTCCTTAGCCTCAA
Tra1	CAAGGTGGAGTGTGGGTCAGA	CCCTGACATGCTGCTTTTCAG
Trb1	GAGTGGTGGATTTCGCCAAA	GAGGGACATGATCTCCATGCA
Ucp2	AAGACCATTGCACGAGAGGAA	TAGGTCACCAGCTCAGTACAGTTGA

2.2. Animals and tissue collection

Animal procedures were approved by the University of Arkansas for Medical Sciences Animal Care and Use Committee. Pregnant Sprague-Dawley rat dams (Charles River Laboratories, Wilmington, MA, USA) were received at gestation Day 4 and immediately placed on CAS, SPI or GEN diets (each animal was housed individually). Animals were maintained in rooms at 24°C, with 40% humidity and a 12-h light/dark cycle and were provided food and water ad libitum. At birth, litters were adjusted to an average of five males and five females per dam. At weaning, males were placed on the same diet as their dam; females were used in unrelated studies. At postnatal Day (PND) 48, five or six males from each diet group (one animal per litter) were weighed and tissues collected. Gastrointestinal tissues were freed of luminal contents, and portions of each one-third region of small intestine (duodenum, jejunum, ileum) removed. Duodenums and jejunums were homogenized in Trizol (Invitrogen, Carlsbad, CA, USA). Retroperitoneal fat was dissected and weighed. The left lateral lobe of liver from each rat was quick frozen. Triglyceride content of liver tissue was determined using reagents from Synermed (Westfield, IN, USA).

2.3. Histological analysis

Hepatic lipid content was assessed by Oil Red O staining of OCT-embedded tissue sections. Cryosections were fixed in 10% neutral-buffered formalin for 15 min, stained with 0.5% Oil Red O in 100% propylene glycol for 15 min and then washed with 85% propylene glycol. Sections were counterstained with Phoenix Blue, washed with deionized water and mounted. Slides were visualized on an Olympus BX50 microscope

and relative area of lipid droplets quantified with image analysis software (MCID Elite Imaging Software 7.0). A minimum of six independent fields per slide were evaluated.

2.4. RNA isolation and real-time quantitative reverse transcriptase-polymerase chain reaction

RNA was extracted using TRIzol reagent, purified using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA), and converted to cDNA using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA). Primers used in quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis are found in Table 1 and were obtained from Integrated DNA Technologies (Coralville, IA, USA). PCR products were amplified using Bio-Rad iTaq SYBR Green Supermix with ROX (Bio-Rad). Messenger RNA abundance was normalized to that for cyclophilin A (*Ppia*) or *Rpl13a* mRNA.

2.5. Iodothyronine deiodinase activity assays

D1, D2 and D3 activities were measured as described previously [41].

2.6. Statistics

Statistical analysis was performed using SigmaStat for Windows (SPSS). Mean values were subjected to analysis by one-way analysis of variance (ANOVA) and pair-wise *t* tests. Differences between treatment means were considered to be significant at $P<.05$ and to represent a tendency at $.05<P<.1$.

3. Results

SPI, but not GEN, elicited significant reductions in body weight and retroperitoneal (abdominal) fat pad weights (normalized to body weight) by PND 48 (Fig. 1). Livers (left lateral lobe) from rats fed SPI had less cumulative area of lipid droplets than did livers from animals fed CAS or GEN (Fig. 2A-D). Livers of SPI-fed rats also had the lowest amount of triglycerides; interestingly, GEN also elicited a reduction in triglyceride content (compared to CAS) though this was less than that of SPI (Fig. 2E).

RNA transcripts that encode selected participants in several pathways of lipid metabolism were examined for potential modulation by dietary protein or GEN (Table 1). In the liver, peroxisome proliferator-activated receptor (PPAR) α (*Ppara*) mRNA was slightly elevated with SPI but not GEN diet (Fig. 3A). No effects of diet on levels of *Ppara* transcripts in the small intestine were found (Fig. 3B, C). Hepatic mRNA abundance of pyruvate dehydrogenase kinase-4 (*Pdk4*) and cytochrome P450 4A10 (*Cyp4a10*) was reduced with SPI. The effect of SPI on hepatic *Cyp4a10* was mimicked by feeding GEN (Fig. 3A). A GEN-specific inhibition of hepatic acyl-CoA thioesterase 1 (*Acot1*) mRNA also was noted (Fig. 3A). SPI elicited reductions in *Pdk4* and 3-hydroxy-3-methylglutaryl-CoA synthase 2 (*Hmgcs2*) gene expression in the duodenum, effects that were not recapitulated by the GEN diet (Fig. 3B). There were no significant ($P<.05$) effects of diet on any examined transcripts in the jejunum (Fig. 3C).

Thyroid receptor mRNAs also exhibited tissue-specific changes in relative abundance with different diets. Liver TRα1 (*Tra1*) mRNA tended ($P=.08$) to increase with SPI but not GEN diet (Fig. 4A). Intestine TRα1 RNA abundance was unaffected by diet (data not shown). Hepatic TRβ1 (*Trb1*) mRNA abundance was increased ($P<.05$) with SPI, but not GEN diet (Fig. 4B); however, intestine TRβ1 expression was unaffected by diet type (data not shown).

Lastly, iodothyronine deiodinase (*Dio1*, *Dio2* and *Dio3*) mRNAs and corresponding enzyme (D1, D2, D3) activities were examined for potential modulation by diet. Liver *Dio1* and *Dio2* mRNA levels were induced with SPI diet (Fig. 5A). The effect of SPI on liver *Dio2*

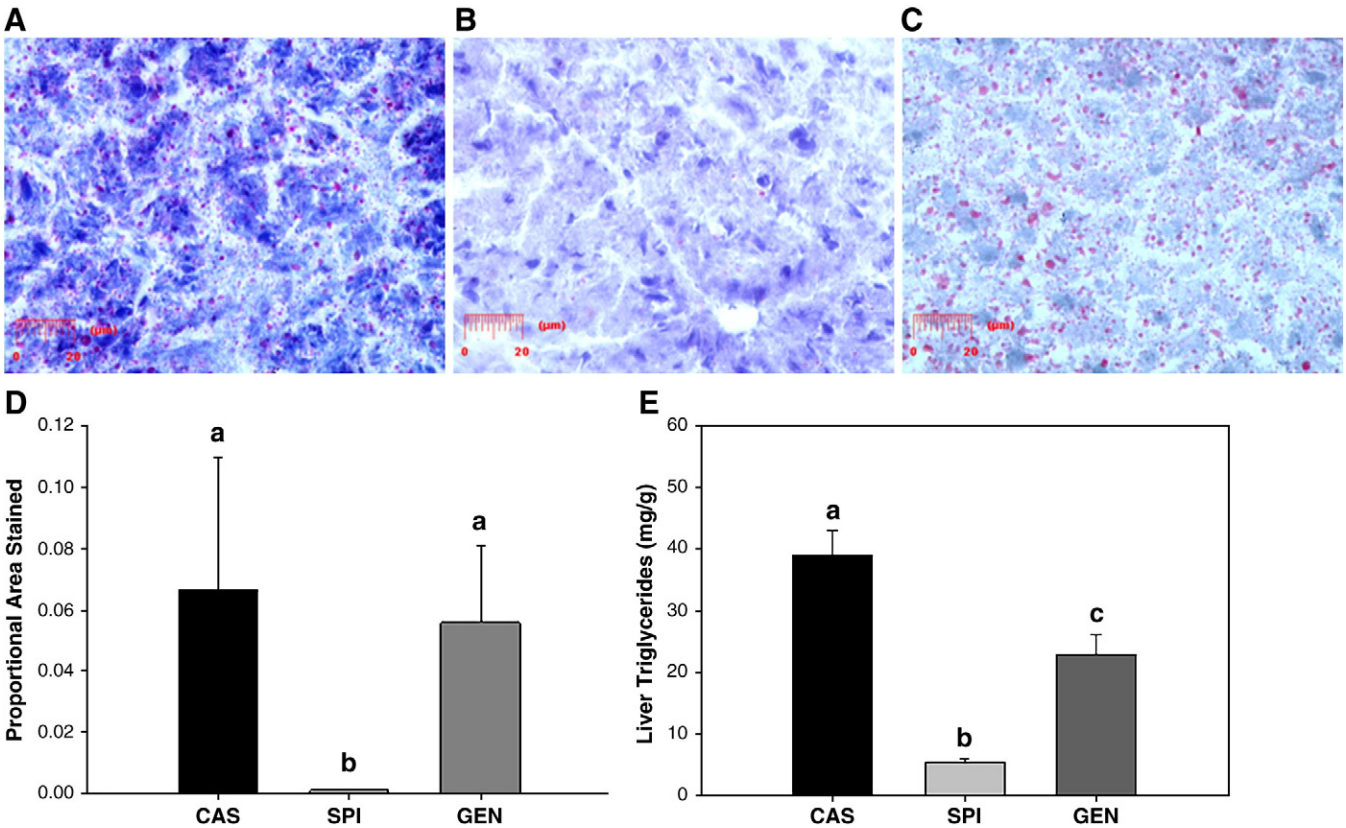

Fig. 2. Effects of dietary protein, but not GEN, on relative amount (number and size) of lipid droplets in PND 48 rat liver. Panels A–C are representative Oil Red O-stained liver sections from CAS-, SPI- and GEN-fed rats, respectively. (D) Bar graphs are means±S.E.M. of the relative proportion of rat liver tissue section stained with Oil Red O (n=5-6 rats per group). Letters a and b designate differences between group means ($P<.01$). *E*, Bar graphs are means±SEM of the total content of triglycerides in rat livers ($n=5–6$ rats per group). Letters a, b and c designate differences between group means ($P<.05$).

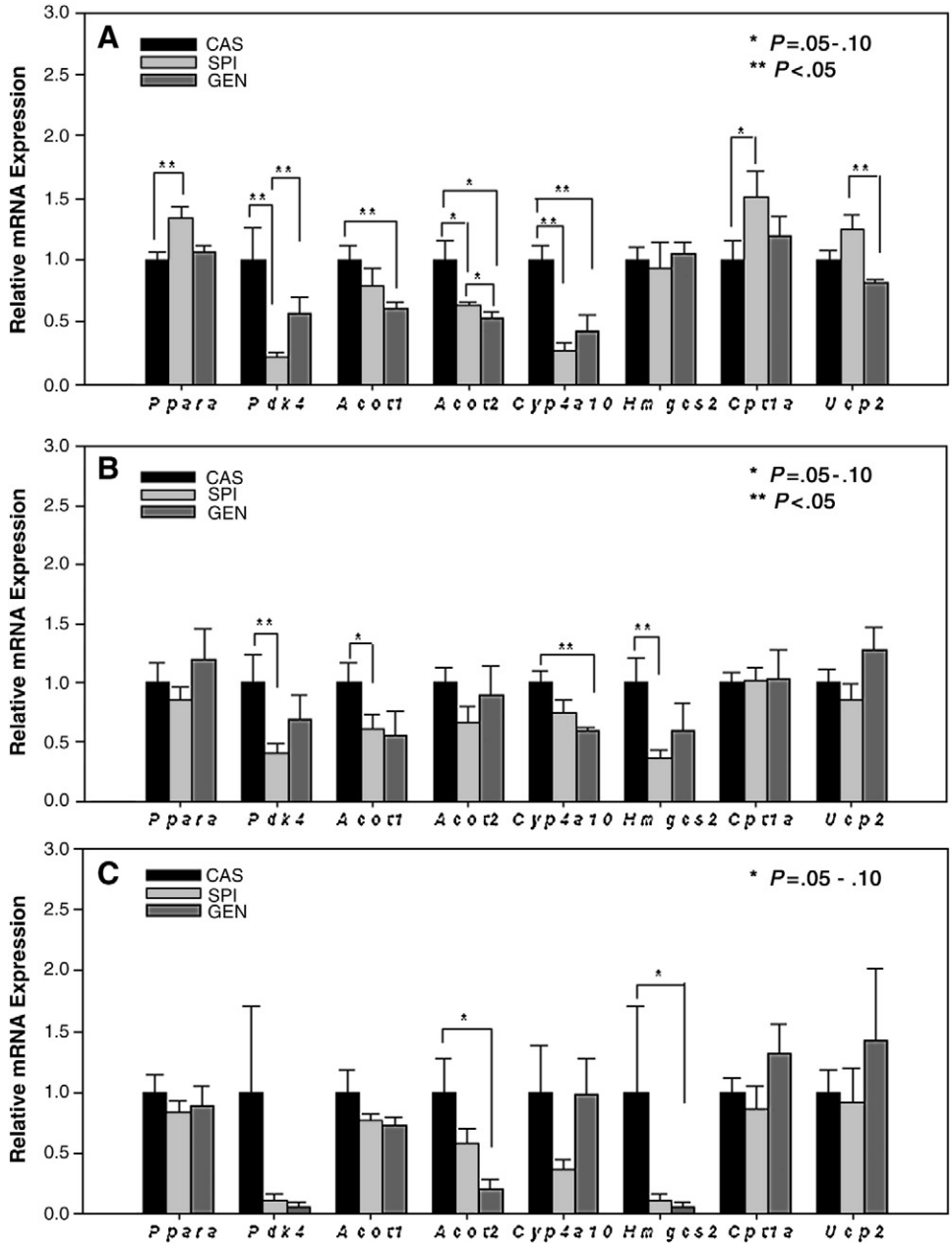

Fig. 3. Effects of dietary proteins and GEN on mRNA abundance of lipid catabolism-related genes in PND 48 animals. (A) liver transcripts normalized to Cyclophilin A mRNA. (B) Duodenum transcripts normalized to that for *Rpl13a* mRNA. (C) jejunum transcripts normalized to Cyclophilin A mRNA. (A and C) n=5–6 animals per group; (B), n=4–6 animals per group. Cyclophilin A mRNA abundance was unaffected by diet in liver and jejunum; however, in duodenum, this transcript tended to be elevated with GEN compared to CAS diet (P=.06). Thus, *Rpl13a* mRNA was used for normalization of duodenum transcripts. *P* value ranges for pairwise *t* tests are indicated.

RNA, but not *Dio1* RNA, was mimicked by GEN. Liver *Dio3* transcript abundance, in contrast, was unaffected by diet. Inductive effects of SPI and GEN on *Dio2* mRNA in the duodenum paralleled that for liver (Fig. 5B). Moreover, GEN induced *Dio3* mRNA in duodenum. In jejunum, SPI, but not GEN, suppressed *Dio1* and *Dio3* transcripts (Fig. 5C). Activities of the iodothyronine deiodinases were evaluated as a measure of corresponding protein abundance (Table 2). SPI and GEN increased D1 and D2 activity in liver. Hepatic D3 activity was greater for SPI than GEN groups (Table 2). Diet had no effect on activities of D1 and D3 in the duodenum or jejunum (Table 2), and there was no detectable D2 activity (i.e., above background) in the small intestine segments. Notably, D1 activity in the small intestine was substantially lower than in liver, whereas D3 activity in duodenum and liver was comparable and exceeded that for jejunum (Table 2).

4. Discussion

This study utilized a "lifetime" nutritional model [37–39,42] in which early pregnant Sprague–Dawley rat dams were fed casein, soy protein, or casein supplemented with genistein, their male progeny were weaned to the same diet as their dam, and tissues were collected at the subsequent young adult stage. This is not an animal model of diet-induced obesity as high fat or high-calorie diets were not fed, but rather a model that allows for the study of developmental effects of

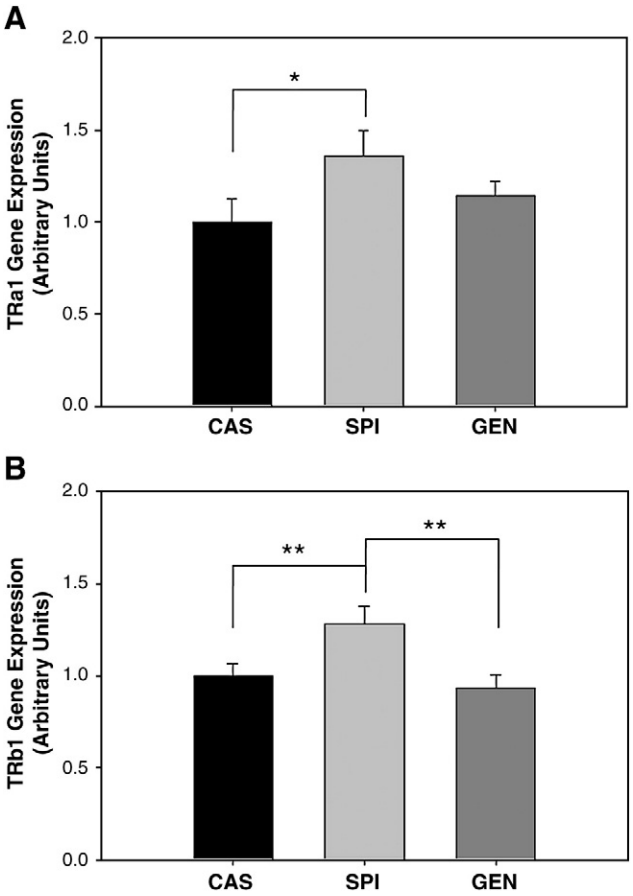

Fig. 4. Effects of dietary protein type on liver thyroid hormone receptor mRNA abundance at PND 48. (A) Liver *Trα1* mRNA expression normalized to Cyclophilin A mRNA (single asterisk, CAS vs. SPI, $P=.08$; $n=5–6$ animals per diet group). (B) Liver *Trβ1* expression normalized to Cyclophilin A mRNA. Double asterisks designate differences between group means ($P<.05$; $n=5–6$ animals per diet group).

different dietary proteins and factors on normal postnatal growth and development and without differences in food intake. It is interesting that differences in body weight and retroperitoneal fat weight were observed with the SPI but not GEN diets. Clearly, the mechanisms that confer the SPI-elicited differences in body and fat weight appear to not solely involve GEN. Results with the present rat model confirmed that soy protein suppresses liver lipid droplet and triglyceride content [9–11,14]. These effects of soy protein are quite consistent between different models; however, the present results show that this effect was observable even in a nonobese model of postnatal growth and again was not solely due to the genistein in SPI. This same lifetime model of SPI consumption conferred reduced colon tumor incidence in adult male rats given the intestinal carcinogen azoxymethane [42]. Future studies in our laboratory will explore possible physiologic relationships of reductions in body fat and colon tumor incidence.

A novel observation was the suppressive effect of SPI, but not GEN, on hepatic and duodenal *Pdk4* mRNA abundance. The mitochondrial pyruvate dehydrogenase complex (PDC) catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA and is an important metabolic control point. The PDC is inactivated by phosphorylations catalyzed by members of the group of pyruvate dehydrogenase kinases 1–4 [43]. Reduction in *Pdk4* mRNA abundance from a steady state with SPI diet would be expected to lower Pdk4 protein levels and increase PDC activity. Our results predict increased hepatic and duodenal PDC activity with SPI diet with resultant decreased blood glucose levels and increased glucose tolerance and insulin sensitivity [44,45]; all are hallmarks of soy consumption in rodent models. It therefore is possible that Pdk4 is an important downstream mediator/participant in the processes and pathways by which soy protein consumption leads to glucose tolerance and insulin sensitivity. However, insulin resistance is known to lead to an increase in hepatic Pdk4 protein abundance in rats [46], and insulin can suppress *Pdk4* gene expression in hepatocytes [47]. Thus, the decrease in *Pdk4* gene expression with soy consumption also may represent a secondary response to insulin sensitization; rather than as a driver of the process. Further studies are required to discriminate these

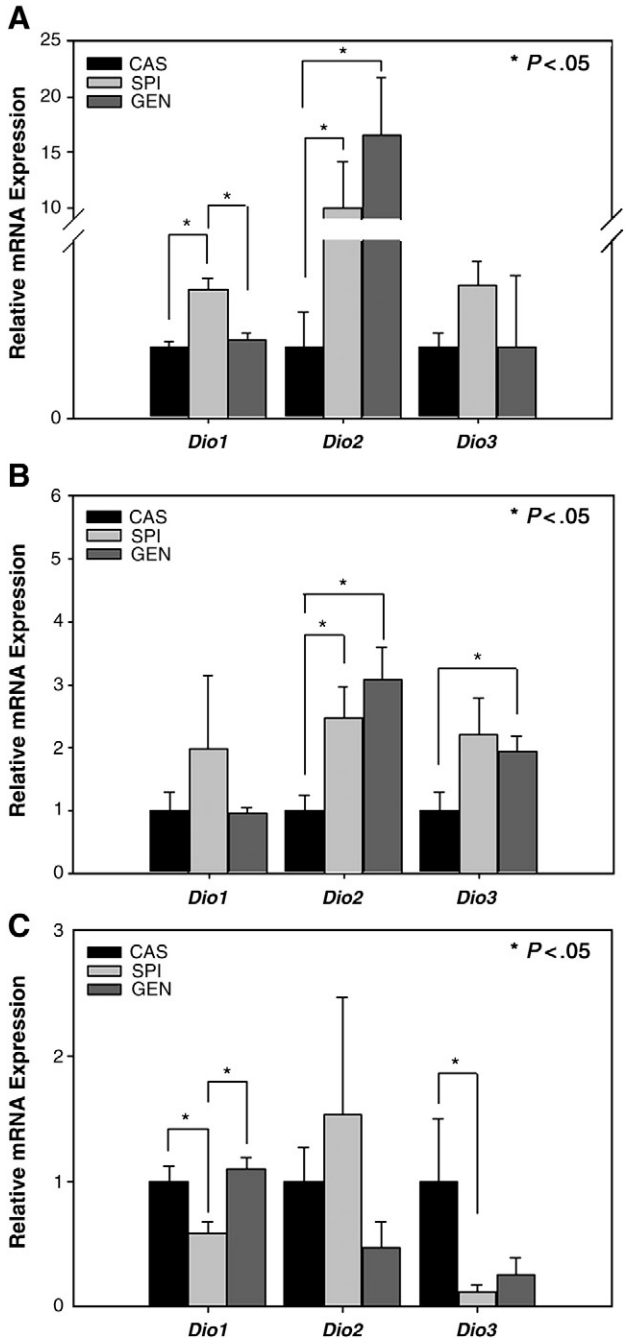

Fig. 5. Tissue-specific effects of dietary proteins and GEN on iodothyronine deiodinase mRNA abundance at PND 48. (A) Liver transcripts normalized to Cyclophilin A mRNA. (B) Duodenum transcripts normalized to *Rpl13a* mRNA. (C) Jejunum transcripts normalized to cyclophilin A mRNA. (A–C) $n=5–6$ animals per group per tissue. Relative differences in expression were compared by pairwise *t* tests ($P<.05$). There were no observed tendencies ($.05<P<.1$) for any differences.

Table 2
Relative iodothyronine deiodinase activities for rat liver and small intestine *

Diet	Liver			Duodenum		Jejunum	
	D1†	D2	D3	D1‡	D3	D1‡	D3§
CAS	90.1±18.1 [a]	15.2±1.3 [a]	6.4±1.0	2.8±0.7	6.7±5.5	5.6±1.9	2.9±1.7
SPI	137.6±25.6 [b]	27.1±2.4 [b]	8.7±1.1 [a]	2.4±1.3	5.6±5.5	3.5±2.3	1.5±0.8
GEN	127.7±23.3 [b]	21.6±4.2 [c]	4.9±1.2 [b]	3.3±1.8	4.8±2.2	5.8±2.0	1.6±1.0
ANOVA†	*P*=.058	*P*<.001	*P*=.087	*P*=.564	*P*=.169	*P*=.157	*P*=.147

* Means±SD; n=6, 6 and 5 of PND 48 animals in CAS, SPI and GEN diet groups, respectively; units of activity of D1 are pmol/min per milligram of protein; units of activity of D2 and D3 are fmol/h per milligram of protein; D2 was undetectable in duodenum and jejunum.

† *P* values obtained from one-way ANOVA of means within each column (overall diet effect) are shown at bottom of each column; superscript letters within a column indicate differences (*P*<.05) between diet groups as determined by *t* tests.

‡ D1 activities of duodenum and jejunum were significantly lower than for liver (*P*<.01).

§ D3 activity of jejunum was significantly lower than for liver and duodenum (*P*<.05).

possibilities. It also is interesting that SPI affected this gene's expression in the duodenum similar to that in liver and that this appears to not be due to GEN. Such observations have bearing on the potential for dietary manipulation, by soy protein and its constituents, of hyperglycemia.

The nuclear receptor PPARα is a well-known inducer of fatty acid and lipid catabolism-related genes and which may involve coparticipation of thyroid hormone receptors [24]. Feeding methanol-washed soy protein to rats increased their hepatic *Ppara* mRNA abundance, irrespective of any isoflavones supplemented to the diet [48]. In Zucker obese diabetic rats, liver *Ppara* mRNA was increased by a soy protein diet [7–9]. The small increase in hepatic *Ppara* mRNA abundance with SPI observed in the present study is consistent with the above findings. Curiously, we did not observe correspondent increases in liver mRNA abundance of the downstream Ppara targets *Acot 1, Acot 2*, *Hmgcs2* and *Pdk4*. Tendencies for small increases in hepatic *Cpt1a* and *Ucp2* mRNAs that were in the same direction as *Ppara* mRNA were noted; however, no transcripts encoding any lipid catabolism-related proteins were induced in small intestine with either SPI or GEN. Supra-physiological levels of isoflavones may enhance fatty acid β-oxidation as in other tissue [48,49]; however, such levels of GEN were not studied here. Contrary to our hypothesis, data did not provide support for inductive effects of SPI or GEN on lipid catabolism-related pathway genes in the small intestine. However, our results do not rule out the occurrence of such actions when high fat diets are fed [33,36].

The ligand T_3 and thyroid hormone receptor agonists induce liver fatty acid oxidation, inhibit hepatosteatosis, and induce energy expenditure [19,21]. The *Ucp2*, *Cpt1a*, *Dio1* and *Trβ1* genes are known gene targets of T_3/TRs [50–53]. These published results prompted us to examine for diet effects on tissue expression of T_3 receptors and iodothyronine deiodinases, the latter a group of proteins that generate or inactivate T_3 in target cells. Trβ1 is the predominant thyroid hormone receptor isoform in rat liver. In work from another group, soy protein, but not soy isoflavones, elevated, in dose-dependent fashion, liver Trβ1 mRNA and protein abundance in male and female Sprague–Dawley rats [26,27]. Our results are in agreement with these earlier findings and reaffirm the induction, by SPI but not GEN, of hepatic Trβ1 mRNA abundance in Sprague–Dawley rats. Trβ actions counter hepatosteatosis [22]; therefore, the effect of SPI to lower liver lipid droplet content may involve the induction of this receptor in concert with that of PPARα. Our data for the small intestine did not indicate diet modulation of thyroid hormone receptor gene expression, thus the induction by SPI is tissue specific. The constituent(s) of soy protein responsible for this unique effect in liver remain to be identified.

Tissue-specific responses of iodothyronine deiodinase genes to type of diet were observed. Interestingly, GEN-induced liver *Dio2* mRNA abundance and liver D1 and D2 activities, thus partially mimicking effects of SPI. D2 is the main enzyme responsible for tissue- and cell type-specific production of T_3 from thyroxine [54]. D1 also may contribute to cellular T_3 production; although this is controversial, since D1 produces other iodothyronines (rT_3 and T_2) as well, and has ~10-fold higher Km for T_4 than does D2. D3 is the major inactivating enzyme of T_3. Taken within the context of these observations, our results point to the enhanced generation of T_3, and perhaps rT_3 and T_2, in hepatocytes in response to consumption of SPI or GEN. The observed increase in the mRNA expression of several thyroid hormone responsive genes, as described earlier, also supports the conclusion of greater local T_3 production in this tissue. Some T_4 metabolites such as T_2 may, in certain physiological contexts, promote energy expenditure and fatty acid oxidation [55]. As the liver is important for processing of lipids and for energy expenditure, it is reasonable to speculate that SPI inductions of liver Trβ1, D1 and D2 collectively contribute to the antiobesity and liver lipid-lowering actions of SPI in rats. This is consistent with an earlier study in which *Dio1* and *Dio2* gene expression and D1 and D2 activities were enhanced in an animal model that is resistant to diet-induced obesity [51]. We therefore speculate that inclusion of SPI or GEN in the diet may, to some degree, augment or supplant the actions of liver-targeted thyroid receptor agonists in countering obesity and hepatosteatosis [19]. Obviously, such possibilities will require confirmation in appropriate animal and clinical models.

Several other observations were of interest: liver D1 levels greatly exceeded those for intestine as described previously [56], duodenum levels of D3, while low, approximated those for liver, intestine D2 activity was undetectable, and relative ratio of D1 to D3 differed in duodenum vs. jejunum. These findings dramatically underscore the tissue-specificity of iodothyronine deiodinase expression and, hence, nature of the iodothyronines generated.

In summary, we have identified several metabolic regulatory genes/proteins to be responsive to SPI and/or GEN-containing diets. The demonstrated inductive effects of dietary SPI on hepatic Ppara and Trβ1 transcripts and iodothyronine deiodinases suggests their associations with diminutions in body weight and liver lipid content in nonobese young adult rats. Genistein may fully account for the inductive effect of SPI on liver D1 activity; however, most other SPI responses (including the diminution in liver triglyceride content) were not recapitulated fully or at all by this isoflavone. Thus, the relevant SPI constituent(s) and underlying pathway(s) mediating these effects of SPI remain to be elucidated. Dietary SPI and GEN may have clinical relevance since hepatosteatosis is becoming ever more prevalent as a consequence of the current epidemic of overweight and obesity.

Acknowledgments

We thank Dr. Rosalia C.M. Simmen for helpful suggestions and review of the manuscript, Samuel Wilkes for measurement of liver

triglyceride content, and Michelle Mulcahey for help with determination of D3 activity.

References

[1] Velasquez MT, Bhathena SJ. Role of dietary soy protein in obesity. Int J Med Sci 2007;4:72–82.
[2] Ørgaard A, Jensen L. The effects of soy isoflavones on obesity. Exp Biol Med (Maywood) 2008;233:1066–80.
[3] Tovar AR, Murguía F, Cruz C, Hernández-Pando R, Aguilar-Salinas CA, Pedraza-Chaverri J, et al. A soy protein diet alters hepatic lipid metabolism gene expression and reduces serum lipids and renal fibrogenic cytokines in rats with chronic nephrotic syndrome. J Nutr 2002;132:2562–9.
[4] Mezei O, Banz WJ, Steger RW, Peluso MR, Winters TA, Shay N. Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese Zucker rats and murine RAW 264.7 cells. J Nutr 2003;133:1238–43.
[5] Ali AA, Velasquez MT, Hansen CT, Mohamed AI, Bhathena SJ. Effects of soybean isoflavones, probiotics, and their interactions on lipid metabolism and endocrine system in an animal model of obesity and diabetes. J Nutr Biochem 2004;15: 583–90.
[6] Ascencio C, Torres N, Isoard-Acosta F, Gómez-Pérez FJ, Hernández-Pando R, Tovar AR. Soy protein affects serum insulin and hepatic SREBP-1 mRNA and reduces fatty liver in rats. J Nutr 2004;134:522–9.
[7] Banz WJ, Davis J, Peterson R, Iqbal MJ. Gene expression and adiposity are modified by soy protein in male Zucker diabetic fatty rats. Obes Res 2004;12: 1907–13.
[8] Davis J, Iqbal MJ, Steinle J, Oitker J, Higginbotham DA, Peterson RG, et al. Soy protein influences the development of the metabolic syndrome in male obese ZDFxSHHF rats. Horm Metab Res 2005;37:316–25.
[9] Tovar AR, Torre-Villalvazo I, Ochoa M, Elías AL, Ortíz V, Aguilar-Salinas CA, et al. Soy protein reduces hepatic lipotoxicity in hyperinsulinemic obese Zucker fa/fa rats. J Lipid Res 2005;46:1823–32.
[10] Gudbrandsen OA, Wergedahl H, Mørk S, Liaset B, Espe M, Berge RK. Dietary soya protein concentrate enriched with isoflavones reduced fatty liver, increased hepatic fatty acid oxidation and decreased the hepatic mRNA level of VLDL receptor in obese Zucker rats. Br J Nutr 2006;96:249–57.
[11] Davis J, Higginbotham A, O'Connor T, Moustaid-Moussa N, Tebbe A, Kim YC, et al. Soy protein and isoflavones influence adiposity and development of metabolic syndrome in the obese male ZDF rat. Ann Nutr Metab 2007;51:42–52.
[12] Torre-Villalvazo I, Tovar AR, Ramos-Barragán VE, Cerbón-Cervantes MA, Torres N. Soy protein ameliorates metabolic abnormalities in liver and adipose tissue of rats fed a high fat diet. J Nutr 2008;138:462–8.
[13] Kim S, Sohn I, Lee YS, Lee YS. Hepatic gene expression profiles are altered by genistein supplementation in mice with diet-induced obesity. J Nutr 2005;135: 33–41.
[14] Iqbal MJ, Yaegashi S, Ahsan R, Lightfoot DA, Banz WJ. Differentially abundant mRNAs in rat liver in response to diets containing soy protein isolate. Physiol Genomics 2002;11:219–26.
[15] Shukla A, Brandsch C, Bettzieche A, Hirche F, Stangl GI, Eder K. Isoflavone-poor soy protein alters the lipid metabolism of rats by SREBP-mediated down-regulation of hepatic genes. J Nutr Biochem 2007;18:313–21.
[16] Dang ZC, Audinot V, Papapoulos SE, Boutin JA, Löwik CW. Peroxisome proliferator-activated receptor γ (PPARγ) as a molecular target for the soy phytoestrogen genistein. J Biol Chem 2003;278:962–7.
[17] Moriyama T, Kishimoto K, Nagai K, Urade R, Ogawa T, Utsumi S, et al. Soybean β-conglycinin diet suppresses serum triglyceride levels in normal and genetically obese mice by induction of β-oxidation, downregulation of fatty acid synthase, and inhibition of triglyceride absorption. Biosci Biotechnol Biochem 2004;68: 352–9.
[18] Duntas LH. Thyroid disease and lipids. Thyroid 2002;12:287–93.
[19] Cable EE, Finn PD, Stebbins JW, Hou J, Ito BR, van Poelje PD, et al. Reduction of hepatic steatosis in rats and mice after treatment with a liver-targeted thyroid hormone receptor agonist. Hepatology 2009;49:407–17.
[20] Weiss RE, Murata Y, Cua K, Hayashi Y, Seo H, Refetoff S. Thyroid hormone action on liver, heart, and energy expenditure in thyroid hormone receptor β-deficient mice. Endocrinology 1998;139:4945–52.
[21] Perra A, Simbula G, Simbula M, Pibiri M, Kowalik MA, Sulas P, et al. Thyroid hormone (T3) and TRβ agonist GC-1 inhibit/reverse nonalcoholic fatty liver in rats. FASEB J 2008;22:2981–9.
[22] Araki O, Ying H, Zhu XG, Willingham MC, Cheng SY. Distinct dysregulation of lipid metabolism by unliganded thyroid hormone receptor isoforms. Mol Endocrinol 2009;23:308–15.
[23] Sjögren M, Alkemade A, Mittag J, Nordström K, Katz A, Rozell B, et al. Hypermetabolism in mice caused by the central action of an unliganded thyroid hormone receptor α1. EMBO J 2007;26:4535–45.
[24] Liu YY, Heymann RS, Moatamed F, Schultz JJ, Sobel D, Brent GA. A mutant thyroid hormone receptor α antagonizes peroxisome proliferator-activated receptor alpha signaling in vivo and impairs fatty acid oxidation. Endocrinology 2007;148: 1206–17.
[25] White HL, Freeman LM, Mahony O, Graham PA, Hao Q, Court MH. Effect of dietary soy on serum thyroid hormone concentrations in healthy adult cats. Am J Vet Res 2004;65:586–91.
[26] Xiao CW, L'Abbé MR, Gilani GS, Cooke GM, Curran IH, Papademetriou SA. Dietary soy protein isolate and isoflavones modulate hepatic thyroid hormone receptors in rats. J Nutr 2004;134:743–9.
[27] Huang W, Wood C, L'Abbé MR, Gilani GS, Cockell KA, Xiao CW. Soy protein isolate increases hepatic thyroid hormone receptor content and inhibits its binding to target genes in rats. J Nutr 2005;135:1631–5.
[28] Kavanagh K, Jones KL, Zhang L, Flynn DM, Shadoan MK, Wagner JD. High isoflavone soy diet increases insulin secretion without decreasing insulin sensitivity in premenopausal nonhuman primates. Nutr Res 2008;28: 368–76.
[29] Milerová J, Cerovská J, Zamrazil V, Bílek R, Lapcík O, Hampl R. Actual levels of soy phytoestrogens in children correlate with thyroid laboratory parameters. Clin Chem Lab Med 2006;44:171–4.
[30] Hampl R, Ostatnikova D, Celec P, Putz Z, Lapcík O, Matucha P. Short-term effect of soy consumption on thyroid hormone levels and correlation with phytoestrogen level in healthy subjects. Endocr Regul 2008;42:53–61.
[31] Teas J, Braverman LE, Kurzer MS, Pino S, Hurley TG, Hebert JR. Seaweed and soy: companion foods in Asian cuisine and their effects on thyroid function in American women. J Med Food 2007;10:90–100.
[32] Field FJ, Born E, Mathur SN. Fatty acid flux suppresses fatty acid synthesis in hamster intestine independently of SREBP-1 expression. J Lipid Res 2003;44: 1199–208.
[33] Kondo H, Minegishi Y, Komine Y, Mori T, Matsumoto I, Abe K, et al. Differential regulation of intestinal lipid metabolism-related genes in obesity-resistant A/J vs. obesity-prone C57BL/6J mice. Am J Physiol Endocrinol Metab 2006;291: E1092–E1099.
[34] Field FJ, Born E, Murthy S, Mathur SN. Regulation of sterol regulatory element-binding proteins in hamster intestine by changes in cholesterol flux. J Biol Chem 2001;276:17576–83.
[35] Lewis GF, Uffelman K, Naples M, Szeto L, Haidari M, Adeli K. Intestinal lipoprotein overproduction, a newly recognized component of insulin resistance, is ameliorated by the insulin sensitizer rosiglitazone: studies in the fructose-fed Syrian golden hamster. Endocrinology 2005;146:247–55.
[36] Mori T, Kondo H, Hase T, Tokimitsu I, Murase T. Dietary fish oil upregulates intestinal lipid metabolism and reduces body weight gain in C57BL/6J mice. J Nutr 2007;137:2629–34.
[37] Linz AL, Xiao R, Parker JG, Simpson PM, Badger TM, Simmen FA. Feeding of soy protein isolate to rats during pregnancy and lactation suppresses formation of aberrant crypt foci in their progeny's colons: interaction of diet with fetal alcohol exposure. J Carcinog 2004;3(1):14.
[38] Xiao R, Su Y, Simmen RCM, Simmen FA. Dietary soy protein inhibits DNA damage and cell survival of colon epithelial cells through attenuated expression of fatty acid synthase. Am J Physiol Gastrointest Liver Physiol 2008;294: G868–G876.
[39] Eason RR, Till SR, Velarde MC, Geng Y, Chatman L, Gu L, et al. Uterine phenotype of young adult rats exposed to dietary soy or genistein during development. J Nutr Biochem 2005;16:625–32.
[40] Kurzer MS, Xu X. Dietary phytoestrogens. Annu Rev Nutr 1997;17:353–81.
[41] Christoffolete MA, Arrojo e Drigo R, Gazoni F, Tente SM, Goncalves V, Amorim BS, et al. Mice with impaired extrathyroidal thyroxine to 3,5,3′-triiodothyronine conversion maintain normal serum 3,5,3′-triiodothyronine concentrations. Endocrinology 2007;148:954–60.
[42] Xiao R, Hennings LJ, Badger TM, Simmen FA. Fetal programming of colon cancer in adult rats: correlations with altered neonatal growth trajectory, circulating IGF-I and IGF binding proteins, and testosterone. J Endocrinol 2007;195:79–87.
[43] Sugden MC, Holness MJ. Recent advances in mechanisms regulating glucose oxidation at the level of the pyruvate dehydrogenase complex by PDKs. Am J Physiol Endocrinol Metab 2003;284:E855–E862.
[44] Roche TE, Hiromasa Y. Pyruvate dehydrogenase kinase regulatory mechanisms and inhibition in treating diabetes, heart ischemia, and cancer. Cell Mol Life Sci 2007;64:830–49.
[45] Jeoung NH, Harris RA. Pyruvate dehydrogenase kinase-4 deficiency lowers blood glucose and improves glucose tolerance in diet-induced obese mice. Am J Physiol Endocrinol Metab 2008;295:E46–54.
[46] Bajotto G, Murakami T, Nagasaki M, Qin B, Matsuo Y, Maeda K, et al. Increased expression of hepatic pyruvate dehydrogenase kinases 2 and 4 in young and middle-aged Otsuka Long-Evans Tokushima Fatty rats: induction by elevated levels of free fatty acids. Metabolism 2006;55:317–23.
[47] Zhang Y, Ma K, Sadana P, Chowdhury F, Gaillard S, Wang F, et al. Estrogen-related receptors stimulate pyruvate dehydrogenase kinase isoform 4 gene expression. J Biol Chem 2006;281:39897–906.
[48] Takahashi Y, Ide T. Effects of soy protein and isoflavone on hepatic fatty acid synthesis and oxidation and mRNA expression of uncoupling proteins and peroxisome proliferator-activated receptor γ in adipose tissues of rats. J Nutr Biochem 2008;19:682–93.
[49] Shin ES, Cho SY, Lee EH, Lee SJ, Chang IS, Lee TR. Positive regulation of hepatic carnitine palmitoyl transferase 1A (CPT1A) activities by soy isoflavones and L-carnitine. Eur J Nutr 2006;45:159–64.
[50] Zavacki AM, Ying H, Christoffolete MA, Aerts G, So E, Harney JW, et al. Type 1 iodothyronine deiodinase is a sensitive marker of peripheral thyroid status in the mouse. Endocrinology 2005;146:1568–75.
[51] Kalaany NY, Gauthier KC, Zavacki AM, Mammen PP, Kitazume T, Peterson JA, et al. LXRs regulate the balance between fat storage and oxidation. Cell Metab 2005;1: 231–44.

[52] Jackson-Hayes L, Song S, Lavrentyev EN, Jansen MS, Hillgartner FB, Tian L, et al. A thyroid hormone response unit formed between the promoter and first intron of the carnitine palmitoyltransferase-Iα gene mediates the liver-specific induction by thyroid hormone. J Biol Chem 2003;278:7964–72.
[53] Hodin RA, Lazar MA, Chin WW. Differential and tissue-specific regulation of the multiple rat c-erbA messenger RNA species by thyroid hormone. J Clin Invest 1990;85(1):101–5.
[54] Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, et al. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. Endocr Rev 2008;29:898–938.
[55] Cimmino M, Mion F, Goglia F, Minaire Y, Géloën A. Demonstration of in vivo metabolic effects of 3,5-di-iodothyronine. J Endocrinol 1996;149:319–25.
[56] Bates JM, St Germain DL, Galton VA. Expression profiles of the three iodothyronine deiodinases, D1, D2, and D3, in the developing rat. Endocrinology 1999;140:844–51.