Hypolipidemic and antiinflammation activities of fermented soybean fibers from meju in C57BL/6 J mice

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Meju, a naturally fermented soy block used to produce soy paste and soy sauce in Korea, is suggested to exhibit hypolipidemic and antiinflammatory activities; however, its mechanisms of action are elusive. Here, we report that the water-soluble fibers but not the amino acids and peptides from meju exhibited hypolipidemic activity in vivo. Feeding of fermented soybean fibers (FSF) from meju reduced plasma cholesterol, triglyceride, adipocyte size, and hepatic lipid accumulation in C57BL/6 J mice. FSF treatment reduced HMG-CoA reductase expression, whereas the expression of genes in the fatty acid uptake and subsequent beta-oxidation were significantly induced in the livers. Hepatic lipogenic genes, including Srebp1c and Lxrα, were unaltered. Feeding with the fermented soybean peptides and amino acids (FSPA) induced the expression of lipogenic genes, which may have canceled the induction of low-density lipoprotein receptor and Cyp7a1 gene expressions in FSPA livers. The plasma concentrations of C-reactive protein, TNF-α, and interleukin-6 were significantly reduced in the FSF, FSPA, and meju groups compared with the control groups, suggesting that both of the fibers and peptides/amino acids from meju may be beneficial. These findings suggest that soluble fibers from meju are critical hypolipidemic components that regulate hepatic gene expressions and reduce proinflammatory cytokines in vivo. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: meju; soybean fibers; lipid metabolism; inflammation.

INTRODUCTION

Dyslipidemia is a major risk factor for cardiovascular disease, and its prevalence has been increasing worldwide (Kromhout, 2001). The mechanism, which dyslipidemia causes coronary heart disease (CHD), has been intensively investigated at the molecular level. Low-density lipoprotein (LDL) cholesterol is one of the major risk factors for CHD, and reductions in LDL cholesterol can prevent the development of CHD. A reduction of approximate 12% in LDL cholesterol reduces the risk of CHD by 19% (Unit, 2005), and this level of reduction in cholesterol is possible with appropriate diets, such as those that include adequate fiber intake.

Epidemiological studies have revealed specific inverse associations between the intake of high-fiber foods and the risk of CHD. The corresponding relative risks for increasing quantities of whole-grain intake are 1.0, 0.86, 0.82, 0.72, and 0.67 (95% confidence intervals comparing two extreme quantities: 0.54 and 0.84; P < 0.001) (Liu et al., 1999; Steffen et al., 2003). Increased consumption of dietary fiber has been suggested to improve serum lipid concentrations (Brown et al., 1999), body weight (Birketvedt et al., 2005), and immune function (Watzl et al., 2005).

Soy is a common staple food in Asia, and its lipid-lowering activity has been intensively studied. The low risk for CHD among Asian populations is, at least in part, explained by high intakes of soy-based foods (Beaglehole, 1990). Accordingly, much research has examined the bioactivity of soy-derived compounds and focused on soy proteins and isoflavones; however, the hypolipidemic activity of soy and soy-based food is not fully understood, indicating that additional compounds may also have effects. Soy and soy-based foods contain significant amounts of fiber that may contribute to hypolipidemic activity. Soybean consumption is effective for the prevention of arteriosclerosis, stroke, and dementia and can reduce the risks of cancer and obesity. Furthermore, fermented soybean foods are effective in preventing and curing adult diseases (Kim et al., 2013).

Epidemiological evidence demonstrating that high-fiber diets are beneficial, coupled with newer evidence of a possible metabolic effect of high-fiber diets on inflammatory markers, suggest that inflammation may be an important mediator in the association between dietary fiber and cardiovascular disease (King, 2005). The proinflammatory cytokines C-reactive protein (CRP), TNF-α, and interleukin-6 (IL-6) are widely recognized markers of vascular inflammation (Rader, 2000; Kern et al., 2001; Rui et al., 2001; Andreozzi et al., 2006). Research has demonstrated an inverse association between
the intake of dietary fibers and plasma concentrations of CRP, a clinical indicator of inflammation (King et al., 2003). Thus, TNF-α, IL-6, and CRP play important roles in insulin resistance and the vascular inflammation process through multiple actions (Sethi and Hotamisligil, 1999; Wellen and Hotamisligil, 2005).

Soy-based foods are commonly consumed in Asia in fermented forms, including soy sauces, doenjang (a.k.a. miso in Japan), tempe, natto, and touchi. Soybeans are fermented with wheat in the presence of NaCl in Japan and China, but soybean block without wheat (i.e., meju) is fermented to produce soy sauce and doenjang. Aspergillus oryzae, lactobacilli, and yeasts are the major microorganisms used in meju fermentation and aging. During fermentation, soy proteins are nearly completely degraded into small peptides and amino acids by microbial proteolytic enzymes to increase digestibility. In contrast, soybean fibers, which are moderately resistant to microbial enzymes, are partially hydrolyzed by mold enzymes and undergo some structural modifications that may confer novel biological activity.

Recent results suggest that the fermented soy in meju exhibits several bioactivities including hypolipidemic activity. Thus, we isolated polysaccharides from meju to examine their activities in mice. Dietary fibers mainly function in the intestine to reduce dietary lipid uptake but also affect blood and liver lipid metabolism. Additionally, it has been suggested that some fibers, particularly pectic-type polysaccharides, may be absorbed by Peyer’s patches and delivered into the circulation (Suh et al., 2013), which suggests a direct effect of fiber on hepatic lipid metabolism.

Although it has been more than 30 years since polysaccharides, such as pectic substances, were first reported to be present in meju, the biological activity of these polysaccharides has not been investigated in detail. In the present study, we investigated the hypolipidemic effects of meju soybean fibers from fermented soybeans. The biological activities of the fibers and amino acids/peptides were examined separately.

MATERIALS AND METHODS

Preparation of the fermented soybean fibers, fermented soybean peptides and amino acids, and meju extract samples. Soybean and NaCl were fermented with mold (Aspergillus oryzae), lactobacilli (Tetragenococcus halophilus), and yeast (Zygosaccharomyces rouxii) to prepare meju at SEMPIO Food Co. (Seoul, Korea). The soybeans were steamed for 3 h, then pressed into a block (19 × 19 × 9.5 cm), and dried for 1 day at room temperature. Then, the meju blocks were hung with rice straw and naturally fermented in a greenhouse at 25°C for 35 days. Then, the second fermentation was placed on rice straw at 18–21°C for 45 days (Lee et al., 2012). Fermented soybean fibers (FSF) and fermented soybean peptides and amino acids (FSPA) were prepared by the electrodialysis, ultrafiltration (1 kDa cutoff), and dialysis membrane (12 kDa cutoff) from the meju extract at SEMPIO Food Co. (Seoul, Korea) (Byung-Suk Hur, 2013).

Animals and diets. C57BL/6 J male mice (8 weeks old) was purchased from Samtako (Seoul, Korea). The mice were randomly assigned to four groups (n = 8/group), fed with a high-fat diet (45% of the calories were from fat; Table S1) for 10 weeks to induce obesity, and then subjected to test diets [control, FSF, FSPA, or meju extract (ME) diet] by oral administration for 12 weeks (compositions of diets were showed in Table S2). Body weights were measured twice per week. At 12 weeks, the mice were sacrificed; blood, liver, epididymal fat, perirenal fat, mesenteric fat, and skeletal muscles were collected according to a protocol approved by the Animal Experimentation Committee of Korea University (Protocol no. KUIACUC-20090420-4).

Blood analysis. The plasma triglycerides (TGs), total cholesterol, LDL cholesterol, and high-density lipoprotein (HDL) cholesterol concentrations were determined using an automated clinical chemistry analyzer (Cobas111, Roche, Basel, Switzerland). Adipokines including CRP, TNF-α, and IL-6 were measured with commercially available ELISA kits (Rockford, IL, USA).

Histological analysis of adipose and liver tissues. Histological analyses of epididymal adipose and liver sections were stained with hematoxylin and eosin as previously described (Jia et al., 2012). The sizes of the adipose cells were determined using AXIO software (Axio Imager M1; Carl Zeiss, Oberkochen, Germany), and microscopic images were obtained from the histopathology department of Anam Korea University Hospital (Seoul, Korea).

Total RNA extraction. Total RNA was isolated from white adipose tissue, intestine, and muscle using a total RNA extraction reagent (RNAiso Plus; Takara Bio Inc., Shiga, Japan) as described previously (Jia et al., 2012).

Quantitative real-time polymerase chain reaction analysis. Synthesis of cDNA was performed as described previously (Jia et al., 2012) and then amplified by quantitative real-time polymerase chain reaction (qPCR) (iCycler iQ5; Bio-Rad) using the rt-PCR Premix solution (SYBR Premix Ex TaqII; Takara Bio) according to the manufacturer’s instructions. Primer sequences are presented in Table S3.

Statistical Analysis. All data are expressed as the mean ± the standard error. Student’s t-tests were performed for two group comparisons. P < 0.05 was considered significant.

RESULTS

Compositions of fermented soybean fibers and fermented soybean peptides and amino acids

Fermented soybean fibers were composed of several monosaccharides, including glucose, arabinose, galactose, and uronic acid, which are components of cellulose
and non-cellulosic polysaccharides (Table 1). The major non-cellulosic polysaccharides of soy fibers are neutral arabinogalactans, which are highly balanced, pectin-related, acidic polysaccharides composed of galacturonic acid and a galactose backbone interspersed with rhamnose, arabinose, xylose, and fucose (Lo, 1989). The oligosaccharides in the raw soybean fiber increased after fermentation. The major monosaccharides of FSF were uronic acid (37.03%), fucose (14.24%), xylose (31.83%), mannose (13.75%), and galactose (21.36%). Isoflavones and soy saponins were not detected by high-performance liquid chromatography (data not shown). The amino acid composition of the FSPA is illustrated in Table S4.

### Table 1. Chemical composition of fermented soybean fibers

<table>
<thead>
<tr>
<th>Chemical Composition (%)</th>
<th>Soybean fiber</th>
<th>Sugar*</th>
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<tbody>
<tr>
<td>Natural</td>
<td>62.97 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Uronic acid</td>
<td>37.03 ± 2.3</td>
<td>0±0</td>
</tr>
<tr>
<td>Protein</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>KOD-liked</td>
<td>0±0</td>
<td>0±0</td>
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<table>
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<tr>
<th>Monosaccharide composition</th>
<th>Rhamnose</th>
<th>Fucose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
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<tr>
<td></td>
<td>9.20 ± 1.0</td>
<td>14.24 ± 0.2</td>
<td>5.12 ± 0.2</td>
<td>31.83 ± 1.0</td>
<td>13.75 ± 0.4</td>
<td>21.36 ± 1.1</td>
<td>4.50 ± 0.2</td>
</tr>
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### Tissue and blood analysis

Body weights increased similarly in all groups over the 12-week feeding period (Table S5). Adipose tissue masses were similar in all groups; however, the average adipocyte size was significantly smaller in the FSF group than in the control group (Fig. 1A). The total plasma cholesterol concentrations of the FSF-fed group were significantly lower (20.4%) than those of the control group at week 12 of feeding. The LDL-cholesterol levels were significantly reduced in the FSF group compared with the control C57BL/6J mouse group, and the HDL-cholesterol-to-LDL-cholesterol ratio was significantly increased in the FSF group compared with the control group (Fig. 1B). FSF and FSPA feeding did not alter TG levels (Fig. 1B) or glucose concentrations (data not shown) in C57BL/6J mice. However, at 12 weeks of feeding, hepatic lipid accumulations were significantly reduced in the FSF and ME groups as assessed by direct

![Figure 1. Adipocyte sizes and plasma lipid concentrations in mice fed fermented soybean fibers (FSF), soybean peptides and amino acids (FSPA), and meju extracts (ME).](image-url)
measurements (Fig. 2). Hepatic cholesterol in the livers of the FSF and ME groups decreased significantly by 20% and 27%, respectively. Additionally, liver TG concentrations decreased by 23%, 16%, and 26% in the FSF, FSPA, and ME groups, respectively (Fig. 2). Liver sections exhibited markedly reduced numbers of lipid droplets in the FSF group compared with controls. FSF feeding reduced hepatic and total blood cholesterol concentrations as *meju* extracts; however, FSPA did not have this effect. Overall, FSF showed consistent hypolipidemic effects in plasma, liver, and adipose tissues, whereas FSPA showed marginal activities. Both FSF and FSPA reduced hepatic TG accumulations as well as ME.

**Expression of genes related to hepatic lipid metabolism**

The expression levels of several lipid metabolism genes were quantified by qPCR in the livers of the mice in each group. Regarding cholesterol metabolism, the expression of HMG-CoA reductase was significantly reduced in the FSF livers compared with controls (Fig. 3A). The expressions of the LDL receptor and *Cyp7a1* were significantly upregulated in the FSPA fed livers. Induction of both LDL receptor and *Cyp7a1* could be the opposite effects on hepatic cholesterol concentrations; the former increases cholesterol levels in the liver, whereas the latter decreases them, thus may result in no net changes in hepatic cholesterol concentrations in the liver by FSPA (Fig. 2). Assessments of the expressions of the cellular fatty acid oxidation related *Cd36* and Acyl CoA synthase (*Acs*) were significantly upregulated in both the FSF and ME groups compared with controls (Fig. 3B). Fatty acid transport protein-5 (*Fatp5*) expression was unaltered. Regarding fatty acid oxidation, carnitine palmitoyl transferase 1a (*Cpt1a*) and acyl-CoA oxidase 1 (*Acox1*) were significantly upregulated in both the FSF and ME groups (Fig. 3C). These data suggest that FSF and ME feeding induced hepatic fatty acid uptake and subsequent oxidation that resulted in reduced lipid accumulation in the liver tissues. The expression of *Insig-2a* gene was significantly increased in all sample groups (Fig. 3D). *Insig-2a* inhibits posttranslational activation of hepatic sterol regulatory element-binding protein (SREBPs), which are key transcription factors in hepatic cholesterol synthesis and lipogenesis, and thus prevented lipogenesis and cholesterol synthesis. Collectively, FSF feeding reduces the key gene in cholesterol biosynthesis, lowering total and LDL cholesterol as well as hepatic cholesterol concentrations while inducing gene expression in fatty acid uptake and subsequent beta-oxidation, thus reducing hepatic TG accumulations.

Meanwhile, the effects of FSPA were mixed and complicated. In the liver, FSPA reduced TG accumulations, but the expression of SREBP-1c, *Lxr*, and *Insig-2a* were all upregulated (Fig. 3D). SREBP1c and *Lxr* are lipogenic transcription factors, whereas *Insig-2a* inhibits SREBP1c processing, thus combined effects of FSPA on SREBP1c, *Lxr*, and *Insig-2a* inductions result in a small TG reduction in the liver but no effect of plasma TG levels (Fig. 2). FSPA feeding did not alter gene expression of fatty acid uptake and oxidation. In addition, upregulation of both LDL receptor and *Cyp7a1* could show no net changes in hepatic cholesterol concentrations in the liver as well as plasma cholesterol by FSPA as described earlier.

**Proinflammatory cytokines**

The expressions of the proinflammatory genes TNF-α and interleukin-6 (IL-6) were marginally reduced; however, plasma concentrations of TNF-α and IL-6 were significantly reduced in all three feeding groups compared with controls (Fig. 4). The concentrations of CRP were also significantly reduced in all three groups.

**DISCUSSION**

We investigated the hypolipidemic activities of *meju* compounds in vivo in normolipidemic obese C57BL/6J...
Lipid accumulations in both adipose and liver tissues were significantly reduced without significantly affecting body weight. Additionally, FSF feeding lowered total and LDL-cholesterol concentrations and increased the plasma HDL-to-LDL cholesterol ratio, which suggests that the lipoprotein profiles of the FSF groups were improved. The hypocholesterolemic activities of soy fibers have been reported previously (Lo et al., 1986), although dietary fiber has not been a major focus of studies of the biological activities of soybeans. Our results

Figure 3. Expressions of lipid metabolism genes in the livers. The mRNA levels were quantified with quantitative real-time polymerase chain reaction. (A) Cholesterol metabolism, (B) fatty acid uptake, (C) fatty acid oxidation, and (D) lipogenesis. Values are presented as the means ± the standard errors. *P < 0.05, **P < 0.01 versus controls.

Figure 4. Expression of proinflammatory proteins and genes. The mRNA and protein expressions of the inflammation-related genes C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). *P < 0.05, **P < 0.01, ***P < 0.001 versus controls.
demonstrate that fermented soy fibers could significantly contribute to the hypolipidemic and antiinflammation effects of soybeans in vivo.

We investigated the expressions of multiple genes that are related to hepatic lipid metabolism. We assessed the expressions of fatty acid uptake genes including Cd36 and Acs in the liver. Fatty acids uptake can be facilitated directly by Cd36 and Acs and can subsequently undergo oxidative metabolism to produce ATP (Schwenk et al., 2010). Our data suggest that the FSF diet may have increased hepatic fatty acid uptake via the upregulation of Cd36 and Acs and subsequently induced beta-oxidation because of increased expressions of Acox1 and Cpt1a. The FSF diet reduced total cholesterol concentrations in mice, and this may have been due to downregulation of HMG-CoA reductase. The gene expression of HMG-CoA reductase is mainly regulated by SREBP-2, which is a master transcription factor in cholesterol biosynthesis. Precursor SREBP-2 forms a complex with SCAP (a SREBP chaperone), whereas Insig-2a retains the SCAP-SREBP-2 complex in the ER. The release of Insig-2a from the complex initiates the translocation of the SCAP-SREBP-2 complex from the ER to the Golgi where two proteases, S1P and S2P, cleave the cytosolic and N-terminal portions of SREBP-2 to release the mature form of SREBP-2. Thus, the induction of Insig-2a blocks the posttranslational processing of SREBP-2 and subsequent lipogenesis (Yellaturu et al., 2009; Yecies et al., 2011). Therefore, the induction of Insig-2a may be responsible for the reduction in HMG-CoA reductase expression via delaying SREBP-2 processing in the FSF group.

LXRα is a nuclear hormone receptor that, upon ligand binding, converts to its active form and functions as a transcription factor that regulates the expression of genes involved in lipogenesis including SREBP-1c in the liver (Zhang and Mangelsdorf, 2002). The FSPA livers exhibited induction of the LDL receptor and Cyp7a1 genes, which contribute to the removal of plasma LDL to the liver and the degradation of cellular cholesterol to bile acids, respectively; however, the FSPA livers also exhibited an upregulation of hepatic lipogenic genes, including LXRα and SREBP-1c; thus, the combined effects may have resulted in the marginal hypolipidemic effects.

Adipose tissue is recognized as a rich source of proinflammatory mediators that may directly contribute to vascular injury, insulin resistance, and atherogenesis. These proinflammatory adipocytokines include TNF-α, IL-6, and CRP (Kershaw and Flier, 2004). The plasma levels of CRP, TNF-α, and IL-6, which are involved in inflammation, were significantly downregulated in the FSF groups. CRP is an acute-phase reactant that is synthesized primarily in the liver and is regulated by circulating levels of IL-6; however, IL-1 and TNF-α can also induce hepatic CRP mRNA expression (Yudkin et al., 1999). Emerging evidence suggests that elevated CRP plasma levels are a strong independent predictor of CHD (Ridker, 2003). TNF-α, an inflammatory cytokine, that is released in greater quantities by obese humans and patients with insulin resistance, both initiates and propagates atherosclerotic lesion formation. Thus, taken together, these results indicate that decreased levels of proinflammatory cytokines and improved lipid profiles may contribute to the prevention of chronic diseases, such as CHD.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

REFERENCES


SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher’s web site:

**Table S1.** Chemical composition of FSF.