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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 interlukin-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 interlukin-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

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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 have 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 was composed of several monosaccharides, including glucose, arabinose, galactose, and uronic acid, which are components of cellulose

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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 galacturonate 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

		Chemical Composition (%)
Soybean fiber		
	Natural	62.97 ±1.0
Sugar*		
	Uronic acid	37.03±2.3
	Protein	0±0
	KOD-liked	0±0
Material		
Monosaccharide composition		
	Rhamnose	9.20±1.0
	Fucose	14.24±0.2
	Arabinose	5.12±0.2
	Xylose	31.83±1.0
	Mannose	13.75±0.4
	Galactose	21.36±1.1
	Glucose	4.50±0.2

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

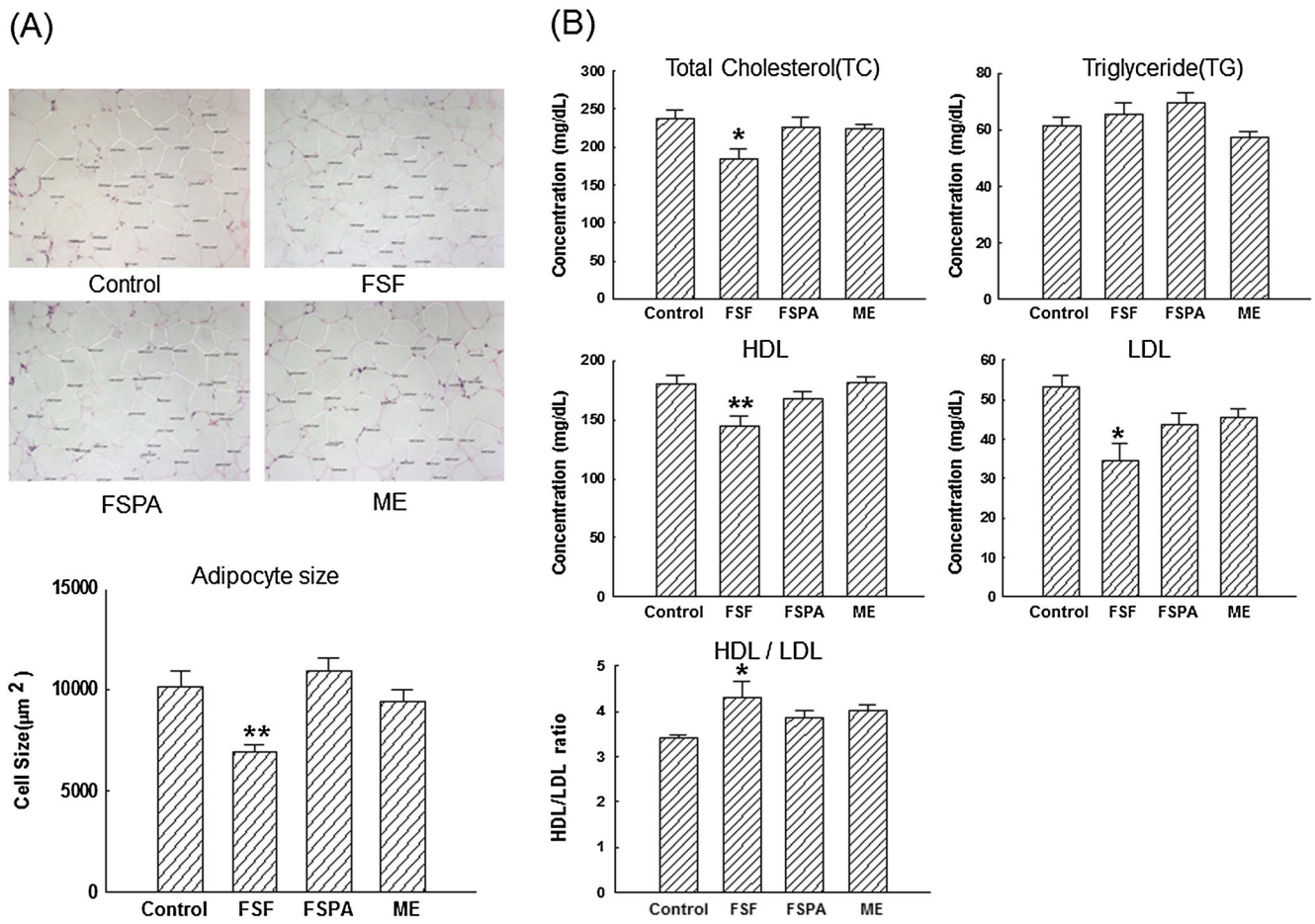

Figure 1. Adipocyte sizes and plasma lipid concentrations in mice fed fermented soybean fibers (FSF), soybean peptides and amino acids (FSPA), and *meju* extracts (ME). (A) Average adipocyte sizes. (B) Plasma total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and the HDL-to-LDL ratio. Values are presented as the mean ± the standard error. $*P < 0.05$, $**P < 0.01$ versus controls. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

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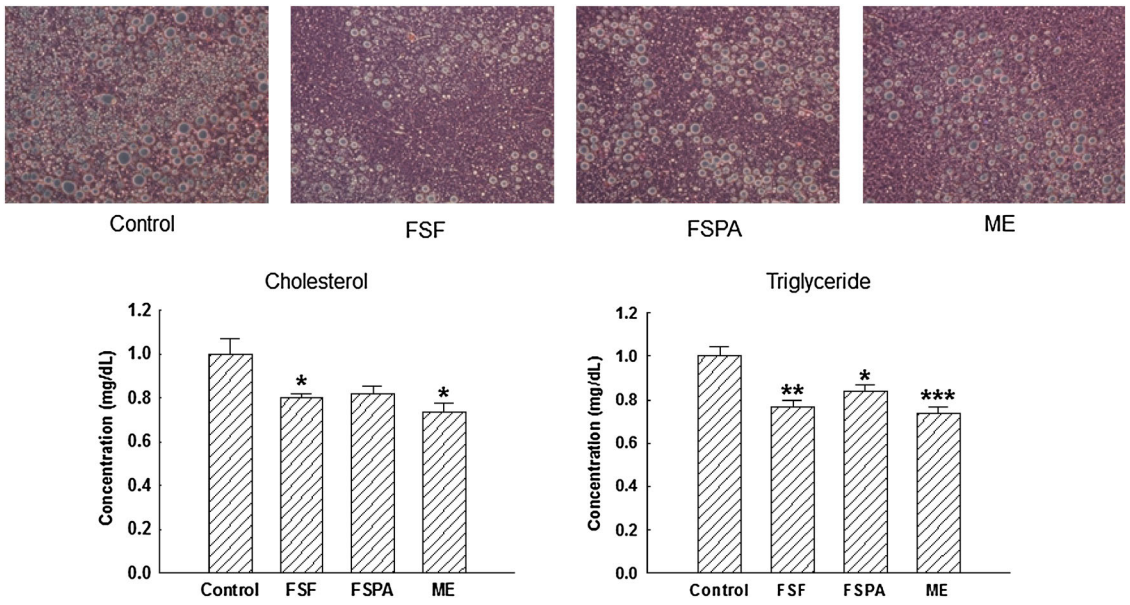

Figure 2. Hepatic lipid accumulations liver sections and hepatic lipid concentrations. Values are presented as the means ± the standard errors. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus controls.

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 1α (*Cpt1α*) 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, *Lxrα*, and *Insig-2a* were all upregulated (Fig. 3D). SREBP1c and *Lxrα* are lipogenic transcription factors, whereas *Insig-2a* inhibits SREBP1c processing, thus combined effects of FSPA on SREBP1c, *Lxrα*, 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 cholesterols 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/6 J

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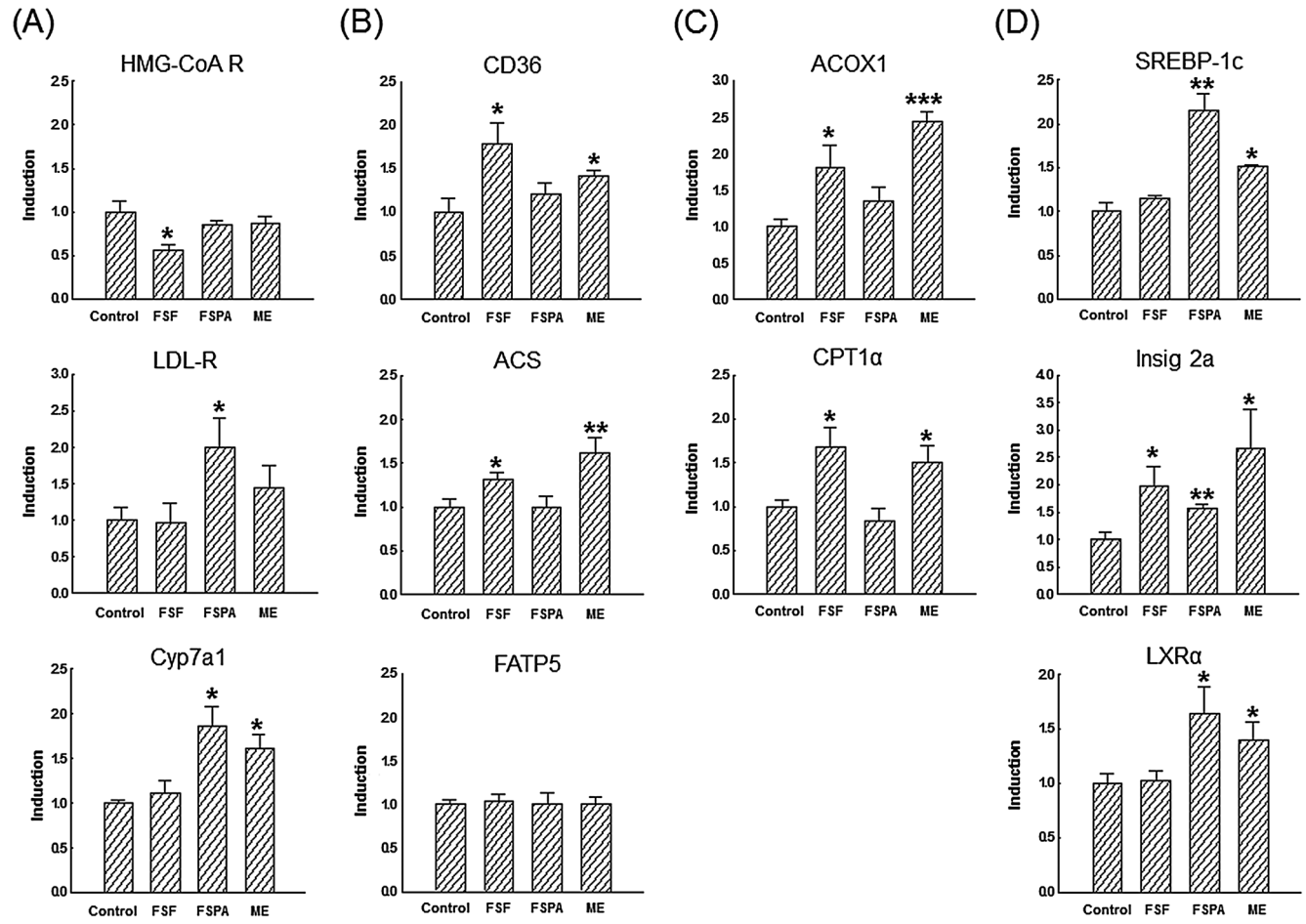

Figure 3. Expressions of lipid metabolism genes in the livers. The mRNA levels were quantified with quantitative real-time polymerase chain reaction. (A) Cholesterol metabolism, (B) fatty acid uptake, (C) fatty acid oxidation, and (D) lipogenesis. Values are presented as the means ± the standard errors.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

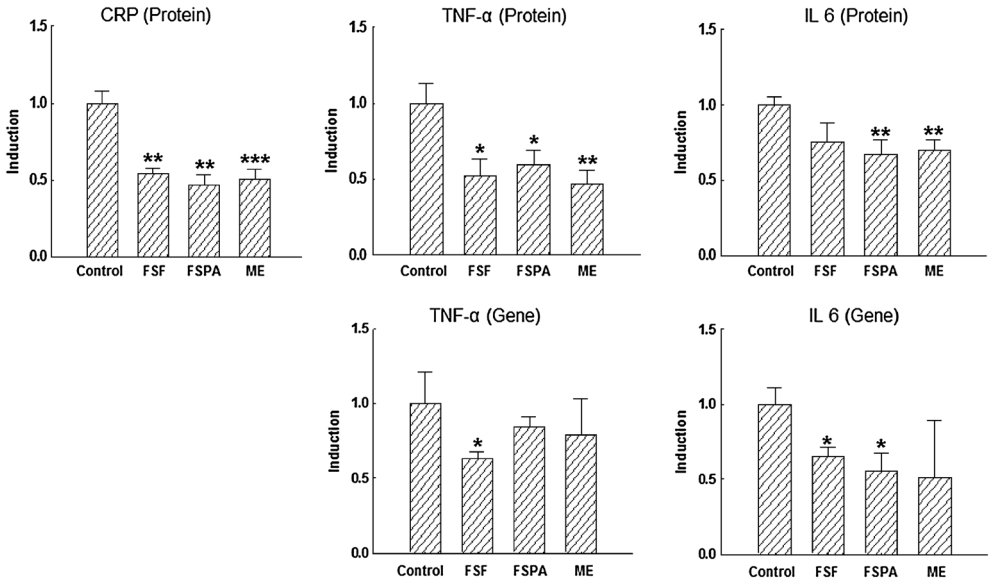

Figure 4. Expression of proinflammatory proteins and genes. The mRNA and protein expressions of the inflammation-related genes C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α) and interlukin-6 (IL-6). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus controls.

mice. 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

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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 *Cpt1α*. 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-SREPB-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

Andreozzi F, Laratta E, Cardellini M, *et al*. 2006. Plasma interleukin-6 levels are independently associated with insulin secretion in a cohort of Italian–Caucasian nondiabetic subjects. *Diabetes* **55**: 2021–2024.

Beaglehole R. 1990. International trends in coronary heart disease mortality, morbidity, and risk factors. *Epidemiol Rev* **12**: 1–15.

Birketvedt GS, Shimshi M, Erling T, *et al*. 2005. Experiences with three different fiber supplements in weight reduction. *Med Sci Monit* **11**: 8.

Brown L, Rosner B, Willett WW, *et al*. 1999. Cholesterol-lowering effects of dietary fiber: a meta-analysis. *Am J Clin Nutr* **69**: 30–42.

Byung-Suk Hur Y-HC, Kim K O, Baik E J. 2013. Production of fermentation microorganisms with starch-hydrolysing activities. Republic of Korea Patent.

Jia Y, Kim JY, Jun HJ, *et al*. 2012. The natural carotenoid astaxanthin, a PPAR-alpha agonist and PPAR-gamma antagonist, reduces hepatic lipid accumulation by rewiring the transcriptome in lipid-loaded hepatocytes. *Mol Nutr Food Res* **56**: 878–888.

Kern PA, Ranganathan S, Li C, *et al*. 2001. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab* **280**: E745–E751.

Kershaw EE, Flier JS. 2004. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* **89**: 2548–2556.

Kim D-H, Kim S-H, Kwon S-W, *et al*. 2013. Mycoflora of soybeans used for *meju* fermentation. *Mycobiology* **41**: 100–107.

King DE. 2005. Dietary fiber, inflammation, and cardiovascular disease. *Mol Nutr Food Res* **49**: 594–600.

King DE, Egan BM, Geesey ME. 2003. Relation of dietary fat and fiber to elevation of C-reactive protein. *Am J Cardiol* **92**: 1335–1339.

Kromhout D. 2001. Epidemiology of cardiovascular diseases in Europe. *Public Health Nutr* **4**: 441–457.

Lee SY, Kim HY, Lee S, *et al*. 2012. Mass spectrometry-based metabolite profiling and bacterial diversity characterization of Korean traditional *meju* during fermentation. *J Microbiol Biotechnol* **22**: 1523–1531.

Liu S, Stampfer MJ, Hu FB, *et al*. 1999. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. *Am J Clin Nutr* **70**: 412–419.

Lo G. 1989. Nutritional and physical properties of dietary fiber from soybeans. *Cereal foods world* **34**: 530–534.

Lo GS, Goldberg AP, Lim A, *et al*. 1986. Soy fiber improves lipid and carbohydrate metabolism in primary hyperlipidemic subjects. *Atherosclerosis* **62**: 239–248.

Rader DJ. 2000. Inflammatory markers of coronary risk. *N Engl J Med* **343**: 1179–1182.

Ridker PM. 2003. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* **107**: 363–369.

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Rui L, Aguirre V, Kim JK, *et al*. 2001. Insulin/IGF-1 and TNF-α stimulate phosphorylation of IRS-1 at inhibitory Ser307 via distinct pathways. *J Clin Invest* **107**: 181–189.

Schwenk RW, Holloway GP, Luiken JJFP, *et al*. 2010. Fatty acid transport across the cell membrane: Regulation by fatty acid transporters. *Prostaglandins Leukot Essent Fatty Acids* **82**: 149–154.

Sethi JK, Hotamisligil GS. 1999. The role of TNF in adipocyte metabolism. In: Seminars in Cell and Developmental Biology, 19–30.

Steffen LM, Jacobs DR, Stevens J, *et al*. 2003. Associations of whole-grain, refined-grain, and fruit and vegetable consumption with risks of all-cause mortality and incident coronary artery disease and ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Clin Nutr* **78**: 383–390.

Suh HJ, Yang HS, Ra KS, *et al*. 2013. Peyer's patch-mediated intestinal immune system modulating activity of pectic-type polysaccharide from peel of *Citrus unshiu*. *Food Chem* **138**: 1079–1086.

Unit ES. 2005. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins. *Lancet* **366**: 1267–1278.

Watzl B, Girrbach S, Roller M. 2005. Inulin, oligofructose and immunomodulation. *Br J Nutr* **93**: 49–56.

Wellen KE, Hotamisligil GS. 2005. Inflammation, stress, and diabetes. *J Clin Invest* **115**: 1111–1119.

Yecies JL, Zhang HH, Menon S, *et al*. 2011. Akt stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and independent pathways. *Cell Metab* **14**: 21–32.

Yellaturu CR, Deng X, Park EA, *et al*. 2009. Insulin enhances the biogenesis of nuclear sterol regulatory element-binding protein (SREBP)-1c by posttranscriptional down-regulation of *Insig-2a* and its dissociation from SREBP cleavage-activating protein (SCAP).SREBP-1c complex. *J Biol Chem* **284**: 31726–31734.

Yudkin JS, Stehouwer C, Emeis J, *et al*. 1999. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* **19**: 972–978.

Zhang Y, Mangelsdorf DJ. 2002. LuXuRies of lipid homeostasis: the unity of nuclear hormone receptors, transcription regulation, and cholesterol sensing. *Mol Interv* **2**: 78–87.

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.

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