JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 8617–8622 8617
DOI:10.1021/jf901878c

Hypolipidemic Effects of *Monascus*-Fermented Soybean Extracts in Rats Fed a High-Fat and -Cholesterol Diet

YOUNG-HEE PYO* AND KI-SEUNG SEONG

Department of Food and Nutrition, Sungshin Women's University, 249-1 Dongsun-Dong Sungbuk-Gu Seoul, 136-742, Republic of Korea, and Korea Food Research Institute, San 46-1, Backhyun-Dong Bundang-Gu Seongnam-Si, Gyeonggi-Do, 463-746, Republic of Korea

We evaluated whether *Monascus*-fermented soybean extracts (MFSE) enriched with bioactive mevinolins (natural statins) and aglycone isoflavones (daidzein, glycitein, and genistein) perform an additive hypolipidemic effect in hyperlipidemic rats than unfermented soybean extracts (UFSE), which have a higher level of glucoside isoflavones (daidzin, glycitin, and genistin) without mevinolin. The oral administration of MFSE (200 and 400 mg kg^{-1} body weight) significantly lowered the serum total cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-C) levels ($p < 0.01$) and raised high-density lipoprotein cholesterol (HDL-C) levels ($p < 0.05$) in hyperlipidemic rats. The MFSE group had a significantly lower 3-hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity and higher atherogenic index (calculated as HDL-C/LDL-C) when compared with the UFSE group (400 mg kg^{-1} body weight) ($p < 0.05$). Treatment with both MFSE200 and MFSE400 groups for 40 days significantly reduced the activities of serum aspartate aminotransferase and alanine aminotransferase by averages of 35.6 and 43.2%, respectively, as compared to the high-fat diet group ($p < 0.01$). The results indicate that MFSE performs a more potent hypolipidemic action via improvement of the lipid profiles and down-regulated HMG-CoA reductase activity than UFSE in hyperlipidemic rats.

KEYWORDS: ***Monascus*-fermented soybean; natural statins; isoflavones; HMG-CoA reductase; hypolipidemic effect**

INTRODUCTION

Atherosclerotic cardiovascular disease (CVD) is the main cause of mortality in humans (*1*). The World Health Organization (WHO) predicted that heart disease and stroke are becoming more deadly, with a projected combined death toll of 24 million by 2030 (*2*). The causal relationship between blood cholesterol level and atherosclerosis is widely accepted, as there is already considerable interest in the possibility of using drugs to decrease cholesterol levels (*3*).

HMG-CoA reductase (EC1.1.1.34) is the rate-limiting enzyme in the biosynthesis of cholesterol in mammals (*4*). Thus, HMG-CoA reductase inhibitors (statin class of drugs; lovastatin, simvastatin, pravastatin, etc.) have become standard medical therapy in the armamentarium available for the prevention and treatment of CVD (*5*). The search for new drugs capable of reducing and regulating serum cholesterol and triglyceride (TG) levels has gained momentum over the years, resulting in numerous reports on significant activities of natural agents (*6*). Natural products are frequently considered to be less toxic and more free from side effects than synthetic agents. Currently, instead of using hypolipidemic medicine to treat abnormal lipidemia in patients who are initially diagnosed with hyperlipidemia, dietary therapy, especially long-term modification of dietary behavior, is offen suggested to decrease and control blood lipids (*7*).

The *Monascus* species have been widely used as a traditional food fungus in Eastern Asia for several centuries (*8*). Since the worthful secondary metabolite—monacolin K (also known as mevinolin and mevacor; natural statins)—was proven as the inhibitor of HMG-CoA reductase (*8*, *9*), *Monascus*-fermented products have been used as functional dietary supplements to reduce the cholesterol levels in the human body (*10*, *11*).

The consumption of soybean has been demonstrated to reduce circulating cholesterol levels, most notably reducing low-density lipoprotein cholesterol levels in hypercholesterolemic individuals (*12*, *13*). However, it is still unclear what component of soybean has a beneficial effect on plasma lipid and how soybeans act to lower cholesterol and lipids. The enhancement of bile excretion, thyroid hormonal changes, and changes in glucose hormonal homeostasis have all been hypothesized (*12*), and special emphasis has been attributed to the soy content of isoflavones, cotyledon fibers, and phospholipids (*13*). Among them, soy isoflavones having both weak estrogenic and antiestrogenic activities may partly be responsible for the cholesterol-lowering and cardioprotective effects (*13*−*15*). In particular, dietary isoflavone aglycones, as compared with isoflavone glucosides, may highly be attributed to a reduction in plasma cholesterol and increase of high-density lipoprotein cholesterol levels (*16*, *17*). Kawakami et al. (*16*) reported that soy isoflavone

*To whom correspondence should be addressed. Tel: +2-920-7588. Fax: +2-920-2076. E-mail: rosapyo@sungshin.ac.kr or rosapyo@hotmail.com.

Published on Web 08/21/2009

aglycones may exert a beneficial effect on lipid metabolism in rats fed cholesterol due to the fact that they are more active and more readily absorbed than their β-glycosides. However, the study concerning the bioavailability of soy isoflavones has remained controversial (*18*).

We recently found that soybean fermented with *Monascus pilosus* has a remarkable content of bioactive isoflavone aglycones [1.52 mg/g dry weight (DW) of soybean] and natural statins, mevinolins (3.64 mg/g DW of soybean) (*19*, *20*). Also, it was observed in vitro that *Monascus*-fermented soybean extracts (MFSEs) not only have the potential for strong free radical scavenging effects but also antihypertensive properties (*20*). Thus, we hypothesized that MFSE enriched with natural statins, mevinolin, and aglycone isoflavones (daidzein, glycitein, and genistein) may have additive hypolipidemic and antiatherosclerotic effects in hyperlipidemic rats when compared with the unfermented soybean extracts (UFSEs), which have only a higher level of glucoside isoflavones (daidzin, glycitin, and genistin; 1.35 mg/g DW of soybean) without mevinolin. Therefore, we evaluated whether MFSE performs a more potent hypolipidemic effect than UFSE in rats fed a high-fat and -cholesterol diet. The serum and liver lipid levels and hepatic HMG-CoA reductase activity were used as the markers of hypolipidemic effects. In addition, to have a measure of the effects of the MFSE on the liver function of the treated rats, the serum aspartate transaminase (AST) and alanine transaminase (ALT) were measured relative to MFSE-untreated control rats.

MATERIALS AND METHODS

Chemicals and Reagents. Mevinolin (statin), daidzein, and genistein were purchased from Sigma (St. Louis, MO). Daidzin, genistin, glycitin, glycitein, and cholesterol were obtained from Funakoshi Chemical Co. (Tokyo, Japan). Liquid chromatography (LC) grade acetonitrile, chloroform, methanol, and dimethyl sulfoxide (DMSO) were purchased from Merk Co. (Darmstadt, Germany). Tryptone, yeast extract, peptone, malt extract, potato dextrose agar (PDA), and general aerobic medium (MRS) were purchased from Difco Co. (Detroit, MI). All other reagents were of the highest grade available unless otherwise indicated.

Preparation of MFSE. MFSE was produced as described elsewhere (*19*, *20*). A two-stage fermentation process was used to produce bioactive compounds, which included a seed culture stage (liquid culture) and a metabolite production stage (solid state fermentation, SSF). The fungal strain was maintained on Petri dishes of PDA and incubated at 30 °C for 7 days. After cultivation, colonies of spores that appeared on the plates were transferred and inoculated into nutrient broth and incubated at 30 °C for 4 days with shaking at 150 rpm for seed culture. Preparation of the soybean medium for SSF proceeded as follows: In brief, whole soybeans were washed, soaked, and autoclaved. After they were cooled, the substrate was inoculated with nutrient broth including *M. pilosus* and incubated at 30 °C for 20 days. Either *Monascus*-fermented or unfermented soybeans were collected, lyophilized, and powdered. A subsample (100 g) was extracted with 1 L of 80% ethanol (v/v) for 5 h, three times, and filtered through Whatman #4 filter paper. The combined extracts were then rotary evaporated at 40 °C and lyophilized. The dried extract was used directly for the study.

Quantification of Isoflavones and Statins. The contents of isoflavones and statins (mevinolins) in MFSE were determined by high-performance liquid chromatography (HPLC) as described by Pyo et al. (*19*) with minor modifications. For the extraction of the isoflavones and mevinolins, 0.1 g of ground sample in 1 mL of 80% ethanol was vigorously shaken and extracted at room temperature for 20 min using an ultrasonicator (Bransonic, Danbury, CT). The extract was centrifuged at 12000*g* for 15 min, and the supernatant was filtered through a syringe filter (0.22 μm, Waters Co., Milford, MA) prior to HPLC analysis. Reversed phase HPLC analysis was carried out with a JASCO system (Tokyo, Japan), using a YMC AM 303 ODS-A column (4.6 mm × 250 mm, Kyoto, Japan). The mobile phase for isoflavones analysis was composed of 0.1% phosphoric acid in acetonitrile (solvent A) and 0.1% phosphoric acid in water (solvent B). Following the injection of 20 μL of sample, solvent A was increased from 15 to 35% over 50 min and then held at 35% for 10 min. The solvent flow rate was 1 mL/min, and the eluted isoflavones were detected at 254 nm. Individual isoflavone standards were used for peak identification according to elution time, UV spectra, and spiking tests. Isoflavone quantification was based on calibration curves for each of the standards. The statins in MFSE were analyzed using an isocratic solvent system with the mixture of 0.1% phosphate buffer (pH 7.7) and acetonitrile (65:35, v/v) as the mobile phase (*19*). The solvent flow rate was 0.8 mL/min, and eluted statins were detected at 238 nm. Quantitative data for statins were obtained by comparison to known standards. Each sample was analyzed in triplicate, and mean values were reported.

Animals and Treatment. SD male rats (Daehan Experiment Animal Center, Eumseong, Korea), 4 weeks of age, were used in the present study. They were housed in plastic cages with wood shavings under controlled conditions (24 ± 0.5 °C; humidity, 55 ± 5%; and 12 h of light from 08:00 to 20:00) and maintained according to the Guide for the Care Use of Laboratory Animals established by Korea Food Research Institute (KFRI, Seongnam, Korea). After acclimation for 1 week, they were randomly assigned into five groups (n = 10): two control and three treatment groups. The control group (regular diet, RD) was fed Purina rat chow diet (Ralston Purina, St. Louis, MO), while the other 40 rats were fed a hyperlipidemic diet [Purina rat chow diet (78.8% w/w) supplemented with pig oil (10% w/w), powdered egg yolk (10% w/w), cholesterol (1% w/w), and bile salt (0.2% w/w)]. After 10 days, animals fed the hyperlipidemic diet were bled to measure their lipid levels. All rats met the hyperlipidemic criteria (*21*) and were enrolled into the study by randomly dividing them into four groups of 10 rats each. The first group served as a high-fat diet control group (HF). The three treatment groups were given daily oral administrations (20 mL/kg) of either UFSE (400 mg kg^{-} body weight, UFSE400) or two MFSE groups (200 and 400 mg kg^{-} body weight, MFSE200 and MFSE400). The normal chow-fed control group (RD) and the untreated hyperlipidemic group (HF) were given oral administrations of distilled water. After 40 days of treatment, the animals were fasted for 12 h, and then, blood was taken from the eye socket for serum lipid analysis. The serum was prepared and frozen at −70 °C until analyzed. The body weights were measured every 3 days, and the daily food intake was recorded.

Serum and Liver Lipid Analysis. Serum total cholesterol (TC), triglyceride (TG) and HDL-C levels were measured in triplicate using commercial enzymatic kits. These kits were as follows: the TC assay kit (CH 200, Randox Laboratories Ltd., Antrim, United Kingdom), the TG assay kit (TR-210, Randox Laboratories Ltd.), and the HDL-C assay kit (CH-203, Randox Laboratories Ltd.). Serum LDL-C levels were gained via the following calculation (*16*): LDL-C (mg/dL) = TC − TG/5 − HDL-C. Liver tissue (0.5 g) was ground in 10 mL of ice-cold Folch (*22*) solution (chloroform:methanol = 2:1; v/v) and incubated for 30 min at room temperature. The aqueous layer was aspirated and discarded, and the fixed volume of the organic layer was then evaporated to dryness. The dried lipid layer was dissolved with an equal volume of DMSO and then used to determine the TC and TG levels using the same enzymatic kit used for the serum analysis.

Determination of Fecal Lipid and Bile Acid. Fecal lipids were extracted according to the method of Folch et al. (*22*), and TC and TG were measured using the same enzymatic kit used for the serum analysis. Fecal bile acid was extracted according to the method of DeWeal et al. (*23*) and determined with the aid of bile acid assay kits (Kyokudo, Tokyo, Japan).

Activities of ALT and AST. The activities of AST and ALT in serum were measured with the aid of an enzymatic kit (Shinyang Chemical, Korea).

HMG-CoA Reductase Activity. Hepatic HMG-CoA reductase activity was indirectly measured based on the HMG Co-A/mevalonate ratio (*24*). HMG Co-A was determined by its reaction with hydroxylamine hydrochloride at alkaline pH and subsequent colorimetric measurement of the resulting hydroxamic acid by formation of complexes with ferric salts at 540 nm. Mevalonate was estimated by reaction with the same reagent but at pH 2.1. At this pH, the lacton form of mevalonate readily reacts with hydroxylamine hydrochloride to form the hydroxamate. The analytical procedure is as follows. Mix equal volumes of the fresh 10% liver tissue

Table 1. Concentrations[a] of Mevinolin, Isomeric Isoflavones, and GABA in MFSEs and UFSEs[b]

	MFSE	UFSE
mevinolins	3635.6 ± 68.4	ND
aglycones isoflavone[c]	1515.1 ± 59.2 a	391.6 ± 18.2 b
glucosides isoflavone[d]	492.8 ± 24.2 c	1559.2 ± 69.7 d
GABA[e]	785.4 ± 32.6 e	315.2 ± 21.5 f

[a] μg/g DW. [b] Each value is the mean ± SD (n = 3). ND, not detected. Within a row, data with different letters are significantly different ($p < 0.01$). [c] Aglyconic isoflavones; daidzein + glycitein + genistein. [d] Glucosidic isoflavons; daidzin + glycitin + genistin. [e] GABA content was cited from ref *25*.

homogenate and diluted perchloric acid. Allow this mixture to stand for 5 min and centrifuge (2000 rpm, 10 min). Treat 1.0 mL of filtrate with 0.5 mL of freshly prepared hydroxylamine reagent (alkaline hydroxylamine reagent in the case of HMG-CoA) and mix, and after 5 min, add 1.5 mL of ferric chloride reagent to the same tube and shake well. Take readings after 10 min at 540 nm vs a similarly treated saline/arsenate blank. The ratio between HMG-CoA and mevalonate is inversely proportional to HMG-CoA reductase activity; that is, an increase in ratio indicates decreased activity.

Statistics. Data were expressed as means ± standard deviations (SDs) from three independent parallel experiments. Statistical analyses were performed using the Statistical Analysis System (SAS Institute, Cary, NC). Analysis of variance (ANOVA) and Duncan's multiple range test were used to determine significant difference among means; a p value (< 0.01 and < 0.05) was selected as the limit of statistical significance.

RESULTS AND DISCUSSION

Isoflavones and Mevinolins Contents in MFSE. The contents of isoflavones and mevinolins in MFSE were analyzed by HPLC. Both isoflavones and mevinolins were determined in the 80% ethanol extracts from *Monascus*-fermented soybean or unfermented soybean. As shown in **Table 1**, MFSE contained more isoflavone aglycones (1515.1 ± 59.2 μg/g DW) as well as mevinolin (3635.6 ± 68.4 μg/g DW) when compared with UFSE (isoflavone aglycones, 391.6 ± 18.2 μg/g DW; mevinolin was not detected). Also, our previous study found that MFSE had more γ-amino butyric acid (GABA) by 2.5 times as compared with that of the UFSE (*25*). GABA is known to be one of the major inhibitory neurotransmitters in the sympathetic nervous system and to play an important role in cardiovascular function (*26*). Accordingly, it is supposed that the cooperative interaction between various functional components in MFSE could play important roles in regulating lipid metabolism. Among them, the major active compounds in MFSE for hypolipidemic effect are assumed mainly mevinolin. The natural statin, mevinolin (also known as lovastatin), is a secondary metabolite of *Monascus* species, which inhibits the activity of HMG-CoA reductase in cholesterol biosynthesis, that is, the conversion of HMG-CoA to mevalonate, catalyzed by HMG-CoA reductase (*5*, *9*).

Effect of MFSE on the Growth and Development of the Rats. The effects of MFSE treatment on food intake and body weight gain are presented in **Table 2**. Rats in the HF group had the highest weight gain, while MFSE400 group had the lowest weight gain ($p < 0.05$). Despite similar food intakes and body weights at the beginning of the study, both HF + MFSE200 and HF + MFSE400 groups weighed less at the end of the study than the HF + UFSE400 group. Among MFSE-treated groups, body weight gain was decreased in a dose-dependent manner. This weight change was accompanied by concomitant decreases in the food efficiency ratio. However, there were no significant differences found in weight gain, indicating that the administration of MFSE to the rats had no toxic effect at the tested doses. The high-fat and -cholesterol diet apparently caused an increase in the body weight of rats despite their lower food intake. This is probably due to supplementation with cholesterol and lipid. The HF control group in the present study was induced in rats by adding cholesterol (1%), egg yolk (10%), pig oil (10%), and bile salt (1%) to the diet for 40 days.

Table 2. Effect of MFSEs on the Body Weight and Food Intake in Rats Fed a High-Fat and -Cholesterol Diet

groups	food intake (g/rat/day)	weight gain (g/rat/day)	feed gain ratio[a]
RD	28.8 ± 2.7 a	8.5 ± 1.1 a	0.29 ± 0.06 a
HF	25.8 ± 5.2 a	10.5 ± 1.7 b	0.41 ± 0.09 b
HF + UFSE400	26.4 ± 3.3 a	7.2 ± 0.8 a	0.27 ± 0.04 a
HF + MFSE200	24.4 ± 4.5 a	6.9 ± 0.9 a	0.28 ± 0.05 a
HF + MFSE400	25.2 ± 2.9 a	6.2 ± 0.8 a	0.25 ± 0.06 a

[a] g weight gain per day/g feed per day. The data are expressed as means ± SDs (n = 3). RD, regular diet control group; HF, high-fat diet control group; UFSE, HF + 400 mg dose (kg^{-1}body weight) of UFSE group; and MFSE200 or 400, HF + 200 or 400 mg dose of MFSE group, respectively. Within a column, data with different letters are significantly different ($p < 0.05$).

Table 3. Effect of MFSEs on Hepatic Enzyme Activities in Rats Fed a High-Fat and -Cholesterol Diet for 40 Days[a]

	AST (karmen units)	ALT (karmen units)	HMG-CoA reductase / mevalonate ratio
RD	25.8 ± 6.3 a	20.6 ± 3.8 a	3.8 ± 0.4 a
HF	41.6 ± 9.5 b	38.4 ± 5.7 b	4.9 ± 0.8 b
HF + UFSE400	30.2 ± 5.2 c	27.7 ± 4.8 c	4.1 ± 0.6 a
HF + MFSE200	27.1 ± 6.1 a	20.5 ± 6.9 a	5.3 ± 0.7 b
HF + MFSE400	26.5 ± 5.7 a	23.1 ± 7.3 a	6.8 ± 0.6 c

[a] The data are expressed as means ± SDs (n = 3). RD, regular diet control group; HF, high-fat diet control group; UFSE, HF + 400 mg dose (kg^{-1} body weight) of UFSE group; and MFSE200 or 400, HF + 200 or 400 mg dose of MFSE, respectively. Within a column, data with different letters are significantly different ($p < 0.05$, and $p < 0.01$).

Effect of MFSE on Hepatic Enzymes. The effect of MFSE on liver function was evaluated by measuring the serum transaminase of ALT and AST. These enzymes leak into the circulation when hepatocytes are damaged (*27*). It is believed that high serum cholesterol levels can cause liver damage. As shown in **Table 3**, animals fed a high-fat and -cholesterol diet exihibited an elevation in serum AST and serum ALT as compared to RD groups ($p < 0.01$). However, rats treated with MFSE had lower serum ALT and AST levels as compared to rats of UFSE ($p < 0.05$) as well as the HF group ($p < 0.01$). Treatment with both MFSE200 and MFSE400 groups for 40 days significantly reduced the activities of AST and ALT by averages of 35.6 and 43.2%, respectively, as compared to the HF group ($p < 0.01$). The hepatic ALT activity was slightly lower in the MFSE200 as compared to the MFSE400 group, but the AST activity was not different among the MFSE groups (**Table 3**). The lowering effect of the MFSE groups on AST and ALT activities suggests that treatment with MFSE prevents the hepatic damage caused by hyperlipidemia.

The HMG-CoA reductase activity was indirectly measured in terms of the ratio between HMG-CoA and mevalonate (*24*). The ratio is inversely proportional to HMG-CoA reductase activity, meaning that an increase in the ratio will indicate a decrease in the enzyme activity. Estimation of hepatic HMG-CoA reductase activity showed a significant decrease (by 1.4 times) in MFSE-treated hyperlipidemic rats ($p < 0.05$) (**Table 3**). In particular, hepatic HMG-CoA reductase was significantly lower by 39.7% in the MFSE400 group than in the UFSE group ($p < 0.01$). The result suggests that MFSE-treated rats showed a further decrease in cholesterol biosynthesis due to its lower HMG-CoA reductase activity, indicating the influence of MFSE on this enzyme. The rats of the UFSE group also showed a slight increase in

Table 4. Effect of MFSEs on the Levels of Serum and Hepatic Lipids of Rats Fed a High-Fat and -Cholesterol Diet for 40 Days

	RD	HF	HF + UFSE400	HF + MFSE200	HF + MFSE400
			serum lipid level		
TC (mg/dL)	49.1 ± 4.4 a	69.7 ± 6.8 b	58.7 ± 7.4 c	46.4 ± 5.2 a	48.6 ± 4.8 a
TG (mg/dL)	49.2 ± 7.7 a	64.1 ± 9.0 b	57.2 ± 7.9 c	48.5 ± 6.1 a	45.7 ± 5.9 a
HDL-C (mg/dL)	17.2 ± 3.5 a	12.0 ± 2.8 b	12.9 ± 3.7 b	16.7 ± 2.1 a	15.2 ± 1.9 a
LDL-C (mg/dL)	10.1 ± 2.4 a	14.8 ± 1.5 b	13.1 ± 1.8 b	10.8 ± 2.4 a	9.9 ± 1.2 a
			liver lipid level		
TC (μmol/g liver)	18.9 ± 4.6 a	25.8 ± 6.7 b	21.6 ± 3.9 b	19.8 ± 6.1 a	16.8 ± 4.1 a
TC (μmol/whole liver)	34.3 ± 5.1 a	43.2 ± 7.1 b	37.4 ± 3.7 c	34.3 ± 3.9 a	31.6 ± 2.9 a
TG (μmol/g liver)	26.4 ± 2.9 a	36.4 ± 5.1 b	30.5 ± 4.3 c	25.7 ± 2.7 a	24.7 ± 3.1 a
TG (μmol/whole liver)	28.3 ± 2.9a	44.4 ± 5.1 b	36.8 ± 4.3 c	31.7 ± 2.7 a	30.7 ± 3.1 a
			hepatosomatic index		
%[a]	4.4 ± 0.2 a	5.1 ± 0.6 b	4.9 ± 0.4 a	4.5 ± 0.1 a	4.3 ± 0.5 a

[a] Relative liver weight: (liver weight/body weight) × 100%. The data are expressed as means ± SDs (n = 3). RD, regular diet control group; HF, high-fat diet control group; UFSE, HF + 400 mg dose (kg^{-1} body weight) of UFSE group; and MFSE200 or 400, HF + 200 or 400 mg dose of MFSE group, respectively. Within a row, data with different letters are significantly different ($p < 0.05$, and $p < 0.01$).

HMG-CoA/mevalonate ratio as compared to rats of RD group after 40 days of treatment. Similar results were observed in Korean soybean paste (Doenjang), which is fermented by diverse microorganisms including fungi and bacilli during manufacture (*28*). Sung et al. (*28*) demonstrated that HMG-CoA reductase inhibitors in soybean paste identified the active components to be genistein, daidzein, and glycitein, aglycone forms of the isoflavones. The discovery of isoflavones as HMG-CoA reductase inhibitors, not yet reported elsewhere, in soy foods is intriguing. There was no significant difference between the UFSE and the RD groups regarding the enzyme activity.

Effect of MFSE on Serum and Liver Lipid Profile. The general effect of MFSE on serum lipid levels was examined in rats fed a high-fat and -cholesterol diet. Before inducing hyperlipidemia, there was no significant difference in serum lipid levels of rats of the five groups. The serum TC and TG in the HF group was higher than the RD group from 10 days ($p < 0.05$), indicating that the high-fat feed had caused the hyperlipidemia. Throughout the study, the TC and TG in both the MFSE and the UFSE groups were lower than the HF group (**Table 4**). Particularly, the TC and TG contents of MFSE200 group decreased by 21.0 and 15.2%, respectively, than those of the UFSE400 group ($p < 0.05$). These results indicated that UFSE reduced serum TC and TG levels in hyperlipidemic rats but did not reach the level of the MFSE groups. By the second week and throughout the study, the MFSE groups showed a decrease in TG and TC. After 40 days (**Table 4**), the serum TC in the groups administered with MFSE decreased by 33.4 (200 dose) and 30.3% (400 dose), and their TG content decreased by 24.3 (200 dose) and 28.7% (400 dose) as compared to the HF group ($p < 0.01$). The TG content in the serum of the MFSE400 group was much lower than that of the UFSE400 group ($p < 0.05$) and was also slightly lower than the RD group. On the basis of our data, the high concentration of LDL-C and TC in hyperlipidemia rats was significantly reduced by oral administration of MFSE ($p < 0.01$).

Another risk factor for developing atherosclerosis is the reduced serum level of HDL-C. This effect of HDL-C is largely attributed to its central function in the reverse cholesterol transport, a process whereby excess cell cholesterol is taken up and processed by HDL particles for further delivery to the liver for metabolism (*29*, *30*). Therefore, it is logical that an increase in HDL level can contribute to a lower risk of atherosclerosis (*30*). In the present study, the serum levels of HDL-C increased by 39.2 and 26.7%, respectively, when compared with HF groups.

Table 5. AI Levels of Each Groups during the Experimental Period

	AI[a] level of the time course (day)			
treatment group	10	20	30	40
RD	0.76 ± 0.09 a	0.80 ± 0.07 a	0.67 ± 0.09 a	1.70 ± 0.14 a
HF	0.78 ± 0.11 a	0.73 ± 0.17 a	0.78 ± 0.16 b	0.81 ± 0.13 b
HF + UFSE400	1.01 ± 0.12 b	1.07 ± 0.20 b	0.99 ± 0.16 c	0.98 ± 0.18 c
HF + MFSE200	1.12 ± 0.19 c	1.24 ± 0.15 c	1.37 ± 0.13 d	1.55 ± 0.16 d
HF + MFSE400	1.15 ± 0.15 c	1.21 ± 0.18 c	1.35 ± 0.19 d	1.54 ± 0.18 d

[a] AI, calculated as HDL-C/LDL-C. The data are expressed as means ± SDs (n = 3). Within a column, data with different letters are significantly different ($p < 0.05$). RD, regular diet control group; HF, high-fat diet control group; UFSE, HF + 400 mg dose (kg^{-1} body weight) of UFSE group; and MFSE200 or 400, HF + 200 or 400 mg dose of MFSE group, respectively.

Also, our results clearly showed that MFSE is capable of increasing the serum level of HDL-C in the treated rats relative to UFSE group rats ($p < 0.05$). In many studies, a small increase in HDL-C is observed after feeding soybean or isoflavones alone (*16*, *31*), whereas in others either no change or a small decrease in HDL-C is observed (*32*, *33*).

Treatment of hyperlipidemic rats with MFSE at a dose of 200−400 mg/kg body weight for 40 days lowered the serum LDL-C levels. The serum levels of LDL-C in the MFSE200 and MFSE400 group decreased by 27.0 and 33.1%, respectively, as compared to HF groups ($p < 0.01$). The UFSE400 group had also performed a significant hypocholesterolemic effect and a hypotriglyceridemia effect in serum ($p < 0.05$), as compared with the HF group. However, the same 400 mg dose (kg^{-1} body weight) of MFSE resulted in a more significant hypocholesterolemic and a hypotriglyceridemic effect of $p < 0.01$. Furthermore, the effect on an increase in the ratio of HDL-C/LDL-C was performed more significantly in MFSE200 than in UFSE400 ($p < 0.05$). The result can suggest that MFSE had a greater hypolipidemic effect than UFSE. As shown in **Table 1**, MFSE includes higher isoflavone aglycones by 3.9-fold than UFSE, which could be one of the reasons why MFSE has a more hypolipidemic effect than UFSE. A similar observation was found in some studies using other animal species such as hamster (*31*) and rabbits (*32*) and in human (*34*) experiments. Especially dietary isoflavone aglycones, as compared with isoflavone glucosides, may highly be attributed to a reduction in plasma cholesterol and an increase of HDL-C levels (*16*). Differences of bioavailability or speed of absorption between isoflavones aglycone and glucoside may be associated

Table 6. Effect of MFSEs on the Fecal Excretions of Lipid and Bile Acid in Hyperlipidemic Rats[a]

	RD	HF	HF + UFSE400	HF + MFSE200	HF + MFSE400
total lipid (mg/day)	158.8 ± 13.8 a	215.5 ± 19.2 b	226.7 ± 19.2 b	295.2 ± 12.9 c	314.8 ± 14.6 c
TC (mg/day)	51.4 ± 8.1 a	89.2 ± 9.9 b	92.3 ± 8.8 b	112.6 ± 17.5 c	120.5 ± 19.2 c
TG (mg/day)	12.5 ± 5.5 a	14.1 ± 2.1 a	15.4 ± 7.9 a	18.3 ± 2.6 b	19.8 ± 6.2 b
bile acid (μmol/day)	42.1 ± 4.9 a	65.9 ± 6.6 b	68.9 ± 9.7 b	81.2 ± 8.8 c	78.1 ± 9.1 c

[a] The data are expressed as means ± SDs (n = 3). RD, regular diet control group; HF, high-fat diet control group; UFSE, HF + 400 mg dose (kg^{-1} body weight) of UFSE group; and MFSE200 or 400, HF + 200 or 400 mg dose of MFSE group, respectively. Within a row, data with different letters are significantly different ($p < 0.05$, and $p < 0.01$).

with differences of regulative function of dietary soy isoflavones on lipid metabolism (*16*, *17*). However, the absorptive rate and speed of isoflavone from the intestine in rats are still well unknown. In general, isoflavones mainly exist in glycone forms in nonfermented soy foods, whereas in fermented soy foods, they exist in aglycone forms. Although MFSE has been proven to exhibit a greater hypolipidemic effect in this study, the dose−response was not obvious.

The atherogenic index (AI), defined as the ratio of HDL-C/LDL-C, is believed to be an important risk factor for the diagnosis of atherosclerosis (*30*). As shown in **Table 5**, the AI was decreased significantly after keeping the animals on a high-fat diet ($p < 0.05$). However, it significantly increased by 1.9 and 1.6 times in MFSE200-treated rats as compared to rats of HF and UFSE groups, respectively. MFSE is capable of potentially increasing the AI levels after 10 days of oral administration. Thus, MFSE might constitute a good candidate for the treatment of atherosclerotic CVD by lowering serum LDL-C levels via improving the serum lipid profile.

MFSE treatment at the levels of 200 and 400 mg dose (kg^{-1} body weight) decreased hepatic cholesterol and TG levels (**Table 4**). Also, MFSE treatment groups decreased relative liver weight expressed as hepatosomatic index (calculated as liver weight/body weight). Thus, we calculated hepatic cholesterol and TG levels in the whole liver and per g liver. Rats in the HF group had significantly higher liver TC and TG as compared to RD ($p < 0.05$). However, MFSE groups could significantly decrease hepatic cholesterol and TG levels expressed as μM/liver ($p < 0.05$). Furthermore, the MFSE groups had all lower liver TG and TC than the UFSE group ($p < 0.05$) and were similar to the RD group. In addition, as shown in **Table 4**, the liver indexes in MFSE groups significantly differed from that in HF group ($p < 0.05$). With an increase in MFSE doses, the TC levels in the whole liver decreased significantly by 20.6 and 26.9% ($p < 0.05$), and the TG levels decreased by 28.6 and 30.9% ($p < 0.01$), respectively, as compared to the HF group. It is hypothesized that the lower liver cholesterol found in rats after MFSE feeding might be related to cholesterol absorption. The decrease in cholesterol balance, which indicates the total change in body pools of cholesterol, may be due to the compensatory mechanisms, such as a decrease in resorption of endogenous cholesterol or an increase in the rate of secretion into the intestinal tract or both (*33*). As shown in **Table 6**, fecal bile acid excretion was significantly greater in MFSE groups than that of UFSE group ($p < 0.05$). Furthermore, higher fecal cholesterol levels were observed after MFSE400 feeding. This suggests that MFSE may influence hepatic cholesterol metabolism by affecting cholesterol absorption, which might lead to a lower liver weight as well as lower lipid and cholesterol levels in rat liver.

Effects of MFSE on Fecal Excretions of Lipids and Bile Acid. At the beginning of the experiment, there were no obvious differences across groups. However, significantly higher fecal total lipid, TC, and TG contents were found in the MFSE group as compared to the HF group (**Table 6**). The excretion of fecal lipid in the UFSE group tended to be higher than in the HF group, although it did not differ significantly between two groups at the end of the experiment. However, fecal excretion of total lipid and TG was markedely increased (by 28.0 and 22.2%, respectively) in the MFSE400 groups as compared with the UFSE group, and more bile acid excretion (15.1% increase) was found in the MFSE200. Fecal cholesterol excretion was 26.0% greater in the MFSE400 group when compared with the HF group ($p < 0.01$). In the recent report, combination therapy with HMG-CoA reductase inhibitor and bile acid sequestrants has been demonstrated to be more effective in human hyperlipidemic patients than either agent in monotherapy (*35*). Clausen et al. (*35*) observed that Abacor, a newly developed soy-based dietary supplement, further reduces plasma cholesterol concentrations when given to hypercholesterolemic patients in statin treatment. Hence, a result of the potential importance in the present study is that additive and synergistic effects between bioactive components such as isoflavones and mevinolin in MFSE may be responsible for the potent hypolipidemic effects in the rat fed a high-fat and -cholesterol diet. Therefore, a probable mechanism for the greater hypolipidemic effect in MFSE-treated rats can be explained on the basis of a dual cholesterol inhibition, that is, the blocking of intestinal absorption by some components such as soy cotyledon fiber (*13*) and the decreasing of endogenous biosynthesis by natural statins and aglyconic isoflavones in MFSE. Further elaborated work is in progress to prove this claim.

In conclusion, the oral administration of MFSE (200 and 400 mg/kg body weight) was able to significantly lower the TC, TG, and LDL-C levels ($p < 0.01$) as well as raise the HDL-C levels ($p < 0.05$) in hyperlipidemic rats. Also, the MFSE group had significantly lower HMG-CoA reductase activity as compared with the UFSE group ($p < 0.05$). It is presumed that a higher hypolipidemic effect of MFSE might be accounted for the combined effect of mevinolin and isoflavones and other bioactive compounds such as GABA. On the basis of these data, it is concluded that the MFSE has definite cardioprotective potential, and these results constitute a valid scientific basis for consuming *Monascus*-fermented soybean for a functional dietary supplement and medicinal application. Further studies are needed to investigate the interaction of soy isoflavones with mevinolin or the synergistic action of these components in their contribution to the hypolipidemic activity of MFSE.

LITERATURE CITED

(1) Castell, W. P. Epidemiology of coronary heart disease. The Framingham study. *Am. J. Med.* **1984**, *76*, 4–12.
(2) Reinhardt, E. Health Watch: The atlas of heart disease and stroke. *UN Chronicle Online* **2005**, 1–4.
(3) Wood, D.; De Backer, G.; Faergeman, O.; Graham, I.; Mancia, G.; Pyorala, K. Prevention of coronary heart disease in clinical practice. Recommendation of the second joint task force of european and other societies on coronary prevention. *Atherosclerosis* **1998**, *140*, 199–270.
(4) Krause, B. R; Newton, R. S. Animal models for the evaluation of inhibitors of HMG-CoA reductase. *Adv. Lipid Res.* **1991**, *1*, 57–72.

(5) Blum, C. B. Comparison of properties of four inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *Am. J. Cardiol.* **1994**, *73*, 3D–7D.
(6) Chen, Z. Y.; Jiao, R.; Ma, K. Y. Cholesterol-lowering nutraceuticals and functional foods. *J. Agric. Food Chem.* **2008**, *56*, 8761–8773.
(7) Anderson, J. W. Diet first, then medication for hypercholesterolemia. *J. Am. Med. Assoc.* **2003**, *290*, 531–533.
(8) Endo, A. Monacolin-K, a new hypocholesterolemic agent produced by *Monascus* species. *J. Antibiot.* **1979**, *32*, 852–854.
(9) Endo, A. HMG-CoA reductase inhibitors. *Nat. Prod. Rep.* **1993**, *10*, 541–550.
(10) Heber, D.; Yip, I.; Ashley, J. M.; Elashoff, D. A.; Go, V. L. W. Cholesterol-lowering effects of a proprietary Chinese red-yeast rice dietary supplement. *Am. J. Clin. Nutr.* **1999**, *69*, 231–236.
(11) Journoud, M.; Jones, P. J. H. Red yeast rice: A new hypolipidemic drug. *Life Sci.* **2004**, *74*, 2675–2683.
(12) Clarkson, T. B. Soy, soy phytoestrogens and cardiovascular disease. *J. Nutr.* **2002**, *132* (Suppl. 3), 566S–569S.
(13) Tikkanen, M. J.; Adlecreutz, H. Dietary soy-derived isoflavone phytoestrogens. Could they have a role in coronary heart disease prevention? *Biochem. Pharmacol.* **2000**, *60*, 1–5.
(14) Hwang, C. S.; Kwak, H. S.; Lim, H. J.; Lee, S. H.; Kang, Y. S.; Choe, T. B.; Hur, H. G.; Han, K. O. Isoflavone metabolites and their *in vitro* dual functions: They can act as an estrogenic agonist or antagonist depending on the estrogen concentration. *J. Steroid Biochem.* **2006**, *101*, 246–253.
(15) Ricketts, M.-L.; Moore, D. D.; Banz, W. J.; Mezei, O.; Shay, N. F. Molecular mechanisms of action of the soy isoflavones includes activation of promiscuous nuclear receptors. A review. *J. Nutr. Biochem.* **2005**, *16*, 321–330.
(16) Kawakami, Y.; Tsurugasaki, W.; Nakamura, S.; Osada, K. Comparison of regulative functions between dietary soy isoflavones aglycone and glucoside on lipid metabolism in rats fed cholesterol. *J. Nutr. Biochem.* **2005**, *16*, 205–212.
(17) Izumi, T.; Piskula, M. K.; Osawa, S.; Obata, A.; Tobe, K.; Saito, M. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J. Nutr.* **2000**, *130*, 1695–1699.
(18) Richellee, M.; PrimoreMerten, S.; Bodenstb, S.; Enslen, M.; Offord, E. A. Hydrolysis of isoflavone glycosides to aglycones by β-glycosidase dose not alter plasma and urine isoflavone pharmacokinetics in postmenopausal women. *J. Nutr.* **2002**, *132*, 2587–2592.
(19) Pyo, Y. H.; Lee, Y. C. Mevinolin production by *Monascus pilosus* KFRI-1140 in solid state fermentation using soymeal. *Food Sci. Biotechnol.* **2006**, *15*, 647–649.
(20) Pyo, Y. H.; Lee, T. C. The potential antioxidant capacity and angiotensin I- converting enzyme inhibitory activity of *Monascus*-fermented soybean extracts: Evaluation of *Monascus*-fermented soybean extracts as multifunctional food additives. *J. Food Sci.* **2007**, *72*, S708–S713.
(21) Yu, H.; Wang, J.; Li, Y. Effects of turtle egg powder mixture on serum lipids of experimental hyperlipidemia rats. *J. Hyg. Res.* **2004**, *33*, 91–93.
(22) Folch, J.; Lees, M.; Sloane Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509.
(23) DeWeal, J.; Raymakers, C. E.; Endeman, H. J. Simple quantitative determination of total fecal bile acid. *Clin. Chem. Acta* **1977**, *79*, 465–470.
(24) Rao, A.; Ramakrishnan, V. S. Indirect assessment of hydroxymethylglutaryl-CoA reductase activity in liver tissue. *Clin. Chem.* **1975**, *21*, 1523–1525.
(25) Pyo, Y. H. Effect of *Monascus*-fermentation on the content of GABA and free amino acods in soybean. *J. Korean Soc. Food Sci. Nutr.* **2008**, *37*, 1208–1213.
(26) Tsuji, K.; Nakagawa, Y.; Ichikawa, T. Effect of beni-koji extracts and pigment produced by *Monascus* spp. On blood pressure, mineral balance and cholesterol levels in spontaneously hypertensive rats. *Nihon Kasei Gakkai Shi* **1993**, *44*, 109–114.
(27) Kew, M. C. Serum aminotransferase concentration as evidence of hepatocellular damage. *Lancet* **2000**, *355*, 591–592.
(28) Sung, J. H.; Choi, S. J.; Lee, S. W.; Park, K. H.; Moon, T. W. Isoflavones found in Korean soybean paste as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 1051–1058.
(29) Martinez, L. O.; Jacquet, S.; Terce, F.; Collet, X.; Perret, B.; Barbaras, R. New insight on the molecular mechanisms of high-density lipoprotein cellular interaction. *Cell. Mol. Life Sci.* **2004**, *61*, 2343–2360.
(30) Nofer, J. R.; Kehrel, B.; Fobker, M.; Levkau, B.; Assmann, G.; Eckardstein, A. V. HDL and atherosclerosis: beyond reverse cholesterol transport. *Atherosclerosis* **2002**, *61*, 1–16.
(31) Song, T.; Lee, S. O.; Murphy, P. A.; Hendrich, S. Soy protein with or without isoflavones, soy germ and soy germ extract, and daidzein lessen plasma cholesterol levels in golden Syrian hamsters. *Exp. Biol. Med.* **2003**, *228*, 1063–1068.
(32) Yousef, M. I.; Kamel, K. I.; Esamail, A. M.; Baghdadi, H. H. Antioxidant activities and lipid lowering effects of isoflavone in male rabbits. *Food Chem. Toxicol.* **2004**, *42*, 1497–1503.
(33) Anthony, M. S.; Clarkson, T. B.; Koudy Williams, J. Effects of soy isoflavones on atherosclerosis potential mechanisms. *Am. J. Clin. Nutr.* **1998**, *68*, 1390s–1393s.
(34) Gardner, C. D.; Newell, K. A.; Cherin, R.; Haskell, W. L. The effect of soy protein with or without isoflavones relative to milk protein on plasma lipids in hypercholesterolmic postmenopausal women. *Am. J. Clin. Nutr.* **2001**, *73*, 728–735.
(35) Clausen, P.; Lindhardsen, J.; Hoie, L.; Stender, S. Treatment with Abacor, a soy-based dietary supplement, further reduces plasma concentrations of total and low-density lipoprotein cholesterol in statin-treated hypercholesterolaemic patients. *Innovative Food Sci. Emerging Technol.* **2004**, *5*, 377–383.

Received June 3, 2009. Revised manuscript received July 21, 2009. Accepted August 06, 2009. This work was supported by a Sungshin Women's University Research Grant (2008).