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Effects of ingredients of Korean brown rice cookies on attenuation of cholesterol level and oxidative stress in high-fat diet-fed mice

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BACKGROUND/OBJECTIVES: Owing to health concerns related to the consumption of traditional snacks high in sugars and fats, much effort has been made to develop functional snacks with low calorie content. In this study, a new recipe for Korean rice cookie, *dasik*, was developed and its antioxidative, lipid-lowering, and anti-inflammatory effects and related mechanisms were elucidated. The effects were compared with those of traditional rice cake *dasik* (RCD), the lipid-lowering effect of which is greater than that of traditional western-style cookies.

MATERIALS/METHODS: Ginseng-added brown rice *dasik* (GBRD) was prepared with brown rice flour, fructooligosaccharide, red ginseng extract, and propolis. Mice were grouped (n = 7 per group) into those fed a normal AIN-76 diet, a high-fat diet (HFD), and HFD supplemented with RCD or GBRD. *Dasik* in the HFD accounted for 7% of the total calories. The lipid, reactive oxygen species, and peroxynitrite levels, and degree of lipid peroxidation in the plasma or liver were determined. The expression levels of proteins involved in lipid metabolism and inflammation, and those of antioxidant enzymes were determined by western blot analysis.

RESULTS: The plasma and hepatic total cholesterol concentrations in the GBRD group were significantly decreased via downregulation of sterol regulatory element-binding protein-2 and 3-hydroxy-3-methylglutaryl-CoA reductase ($P < 0.05$). The hepatic peroxynitrite level was significantly lower, whereas glutathione was higher, in the GBRD group than in the RCD group. Among the antioxidant enzymes, catalase (CAT) and glutathione peroxidase (GPx) were significantly upregulated in the GBRD group ($P < 0.05$). In addition, nuclear factor-kappaB (NF-κB) expression in the GBRD group was significantly lower than that in the RCD group.

CONCLUSIONS: GBRD decreases the plasma and hepatic cholesterol levels by downregulating cholesterol synthesis. This new *dasik* recipe also improves the antioxidative and anti-inflammatory status in HFD-fed mice via CAT and GPx upregulation and NF-κB downregulation. These effects were significantly higher than those of RCD.

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INTRODUCTION

The western diet has become popular in Korea since the late 1970s, which has concomitantly increased the Korean population's consumption of western-style cookies. Although Korean traditional confectionaries are not as diverse as western-style cookies, they are generally higher in nutritional value. In particular, the Korean rice cookie (*dasik*; literally "tea food") does not require baking or frying process, and sugar, butter, milk, and eggs are not contained. Rice cake *dasik* (RCD), a representative *dasik*, is made by mixing rice cake flour, mung bean starch, and honey, and then forming the dough into a certain shape by using a special-purpose mold. Therefore, the nutritional value of RCD is higher than that of western-style cookies. In our previous study, the plasma and hepatic lipid levels in mice fed a high-fat diet (HFD) supplemented with RCD were lower than those in mice fed a western-style cookie [1]. In addition, the plasma cholesterol levels of middle-aged women who consumed ginseng-added brown rice *dasik* (GBRD; newly developed by our team) for 6 weeks were significantly lower than the levels in women who consumed a western-style cookie [2].

Consumption of whole grains in a diet is strongly advised [3] to reduce the prevalence of obesity, which can cause cardiovascular diseases, diabetes, or cancer. Natural sweeteners such as fructooligosaccharide (FOS), stevia, and short-chain FOS have been tested as sugar alternatives in the food manufacturing industry to minimize the detrimental health effects of cane sugar [4]. Recently, the prebiotic effects of FOS have been addressed [5], which has shown protection against colon cancer as well as immunomodulatory [6], anti-obesity [7], anti-inflammatory [8], and lipid-lowering effects [8]. Moreover,

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functional ingredients with pharmacological properties, such as herbs, essential oils, and ginseng, are being used to develop healthier recipes. The health-promoting effects of ginsengs are well established with respect to their antioxidative [9], anti-inflammatory [10], antihypertensive [11], hypolipidemic [12], anti-diabetic [13], and cancer preventive activities [14].

A HFD is an underlying cause of hyperlipidemia, which is characterized by high levels of total cholesterol (TC) and triglyceride (TG) [15]. Under prolonged hyperlipidemia, liver-damaging effects, including hepatic steatosis, are often observed [16]. In addition, a HFD might contribute to the overproduction of reactive oxygen species (ROS) during energy production in the mitochondrial respiratory chain [17-19]. These highly reactive ROS molecules have detrimental effects on DNA, lipids, and proteins in the biological system [20,21]. Moreover, ROS activate the nuclear factor-kappaB (NF-κB) pathway [17,18], which further augments oxidative stress and inflammation [18,19]. It is a well-known fact that any agent with lipid-lowering, antioxidative, and/or anti-inflammatory effects may attenuate HFD-induced dyslipidemia and its associated injuries.

In our previous studies, GBRD exerted a plasma cholesterol-lowering effect in middle-aged women [2], and RCD demonstrated a better lipid-lowering effect than that of a western-style cookie in HFD-fed mice [1]. Therefore, in this study, we investigated the mechanism of action of GBRD to reveal its lipid-lowering, antioxidative, and anti-inflammatory effects in HFD-fed mice, for comparison with RCD.

MATERIALS AND METHODS

Dasik ingredients

All *dasik* ingredients were purchased, except for the rice cake flour. To prepare the rice cake flour, rice was first soaked in water for 2 h, following which it was ground and steamed. The steamed rice cake was then powdered using a household mixer and sieved (40-mesh) twice. Mung bean starch, brown rice powder, honey, ginseng extracts (Korea Ginseng Co., Daejeon, Republic of Korea), FOS (Cheiljedang Co., Seoul, Republic of Korea), and propolis (Withealth Co., Gochang, Republic of Korea) were purchased from a local market.

Animal study

The AIN-76 synthetic diet (normal diet, ND), HFD, and various *dasik*-containing HFDs were prepared as shown in Table 1. The HFD was prepared by adding lard (21.7%, w/w) and cholesterol (0.4%, w/w) to the ND to provide 46.72% of the total calories from the fats. For the *dasik* diet preparation, each ingredient of RCD and GBRD was directly added according to the respective recipes shown in Table 2. The calories supplemented from *dasik* accounted for 7% of the total calories. All the experimental diets were isocaloric (5.0 kcal/kg diet) except for the ND (3.9 kcal/kg diet).

Male ICR mice (4-week old) were purchased from Orient Bio Inc. (Seongnam, Korea). The animals were kept in individual cages during the entire experimental period, under controlled conditions of 23 ± 1°C and 50% humidity with a 12 h light-dark cycle. After 1 week of acclimatization, the mice were randomly assigned into four groups (n = 7 per group) on the basis of body weight. The experimental groups were mice fed the ND (NOR group) or the HFD only (HFD group) or *dasik*-supplemented HFDs (RCD and GBRD groups). The animals were raised for 9 weeks with free access to the diet and water. The food consumption and body weight of each mouse were measured weekly. The animal protocols were reviewed for their ethical procedures and scientific care and approved by the Institutional Animal Care and Use Committee of Pusan National University (PNU-IACUC; Approval No. PNU-2012-0118).

Plasma, liver, and epididymal fat pad collection

After completion of the feeding period, the mice were fasted for 12 h and then anesthetized by intraperitoneal administration

Table 1. Composition of the experimental diets

Ingredient (g)	Normal[1)]	HFD[2)]	HFD + *dasik*[3)]	
			RCD	GBRD
Casein	200	171	171	171
dl-Methionine	3	3	3	3
Corn starch	350	203	124	124
Sucrose	300	280	257	256
Cellulose	50	43	43	43
Corn oil	50	39	39	39
Lard		217	217	217
Cholesterol		4	4	4
Mineral mix	35	30	30	30
Vitamin mix	10	9	9	9
Choline bitartrate	2	2	2	2
Rice cake flour			58	
Mung bean starch			21	
Brown rice flour				79
Honey			23	
Fructooligosaccharide				23
Red ginseng extract				2
Propolis				0.1

HFD, High-fat diet; RCD, Rice cake *dasik*; GBRD, ginseng-added brown rice *dasik*.
[1)] The normal diet was prepared synthetically according to AIN-76A guidelines.
[2)] Calories from fat in the HFD amounted to 46.53% of the total calories.
[3)] Calories from RCD and GBRD in the HFD amounted to 7% of the total calories, respectively.

Table 2. Recipes and nutritional value of the *dasiks* tested in this study

	RCD	GBRD
Ingredient (g)		
Rice cake flour	56.8	
Mung bean starch	20.6	
Brown rice flour		75.6
Honey	22.6	
Fructooligosaccharide		22.0
Red ginseng extract		2.3
Propolis		0.1
Nutritional value		
Calories (kcal/100 g)	347.9	344.0
Carbohydrate (g/100 g)	75.1	77.4
Fiber (g/100 g)	1.7	10.7
Protein (g/100 g)	8.0	5.8
Lipid (g/100 g)	1.0	2.1

RCD, Rice cake *dasik*; GBRD, ginseng-added brown rice *dasik*.

of 30 mg/kg of a combination of zolazepam and tiletamine (Zoletil 50; Virbac Laboratories, Carros, France) and 10 mg/kg of xylazine (Rompun; Bayer Korea, Seoul, Korea). Blood was collected from the inferior vena cava into a heparin tube and then centrifuged at 3,012 × g and 4°C for 20 min to obtain the plasma. The liver was excised after perfusion with ice-cold phosphate-buffered saline (PBS) and weighed after it had been cleared of impurities and rinsed several times with PBS. The liver was then immediately placed in liquid nitrogen and stored at -80°C. Epididymal fat pads were also removed and weighed.

Determination of transaminase activities

Plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities were measured with a commercially available kit (AM101-K; Asan Pharm., Seoul, Korea).

Determination of plasma and hepatic lipid concentrations

Plasma TG and TC concentrations were measured using commercially available kits (AM157S-K and AM202-K, respectively; Asan Pharm.). The hepatic lipids were extracted from the liver tissue using a modified method of Folch *et al*. [22]. Hepatic TG and TC concentrations were measured with the same kits used for the plasma lipid analysis.

Preparation of liver homogenates, post-mitochondrial fractions, and whole cells

Liver homogenates for the lipid peroxidation and glutathione (GSH) assays were prepared using a homogenizer (PT-MR 3100; Polytron, Kinematica, Lucerne, Switzerland). Post-mitochondrial fractions of liver for ROS and peroxynitrite ($ONOO^-$) analyses were prepared by centrifugation of the liver homogenates at 18,627 × g and 4°C for 20 min.

Determination of hepatic oxidative stress-related parameters

Hepatic lipid peroxidation was determined as thiobarbituric acid-related substances (TBARS) and expressed as the malondialdehyde concentration [23]. The hepatic GSH concentration was measured using the GSH standard curve [24]. The hepatic ROS level was measured with 2′,7′-dichlorofluorescein diacetate, whereas the $ONOO^-$ level was determined using dihydrorhodamine buffer [25]. Changes in the fluorescence of the reaction mixtures of ROS and $ONOO^-$ were measured for 30 min at an excitation wavelength of 485 nm and emission wavelength of 530 nm.

Immunoblot analysis

Liver tissue was homogenized in ice-cold lysis buffer (50 mM Tris, pH 8.0, 5 mM ethylenediaminetetraacetic acid, 150 mM NaCl, and 1% nonidet-P40 containing a protease inhibitor cocktail) using a homogenizer (PT-MR 3100; Polytron), placed on ice for 1 h, and then centrifuged at 18,627 × g and 4°C for 20 min. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed using a method previously reported [26]. The primary antibodies used for the western blot assay were those for sterol regulatory element-binding protein (SREBP)-1 (H-160; sc-8984), acetyl-CoA carboxylase alpha (ACCα; T-18; sc-26817), peroxisome proliferator-activated receptor alpha (PPARα; H-98; sc-9000), carnitine palmitoyltransferase 1 (CPT1; S-17; sc-139482), peroxisomal acyl-CoA oxidase 1 (ACOX1; H-140; sc-98499), SREBP-2 (H-164; sc-5603), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR; H-300; sc-33827), microsomal cytochrome P450 family 7 subfamily A member 1 (CYP7A1; H-58; sc-25536), superoxide dismutase (SOD; FL-154; sc-11407), catalase (CAT; F-17; sc-34285), glutathione peroxidase (GPx; B-6; sc-133160), NF-κB p65 (A; sc-109), nitric oxide synthase 2 (iNOS; C-11; sc-7271), and cyclooxygenase-2 (COX-2; M-19; sc-1747) from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA), and fatty acid synthase (FAS; ab22759) from Abcam Inc. (Cambridge, UK). The horseradish peroxidase-conjugated secondary antibodies used (all procured from Abcam Inc.) were rabbit polyclonal secondary antibody to mouse IgG-H&L (ab6728), donkey polyclonal secondary antibody to rabbit IgG-H&L (ab6802), and rabbit polyclonal secondary antibody to goat IgG-H&L (ab6741). Protein expression was visualized by enhanced chemiluminescence-based detection using the CAS-400 imaging system (Core Bio, Seoul, Korea). The band densities were measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA), where corresponding protein amounts were normalized to the value of β-actin.

Statistical analysis

All data are presented as the mean ± standard deviation (SD). Statistical significance of differences in mean values of four groups were assessed by one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test. Significance was considered at $P < 0.05$.

RESULTS

Body weight gain, epididymal fat mass, and liver weights

As shown in Table 3, the body weight gain, epididymal fat mass, and liver weights of the HFD group were significantly higher than those of the NOR group ($P < 0.05$), although food intakes among the experimental groups were not different. Compared with the HFD group, however, the body weight gain, and epididymal fat mass of the RCD and GBRD groups were significantly lower. The epididymal fat mass was significantly lower in the GBRD group than in the RCD group ($P < 0.05$). In addition, liver weights of the RCD and GBRD groups were lower than the HFD group.

Plasma and hepatic lipid concentrations

As shown in Table 4, both the plasma TG and TC concentra-

Table 3. Food intake, body weight gain, and epididymal fat mass in mice fed a high-fat diet supplemented with different *dasiks* for 9 weeks

Group[1)]	Food intake (g)	Body weight gain (g)	Epididymal fat mass (g/100g bw)	Liver weights (g/100g bw)
NOR	4.63 ± 0.27[NS]	7.95 ± 1.16[c]	0.91 ± 0.24[d]	5.37 ± 1.32[b]
HFD	4.52 ± 0.20	20.10 ± 0.60[a]	4.36 ± 0.98[a]	7.61 ± 1.66[a]
RCD	4.36 ± 0.18	14.72 ± 1.73[b]	3.38 ± 0.73[b]	7.20 ± 1.86[ab]
GBRD	4.47 ± 0.39	12.57 ± 4.36[b]	2.49 ± 0.24[c]	6.58 ± 1.53[ab]

Data are the mean ± SD (n = 7 / group).
[1)] NOR, mice fed the AIN-76 diet; HFD, mice fed the high-fat diet (HFD) only; RCD, mice fed the HFD supplemented with rice cake *dasik*; GBRD, mice fed the HFD supplemented with ginseng-added brown rice *dasik*.
[a-d] Data with different letters in the column are significantly different based on ANOVA followed by Duncan's multiple-range test at $P < 0.05$.
[NS] Not significantly different at $P < 0.05$.

Table 4. Biochemical parameters in the plasma and liver of mice fed a high-fat diet supplemented with different *dasiks* for 9 weeks

Group[1)]	NOR	HFD	RCD	GBRD
Plasma				
AST	62.32 ± 17.62^{NS}	71.15 ± 17.01	62.19 ± 4.26	59.17 ± 11.97
ALT	53.51 ± 14.29^{NS}	60.66 ± 13.78	53.43 ± 3.45	50.97 ± 9.71
TG	28.52 ± 1.30^{c}	42.37 ± 4.43^{a}	35.33 ± 4.67^{b}	29.96 ± 5.20^{bc}
TC	75.00 ± 15.58^{d}	157.64 ± 17.31^{a}	134.07 ± 16.16^{b}	108.00 ± 14.44^{c}
Liver				
TG	18.57 ± 7.46^{c}	85.37 ± 8.68^{a}	62.38 ± 17.17^{b}	61.97 ± 14.12^{b}
TC	2.42 ± 0.33^{d}	16.66 ± 1.46^{a}	12.41 ± 1.91^{b}	7.76 ± 1.15^{c}
ROS	0.74 ± 0.22^{c}	2.41 ± 0.76^{a}	1.59 ± 0.18^{b}	1.47 ± 0.02^{b}
$ONOO^-$	0.96 ± 0.10^{c}	2.50 ± 0.87^{a}	1.76 ± 0.37^{b}	1.03 ± 0.19^{c}
TBARS	6.20 ± 0.10^{b}	11.25 ± 1.94^{a}	7.40 ± 0.03^{b}	6.33 ± 1.26^{b}
GSH	0.14 ± 0.03^{a}	0.02 ± 0.01^{c}	0.05 ± 0.03^{c}	0.09 ± 0.02^{b}

Data are mean ± SD (n = 7 / group).
AST, aspartate transaminase; ALT, alanine transaminase; TG, triglycerides; TC, total cholesterol; ROS, reactive oxygen species; $ONOO^-$, peroxynitrite; TBARS, thiobarbituric acid-related substances; GSH, glutathione.
[1)] NOR, mice fed the AIN-76 diet; HFD, mice fed the high-fat diet (HFD) only; RCD, mice fed the HFD supplemented with rice cake *dasik*; GBRD, mice fed the HFD supplemented with ginseng-added brown rice *dasik*.
[a-d] Data with different letters in the row are significantly different based on one-way ANOVA followed by Duncan's multiple-range test at $P < 0.05$.

tions were higher in the GBRD, RCD, and HFD groups than in the NOR group, but varied among the groups in the order of HFD > RCD > GBRD. The hepatic TG and TC concentrations in the RCD and GBRD groups were lower than those in the HFD group ($P < 0.05$). The plasma and hepatic TC levels in the GBRD group were significantly lower, by 19.44% and 37.48%, respectively ($P < 0.05$), than those in the RCD group. However, both the plasma and hepatic TG concentrations between the RCD and GBRD groups were not significantly different. The plasma AST and ALT activities from all experimental groups were in the normal range.

Hepatic oxidative stress-related markers

As shown in Table 4, hepatic ROS, $ONOO^-$, and TBARS levels in the RCD and GBRD groups were lower than those in the HFD group ($P < 0.05$). Moreover, the hepatic $ONOO^-$ concentration in the GBRD group was significantly reduced by 41.77% compared with that in the RCD group. On the other hand, the hepatic GSH level in the GBRD group was increased by 395.06% and 178.44%, respectively, relative to that in the HFD and RCD groups ($P < 0.05$).

Expression of proteins related to fatty acid synthesis and oxidation

The expression of proteins related to fatty acid synthesis (SREBP-1 and FAS) was not significantly different among the HFD-fed groups (Fig. 1). However, the expression of ACCα in the RCD and GBRD groups was significantly decreased compared with that in the HFD group ($P < 0.05$). On the other hand, the hepatic expression levels of PPARα, CPT1, and ACOX1 were increased in the RCD and GBRD groups ($P < 0.05$) compared with those in the HFD group. However, there were no differences in PPARα, CPT1, and ACOX1 expression levels between the RCD and GBRD groups.

Expression of proteins related to cholesterol synthesis and catabolism

Protein expression of the mature form of SREBP-2 and HMGCR was significantly decreased in the RCD and GBRD groups compared with that in the HFD group ($P < 0.05$, Fig. 2). Moreover, the hepatic expression levels of SREBP-2 and HMGCR in the GBRD group were significantly reduced by 30.37% and 28.07%, respectively, relative to the levels in the RCD group ($P < 0.05$). The protein expression level of cholesterol CYP7A1 was significantly higher in the RCD and GBRD groups than in the HFD group; however, no significant difference in gene expression was found between the RCD and GBRD groups.

Expression of antioxidant enzymes

As shown in Fig. 3, expression of the antioxidant enzymes was significantly downregulated in the three HFD groups compared with that in the NOR group ($P < 0.05$, Fig. 3). Compared with the HFD group, the CAT and GPx expression levels in both the RCD and GBRD groups were significantly increased ($P < 0.05$). Moreover, GPx protein expression in the GBRD group was significantly increased by 116.52% relative to the level in the RCD group ($P < 0.05$).

Inflammation-related markers

As shown in Fig. 4, the GBRD group had overall lower inflammatory transcription factor and enzyme expression levels

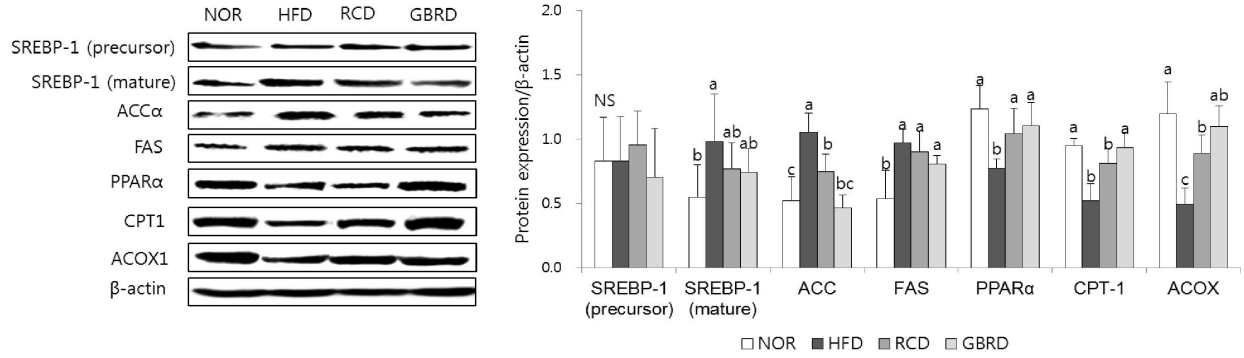

Fig. 1. Protein expression of transcription factors and enzymes in fatty acid metabolism in the liver of mice fed a high-fat diet (HFD) supplemented with different *dasiks* for 9 weeks. Data are the mean ± SD (n = 7 /group). NOR, mice fed the AIN-76 diet; HFD, mice fed the HFD only; RCD, mice fed the HFD supplemented with rice cake *dasik*; GBRD, mice fed the HFD supplemented with ginseng-added brown rice *dasik*. [a-c]Data with different letters are significantly different based on one-way ANOVA followed by Duncan's multiple-range test at $P < 0.05$. [NS]Not significantly different at $P < 0.05$.

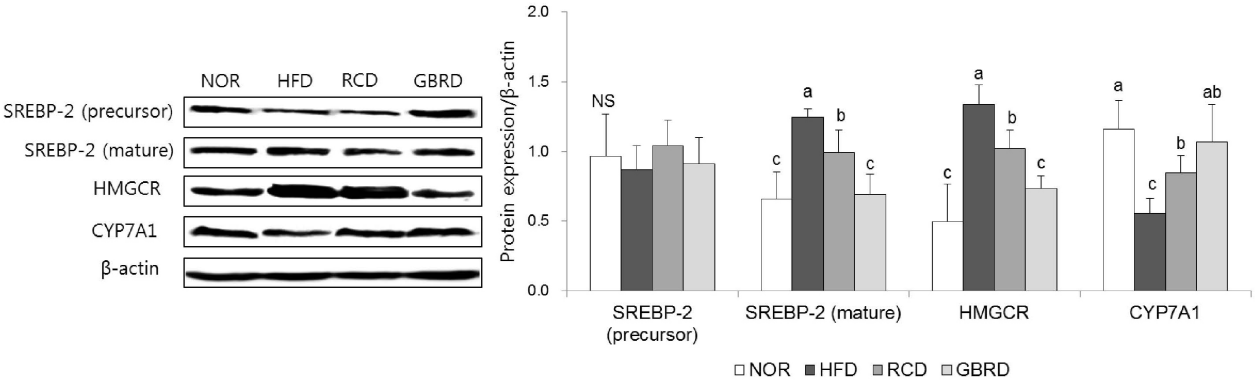

Fig. 2. Protein expression of transcription factors and enzymes in cholesterol metabolism in the liver of mice fed a high-fat diet (HFD) supplemented with different *dasiks* for 9 weeks. Data are the mean ± SD (n = 7 /group). NOR, mice fed the AIN-76 diet; HFD, mice fed the HFD only; RCD, mice fed the HFD supplemented with rice cake *dasik*; GBRD, mice fed the HFD supplemented with ginseng-added brown rice *dasik*. [a-c]Data with different letters are significantly different based on one-way ANOVA followed by Duncan's multiple-range test at $P < 0.05$. [NS]Not significantly different at $P < 0.05$.

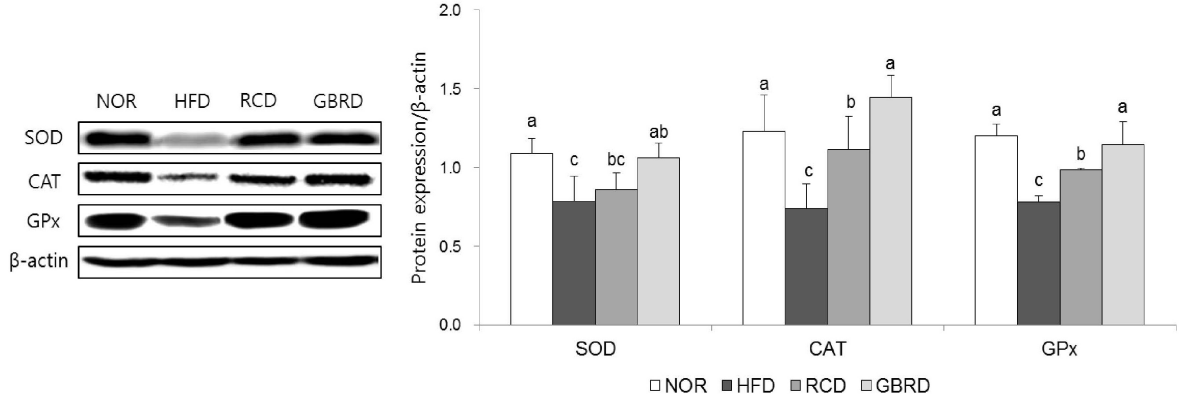

Fig. 3. Protein expression of antioxidant enzymes in the liver of mice fed a high-fat diet (HFD) supplemented with different *dasiks* for 9 weeks. Data are the mean ± SD (n = 7 /group). NOR, mice fed the AIN-76 diet; HFD, mice fed the HFD only; RCD, mice fed the HFD supplemented with rice cake *dasik*; GBRD, mice fed the HFD supplemented with ginseng-added brown rice *dasik*. [a-c]Data with different letters are significantly different based on one-way ANOVA followed by Duncan's multiple-range test at $P < 0.05$.

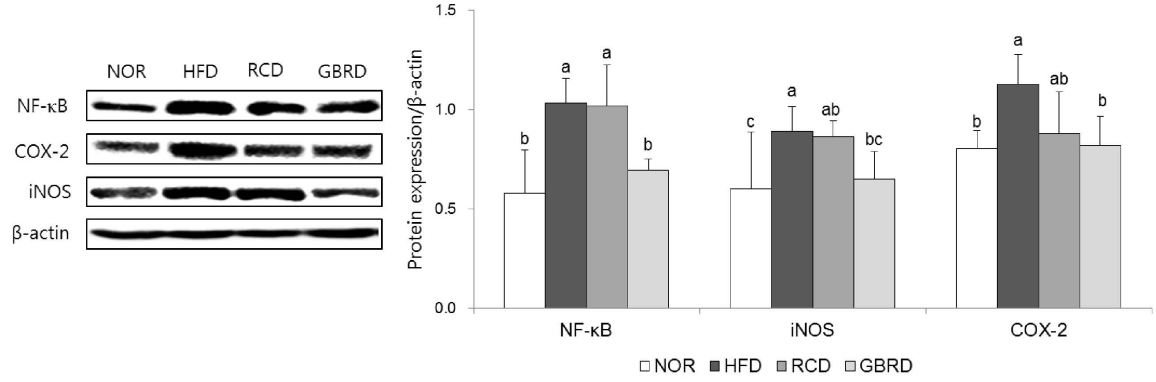

Fig. 4. Protein expression of inflammation-related transcription factors and enzymes in the liver of mice fed a high-fat diet (HFD) supplemented with different *dasiks* for 9 weeks. Data are the mean ± SD (n = 7 /group). NOR, mice fed the AIN-76 diet; HFD, mice fed the HFD only; RCD, mice fed the HFD supplemented with rice cake *dasik*; GBRD, mice fed the HFD supplemented with ginseng-added brown rice *dasik*. [a-c]Data with different letters are significantly different based on one-way ANOVA followed by Duncan's multiple-range test at $P < 0.05$.

($P < 0.05$). Compared with the HFD group, the expression of NF-κB, iNOS, and COX-2 in the GBRD group was downregulated by 32.68%, 27.14%, and 27.54%, respectively ($P < 0.05$). Moreover, the expression of NF-κB in the GBRD group was decreased by 31.83% relative to the level in the RCD group ($P < 0.05$).

DISCUSSION

The ingredients in snacks are important because each calorie from snacking contributes significantly to the total daily calorie intake of an individual. There is therefore a demand for new

snack varieties with better nutritional value than that available in current snacks. *Dasik*, a Korean traditional cookie, is a snack prepared with plant origin ingredients such as rice, starch, pine pollen, black sesame seeds, and honey as a sweetener. In our previous study, RCD (a representative *dasik*) showed higher antioxidative and lipid-lowering effects than a western-style cookie in mice fed a HFD, and these effects were significant despite that the calories from the two snacks were the same [1]. Moreover, GBRD revealed a plasma cholesterol-lowering effect in middle-aged women [2]. In this present study, the functional properties of GBRD with regard to its lipid-lowering, antioxidative, and anti-inflammatory effects were examined and their mechanisms of action were elucidated in mice fed a HFD resembling the diet condition of overweight individuals. The important findings of this study that the GBRD group inhibited cholesterol synthesis through a decrease of the plasma and hepatic TC levels, compared with the RCD group. Moreover, the expression of antioxidant enzymes was upregulated, whereas that of inflammatory transcription factors was downregulated.

Grains are a major carbohydrate source for energy production in the body. People usually prefer polished grains to unpolished ones, although the nutritional value of whole grains is higher. Whole grains are rich in dietary fiber, which decreases the plasma lipids, in particular cholesterol. Hepatic lipid metabolism is mainly regulated by SREBPs, which mediate the synthesis and uptake of TG, TC, and fatty acids in the liver [27]. Precursor SREBPs (inactivated form) are cleaved by binding sterol regulatory elements and activated to mature SREBPs (activated form), which regulates expression of target genes. Numerous studies have indicated that HFD-fed mice were increased SREBPs levels in the liver [28,29]. SREBP-1 plays a key role in TG synthesis by regulating lipogenesis genes such as FAS and stearoyl-CoA desaturase 1, whereas SREBP-2 plays a key role in cholesterol synthesis by regulating genes such as HMGCR and low-density lipoprotein receptor [30]. Moreover, lipolysis genes such as PPARα and CD36 also play a role in fatty acid oxidation [31,32]. In the current study, GBRD decreased the plasma and hepatic TC concentrations through inhibition of cholesterol synthesis. The SREBP-2 and HMGCR expression levels elevated by the HFD were decreased in the GBRD group, whereas CYP7A1 expression was increased. The plasma and hepatic cholesterol-lowering effects of GBRD were significantly higher than those of RCD ($P < 0.05$). Our results are in line with previous studies that brown rice or pre-germinated brown rice suppressed hypercholesterolemia by stimulating bile acid synthesis via the increase of CYP7A1 [33]. γ-oryzanol in brown rice contributed to the plasma cholesterol level reduction by inhibiting cholesterol absorption [34], in addition to the enhanced fecal excretion by dietary fiber [35]. The γ-oryzanol content in brown rice is approximately 21-fold higher than that in polished rice [35]. Furthermore, the lipid-lowering effects of GBRD might, in part, be from the red ginseng. Korean red ginseng extract exerted plasma lipid-lowering effects in HFD-fed mice by regulating genes associated with lipid metabolism [11]. In particular, the mechanisms of action of ginsenosides Rb1 [36], Re [37], Rg1 [38], and Rg3 [39] in lowering lipid and cholesterol levels are well established as being through the downregulation of SREBPs and associated enzymes and the upregulation of fatty acid oxidation-related factors. The ginsenosides Ro, Rg3, Re, Rg1, and Rg2 increased the mRNA levels of CYP7A1 in hypercholesterolemic rats, which increased bile salt synthesis [40]. Moreover, the FOS used as a honey replacement in GBRD might also reveal lipid-lowering effects. FOS was found to have decreased the plasma and hepatic lipid levels and epididymal fat mass, but increased the fecal TG, TC, and nonesterified fatty acids, in C57BL/6J mice fed a HFD [8]. There is a natural demand for sugar alternatives with fewer calories but with sweet taste, because the daily intake of calorie-dense snacks is culpable for the development of obesity, especially in youngsters. Although FOS has 30-60% of sweetness relative to sugar, it produces only 0-3 kcal per gram [5].

Oxidative stress is one of the major contributors to the development of a variety of diseases, including hepatic steatosis, by damaging the normal cell condition [40]. Overloaded energy production by a HFD produces a large amount of ROS relative to that induced during normal energy production, which subsequently elevates oxidative stress. Antioxidant enzymes such as SOD, CAT, and GPx, and reduced-GSH scavenge ROS and free radicals and/or prevent their formation. In our current study, among the experimental groups, GBRD demonstrated the lowest oxidative stress levels by diminishing lipid peroxidation, ROS, and $ONOO^-$ production. However, GBRD upregulated antioxidant enzyme expression. It might be due to the beneficial effects of Korean red ginseng, which has revealed preventive effects against free radical-induced oxidative damage [41,42]. Korean red ginseng has demonstrated DPPH radical-scavenging [9] and lipid peroxidation inhibition activities [43]. Moreover, it augmented SOD activity to near-normal levels in various organs of aged rats compared with the effects in young rats [44]. This might be due to the effects of the nutrients in ginseng, including ginsenosides, antioxidant vitamins, sulfur-containing amino acids, trace elements, and other active compounds [45]. Furthermore, the antioxidant γ-oryzanol [46] in brown rice may, in part, have contributed to the decrease in the HFD-induced oxidative stress.

High dietary fat intake is strongly associated with the development of degenerative diseases due to oxidative stress elevation, a fact that is confirmed from epidemiological and experimental studies. In this study, using brown instead of white rice, adding ginseng extract, and replacing honey with FOS further promoted the health benefits of *dasik* relative to those of RCD that had already shown greater health benefits than western-style cookies [1]. The lipid-lowering effect of GBRD was more apparent on TC, mediated via SREBP-2 downregulation. It also decreased hepatic oxidative stress and demonstrated anti-inflammatory effects via NF-κB downregulation. Taken together, our finding concludes that intake of GBRD, a functional *dasik*, would be better for health benefits. Further studies are required to optimize the automated production of *dasik* products, including GBRD.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interests.

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