Consumption of Soy Protein Isolate Modulates the Phosphorylation Status of Hepatic ATPase/ATP Synthase β Protein and Increases ATPase Activity in Rats1–3

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Abstract

ATPase/ATP synthase plays important roles in the regulation of carbohydrate, protein, and lipid metabolism through modulating energy homeostasis. The purpose of this study was to examine the effects of feeding soy proteins and isoflavones (ISF) on the enzymatic activity and protein modification of hepatic mitochondrial ATPase/ATP synthase. In Expt. 1, Sprague-Dawley rats aged 50 d were fed diets containing either 20% casein or 20% alcohol-washed soy protein isolate (SPI) with or without supplemental ISF (770.7 μmol/kg diet) for 70 d. In Expt. 2, weanling Sprague-Dawley rats were fed diets containing 20% casein with or without added ISF (154.1 μmol/kg diet) or 20% SPI for 90 d. Hepatic mitochondrial ATPase activity was significantly higher in the rats fed SPI than in those fed casein. Addition of ISF to SPI eliminated the action of SPI. ATPase/ATP synthase β protein contents in the liver were unchanged; however, its patterns measured by 2-dimensional Western blot were different among dietary groups. The rats fed SPI or SPI plus ISF had 3 more major protein spots with the same molecular weights (80 kDa and 55 kDa) as those presented in the rats fed casein but with different isoelectric points. Pretreatment of hepatic mitochondrial proteins from the rats fed casein with alkaline phosphatase produced the same ATPase/ATP synthase β patterns as observed in the SPI-fed rats and significantly elevated the ATPase activity. These results suggest that consumption of soy proteins increases hepatic ATPase activity, which might be a consequence of increased dephosphorylation or decreased phosphorylation of the mitochondrial ATPase/ATP synthase β protein. J. Nutr. 137: 2029–2035, 2007.

Introduction

Soy consumption has been linked to decreased risk of cardiovascular disease (1,2), type 2 diabetes (3–6), and certain types of carcinogenesis such as breast cancer (7,8) and prostate cancer (9–11). However, the molecular events involved have not been fully elucidated. Improvement of the blood lipid profiles such as reduction of total cholesterol, LDL cholesterol, triglyceride levels, and elevation of HDL cholesterol by intake of soy products has been reported in both human and animal studies (12,13) and are thought to be one of the major benefits of soy. Meanwhile, many other effects of soy components have also been unveiled, such as modulation of ATPase activity (14,15).

ATPase/ATP synthase, an enzymatic complex responsible for ATP synthesis and hydrolysis in mitochondria, is comprised of a membrane-bound F0 portion and a soluble F1 portion. F1 consists of 5 subunits (α, β, γ, δ, and ε). The catalytic sites of the ATP synthesis are present on the β subunit. F1 catalyzes the synthesis of ATP from ADP and inorganic phosphate through utilizing the transmembrane proton gradient and membrane potential generated during substrate oxidation. This reaction can be reversed through pumping protons in the opposite direction and results in ATP hydrolysis (16).

A proper functioning of ATPase/ATP synthase is essential for maintaining energy homeostasis and normal physiological performance. Dysfunction of ATPase/ATP synthase protein (17,18) or altered enzymatic activity is associated with various diseases (19–21). For example, downregulation of ATP synthase β subunit messenger RNA levels by small interfering RNA decreased glucose-induced insulin secretion in cultured pancreatic β cells (18,21). In insulin-resistant or type 2 diabetic patients, the ATPase/ATP synthase β subunit protein and insulin-stimulated rates of mitochondrial ATP synthesis in skeletal muscle were suppressed (17,22). Mitochondrial ATP synthase activity was markedly decreased in type 1 diabetic rat liver (21). Steatotic

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liver of rats had significantly lower mitochondrial ATPase activity compared with that of normal rats (20). Moreover, the ATPase/ATP synthase β subunit on hepatocyte plasma membrane was recently identified as a high-affinity HDL receptor (23). HDL mediates the efflux and transport of cholesterol from peripheral cells to the liver for further metabolism, suggesting ATPase/ATP synthase may play a role in the regulation of cholesterol homeostasis. In addition, ATP synthase β subunit has also been found on the cell surface of several human tumor cell lines and mediates the killing of tumor cells by lymphocytes (24,25). Blocking ATP production in hepatocellular carcinoma cells seeded into rats resulted in complete eradication of cancer (26). These results suggest that modulation of ATPase/ATP synthase may be an important strategy for the prevention or improvement of certain types of chronic diseases.

Dietary supplementation with soy isolated protein containing isoflavones (ISF)7 has been shown in diabetic rats to prevent reduction of Na⁺, K⁺-ATPase activity, another member of ATPase family that is responsible for transporting Na⁺, K⁺ ions and maintaining Na⁺, K⁺ gradients across the membranes using ATP hydrolysis energy (14). Genistein, one of the main soy ISF, was also shown to inhibit the brain and hepatic mitochondrial ATP synthase activity in vitro (15).

However, whether consumption of soy components (proteins or ISF) affects the ATPase/ATP synthase activity in vivo and the potential mechanism(s) involved is not fully understood. The objective of this study was to examine the effects of feeding alcohol-washed soy protein isolates (SPI) (containing a minimal amount of ISF) or with supplemental ISF on the enzymatic activity and protein modification of hepatic mitochondrial ATPase/ATP synthase in rats.

Materials and Methods

Chemicals and reagents. Alcohol-washed SPI (Pro Fam 930 containing 90% protein) and Novasoy (soy ISF concentrate) were purchased from Archer Daniels Midland. Casein (90% total protein) was from ICN Biomedicals and Harlan Teklad. Alkaline phosphatase (AP) from Escherichia coli, ATPase, aminomethyl benzene-sulfonyl fluoride hydrochloride, bacitracin, chlomodiopropyl dimethyl-ammonio-propane sulphonate (CHAPS), and Tris were from Sigma Chemical. Protease inhibitor cocktail was from Roche. Zoom Strips, sample loading buffer, and Bis-Tris gels were from Invitrogen Life Technologies. Affinity purified mouse monoclonal antibody against human ATP synthase β was from BD Biosciences. Goat anti-mouse IgG (H+L)-horseradish peroxidase conjugated antibody and Bio-Rad protein assay kits were purchased from Bio-Rad Laboratories. ECL Western blotting detection kit was obtained from Amersham. X-ray film was from Pierce Biotechnology. ATPase assay kit and PiBind resin were from Innova Biosciences.

Animals, diets, and tissue samples. The animal experimental protocols were approved by the Health Canada Ottawa Animal Care Committee and all animal handling and care followed the guidelines of the Canadian Council for Animal Care. In Exp. 1, Sprague-Dawley male rats (Charles River) aged 50 d were randomly divided into 3 groups (8 rats per group) and fed 1 of the diets (20% casein, 20% alcohol-washed SPI, or 20% SPI supplemented with 770.7 μmol ISF/kg diet) for 70 d (Table 1). In Exp. 2, Sprague-Dawley male rats (28 d old) were fed diets containing 20% casein with or without ISF supplementation (154.1 μmol/kg diet) or 20% alcohol-washed SPI for 90 d (Table 1). All diets were formulated according to the specifications of AIN93G (27). The actual content of ISF was determined by Waters HPLC linear gradient with UV detection monitored at 254 nm (28) and reported previously (29). At the end of the feeding period, rats were necropsied; tissues were collected and immediately frozen in liquid nitrogen and stored at −80°C until analysis.

Mitochondrial preparation. The mitochondrial fractions of the liver were isolated as described (15). Briefly, liver tissue (150 mg) was homogenized at 4°C in lysis buffer (50 mmol/L Tris, 120 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L MgSO₄, 1 mmol/L CaCl, 10% glycerol, 0.5 mmol/L aminothyl benzene-sulfonyl fluoride hydrochloride, 0.1 mmol/L bacitracin, pH 7.4) with a glass homogenizer (10 strokes in 1 min). Homogenates were centrifuged at 600 × g; 10 min to separate the cell debris. The supernatant was further centrifuged at 15,000 × g; 5 min and the precipitated mitochondrial fractions were collected and quantified for their protein content. Equal amounts of the mitochondrial fraction from each animal were solubilized in lysis buffer containing 1% CHAPS, sonicated for 6 × 10 s, and then centrifuged at 15,000 × g; 5 min at 4°C. Supernatant was collected for the analysis of ATPase/ATP synthase.

Determination of ATPase/ATP synthase activity. ATPase/ATP synthase activity was measured in the direction of ATP hydrolysis as ATPase activity using an ATPase assay kit. Briefly, mitochondrial proteins (15 μg) were incubated with the PiBind resin to remove the free inorganic phosphates from the samples and then substrate solutions containing 1 mmol/L ATP were added. The reaction was stopped after 30-min incubation at room temperature and absorbance at 650 nm was measured to determine the released inorganic phosphate. The specific activity of the enzyme was expressed as μmol·mg protein−1·min−1. One unit of activity was defined as the amount of enzyme that catalyzes the reaction of 1 μmol of ATP per minute. A blank was assayed under the same conditions, except stop mix was added to samples before the substrate solution.

To determine the effect of dephosphorylation of ATPase/ATP synthase protein on the enzymatic activity, 15 μg mitochondrial protein from the rats fed 20% casein was incubated at 37°C with either 0.5 units AP in the buffer (50 mmol/L Tris, 0.1 mmol/L EDTA, pH 8.5) or the same amount of heat-inactivated AP (boiled for 5 min) for 2 h. The free inorganic phosphates produced from the dephosphorylation reaction were eliminated by PiBind resin and then the ATPase activity was measured as described above.

Protein extraction and Western blot analysis. Total protein extraction from rat livers and Western blot analysis were conducted as described previously (29) with minor modifications. Total proteins (60 μg) were resolved by 12% SDS-PAGE and electrotransferred (25 V, 4°C, 90 min) onto nitrocellulose membranes. After blocking, membranes were incubated overnight at 4°C with mouse anti-human ATP synthase β monoclonal antibody (1:1000) and subsequently with horseradish peroxidase-conjugated secondary antibody (1:5000) at room temperature for 45 min. Immunoactivity was detected by chemiluminescence autoradiography according to the manufacturer’s instructions and the images were scanned. The intensities of the protein bands of interest and the Ponceau®-stained proteins were determined densitometrically using Scion Image software. The intensities of the target proteins were normalized by the respective Ponceau-stained total protein (30).

Two-dimensional Western blot analysis of ATPase/ATP synthase. Liver tissues were ground with mortar and pestle in liquid nitrogen and exposed to prechilled lysis buffer containing urea (8 mol/L), thiourea (2 mol/L), CHAPS (4%), ampholytes 3–10 (0.2%), dithiothreitol (1%), and protease inhibitor cocktail (1 tablet/10 mL). After incubating for 90 min at room temperature, the samples were centrifuged at 15,000 × g; 15 min at 4°C. The supernatant was assayed for protein concentration using Quant-it protein assay kit (Invitrogen Life Technologies) and stored at −80°C.

Liver proteins (40 μg) were applied to Zoom Strips (pH 4–7) to rehydrate overnight in rehydration buffer (8 mol/L urea, 2 mol/L thiourea, 4% CHAPS, 0.5% ampholytes 3–10, 1% DTT) and then focused at 2000 V for 3 h. After completion of the focusing, the isoelectric focusing strips were equilibrated in sample loading buffer for 30 min, and then separated on 4–12% SDS-PAGE at 200 V for ~45 min. The gel was transblotted onto polyvinylidene difluoride membrane and stained with

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7 Abbreviations used: AP, alkaline phosphatase; CHAPS, chlomodiopropyl dimethylammonio-propane sulphonate; 2D, 2-dimensional; ISF, isoflavone; pI, isoelectric point; SPI, soy protein isolate.
Coimmunostained with mouse anti-human ATP synthase β monoclonal antibody (1:750 dilution) and detected using ECL kit.

To investigate the effect of dephosphorylation on 2-dimensional (2D) pattern of ATPase/ATP synthase proteins, hepatic protein (40 μg) from the rats fed 20% casein was incubated with either 2 units active or heat-inactivated AP (boiled for 5 min) at 37°C for 2 h. The ATP synthase β protein in the treated samples was analyzed using 2D Western blot.

**Statistical analyses.** Data are means ± SEM. Effects of diets on hepatic ATPase/ATP synthase β protein content and ATPase activity were analyzed by 1-way ANOVA. Differences between individual means were determined by Fisher’s least significant differences test. Differences of P < 0.05 were considered significant. Data were analyzed using STATISTICA version 7.1 (StatSoft).

**Results**

**Food consumption, body weight, and plasma insulin level.** Food consumption, body weight, and the plasma insulin concentration did not differ among the dietary groups (data not shown).

**Hepatic mitochondrial ATPase/ATP synthase activity.** Consumption of the diet containing 20% alcohol-washed SPI increased hepatic mitochondrial ATPase activity (P < 0.05) in both Expt. 1 and Expt. 2 (Table 2). Addition of ISF (770.7 μmol/kg diet) to the SPI-based diet greatly suppressed the SPI-induced increase in ATPase activity. However, a smaller amount of ISF (154.1 μmol/kg diet) added into the casein-based diet did not affect ATPase activity.

**Hepatic total ATPase/ATP synthase β protein content.** Hepatic ATPase/ATP synthase β protein contents measured by Western blot were not affected by either source of dietary proteins (casein and SPI) or addition of ISF (data not shown).

**Isoelectric points of the hepatic ATPase/ATP synthase β protein.** The Coomassie Blue-stained 2D images (Fig. 1A,C,E) showed similar intensities in most of the protein spots, indicating that the loading and transfer of the sample proteins were even. Two major spots were detected in the liver proteins of the rats fed casein diet using ATP synthase β antibody. One is 80 kDa and has an isoelectric point (pI) of 4.88, and another is 55 kDa with a pI of 5.28 (Fig. 1B). Except for these 2 spots, 3 extra ones that the loading and transfer of the sample proteins were even.

### Table 1 Composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Casein</th>
<th>SPI</th>
<th>SPI + ISF</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/kg diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein¹</td>
<td>222.2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Soy protein²</td>
<td>–</td>
<td>222.2</td>
<td>222.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>375.3</td>
<td>375.3</td>
<td>374.5</td>
</tr>
<tr>
<td>Dextrose cornstarch</td>
<td>132.0</td>
<td>132.0</td>
<td>132.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70.0</td>
<td>70.0</td>
<td>70.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Mineral mix³</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
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<tr>
<td>Vitamin mix³</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Choline Bitartrate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>–</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Ter-Butylhydroquinone</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td>Novasoy²</td>
<td>–</td>
<td>–</td>
<td>0.833</td>
</tr>
<tr>
<td>Total ISF³</td>
<td>ND</td>
<td>119.2</td>
<td>889.9</td>
</tr>
<tr>
<td>Added ISF</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Genistein⁴</td>
<td>ND</td>
<td>68.8</td>
<td>460.3</td>
</tr>
<tr>
<td>Daidzein⁴</td>
<td>ND</td>
<td>41.3</td>
<td>357.5</td>
</tr>
<tr>
<td>Glycitein⁴</td>
<td>ND</td>
<td>9.1</td>
<td>72.1</td>
</tr>
</tbody>
</table>

1 Casein from ICN Biomedicals contained 90% crude protein.
2 Alcohol-washed SPI containing 90% crude protein and Novasoy ISF concentrate containing 30% total ISF were purchased from Archer Daniels Midland.
3 AIN-93-G mineral mix (27) and AIN-93G vitamin mix (27) were from ICN Biomedicals.
4 The actual concentration of ISF was determined by HPLC linear gradient with UV detection monitored at 354 nm (28). The total ISF content was the sum of molar amounts of total daidzein, genistein, and glycitein. ND, Not detectable.

### Table 2 Hepatic mitochondrial ATPase activity

<table>
<thead>
<tr>
<th>Expt. 1</th>
<th>Casein</th>
<th>SPI</th>
<th>SPI + ISF</th>
</tr>
</thead>
<tbody>
<tr>
<td>mU/mg protein⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 6)</td>
<td>59.48 ± 8.37a</td>
<td>103.74 ± 15.25b</td>
<td>60.44 ± 9.65a</td>
</tr>
<tr>
<td>Expt. 2</td>
<td>Casein</td>
<td>Casein + ISF</td>
<td>SPI</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>56.78 ± 7.78a</td>
<td>55.63 ± 5.49a</td>
<td>76.72 ± 7.42a</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. Means in the same experiment without a common letter differ, P < 0.05.
2 Casein, 20% casein; SPI, 20% alcohol-washed SPI; SPI + ISF, SPI supplemented with 770.7 μmol ISF/kg diet.
3 Casein, 20% casein; Casein + ISF, 20% casein supplemented with 154.1 μmol ISF/kg diet; SPI, 20% alcohol-washed SPI.
proteins of the rats fed diets containing either SPI alone or SPI supplemented with ISF (770.7 μmol/kg diet) (Fig. 1D,F).

**Impact of in vitro dephosphorylation on 2D patterns and enzymatic activity of the hepatic ATPase/ATP synthase β protein.** Preincubation of the liver proteins from the rats fed casein diet with AP produced 3 new protein spots that were immunostained by ATPase/ATP synthase β antibody (Fig. 2C) compared with the control group (Fig. 2A). Their molecular weights and pI were 80 kDa and 5.32; 55 kDa and 6.14; 55 kDa and 6.16, respectively (Fig. 2C). However, pretreatment of the liver proteins with the same amount of heat-inactivated AP did not affect the 2D patterns of the ATPase/ATP synthase β protein (Fig. 2B). Interestingly, AP treatment dramatically increased the mitochondrial ATPase catalytic activity (buffer control, 57.69 ± 6.45; boiled AP, 67.34 ± 5.17; AP, 91.06 ± 11.31 mU·mg protein⁻¹·min⁻¹; P < 0.05).

**Discussion**

In this study, we demonstrated for the first time, to our knowledge, that dietary SPI causes the dephosphorylation of hepatic ATPase/ATP synthase β protein and increases the mitochondrial ATPase activity, whereas supplementation with ISF (770.7 μmol/kg diet) completely eliminates the actions of SPI on ATPase activity.

The SPI used in our studies was alcohol washed; however, it still contained a minimal amount of ISF (119.2 μmol/kg diet) completely eliminates the actions of SPI on ATPase activity. The results suggested that the amount of added ISF had no effect on hepatic mitochondrial ATPase activity, indicating that protein fraction rather than the contained ISF may play a major role in the regulation of the enzymatic activity.

To determine the potential mechanism(s) involved in the actions of SPI and ISF on the ATPase activity, we further measured the hepatic ATPase/ATP synthase β protein content and showed that neither dietary SPI nor added ISF had any effect on the protein content of the enzyme measured by immunostaining with specific antibody. This suggests that regulation of the hepatic mitochondrial ATPase activity by dietary SPI and ISF might be due to the posttranslational modification of the enzymatic proteins. To test this hypothesis, we used 2D Western blot to analyze the dietary effects on the pI of the ATPase β subunit that contains the catalytic sites of the enzyme. Results indicated that the rats fed SPI with or without supplemental ISF had 3 extra protein spots with the same molecular weights (1 at 80 kDa and 2 at 55 kDa) as those presented in the rats fed casein but with different pI. These proteins might be the modified forms of ATPase/ATP synthase β as they cross-reacted with the anti-ATP synthase β monoclonal antibody.

Phosphorylation/dephosphorylation is one of the most common protein modifications in animal cells (31). It has been shown that the mitochondrial ATPase/ATP synthase β subunits in human skeletal muscle have multiple phosphorylation sites and can be phosphorylated in vivo (17). The pI of the 3 extra protein spots found in the rats fed the SPI-based diet were 5.32, 6.14, and 6.16, respectively, which may represent the dephosphorylated isoforms of ATPase/ATP synthase β. To verify this assumption, we pretreated the hepatic mitochondria or total protein extracts from Novasoy was added to the casein-based diet in Expt. 2. The

![FIGURE 1](https://academic.oup.com/jn/article-abstract/137/9/2029/4664846)

2D Western blot analysis of hepatic ATPase/ATP synthase β protein in rats fed diets containing either 20% casein (A and B) or 20% alcohol-washed SPI in the absence (C and D) or presence (SPI+ISF, E and F) of supplemental ISF (770.7 μmol/kg diet) for 70 d in Expt. 1. The pI were calculated using a linear regression. The images shown are representative of 3 replicates. The spots of interest are marked with arrows.
The bioactive component(s) in the SPI and the cellular mechanisms by which dietary SPI modulate the phosphorylation status of ATPase/ATP synthase β subunit are unclear.

In vitro study showed that soy-derived protease inhibitors increased the activity of cellular tyrosine phosphatase in human skin fibroblasts (33) and mitogen-activated protein kinase phosphatase-1 in MCF7 breast cancer cells (34). In addition, the trypsin inhibitor derived from soybeans has been shown to suppress the activity of extracellular signal-regulated kinase 1/2, p38 mitogen-activated protein kinase, and c-Jun N-terminal kinase in human ovarian tumor cells (35), fibroblasts (36), and keratinocytes (37).

Activation of p38 results in inhibition of ATPase activity in the heart of mouse (38). Stimulation of Na$^+$ K$^+$ ATPase phosphorylation through extracellular signal-regulated kinase-dependent protein kinase C pathway decreases the activity of Na$^+$ K$^+$ ATPase in opossum kidney cells (39). Furthermore, casein kinase II is able to phosphorylate the β subunit of chloroplast ATP synthase (40). However, the pathway through which soy component(s) modulate the phosphorylation status of ATPase/ATP synthase β subunit remains to be investigated.

Insulin is an important regulator of energy metabolism and is shown to be associated with the regulation of ATP synthase gene expression and enzymatic activity. For instance, the ATP synthase β mRNA levels were decreased 67–75% in skeletal muscle of the patients with type 2 diabetes (41) and the hepatic ATPase activity was lower in diabetic rats than in healthy rats (21). Furthermore, the phosphorisoform of ATP synthase β subunit was downregulated in diabetic human muscle (17). However, the plasma insulin levels were not changed by diets in our study, indicating that insulin may not be a major contributing factor to the observed actions of soy protein or ISF.

This study also showed that addition of ISF into the SPI diet (770.7 μmol/kg) inhibited the SPI-induced increase of the ATPase activity. This appears to be consistent with other researchers’ discoveries showing that estradiol and other estrogen analogs inhibited ATPase activity in the brain (42) or liver (43) of rats and that quercetin, a flavone phytochemical, inhibited ATPase activity in rat liver (15) and bovine heart (44).

Although supplementation of soy ISF into the SPI diet eliminated the SPI-induced elevation of the ATPase activity in the liver, the 2D patterns of the ATPase/ATP synthase β protein were not altered, indicating that ISF may regulate the enzymatic activity via different mechanism(s). It has been recently identified that one of the subunits in ATPase/ATP synthase functions as an estradiol-binding protein. Through this protein, estradiol can rapidly inhibit the mitochondrial ATP synthase activity (45). Soy ISF are structurally similar to estradiol. Polyphenolic phytochemicals have 2 potential binding sites in ATPase/ATP synthase, 1 at F1 and 1 at F0 (46), and different phytochemicals appear to inhibit the ATPase activity through binding to different parts of the enzyme.

For instance, quercetin targets F1, whereas genistein, one of the major soy ISF, most likely targets F0 to inhibit the proton transport via binding to the estradiol-binding protein (46). Different portions (i.e. proteins and ISF) of the soybeans have been shown to affect the hepatic mitochondrial ATPase/ATP synthase activity differently in our study. However, the physiological importance of the protein modification and altered enzymatic activity of the ATPase/ATP synthase by dietary SPI and ISF remains to be determined. Many effects of soy have been consistently shown in both human and rat studies, such as the lipid-lowering actions and involved alteration of gene expression. Moreover, the amino acid sequences of human and rat ATP synthase β subunits are highly homologous (99%). This suggests that consumption of soy foods may have similar effects on ATPase/ATP synthase in humans.
In summary, this study demonstrates for the first time, to our knowledge, that consumption of SPI significantly raised mitochondrial ATPase activity in the liver. Addition of ISF (770.7 μmol/kg diet) into the SPI diet abrogated this effect. Hepatic ATPase/ATP synthase β protein contents were unaffected by either SPI or ISF. However, 2D Western blot demonstrated that 3 new protein spots thought to be dephosphorylated isoforms of ATPase/ATP synthase β protein were present in the liver of the rats fed SPI-based diets. Dephosphorylation of hepatic protein extract of the rats fed casein with AP produced the same ATPase/ATP synthase β protein pattern as that in the rats fed SPI and significantly elevated the ATPase/ATP synthase activity. Taken together, our results show that consumption of SPI increases hepatic mitochondrial ATPase activity, which might be due to elevated dephosphorylation or decreased phosphorylation of the ATPase/ATP synthase β subunit.

Literature Cited


