Comparative effects of doenjang prepared from soybean and brown rice on the body weight and lipid metabolism in high fat-fed mice

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The comparative effects of doenjang prepared from fermented soybean or brown rice on the body weight and lipid metabolism in C57BL/6N mice fed with high fat diet were investigated. The animals were randomly divided and given experimental diets for eight weeks: normal control diet, high fat diet, and high fat diet supplemented with soybean doenjang, brown rice doenjang, brown rice-rice bran doenjang, or brown rice-red ginseng marc doenjang. At the end of the experimental period, the HF group exhibited a marked increase in body weight, body fat, plasma triglyceride concentration, and atherogenic index relative to the normal control diet group. However, diet supplementation of doenjang counteracted this high fat-induced hyperlipidemia through modulation of lipogenesis and adipokine production. In general, compared with soybean doenjang, the brown rice-rice bran doenjang and brown rice-red ginseng marc doenjang were similarly effective in improving the lipid metabolism under high fat diet condition. These findings demonstrate that brown rice, in combination with rice bran or red ginseng marc, may be useful as a functional biomaterial for the preparation of doenjang with strong anti-obesity effect and hypolipidemic action.

Key Words: brown rice, soybean, doenjang, hypolipidemic effect, obesity

Doenjang, which literally means “thick paste”, is a Korean fermented soybean paste. It is produced through fermentation of soybeans using various microorganisms including *Aspergillus* species and *Bacillus subtilis*.^(1,2) Due to its unique and distinct flavor, doenjang is primarily used as a dipping condiment and flavor seasoning in soups and stews in Korea. In 2009, doenjang became an internationally accepted food when it was registered in CODEX Alimentarius,⁽³⁾ a collection of international food standards, guidelines, and practices. Since then, the availability and popularity of doenjang in the market worldwide have increased. For the past years, doenjang has received much attention due to its reported health benefits, such as stronger anti-mutagenic and anti-cancer activities compared to raw and cooked soybeans.^(4,5) It is also a good source of essential amino acids, minerals, and vitamins.⁽⁶⁾ Moreover, fermented soybean products were shown to have higher phenolic and protein contents and greater antioxidant activity than non-fermented ones.⁽⁷⁾ The increase in genistein and linoleic acid contents during fermentation of soybean is believed to be mainly responsible for the high biological activity of doenjang.^(4,5)

Rice is one of the most important food crops in the world and is mostly produced and consumed in Asia. In Korea, however, the consumption of rice has been declining due to the changing dietary habits. To increase the market value of rice, value-added rice products are continuously being developed and produced in

the country. In the present study, we prepared doenjang using brown rice as the main component instead of soybean. Fermented brown rice and rice bran has been reported to exhibit strong anti-tumor and anti-cancer activities.^(8–11) It would be interesting to know whether doenjang made from brown rice only or in combination with other food crop would have comparable physiological effect with that of soybean doenjang.

Nowadays, because of high dietary fat intake and sedentary lifestyle, the incidence of obesity is rapidly increasing in epidemic proportions in both developed and developing countries.⁽¹²⁾ The need for functional foods that have body fat-lowering effect and hypolipidemic action has become stronger and more urgent. While a number of studies have been previously conducted on the anti-obesity and hypolipidemic effects of soybean, there were limited studies on the effect of doenjang on the lipid metabolism. Furthermore, doenjang prepared from brown rice has not been studied yet. Hence, this study was conducted to evaluate the comparative effects of doenjang made from fermented soybean, brown rice, or brown rice in combination with rice bran and red ginseng on the body weight and lipid metabolism in mice fed with high fat diet.

Materials and Methods

Materials and chemicals. The soybean (SB) doenjang and brown rice were purchased from a local market in Daegu, Korea. The rice bran and red ginseng marc were provided by Rice Processing Complex (RPC, Gimcheon, Korea) and Punggi Ginseng Farming Corp. (Yeongju, Gyeongbuk, Korea), respectively. The *Aspergillus oryzae* was obtained from NUC Electronics Co., Ltd. (Daegu, Korea). Chemicals such as ethanol, ketamine-HCl, magnesium chloride, calcium chloride, potassium phosphate buffer, triethanolamine, and Triton X-100 were purchased from Merck KGaA (Darmstadt, Germany). All other chemicals used were obtained from Sigma-Aldrich, Inc. (Steinheim, Germany).

Preparation of brown rice doenjang samples. The brown rice (BR) doenjang was prepared based on the manufacturing process for commercial doenjang as described by Park *et al.*⁽¹³⁾ with some modifications. Briefly, the brown rice was washed, soaked in water for 3 h at 4°C, and cooked using an electric rice cooker. After cooling, the rice was inoculated with *Aspergillus oryzae* (0.2%, w/w) and incubated for 3 days at 30°C. The resulting koji was added with salt (12%, w/w) and mixed using a food processor. The mixture was then fermented and ripened for 30 days at 30°C. For the preparation of brown rice-rice bran (BRB) and brown rice-red ginseng marc (BRG) doenjang

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Table 1. Proximate composition (% dry basis) of the doenjang samples

Composition	Doenjang ¹			
	SB	BR	BRB	BRG
Moisture	4.52	4.91	4.96	5.23
Carbohydrate	45.55	74.72	68.77	70.79
Crude protein	23.42	7.82	9.45	8.15
Crude fat	0.91	0.41	1.13	0.16
Dietary fiber	4.36	2.47	4.16	3.04
Ash	21.24	9.67	11.53	12.63

¹SB, soybean doenjang; BR, brown rice doenjang; BRB, brown rice and rice bran doenjang; BRG, brown rice and red ginseng marc doenjang.

samples, the same method was used. The rice bran and red ginseng marc were washed and steamed separately for 40 min. The cooked brown rice was added with rice bran (20%, w/w) or red ginseng (30%, w/w) prior to inoculation with *A. oryzae*. All doenjang samples were freeze-dried at -70°C prior to use. Their proximate compositions are shown in Table 1.

Animals and diets. Forty-eight male C57BL/6N mice of 4 weeks of age, weighing 12 g, were purchased from Orient Inc. (Seoul, Korea). Each mouse was housed in a stainless steel cage in a room maintained at 25°C with 50% relative humidity and 12/12 h light/dark cycle. The animals were fed with pelletized chow diet for 2 weeks upon arrival and randomly divided into 6 dietary groups ($n = 8$). The first group was fed with a normal control diet (NC group), while the second group was fed with a high fat diet (17%, w/w, HF group). The other 4 groups were fed with a high fat diet supplemented with soybean doenjang (HF + SB group), brown rice doenjang (HF + BR group), brown rice-rice bran doenjang (HF + BRB group), or brown rice-red ginseng marc doenjang (HF + BRG group). The composition of the experimental diet (Table 2) was based on the AIN-76 semisynthetic diet.⁽¹⁴⁾ The mice were fed for eight weeks and allowed free access to food and water. The feed intake and weight gain were measured daily and weekly, respectively. At the end of the experimental period, the mice were anaesthetized with ketamine-HCl following a 12-h fast. Blood samples were drawn from the inferior vena cava into a heparin-coated tube and centrifuged at $1,000 \times g$ for 15 min at 4°C to obtain the plasma. The body organs (liver, heart, and kidney) and adipose tissues (epididymal, perirenal, and inguinal) were removed, rinsed with physiological saline, weighed, and stored at -70°C until analysis. The current study

protocol was approved by the Ethics Committee of Kyungpook National University for animal studies.

Determination of plasma lipid profile. The plasma total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol concentrations were determined using a commercial kit (Asan Pharmaceutical, Seoul, Korea).

Determination of free fatty acid and phospholipid contents in plasma. The plasma free fatty acid and phospholipid levels were measured using commercial assay kits (Enzychrom, Bio-Assays Systems, CA) following the instruction manual.

Measurement of lipid-regulating enzyme and β -oxidation activities. The liver was homogenized according to the method described by Hulcher and Olson.⁽¹⁵⁾ Briefly, the tissue was homogenized in 3–5 mL of 0.1 M triethanolamine, 0.02 M ethylenediaminetetraacetic acid (EDTA), and 0.002 M dithiothreitol (DTT) per gram tissue and centrifuged at $1,000 \times g$ at 4°C for 15 min. The pellet was removed and the supernatant was centrifuged at $10,000 \times g$ at 4°C for 15 min. After centrifugation, the pellets were resuspended in the same buffer used in homogenization and analyzed for carnitine palmitoyl transferase (CPT) and β -oxidation activities, and the supernatant was further centrifuged at $105,000 \times g$ at 4°C for 1 h. The resulting supernatant was measured for glucose-6-phosphate dehydrogenase (G6PD) activity, while the pellets were resuspended in the same buffer containing triethanolamine, EDTA, and DTT and analyzed for fatty acid synthase (FAS) and malic enzyme (ME) activities. The protein content was measured using Bradford protein assay.⁽¹⁶⁾

The FAS activity was determined based on the spectrophotometric method of Gibson and Hubbard.⁽¹⁷⁾ The assay mixture contained 125 mM potassium phosphate buffer (pH 7.0), 10 mM EDTA, 10 mM β -mercaptoethanol, 33 μM acetyl-CoA, 100 μM malonyl-CoA, and 100 μM nicotinamide adenine dinucleotide phosphate (NADPH). The mixture was added with malonyl CoA and the change in absorbance at 340 nm at 30°C was recorded.

The ME activity was measured using the method described by Ochoa.⁽¹⁸⁾ The reaction mixture was composed of 0.4 M triethanolamine (pH 7.4), 30 mM malic acid, 0.12 M MgCl_2 , and 3.4 mM NADP. The absorbance was measured at 340 nm at 27°C .

The G6PD activity was determined based on the reduction of 6 mM NADP^+ by G6PD in the presence of glucose-6-phosphate.⁽¹⁹⁾ The enzyme activity was measured by monitoring the increase in absorption of the reaction mixture at 340 nm at 37°C . The activities of FAS, ME, and G6PD were expressed as nmol or μmol

Table 2. Composition of the experimental diets (%)

Component	NC ¹	HF	HF + SB	HF + BR	HF + BRB	HF + BRG
Casein	20	20	17.58	19.12	18.96	19.09
DL-Methionine	0.3	0.3	0.3	0.3	0.3	0.3
Sucrose	50	50	45.24	42.32	42.91	42.7
Corn starch	15	—	—	—	—	—
Cellulose	5	5	4.54	4.73	4.56	4.67
Corn oil	5	3	3	3	3	3
Cholinbitartrate	0.2	0.2	0.2	0.2	0.2	0.2
Mineral mixture ²	3.5	3.5	1.37	2.52	2.34	2.23
Vitamin mixture ³	1	1	1	1	1	1
Lard	—	17	16.83	16.88	16.8	16.9
SB	—	—	10	—	—	—
BR	—	—	—	10	—	—
BRB	—	—	—	—	10	—
BRG	—	—	—	—	—	10
Total (%)	100	100	100	100	100	100

¹NC, normal control diet; HF, high fat diet; HF + SB, high fat diet + soybean doenjang; HF + BR, high fat diet + brown rice doenjang; HF + BRB, high fat diet + brown rice and rice bran doenjang; HF + BRG, high fat diet + brown rice and red ginseng marc doenjang. ²AIN-76 mineral mixture. ³AIN-76 vitamin mixture.

reduced NADPH/min/mg protein.

The CPT activity was measured following the method of Bieber *et al.*⁽²⁰⁾ The assay mixture contained 116 mM Tris-HCl (pH 8.0), 1.1 mM EDTA, 2.50 mM L-carnitine, 0.5 mM 5,5-dithiobis-2-nitrobenzoic acid, 75 mM palmitoyl-CoA, 0.2% Triton X-100. The reaction was initiated by the addition of 50 μ L cytosol and incubated at 25°C for 2 min. The change in absorbance at 412 nm was measured and the activity was expressed as nmol or μ mol CoASH or oxidized CoA/min/mg protein.

The hepatic β -oxidation activity was measured from the final product of NADH by palmitoyl substrates.⁽²¹⁾ The assay mixture contained 50 mM Tris-HCl (pH 8.0) 20 mM NAD⁺, 0.33 M DTT, 1.5% BSA (1.5 g/100 mL), 2% Triton X-100 (2 g/100 mL), 10 mM CoA, 1 mM FAD, 100 mM KCN and 5 mM palmitoyl-CoA. The reaction was initiated by the addition of 10 μ L cytosol and incubated at 37°C for 5 min. The change in absorbance at 340 nm was measured and the activity was expressed as nmol reduced NADPH/min/mg protein.

Measurement of plasma adipokine concentrations. The leptin concentration was measured using an enzyme immunoassay (EIA) kit (Spi-Bio, Montigny le Bretonneux, France). The levels of resistin, adiponectin, and tumor necrosis factor (TNF)- α were determined using enzyme-linked immunosorbent assay (ELISA) kits (Shibayagi Co., Gunma, Japan).

Statistical analysis. All data are presented as the mean $\pm$ SE. The data were evaluated by one-way ANOVA using a Statistical Package for Social Sciences software program (SPSS Inc., Chicago, IL) and the differences between the means were assessed using Tukey's test. Statistical significance was considered at $p < 0.05$.

Results

Body weight gain and organ weights. All animal groups exhibited similar body weight prior to feeding them with experi-

mental diets (Table 3). The feed intake was also similar in all groups. At the end of the experimental period, a marked increase in the body weight was found in HF mice relative to that of the control group. However, diet supplementation of doenjang significantly suppressed this high fat diet-induced body weight gain. In particular, the HF + SB and HF + BRG groups showed similar final body weight and body weight gain with the control group. Although the HF + BR and HF + BRB showed higher body weight than the NC group, their final weights were still considerably lower than that of the HF group. Accordingly, the food efficiency ratio (FER) was highest in HF group and lowest in NC, HF + SB, and HF + BRG groups. There was no significant change in the weights of liver, heart, and kidney among the animal groups (Table 4). On the other hand, a substantial increase in the white adipose tissue weight was observed in HF group. Addition of SB, BR, and BRB doenjang in the diet resulted in a slight decrease in the weight of adipose tissues relative to the HF mice. The HF + BRG group, however, showed similar body fat weight with the NC group.

Plasma lipids. A substantial elevation in the plasma triglyceride concentration was found in HF group (Table 5). On the other hand, the triglyceride level decreased in all doenjang-fed mice, with the HF + BRG group having the lowest concentration. Significantly lower plasma total cholesterol level was also observed in HF + BRG group than that of the HF group. The HDL-cholesterol concentration markedly increased in all doenjang-fed groups relative to that of the NC group. Accordingly, the doenjang-fed groups showed significantly higher and lower HDL-cholesterol/total cholesterol ratio (HTR) and atherogenic index (AI), respectively, compared with the HF group. Moreover, the HF + SB, HF + BRB, and HF + BRG groups exhibited considerably lower free fatty acid concentration than the HF mice. No significant difference was found in the phospholipid content between the control and experimental-fed groups.

Table 3. Body weight gain and feed intake in mice fed with high fat diet supplemented with brown rice doenjang

Dietary group ¹	Body weight ²			Feed intake (g/day)	FER ³
	Initial weight (g)	Final weight (g)	Weight gain (g/day)		
NC	21.69 $\pm$ 0.30 ^a	32.29 $\pm$ 0.18 ^a	0.19 $\pm$ 0.01 ^a	3.23 $\pm$ 0.05 ^a	0.060 $\pm$ 0.005 ^a
HF	21.72 $\pm$ 0.46 ^a	38.82 $\pm$ 0.25 ^d	0.31 $\pm$ 0.01 ^c	3.19 $\pm$ 0.03 ^a	0.090 $\pm$ 0.003 ^c
HF + SB	21.55 $\pm$ 0.31 ^a	32.69 $\pm$ 0.38 ^a	0.20 $\pm$ 0.02 ^{ab}	3.28 $\pm$ 0.05 ^a	0.061 $\pm$ 0.001 ^a
HF + BR	21.79 $\pm$ 0.33 ^a	34.91 $\pm$ 0.28 ^c	0.23 $\pm$ 0.02 ^b	3.27 $\pm$ 0.06 ^a	0.072 $\pm$ 0.005 ^b
HF + BRB	21.65 $\pm$ 0.42 ^a	33.73 $\pm$ 0.67 ^b	0.22 $\pm$ 0.02 ^{ab}	3.25 $\pm$ 0.07 ^a	0.067 $\pm$ 0.001 ^{ab}
HF + BRG	21.56 $\pm$ 0.17 ^a	32.59 $\pm$ 0.43 ^a	0.20 $\pm$ 0.01 ^{ab}	3.25 $\pm$ 0.05 ^a	0.061 $\pm$ 0.002 ^a

¹NC, normal control diet; HF, high fat diet; HF + SB, high fat diet + soybean doenjang; HF + BR, high fat diet + brown rice doenjang; HF + BRB, high fat diet + brown rice and rice bran doenjang; HF + BRG, high fat diet + brown rice and red ginseng marc doenjang. ²Values are means $\pm$ SE ($n = 8$). Means in the same column not sharing a common superscript are significantly different at $p < 0.05$. ³FER, food efficiency ratio = body weight gain/feed intake.

Table 4. Weights of organs and adipose tissues in mice fed with high fat diet supplemented with brown rice doenjang

	NC ¹	HF	HF + SB	HF + BR	HF + BRB	HF + BRG
Organ weight (g)²						
Liver	3.81 $\pm$ 0.06 ^a	3.99 $\pm$ 0.21 ^a	3.79 $\pm$ 0.12 ^a	3.81 $\pm$ 0.16 ^a	3.79 $\pm$ 0.16 ^a	3.58 $\pm$ 0.13 ^a
Heart	0.46 $\pm$ 0.01 ^a	0.48 $\pm$ 0.01 ^a	0.48 $\pm$ 0.01 ^a	0.50 $\pm$ 0.02 ^a	0.46 $\pm$ 0.02 ^a	0.47 $\pm$ 0.01 ^a
Kidney	1.20 $\pm$ 0.02 ^a	1.25 $\pm$ 0.01 ^a	1.22 $\pm$ 0.03 ^a	1.20 $\pm$ 0.02 ^a	1.23 $\pm$ 0.03 ^a	1.17 $\pm$ 0.02 ^a
White adipose tissue weight (g)						
Epididymal	4.76 $\pm$ 0.28 ^a	6.22 $\pm$ 0.17 ^b	5.59 $\pm$ 0.30 ^{ab}	5.47 $\pm$ 0.19 ^{ab}	5.53 $\pm$ 0.19 ^{ab}	4.81 $\pm$ 0.26 ^a
Perirenal	1.78 $\pm$ 0.14 ^a	3.01 $\pm$ 0.08 ^b	2.47 $\pm$ 0.33 ^{ab}	2.39 $\pm$ 0.10 ^{ab}	2.32 $\pm$ 0.13 ^{ab}	1.97 $\pm$ 0.12 ^a
Inguinal	1.70 $\pm$ 0.07 ^a	2.81 $\pm$ 0.19 ^b	2.27 $\pm$ 0.24 ^{ab}	2.39 $\pm$ 0.19 ^{ab}	2.24 $\pm$ 0.18 ^{ab}	1.90 $\pm$ 0.30 ^a

¹NC, normal control diet; HF, high fat diet; HF + SB, high fat diet + soybean doenjang; HF + BR, high fat diet + brown rice doenjang; HF + BRB, high fat diet + brown rice and rice bran doenjang; HF + BRG, high fat diet + brown rice and red ginseng marc doenjang. ²Values are means $\pm$ SE ($n = 8$). Means in the same row not sharing a common superscript are significantly different at $p < 0.05$.

Lipid-regulating enzyme and β -oxidation activities. The high fat diet significantly increased the activity of hepatic FAS, but diet supplementation with doenjang markedly decreased the enzyme activity (Table 6). The activities of ME, G6PD, and adipocyte FAS enzymes also decreased in doenjang-fed groups relative to those of the HF group. No significant difference was found in the CPT activity among the animal groups. All experimental-fed groups showed significantly higher β -oxidation activity than the control group.

Plasma adipokine concentrations. The concentrations of leptin, resistin, and TNF- α substantially increased with high fat feeding (Table 7). However, diet supplementation of doenjang counteracted the elevation in resistin level. Furthermore, the leptin concentration significantly decreased to normal level in HF + BRG group. The TNF- α concentration was also reduced to normal level with the addition of SB, BR, and BRB doenjang in the diet. The adiponectin markedly decreased in HF mice, but increased in HF + BRG group.

Discussion

The present study investigated the effect of dietary feeding of doenjang prepared from soybean, brown rice, or brown rice combined with rice bran and red ginseng marc on the body weight and lipid metabolism in mice under high fat diet condition. The results showed that doenjang could suppress the body weight gain, reduce the amount of body fat, and improve the plasma lipid profile in high fat-fed mice. A recent investigation on the anti-obesity effects of soybean doenjang and non-fermented soybeans revealed that the former was more effective in decreasing visceral fat accumulation and adipocyte size in high fat-fed rats, which was attributed to the higher content of aglycone isoflavones in doenjang.⁽²²⁾ The fat-lowering effect of soybean doenjang could also be partly due to its high protein content. Soy protein was previously shown to reduce the body fat in obese mice and rats.^(23,24) Velasquez and Bhatena⁽²⁵⁾ reported that soy protein and its isoflavones reduced body weight and fat mass in both humans and animal models, suggesting their beneficial role in obesity. Moreover, soybean doenjang was found to reduce the triglyceride and total cholesterol levels and increase the HDL-cholesterol

Table 5. Plasma lipid profile in mice fed with high fat diet supplemented with brown rice doenjang

	NC ¹	HF	HF + SB	HF + BR	HF + BRB	HF + BRG
Triglyceride (mg/dL) ²	116.39 $\pm$ 6.48 ^{ab}	146.17 $\pm$ 8.92 ^c	121.80 $\pm$ 4.70 ^{ab}	131.58 $\pm$ 4.43 ^{bc}	122.37 $\pm$ 6.22 ^{ab}	107.52 $\pm$ 2.63 ^a
Total cholesterol (mg/dL)	137.24 $\pm$ 5.26 ^{ab}	158.66 $\pm$ 3.43 ^b	148.03 $\pm$ 5.80 ^{ab}	149.21 $\pm$ 7.54 ^{ab}	138.98 $\pm$ 4.17 ^{ab}	132.28 $\pm$ 1.80 ^a
HDL-cholesterol (mg/dL)	83.33 $\pm$ 2.00 ^a	86.41 $\pm$ 2.39 ^{ab}	94.75 $\pm$ 1.00 ^{bc}	92.39 $\pm$ 2.88 ^{bc}	93.48 $\pm$ 1.45 ^{bc}	95.47 $\pm$ 2.41 ^c
HTR ³	60.72 $\pm$ 1.39 ^b	54.46 $\pm$ 1.51 ^a	64.00 $\pm$ 0.67 ^{bc}	61.92 $\pm$ 1.93 ^{bc}	67.26 $\pm$ 1.04 ^{cd}	72.17 $\pm$ 1.82 ^d
AI ⁴	0.65 $\pm$ 0.04 ^c	0.84 $\pm$ 0.05 ^d	0.56 $\pm$ 0.02 ^{bc}	0.62 $\pm$ 0.05 ^{bc}	0.49 $\pm$ 0.02 ^{ab}	0.39 $\pm$ 0.04 ^a
Free fatty acid (mmol/L)	0.60 $\pm$ 0.10 ^{abc}	0.80 $\pm$ 0.05 ^c	0.51 $\pm$ 0.02 ^{ab}	0.68 $\pm$ 0.07 ^{bc}	0.56 $\pm$ 0.03 ^{ab}	0.46 $\pm$ 0.08 ^a
Phospholipid (mmol/L)	1.30 $\pm$ 0.04 ^{ab}	1.25 $\pm$ 0.03 ^a	1.34 $\pm$ 0.02 ^{ab}	1.28 $\pm$ 0.02 ^{ab}	1.30 $\pm$ 0.04 ^{ab}	1.36 $\pm$ 0.01 ^b

¹NC, normal control diet; HF, high fat diet; HF + SB, high fat diet + soybean doenjang; HF + BR, high fat diet + brown rice doenjang; HF + BRB, high fat diet + brown rice and rice bran doenjang; HF + BRG, high fat diet + brown rice and red ginseng marc doenjang. ²Values are means $\pm$ SE (n = 8). Means in the same row not sharing a common superscript are significantly different at p < 0.05. ³HTR = (HDL-cholesterol/total cholesterol) $\times$ 100. ⁴AI, atherogenic index = (total cholesterol - HDL-cholesterol)/HDL-cholesterol.

Table 6. Lipid-regulating enzyme and β -oxidation activities in mice fed with high fat diet supplemented with brown rice doenjang

	NC ¹	HF	HF + SB	HF + BR	HF + BRB	HF + BRG
<i>Hepatic lipid-regulating enzyme (nmol/min/mg protein)²</i>						
FAS	18.70 $\pm$ 0.24 ^c	22.43 $\pm$ 0.61 ^d	14.99 $\pm$ 0.83 ^{ab}	15.99 $\pm$ 0.79 ^b	12.91 $\pm$ 0.43 ^a	12.68 $\pm$ 0.46 ^a
ME	29.76 $\pm$ 1.31 ^b	30.65 $\pm$ 1.75 ^b	23.87 $\pm$ 1.09 ^a	27.13 $\pm$ 0.89 ^{ab}	24.01 $\pm$ 0.77 ^a	22.58 $\pm$ 1.30 ^a
G6PD	8.54 $\pm$ 0.41 ^b	8.71 $\pm$ 0.22 ^b	4.93 $\pm$ 0.20 ^a	4.81 $\pm$ 0.22 ^a	4.49 $\pm$ 0.26 ^a	4.29 $\pm$ 0.07 ^a
CPT	24.60 $\pm$ 0.96 ^a	21.90 $\pm$ 0.75 ^a	25.88 $\pm$ 1.20 ^a	25.66 $\pm$ 0.77 ^a	27.13 $\pm$ 1.79 ^a	26.45 $\pm$ 1.83 ^a
β -oxidation	1.75 $\pm$ 0.09 ^a	2.35 $\pm$ 0.17 ^b	2.62 $\pm$ 0.20 ^b	2.43 $\pm$ 0.08 ^b	2.53 $\pm$ 0.11 ^b	2.85 $\pm$ 0.12 ^b
<i>Adipocyte lipid-regulating enzyme (μmol/min/mg protein)</i>						
FAS	37.47 $\pm$ 2.50 ^{ab}	47.03 $\pm$ 2.92 ^b	28.23 $\pm$ 2.54 ^a	34.72 $\pm$ 3.73 ^{ab}	32.73 $\pm$ 3.25 ^{ab}	30.48 $\pm$ 4.20 ^a
ME	300.60 $\pm$ 8.15 ^{ab}	343.32 $\pm$ 19.44 ^b	277.43 $\pm$ 1.89 ^a	294.69 $\pm$ 9.26 ^a	278.79 $\pm$ 9.92 ^a	258.80 $\pm$ 2.62 ^a
G6PD	121.41 $\pm$ 5.11 ^{ab}	138.90 $\pm$ 6.12 ^b	109.75 $\pm$ 4.21 ^a	118.91 $\pm$ 8.58 ^{ab}	111.58 $\pm$ 2.37 ^a	108.92 $\pm$ 2.41 ^a
CPT	80.38 $\pm$ 10.54 ^a	72.24 $\pm$ 10.02 ^a	118.60 $\pm$ 7.38 ^a	95.92 $\pm$ 2.37 ^a	120.08 $\pm$ 13.14 ^a	121.89 $\pm$ 12.51 ^a

¹NC, normal control diet; HF, high fat diet; HF + SB, high fat diet + soybean doenjang; HF + BR, high fat diet + brown rice doenjang; HF + BRB, high fat diet + brown rice and rice bran doenjang; HF + BRG, high fat diet + brown rice and red ginseng marc doenjang. ²Values are means $\pm$ SE (n = 8). Means in the same row not sharing a common superscript are significantly different at p < 0.05.

Table 7. Plasma adipokine concentrations in mice fed with high fat diet supplemented with brown rice doenjang

	NC ¹	HF	HF + SB	HF + BR	HF + BRB	HF + BRG
Leptin (ng/mL) ²	1.32 $\pm$ 0.10 ^a	1.84 $\pm$ 0.06 ^b	1.50 $\pm$ 0.13 ^{ab}	1.58 $\pm$ 0.11 ^{ab}	1.41 $\pm$ 0.13 ^{ab}	1.26 $\pm$ 0.10 ^a
Resistin (ng/mL)	15.47 $\pm$ 0.06 ^b	16.18 $\pm$ 0.05 ^d	15.46 $\pm$ 0.09 ^b	15.74 $\pm$ 0.05 ^c	14.75 $\pm$ 0.02 ^a	15.33 $\pm$ 0.07 ^b
Adiponectin (μ g/mL)	3.23 $\pm$ 0.11 ^b	2.31 $\pm$ 0.16 ^a	3.82 $\pm$ 0.95 ^{ab}	3.50 $\pm$ 0.09 ^{ab}	4.00 $\pm$ 0.26 ^{ab}	4.13 $\pm$ 0.23 ^b
TNF- α (ng/mL)	1.29 $\pm$ 0.04 ^a	1.55 $\pm$ 0.05 ^b	1.21 $\pm$ 0.03 ^a	1.30 $\pm$ 0.02 ^a	1.22 $\pm$ 0.08 ^a	1.35 $\pm$ 0.02 ^{ab}

¹NC, normal control diet; HF, high fat diet; HF + SB, high fat diet + soybean doenjang; HF + BR, high fat diet + brown rice doenjang; HF + BRB, high fat diet + brown rice and rice bran doenjang; HF + BRG, high fat diet + brown rice and red ginseng marc doenjang. ²Values are means $\pm$ SE (n = 8). Means in the same column not sharing a common superscript are significantly different at p < 0.05.

concentration in rats fed with high fat and high cholesterol diet.⁽²⁶⁾ Similarly, dietary feeding of fermented soybean and rice bran resulted in a significant decrease in the serum and hepatic triglyceride levels in high fat-fed rats.⁽²⁷⁾ Baek *et al.*⁽²⁸⁾ reported that fermented brown rice also exhibited hypocholesterolemic action in cholesterol-fed rats. They suggested that the cholesterol-lowering effect of fermented brown rice could be related to its high amount of carbohydrates that could be metabolized into short chain fatty acids, which were demonstrated to lower plasma cholesterol level. Results of the present study showed, for the first time, that doenjang made from brown rice in combination with rice bran or red ginseng marc has hypolipidemic effect and may be useful in the management of high fat-induced hyperlipidemia. Further studies on the composition and bioactive components of the doenjang samples are needed in order to better understand their therapeutic potential against high fat diet-induced hyperlipidemia and obesity.

While the BR doenjang effectively reduced the body weight and slightly decreased the body fat, the addition of red ginseng marc further enhanced its body weight-lowering action. It is interesting to note that the combination of brown rice and red ginseng marc in doenjang showed similar body weight-lowering effect with that of soybean doenjang. Furthermore, among the doenjang samples analyzed, the BRG doenjang appeared to be the most effective in improving the lipid metabolism and enhancing the lipid profile in mice. The BR doenjang showed a marginal effect only on the plasma lipid profile in mice, but its hypolipidemic action became more potent with the inclusion of red ginseng marc. Red ginseng marc, a fibrous by-product that is often discarded as a waste after the production of ginseng extract, contains lipid-soluble components that have various pharmacological properties, such as anti-cancer and immunomodulatory effect.^(29,30) In addition, fermented red ginseng marc was found to possess biologically active compounds that have strong anti-oxidant effect.⁽³¹⁾ Kim *et al.*⁽³²⁾ reported that extracts from fermented red ginseng could reduce the triglyceride and total cholesterol levels in diabetic rats. They found that fermentation of red ginseng resulted in increased ginsenoside content. The triterpene saponins, such as ginsenosides Rg3 and Rh2, from red ginseng extract have been recently shown to exert anti-obesity effect in high fat-fed mice through modulation of the lipid metabolism.⁽³³⁾ This could be partly responsible for the strong hypolipidemic activity of BRG doenjang. Based on the results of this study, it seemed that the fermented red ginseng marc may have greater hypolipidemic activity compared with the fermented brown rice. However, the development of doenjang product from red ginseng marc alone may have low possibility due to the strong and distinct ginseng smell that doenjang consumers may find unfavorable.

The reduction in plasma triglyceride and total cholesterol levels found in doenjang-fed groups, particularly the HF + BRG mice, could be partly due to the enhanced fatty acid oxidation and suppression of lipogenesis through the regulation of the lipogenic enzyme activities. The lipogenic enzymes FAS, ME, G6PD, and CPT are important for the biosynthesis of cholesterol and fatty acid, and reduced activities of FAS, ME and G6PD could limit the availability of fatty acids required for the synthesis of triglycerides.^(34,35) On the other hand, an increase in the CPT activity could result in an increase in fatty acid oxidation, thereby reducing triglyceride accumulation.⁽³⁶⁾ Although the CPT activity did not significantly differ among the animal groups, the decrease in the activities of FAS, ME, and G6PD in doenjang-fed mice relative to that of the HF group might have led to the decreased levels of triglyceride, total cholesterol, and fatty acids observed in these animal groups. Furthermore, the marked increase in the body fat of HF mice could also be related to the increased activity of the FAS enzyme. It was previously shown that enhanced FAS gene expression was significantly associated with visceral fat

accumulation in human subjects.⁽³⁷⁾ Similarly, the down regulation of ME and G6PD gene expressions has been linked to decreased fat accumulation.⁽³⁸⁾ Hence, the decrease in the amount of body fat in doenjang-fed groups, particularly the HF + BRG group, was probably due to the reduced activities of FAS, ME, and G6PD enzymes.

The reduction in plasma leptin, resistin, and TNF- α and elevation in plasma adiponectin by the doenjang samples could also be partly responsible for their hypolipidemic effect in mice under high fat diet condition. The adipokines are protein hormones secreted by adipose tissues that are involved in the regulation of lipid and glucose metabolism.⁽³⁹⁾ The production and release of leptin, resistin, and TNF- α are associated with the progression of obesity.⁽⁴⁰⁾ The adiponectin, on the other hand, is inversely related with obesity and has anti-atherosclerotic and anti-inflammatory properties.⁽⁴¹⁾ The marked increase in the amount of adipose tissues could have resulted in the elevation in the leptin, resistin, and TNF- α concentrations, and decrease in adiponectin level in high fat-fed mice. On the other hand, the reduced leptin, resistin, and TNF- α levels and enhanced adiponectin concentration found in doenjang-fed mice relative to the HF group suggest that the expressions and secretion of these adipokines were influenced by the reduced amount of body fat, leading to decreased concentrations of plasma triglyceride and cholesterol in these animals.

In conclusion, results of this study illustrate that dietary feeding of doenjang markedly suppressed the body weight gain, decreased the amount of body fat, and improved the lipid profile through inhibition of hepatic and adipocyte lipogenesis and modulation of adipokine production in high fat-fed mice. Compared with soybean doenjang, the brown rice doenjang showed relatively lower hypolipidemic activity. However, addition of rice bran or red ginseng marc in doenjang preparation significantly enhanced its physiological effect that was comparable with that of soybean. Brown rice, in combination with rice bran or red ginseng marc, may be useful as a functional biomaterial for the preparation of doenjang with strong preventive action against high fat diet-induced hyperlipidemia and obesity.

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Abbreviations

AI	atherogenic index
BR	brown rice
BRB	brown rice-rice bran
BRG	brown rice-red ginseng marc
CPT	carnitine palmitoyl transferase
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
FAS	fatty acid synthase
G6PD	glucose-6-phosphate dehydrogenase
HDL	high density lipoprotein
HF	high fat
HTR	HDL-cholesterol/total cholesterol ratio
ME	malic enzyme
NADPH	nicotinamide adenine dinucleotide phosphate
NC	normal control
SB	soybean
TNF	tumor necrosis factor

Conflict of Interest

No potential conflicts of interest were disclosed.

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