Consumption of Soy Protein Isolate Reduces Hepatic SREBP-1c and Lipogenic Gene Expression in Wild-Type Mice, but Not in FXR-Deficient Mice

Tsutomu Hashidume, Takashi Sasaki, Jun Inoue, and Ryuichiro Sato

Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

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We examined to determine whether hepatic gene expression is affected in mice in which blood lipid levels remain unchanged fed soy protein isolate (SPI) for a short time. We also examined SPI-mediated effects in farnesoid X receptor (FXR)-deficient mice. Compared with casein, SPI affected the expression of various hepatic genes related to lipid metabolism in the wild-type mice. No effects of SPI were observed in the FXR-deficient mice, suggesting the importance of FXR. Hepatic peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) gene expression was reduced by SPI, and this might be associated with a decrease in FXR expression. Decreased FXR led to decreased expression of its target, the bile-salt export pump necessary for bile acid secretion and dietary lipid absorption. The earliest response to SPI was a decrease in hepatic sterol regulatory element-binding protein (SREBP)-1c mRNA, on day 3. SPI activated hepatic adenosine monophosphate-activated protein kinase (AMPK), which can lead to a reduction in SREBP-1c mRNA. These data indicate the importance of SREBP-1c and PGC-1α/FXR in SPI-mediated alterations in hepatic gene expression.

Key words: soy protein isolate; sterol regulatory element-binding protein (SREBP)-1c; farnesoid X receptor (FXR); peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α); adenosine monophosphate-activated protein kinase (AMPK)

Obesity has increased worldwide and causes insulin resistance, resulting in various diseases, including metabolic syndrome, diabetes, and atherosclerosis. The primary alteration triggered by obesity is dysfunction of the liver or adipose tissue, which results in lipid metabolism disorder, but the molecular mechanism underlying insulin resistance and obesity is not yet fully understood, and effective dietary treatment is currently of great interest.

Soy protein is recognized as a functional food ingredient with various functions, including appetite suppression and anti-tumor activity. Lipid metabolism in particular has been reported to improve on soy protein consumption not only in laboratory animals but also in humans. Furthermore, it has been found that β-conglycinin, a soy protein, effectively decreases blood triacylglycerol (TG) in humans, and soy protein treatment is believed to be beneficial for patients with metabolic syndrome.

A plausible explanation of the improved lipid metabolism resulting from soy protein consumption is that peptides generated by the intestinal digestion of soy protein bind to the lipid micelle formed in a mixture with bile acids and dietary lipids, preventing their absorption from the small intestine and increasing lipid excretion in the feces. A decline in bile acid uptake leads to reduced activities of the farnesoid X receptor (FXR), which is activated by bile acids in the small intestine and the liver. In these tissues, FXR directly or indirectly regulates the expression of several key genes related to lipid metabolism, including the ideal bile acid-binding protein, fibroblast growth factor 15/19, CYP7A1, and the small heterodimer partner (SHP). Nevertheless, the effect of soy protein on FXR functions has yet to be elucidated fully.

A number of nutritional experiments have been performed in order to observe favorable changes in blood lipids through long-term administration of test diets, but it appears that preceding alterations in gene expression in some tissues ought to be observed prior to changes in blood components. Based on this concept, in this study, mice were fed soy protein isolate (SPI) for 4 weeks, and alterations in gene expression in the liver were analyzed before the onset of changes in blood components. At the same time, to examine the effect of soy protein on bile acid metabolism, FXR-deficient (FXR KO) and wild-type (WT) mice were fed SPI for 4 weeks. Furthermore, after the mice were fed SPI for a shorter duration (3 or 10 d), we searched for subsets of the genes that responded to dietary SPI in the earliest part of this period to identify the initial trigger of the soy protein function.

Abbreviations: ACC, acetyl-CoA carboxylase; AMPK, adenosine monophosphate-activated protein kinase; BSEP, bile salt export pump; FAS, fatty acid synthase; FXR, farnesoid X receptor; PEPCK, phosphoenolpyruvate carboxykinase; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; SCG1, stearoyl CoA desaturase 1; SHP, small heterodimer partner; SPI, soy protein isolate; SREBP, sterol regulatory element-binding protein; TG, triacylglycerol

1 To whom correspondence should be addressed. Tel: +81-3-5841-5136; E-mail: aroysato@mail.ecc.u-tokyo.ac.jp
Materials and Methods

Animals and experimental protocol. Male C57BL/6N and FXR KO mice (C57BL/6N mouse background) were obtained from Clea Japan (Tokyo, Japan) and kindly provided by Dr. Frank J. Gonzalez respectively. The mice were maintained in the animal care facility at The University of Tokyo. All the mice were housed on a 12 h light/ dark cycle, and they were divided into two groups. They were fed a high-fat diet containing 27% fat (50% of total energy) with either casein or SPI (provided by Fuji oil, Osaka, Japan) as the protein source. The protein concentration of the diet was 20%, was adjusted on the basis of the protein content of the isolated protein (casein 86.2%, SPI 90.6%). The mice were fed for 3, 10, or 28 d and had free access to diet and water. Table 1 shows the composition of the experimental diet.

Body weight and food intake were measured every 3 d. After 6 h of food deprivation, five mice per group were killed under anesthesia. We determined the body composition and collected blood and the liver. Blood was collected in tubes with 0.1 mM EDTA and centrifuged at 3,500 rpm for 30 min to obtain plasma. The tissues were rapidly excised, frozen in liquid nitrogen, and stored at −80°C. All experiments, repeated several times, were performed according to the guidelines of the Animal Usage Committee of The University of Tokyo.

Plasma measurements. Plasma glucose, TG, total cholesterol, HDL cholesterol and bile acid concentrations were determined using kits purchased from Wako Chemicals (Tokyo, Japan). The plasma insulin concentration was determined using an ELISA kit (Shibayagi, Gunma, Japan).

Real-time quantitative PCR. Total RNA from the liver was extracted with an RNeasy kit (Qiagen, Valencia, CA) and reverse transcribed using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Fluorescence real-time PCR was performed by the SYBR Green procedure (Roche Applied Biosciences, Tokyo, Japan) on an ABI PRISM 7000 system, as previously described.14,15) The livers were obtained from male C57BL/6N mice fed for 24 h or fasted/refed normal chow. The livers of male C57BL/6N mice fed the test diets for 28 d were obtained as described above.

Western blot. Frozen liver pooled samples (n = 3–5) were homogenized in ice-cold RIPA buffer (50 mM Tris-HCl pH 7.4, 1 mM EDTA, 150 mM NaCl, 1% Nonidet P-40, and 0.25% sodium deoxycholate) containing a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO), and centrifuged. The supernatant (10 μg protein/lane) was subjected to SDS–PAGE (8%), transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA), and probed with anti-adenosine monophosphate-activated protein kinase (AMPK) γ (Thr172) (#2531; Cell Signaling Technology, Beverly, MA) antibodies, as previously described.14,15) The primer sequences and TaqMan probes are given in Supplemental Table 1 (see Biosci. Biotechnol. Biochem. Web site). 36B4 mRNA was employed as internal control.

Results

Body weight and blood components of mice fed one of the test diets for 28 d

The WT and FXR KO mice fed the casein diet were heavier than those fed the SPI diet for 28 d (Table 2). The body weight and weight gain of the FXR KO mice were smaller than the WT mice. Food consumption, plasma glucose, insulin, and TG were unchanged in the mice from both groups fed the SPI diet. Consistently with a previous report,17) the plasma bile acid and HDL cholesterol concentrations in the FXR KO mice were significantly higher than those in the WT mice.

Analysis of hepatic mRNA

Total RNA was extracted from the liver of the mice after food deprivation for 6 h. Real-time PCR analyses were performed using gene-specific TaqMan probes or primer sets for more than 10 genes related to lipid and carbohydrate metabolism. Although there were no significant diet-induced differences in blood components, the mRNA levels of sterol regulatory element-binding protein-1c (SREBP-1c), a predominant isoform in the liver, were significantly lower in the WT mice fed the SPI diet for 28 d (Table 3). This decrease resulted in a slight decline in its targets, FAS, acetyl-CoA carboxylase1 (ACC1), and SCD1, in the WT mice (no significant differences). In contrast, SREBP-1c mRNA abundance was low, unchanged by diet, in the FXR KO mice. No changes in the mRNA levels of SREBP-2 or its targets were observed in either group of mice (data not shown). Gene expression of FXR and its target bile-salt export pump (BSEP) was reduced in the WT mice. On the other hand, CYP7A1 gene expression, which is negatively regulated by FXR, was unchanged. Furthermore, the mRNA levels of the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), a pivotal coactivator of various transcription factors that control the expression of a subset of the genes related to gluconeogenesis and lipoprotein metabolism, were

Table 1. Food Composition

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Casein</th>
<th>SPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein1</td>
<td>229</td>
<td></td>
</tr>
<tr>
<td>SPI2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornstarch1</td>
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<td>218</td>
</tr>
<tr>
<td>α-Cornstarch1</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td>Sucrose1</td>
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<td>Soybean oil3</td>
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<tr>
<td>Fiber1</td>
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<td>62</td>
</tr>
<tr>
<td>Mineral Mix (AIN-93)3</td>
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<td>43</td>
</tr>
<tr>
<td>Vitamin Mix (AIN-93)1</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>t-Cystine4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Choline Bitartrate4</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

1Oriental Yeast (Tokyo, Japan)
2Fuji Oil (Osaka, Japan)
3Wako Chemical (Tokyo, Japan)
4Hayashi Chemical (Osaka, Japan)
hepatic SREBP-1c gene expression in the WT mice. That SPI consumption initially preferentially affected reduced by the SPI diet (Fig. 1B). These results indicate that SPI-mediated effects on hepatic gene expression to be alterations in hepatic gene expression prior to changes in the plasma glucose and lipid concentrations.

**Analysis of hepatic RNA in WT mice fed the test diet for 3 or 10 d**

To examine further the number of days required for SPI-mediated effects on hepatic gene expression to be exerted, WT mice were fed the test diet for a shorter period of time, 3 or 10 d. Body weights and plasma lipid profiles did not differ between the diets (data not shown). In the livers of the mice fed the SPI diet for 10 d, gene expression of SREBP-1c and its targets (FAS, ACC1, and SCD1) was suppressed (Fig. 1A). The mRNA levels of FXR and PGC-1α were again lower after the 10-d treatment. Strangely, expression of the gene for the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) and CYP7A1 was significantly lower in the mice fed the SPI diet only for 10 d. Given the PGC-1α-mediated enhancement of gluconeogenic gene expression, the decrease in PEPCK mRNA appeared to be plausible. However, why this effect was lost despite a decrease in PGC-1α mRNA after the 28-d treatment (Table 3) remains unclear. Following the 3-d treatment, SREBP-1c and its target, SCD1, were the only genes the expression of which was significantly reduced by the SPI diet (Fig. 1B). These results indicate that SPI consumption initially preferentially affected hepatic SREBP-1c gene expression in the WT mice.

### Table 2. Body Weights, Weight Gain, Food Consumption, and Blood Components of Mice Fed the Casein or the SPI Diet for 28 d

<table>
<thead>
<tr>
<th></th>
<th>WT mice</th>
<th>FXR KO mice</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>casein</td>
<td>SPI</td>
<td>diet mice</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>30.7 ± 1.1</td>
<td>27.5 ± 1.1</td>
<td>24.1 ± 0.2</td>
</tr>
<tr>
<td>Weight gain, g/28 d</td>
<td>13.4 ± 1.2</td>
<td>10.3 ± 1.4</td>
<td>9.0 ± 0.8</td>
</tr>
<tr>
<td>Food consumption, g/28 d</td>
<td>73.7 ± 1.6</td>
<td>71.2 ± 3.6</td>
<td>66.2 ± 4.6</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>279 ± 13</td>
<td>271 ± 11</td>
<td>251 ± 18</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>11.9 ± 2.5</td>
<td>11.1 ± 3.8</td>
<td>5.4 ± 2.7</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>96.6 ± 36.6</td>
<td>47.1 ± 5.5</td>
<td>73.0 ± 5.4</td>
</tr>
<tr>
<td>HDL-cholesterols, mg/dL</td>
<td>40.4 ± 3.4</td>
<td>32.8 ± 2.0</td>
<td>129 ± 5</td>
</tr>
<tr>
<td>Bile acid, μmol/L</td>
<td>3.9 ± 0.8</td>
<td>5.1 ± 1.1</td>
<td>26.2 ± 5.5</td>
</tr>
</tbody>
</table>

Values are means ± SEM, n = 5. Means in a row with superscripts without a common letter differ, p < 0.05.

### Table 3. Hepatic Gene Expression of Mice Fed the Casein or the SPI Diet for 28 d

<table>
<thead>
<tr>
<th></th>
<th>WT mice</th>
<th>FXR KO mice</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>casein</td>
<td>SPI</td>
<td>diet mice</td>
</tr>
<tr>
<td>SREBP-1a</td>
<td>1.00 ± 0.19</td>
<td>0.91 ± 0.06</td>
<td>1.64 ± 0.08</td>
</tr>
<tr>
<td>SREBP-1c</td>
<td>1.00 ± 0.12a</td>
<td>0.60 ± 0.03b</td>
<td>0.26 ± 0.02c</td>
</tr>
<tr>
<td>FAS</td>
<td>1.00 ± 0.12</td>
<td>0.86 ± 0.06</td>
<td>2.88 ± 0.39</td>
</tr>
<tr>
<td>ACC1</td>
<td>1.00 ± 0.09</td>
<td>0.69 ± 0.03</td>
<td>1.77 ± 0.17</td>
</tr>
<tr>
<td>SCD1</td>
<td>1.00 ± 0.09</td>
<td>0.54 ± 0.07</td>
<td>1.81 ± 0.25</td>
</tr>
<tr>
<td>FXR</td>
<td>1.00 ± 0.04</td>
<td>0.79 ± 0.04ab</td>
<td>0.74 ± 0.08b</td>
</tr>
<tr>
<td>BSEP</td>
<td>1.00 ± 0.10c</td>
<td>0.68 ± 0.05b</td>
<td>0.74 ± 0.08b</td>
</tr>
<tr>
<td>SHP</td>
<td>1.00 ± 0.22</td>
<td>0.85 ± 0.14</td>
<td>0.46 ± 0.11</td>
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<tr>
<td>CYP7A1</td>
<td>1.00 ± 0.30</td>
<td>1.04 ± 0.44</td>
<td>2.90 ± 0.86</td>
</tr>
<tr>
<td>PGC1α</td>
<td>1.00 ± 0.12</td>
<td>0.50 ± 0.03</td>
<td>1.59 ± 0.25</td>
</tr>
<tr>
<td>PEPCK</td>
<td>1.00 ± 0.22</td>
<td>1.68 ± 0.27</td>
<td>0.14 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SEM, n = 5. Means in a row with superscripts without a common letter differ, p < 0.05.
expression was reduced by soy protein, which is gene expression,20,21) is enhanced in the liver in response activity, which has been found to reduce SREBP-1c present study prompted us to examine whether AMPK SREBP-1c gene expression throughout 28 d in the hepatic SREBP-1c expression in response to SPI con-
bands observed (Fig. 2) appear to correspond to an in these assays recognizes both SREBP-1a and -1c, the (Fig. 2B). Although the antibody against SREBP-1 used SREBP-1 bound to the FAS or SCD1 promoter (Fig. 2A). Reflecting decreased hepatic SREBP-1c expression in the mice fed the SPI diet, less SREBP-1b to the promoter of FAS and SCD1 in these mice (Fig. 2B). Although the antibody against SREBP-1 used in these assays recognizes both SREBP-1a and -1c, the bands observed (Fig. 2) appear to correspond to an active form of SREBP-1c, the predominant form in the liver. These results clearly indicate that decreased hepatic SREBP-1c expression in response to SPI consumption resulted in reduced promoter activities of the FAS and SCD1 genes in the liver.

AMPK activation in the liver

The finding that SPI consumption suppressed hepatic SREBP-1c gene expression throughout 28 d in the present study prompted us to examine whether AMPK activity, which has been found to reduce SREBP-1c gene expression,20,21) is enhanced in the liver in response to SPI consumption. Western blot analyses were performed using both anti-phosphorylated AMPK, an active form, and anti-AMPK antibodies. As shown in Fig. 3, the protein levels of phosphorylated AMPK but not of total AMPK increased in the mice fed the SPI diet for 3 to 28 d. No elevation was observed in the livers of the FXR KO mice fed SPI for 28 d. This might in part provide an explanation of the SPI-mediated decrease in hepatic SREBP-1c mRNA.

Discussion

In the present study, we found the following: (i) As compared to casein, soy protein consumption by mice affected the expression of various genes related to lipid metabolism in the liver prior to causing noticeable changes in blood lipid components. (ii) The effects of soy protein consumption were not observed in the FXR KO mice, suggesting the importance of FXR and its physiological ligands, bile acids. (iii) PGC-1α gene expression was reduced by soy protein, which is believed to be responsible for decreases in the expression of FXR, thereby reducing BSEP gene expression. (iv) The earliest response to soy protein consumption was a decrease in hepatic SREBP-1c mRNA, observed on day 3. (v) Soy protein activated hepatic AMPK only in the WT mice, which can lead to reductions in SREBP-1c gene expression. These results are illustrated in Fig. 4.

FXR KO mice were first generated almost 10 years ago, and have been used in various experiments to determine the biological function of this nuclear receptor.17,22–24) FXR KO mice were distinguished from WT mice by increased serum bile acid, hepatic cholesterol, and TG, and a decreased bile acid pool. Furthermore, the toleration of FXR KO mice of dietary administration of bile acid (1%) is poor, as evidenced by the death of approximately 30% of them by day 7,17) and their tendency to spontaneously develop liver tumors due to chronic liver injury.25) These findings prompted us to expect different responses to dietary proteins in the null mice as compared to those in the WT mice. Indeed, we observed no diet-related alterations in gene expression in the FXR KO mice, whereas the SPI diet affected the expression of the genes related to lipid and carbohydrate metabolism in the WT mice (Table 3). The absence of hepatic AMPK activation in the FXR KO mice fed soy protein (Fig. 3) may also have resulted from the above-mentioned abnormalities in the hepatic metabolism of lipids. It is interesting that in the WT mice, the impaired function of FXR in response to soy protein consumption might be associated with the beneficial effects of soy protein on lipid metabolism, because such effects were not observed in the null mice. The molecular mechanism of this effect remains unclear, but it appears that a slight inhibition of FXR function along with a reduction in SREBP-1c and PGC-1α gene

Fig. 2. Promoter Pull-Down Assays Using Nuclear Fractions Prepared from the Livers of Mice Fasted, Refed Normal Chow (A), and Fed the Casein or SPI Diet for 28 d (B).

Using biotinylated PCR fragments of the promoter of mouse FAS or SCD1 containing an SREBP-binding site, nuclear SREBP-1 associated with these fragments was detected by Western blotting using anti-SREBP-1 antibody. C, casein; S, SPI

Fig. 3. AMPK Activation in the Livers of WT and FXR KO Mice Fed the Casein or the SPI Diet for the Indicated Durations.
Pooled liver lysates were analyzed by Western blotting using anti-phosphorylated AMPK and anti-AMPK antibodies. C, casein; S, SPI

Fig. 4. A Model of SPI Functions in the Mouse Liver.
expression favors an improvement in lipid metabolism when WT mice are fed soy protein for a longer period of time. 3

PGC-1α has been found to control a number of biological pathways associated with energy production and utilization. 26) One important function of PGC-1α appears to be the promotion of hepatic gluconeogenic gene expression in response to fasting. 27) Therefore, the reduction in PEPCK gene expression observed after the 10-d treatment with soy protein is to be explained by reduced PGC-1α gene expression. However, the reason with this decrease did not continue up to day 28, despite the sustained decrease in PGC-1α mRNA, is unclear. It is possible that hepatic PEPCK gene expression was primarily regulated by a transcription factor or co-activator other than PGC-1α on day 28. Given that PGC-1α enhances FXR gene expression, 28) the decrease in FXR mRNA in the WT mice in response to soy protein consumption was probably due to reduced PGC-1α gene expression. In that sense, it is possible that PGC-1α downregulation is important to the effects of soy protein. Indeed, a recent study clearly demonstrated that impaired gene expression of BSEP caused by decreased FXR transcriptional activity resulted in lipid malabsorption from the intestine. 29) This might indicate in part the importance of the PGC-1α-FXR-BSEP pathway in SPI-mediated improvement of lipid metabolism. The transcription of PGC-1α is highly regulated by a transcription factor, the cAMP-dependent response element-binding protein, that is activated by cAMP-dependent protein kinase. 30) Further studies are required to elucidate the molecular mechanism by which soy protein consumption affects PGC-1α gene expression.

Contrary to our expectations, the hepatic CYP7A1 mRNA levels were almost unchanged, or rather were decreased on day 10 in the mice fed soy protein (Fig. 1A). FXR is heavily involved in the transcriptional regulation of this gene through the function of FXR target SHP. The reduction in FXR gene expression by soy protein was theoretically assumed to bring about an increase in CYP7A1 mRNA. On the other hand, PGC-1α is also involved in the transcription of the CYP7A1 gene through enhancement of the transcriptional activities of several nuclear receptors, including the liver X receptor, liver receptor homolog 1, and hepatocyte nuclear factor 4, which directly promote the transcription of this gene. It is thus possible that these opposing effects due to soy protein consumption canceled each other, resulting in an absence of alteration in gene expression on days 3 and 28, while decreased PGC-1α strongly affected CYP7A1 gene expression only on day 10.

Because transcription factor SREBP-1c plays a central role in whole-body lipid metabolism, particularly fatty acid and TG metabolism, suppression of SREBP-1c gene expression in the liver, observed in the WT mice fed soy protein, almost certainly results in a decrease in the serum TG concentration and hepatic TG content at a later date, consistently with previous reports. 31,32) Indeed, expression of the SREBP-1 targets was reduced throughout the 28 d, suggesting that fatty acid synthesis started to diminish on day 28 in the present study. Some studies have found that the consumption of soy protein delays or inhibits insulin secretion, thereby reducing the expression of SREBP-1c, which is tightly regulated by insulin. 33,34) However, in the present study no significant decrease in the serum insulin concentration was observed in the WT or FXR KO mice fed soy protein. We cannot rule out the possibility that the slight decrease in serum insulin found in this study (Table 2) contributed to reduced SREBP-1c gene expression. Alternatively, significant activation of hepatic AMPK, which has been reported to decrease SREBP-1c mRNA levels, 20,21) suggests a direct soy protein effect on SREBP-1c gene expression. It has been reported that a soy protein diet induced the expression of adiponectin in adipose tissue. 35) In addition, adiponectin promotes intracellular AMPK activity by binding to an adiponectin-specific receptor, Adipo R1 or R2. 36) However, we failed to observe any changes in adiponectin mRNA levels in white adipose tissue throughout the 28 d in the present study (data not shown). No inducing effect of soy protein on hepatic AMPK activity has yet been demonstrated. Investigation as to whether soy protein consumption activates hepatic AMPK is now in progress.

Although it is well known that isoflavones have a beneficial impact on lipid metabolism, we think the results presented here were scarcely caused by these contaminants in SPI. Two previous studies using soy protein isolate prepared by an identical method to ours have demonstrated that isoflavones contaminating SPI are negligible both in quantitative and qualitative terms. 37,38) Another study using isoflavone-poor soy protein also found an alteration in the lipid metabolism by SREBP-mediated downregulation of hepatic genes, 31) but we cannot rule out the possibility that the tiny amount of isoflavones in SPI, if any, affected the expression of the several genes determined in the present study.

In conclusion, the administration of a soy protein diet to mice resulted in alterations in the expression of various genes in the liver prior to noticeable changes in blood lipid components. This effect was not observed when FXR KO mice were fed the same diet, indicating the importance of FXR function for it. The earliest response to soy protein consumption was a decrease in hepatic SREBP-1c mRNA, observed on day 3. Investigation as to the determinants of the soy protein functions, for example its amino acid composition and specific peptides derived from SPI, is now underway. Taken together, the decrease in SREBP-1c mRNA in combination with reduced PGC-1α, FXR, and BSEP gene expression appears to be associated with the beneficial effects of soy protein on lipid metabolism. This study contributes to an understanding of the molecular mechanism by which soy product intake provides nutritional benefits at least partly through the functioning of soy protein.

Acknowledgments

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