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Rice Cookie Decreases Plasma and Hepatic Lipid Levels in High-Fat Diet-fed Mice: A Comparison Study with Traditional Western Style Cookies

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Abstract The lipid-lowering and antioxidant effects of the traditional Korean rice cookie (KRC), *dasik*, were compared with those of a western style cookie (WSC) in mice fed a high-fat diet (HFD). The KRC or WSC was supplemented to the HFD as 7% of the total calories. The experimental groups (n = 7) were the normal diet group, HFD group, HFD-KRC group, and HFD-WSC group. The plasma and hepatic triglyceride concentrations of the HFD-KRC group were found to be lower than those of the HFD-WSC group as a result of sterol regulatory element-binding protein 1 and fatty acid synthase expression downregulation, and concomitant peroxisome proliferator-activated receptor-alpha, carnitine palmitoyltransferase 1, and acyl-coenzyme A oxidase 1 expression upregulation ($p<0.05$). The hepatic reactive oxygen species and peroxynitrite levels were also diminished in the HFD-KRC group of mice, whereas their catalase and glutathione peroxidase protein expression levels were higher than those in the HFD and HFD-WSC groups ($p<0.05$). In conclusion, the lipid-lowering effects and antioxidant property of the KRC were greater than those of the WSC in mice fed a HFD. Thus, the choice of KRCs as a snack would be preferable to choosing WSCs.

Keywords: *rice cookie, high-fat diet, lipid, antioxidant, transcription factor*

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1. Introduction

Calorie-heavy snacks have become a major concern in regard to public health. In the USA, calories from snacking have increased steadily to approximately 25% of the daily calorie intake [1]. Moreover, the nutritional value of traditional western style cookies (WSCs), made using refined flour, butter, eggs, and sugar, is considered to be poor because of their high sugar and fat, but low fiber, vitamin, and mineral, contents [2]. It is a well-known fact that these ingredients, particularly fat and sugar, are positively associated with plasma lipid elevation [3]. In contrast, the Korean traditional rice cookie (KRC), *dasik* (meaning "a tea food"), is made from steamed rice-cake flour, mung bean starch, and honey. Other sugar and fat sources are not used and further baking or frying is not required for *dasik* preparation.

Steamed rice has shown hypolipidemic effects in hamsters fed a high-fat diet (HFD), by downregulating the expression of transcription factors and genes related to cholesterol and fatty acid synthesis and upregulating those related to lipid oxidation [4]. The glycemic index (GI) of mung bean starch is low, which is advantageous since diets with a low GI have revealed serum lipid-lowering effects [5]. Therefore, these carbohydrate sources of KRCs might have a more beneficial effect on plasma lipid profiles than the wheat flour of WSCs. Aside from the carbohydrate source; the type of sweetener used for cookie preparation might also change the plasma lipid concentration. Sugar, the main sweetener in WSCs, is cited as a positive attributor of hyperlipidemia [6], whereas honey has exerted triglyceride (TG)- and total cholesterol (TC)-lowering effects in humans [7].

Hyperlipidemia, a condition characterized by a high level of TG or cholesterol in the blood [8], is a reflection of increased fatty acid synthesis and decreased hepatic lipid oxidation. The biosyntheses of cholesterols, fatty acids, and TGs are tightly regulated by a family of transcription factors named sterol regulatory element-binding proteins (SREBPs) [9]. SREBP-1 mainly stimulates fatty acid synthesis via the upregulation of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). Transcription factor SREBP-2 regulates the enzymes involved in cholesterol biosynthesis, including 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) [10]. Patients with hyperlipidemia are usually treated with a drug or functional food that inhibits SREBP expression [10]. Lipid catabolism, in particular fatty acid oxidation, is principally regulated by peroxisome proliferator-activated receptor-alpha (PPAR-α) through the upregulation of enzymes such as carnitine palmitoyltransferase 1 (CPT1) and acyl-coenzyme A oxidase 1 (ACOX1) [11]. The major route for plasma

cholesterol elimination is via bile acid secretion. The microsomal cytochrome P450 family 7 subfamily A member 1 (CYP7A1; also known as cytochrome P450 cholesterol 7 alpha-hydroxylase or cytochrome P450 cholesterol 7 alpha-monooxygenase) is the rate-limiting enzyme that catalyzes the conversion of cholesterol into bile acids in the liver [12].

Under the condition of hyperlipidemia, oxidative stress is commonly observed. Reactive oxygen species (ROS) are produced in living organisms as a result of normal cellular metabolism and environmental factors [13]. Aerobic organisms have integrated antioxidant systems (including enzymatic and non-enzymatic antioxidants) that prevent the oxidative damage from ROS [13]. However, oxidative stress is elevated if the antioxidant systems are not properly activated, which can lead to the development of chronic diseases such as atherosclerosis, hypertension, cancer, neurological disorders, diabetes, and acute respiratory distress syndrome [13,14]. Postprandial oxidative stress is characterized by an increased susceptibility of organisms toward oxidative damage after consumption of a meal rich in lipids and/or carbohydrates [15]. Thus, the macronutrients in foods can have effects on the redox balance in the body, where they either are a target of oxidative modifications after absorption or are present in a prooxidant form in the diet. Hyperlipidemia and hyperglycemia are cited as positive contributors to elevated oxidative damage, through their effects on lipoprotein levels and the antioxidant status [16]. Postprandial increases of plasma lipid and carbohydrate levels therefore increase the risk of atherosclerosis and related disorders by increasing oxidative stress [17].

In this study, the health benefits of the KRC and of a traditional WSC were compared by evaluating their effects on the plasma and hepatic lipid concentrations, ROS production, lipid peroxidation, and antioxidant status (and its related mechanisms) in HFD-fed mice.

2. Materials and Methods

2.1. Preparation of the Cookie Samples

The KRC was made up of 55.6% rice cake flour, 22.2% mung bean starch, and 22.2% honey. The ingredients were mixed thoroughly and an exact amount of the dough was then placed into a special-purpose mold. Commercially available WSCs (Lotus Bakeries, Kaprijke, Belgium) were purchased at a local market, and according to the manufacturer's information, the ingredients were wheat flour, sugar, vegetable oils, brown sugar, sodium bicarbonate, soy flour, salt, and cinnamon.

2.2. Evaluation of the Nutritional Value of the Cookie Samples

The nutritional value of both types of cookies, including calories and contents of protein, fat, carbohydrate, and fiber, were determined using a diet-analyzing program (CAN-pro 3.0; Korean Nutrition Society, Seoul, Korea). For the KRC, the recipe was used. For the WSC, the nutrition information on the product label was used. The nutritional value was expressed as kcal/100 g or g/100 g.

2.3. Experimental Diets and Animals

As shown in Table 1, an AIN-76 synthetic diet, a HFD, and a HFD containing KRC or WSC were prepared. The HFD was prepared by adding lard and cholesterol to the AIN-76 diet to provide 46.53% of the total calories from fats. For the dessert diet, the KRC or WSC was supplemented to the HFD as 7% of the total calories, which were calculated as follows; calories from daily snack consumption has been reported approximately 25% to total calorie intake and calorie from carbohydrate-dessert is accounted as 26% to total calorie from all snacks [1] (25% * 0.26 ≒ 7%). The normal diet contained 3.9 kcal/g, whereas the HFD, HFD-KRC, and HFD-WSC contained 5.0 kcal/g.

Table 1. Composition of the experimental diets

Ingredient (g)	ND[1)]	HFD[2)]	HFD + dessert[3)]	
			KRC	WSC
Casein	200	171	175	176
DL-Methionine	3	3	3	3
Corn starch	350	203	127	128
Sucrose	300	279	262	264
Cellulose	50	43	44	44
Corn oil	50	39	41	41
Lard		217	222	224
Cholesterol		5	5	5
Mineral mix	35	30	31	31
Vitamin mix	10	9	9	9
Choline bitartrate	2	2	2	2
Cookie				73
Rice cake flour			59	
Mung bean starch			22	
Brown rice flour				
Honey			24	

HFD, high-fat diet; KRC, Korean rice cookie; WSC, Western style cookie.
[1)]Normal diet (ND) was prepared synthetically according to AIN-76A guidelines.
[2)]The calories from fat in the HFD was 46.53% of the total calories.
[3)]The calories from the KRC or WSC in the HFD was 7% of the total calories.

Male ICR mice (n = 28; 4 weeks old) were purchased from Orient Bio Inc. (Seongnam, Korea). The animals were kept in individual cages during the entire experimental period under the controlled condition 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 the four experimental groups: normal diet (NOR) group, HFD group, KRC-containing HFD (HFD-KRC) group, and WSC-containing HFD (HFD-WSC) group. The animals had free access to the diet and water for 9 weeks. The food consumption and body weight of the mice were measured every week. The animal protocol was reviewed for ethical procedures and scientific care and approved by the Pusan National University–Institutional Animal Care and Use Committee (Approval No. PNU-2012-0118).

2.4. Plasma, Liver, and Epididymal Fat Pad Collection

After 12 h of fasting, the mice were anesthetized by intraperitoneal administration of 30 mg/kg of a zolazepam and tiletamine combination (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 heparin tubes, which were then centrifuged at 3,012 ×*g* and 4°C for 20 min to obtain the plasma. The liver was removed after perfusion with ice-cold phosphate-buffered saline (PBS), and after impurities had been detached, it was rinsed several times with PBS and then weighed. Epididymal fat pads were excised and weighed. The plasma and liver samples were placed immediately on dry ice and stored at –80°C for further analysis.

2.5. Determination of Biochemical Parameters in the Plasma and Liver

The plasma leptin concentration was measured using a leptin ELISA kit (Cat. No. KMC2281; Invitrogen Co., Camarillo, CA, USA) according to the manufacturer's protocol. The detection sensitivity of the assay is <50 pg/mL. Plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities were measured with a commercially available kit (AM101-K; Asan Pharm., Seoul, Korea).

Plasma TG and TC concentrations were measured using commercial 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. [18]. Hepatic TG and TC concentrations were measured with the same kits used for the plasma lipid analyses.

2.6. Determination of Hepatic Oxidative Stress-related Parameters

Liver homogenates used for thiobarbituric acid-related substances (TBARS) and glutathione (GSH) concentration determination were prepared by homogenizing hepatic tissue with ice-cold PBS (1:9, w/w). The post mitochondrial fraction of liver used for determining the ROS and peroxynitrite ($ONOO^-$) levels was obtained by centrifugation of the liver homogenates at 18,627 ×*g* and 4°C for 20 min. Hepatic lipid peroxidation was determined as TBARS and expressed as the malondialdehyde (MDA) concentration [19]. The hepatic GSH concentration was measured using the method of Ellman [20]. Hepatic ROS and $ONOO^-$ levels were measured by 2′,7′-dichlorofluorescein diacetate and dihydrorhodamine 123 assays [21], respectively. Changes in fluorescence of the reaction mixture were measured for 30 min at an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

2.7. Western Blot Analysis

To obtain a whole-cell hepatic fraction, liver tissue was homogenized in ice-cold lysis buffer (50 mM Tris, pH 8.0, 5 mM EDTA, 150 mM NaCl, and 1% nonidet-P40 containing protease inhibitor cocktail) using a Polytron homogenizer (PT-MR 3100; Polytron, Kinematica, Lucerne, Switzerland), 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 of the fraction was performed using a previously reported method [22], in preparation for western blot analysis. The primary antibodies used for the western blot assay were those for SREBP-1 (H-160; sc-8984), ACCα (T-18; sc-26817), PPARα (H-98; sc-9000), CPT1-C (S-17; sc-139482), ACOX1 (H-140; sc-98499), SREBP-2 (H-164; sc-5603), HMGCR (H-300; sc-33827), CYP7A1 (H-58; sc-25536), SOD-1 (FL-154; sc-11407), catalase (F-17; sc-34285), and glutathione peroxidase (GPx-1/2) (B-6; sc-133160) from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA), and anti-fatty acid synthase (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.

2.8. Statistical Analysis

Data are presented as the mean ± standard deviation. Statistical significance of differences in the mean values among the four groups was assessed by one-way analysis of variance, followed by Duncan's multiple-range test as a post hoc analysis to determine where the significance exists at $p<0.05$. Student's *t*-test was used to determine differences between two groups.

3. Results and Discussion

3.1. Nutritional Evaluation of the Two Types of Cookies

In 100 g of the KRC versus 100 g of the WSC, the calorie amount was 347.9 kcal versus 496.0 kcal, the carbohydrate content was 75.1 g versus 73.3 g, the protein content was 8.0 g versus 6.7 g, and the fat content was 1 g versus 20 g, respectively.

3.2. Body Weight, Epididymal Fat Mass, and Leptin Level

As shown in Table 2, the body weight gain, epididymal fat mass, and leptin level of the HFD-KRC group were significantly lower than those of the HFD-WSC group ($p<0.05$). This might be due to the different ingredients used in each type of cookie, even though the calories provided to the experimental groups were the same. Although the carbohydrate contents in the KRC and WSC were the same, the grain source for the KRC is rice and that for the WSC is wheat. As mentioned before, cooked rice was reported to lower the epididymal and retroperitoneal fat mass of hamsters fed a HFD [4]. In addition, the lipid content in the WSC was 20-fold higher than that in the KRC (20 g/100 g vs 1 g/100 g). The lack of fiber in the WSC might have attributed to the body weight gain in the HFD-WSC group.

Table 2. Food intake, body weight gain, epididymal fat mass, and leptin level in mice fed a cookie-supplemented high-fat diet for 9 weeks

Group[1)]	Food intake (g)	Body weight gain (g)	Epididymal fat mass (g/100 g bw)	Leptin (μg/mL)
NOR	4.53 ± 0.14[NS]	7.95 ± 1.16[c]	0.90 ± 0.24[c]	1.89 ± 0.81[c]
HFD	4.51 ± 0.17	20.10 ± 0.60[a]	3.95 ± 0.24[a]	7.62 ± 0.76[a]
HFD-KRC	4.56 ± 0.45	15.05 ± 1.68[b]	2.98 ± 0.54[b]	6.01 ± 0.66[b]
HFD-WSC	4.67 ± 0.24	20.50 ± 3.85[a]	3.80 ± 0.85[a]	7.53 ± 0.91[a]

Data are the mean ± SD (n = 7 per group).
[1)]NOR, mice fed the AIN-76 diet; HFD, mice fed the high-fat diet (HFD) only; HFD-KRC, mice fed the HFD supplemented with the Korean rice cookie (KRC); HFD-WSC, mice fed the HFD supplemented with the Western style cookie (WSC).
[a-c]Data with different letters in the column are significantly different according to one-way ANOVA followed by Duncan's multiple range test at $p<0.05$.
[NS]Not significantly different at $p<0.05$.

A HFD increases the body weight and results in adipose tissue accumulation [23]. Leptin, a hormone secreted primarily by adipocytes, controls the body weight by regulating food intake and increasing energy expenditure [24]. Leptin levels are positively correlated with body weight and epididymal fat mass [25].

3.3. Lipid-lowering Effects of Korean Rice Cookie

Compared with the levels in the NOR group, the plasma TG and TC concentrations of the HFD group had increased by 151.96% and 151.39%, respectively ($p<0.05$, Table 3), and the hepatic concentrations were likewise augmented by 474% and 609%, respectively ($p<0.05$, Table 3). These results are in line with other studies that showed a significant increase in plasma and hepatic lipid levels induced by a HFD [26]. Among the HFD groups, the plasma and hepatic TG concentrations of the HFD-KRC group were significantly lower than those of the HFD group ($p<0.05$), but no differences were found in the plasma and hepatic TC levels. However, when the plasma and hepatic lipid levels were compared between the HFD-KRC and HFD-WSC groups, significant differences were observed ($p<0.05$ by Student's *t*-test). In particular, the plasma and hepatic TG concentrations of the HFD-KRC group were significantly lower (by 23.35% and 21.36%, respectively, $p<0.05$) than those of the HFD-WSC group. Since the lipid content in the WSC was 20-fold higher than that in the KRC, the lipid-lowering effect of the KRC might be due to its lower lipid content as well as its carbohydrate sources, which were different from those of the WSC. A diet supplemented with cooked rice decreased the plasma and hepatic lipid concentrations in HFD-fed hamsters [4]. Moreover, honey was demonstrated to have plasma TG- and TC-lowering effects in humans [27], whereas sugar is a known attributor of hyperlipidemia [28]. Mung bean starch with its low GI might be another contributor of the lower lipid concentration, as diets with a low GI have demonstrated serum lipid-lowering effects [5].

3.4. Inhibition of Hepatic Triglyceride Metabolism by Korean Rice Cookie

Protein expression of the mature forms of SREBP-1, ACC, and FAS in the HFD group was increased by 185.48%, 168.42%, and 174.07%, respectively, relative to the levels in the NOR group ($p<0.05$; Figure 1), which were in good agreement with the hepatic TG results observed in this study. The expression levels of the lipogenic transcription factor and enzymes were similar between the HFD-WSC and HFD groups, but were comparatively lower in the HFD-KRC group ($p<0.05$). In particular, the SREBP-1 and FAS expression levels of the HFD-KRC group were significantly decreased by 35.00% and 31.87%, respectively, compared with those of the HFD-WSC group. In the case of lipid oxidation factors, the expression levels of PPARα, CPT1, and ACOX1 were lower in the HFD group than in the NOR group ($p<0.05$). Among the HFD-fed groups, the HFD-KRC group had higher expression of transcription factor and enzymes related to fatty acid oxidation than the HFD and HFD-WSC groups ($p<0.05$). In the HFD-KRC group, the expression of PPARα, CPT1, and ACOX1 was increased by 131.09%, 175.20%, and 202.18%, respectively, compared with the HFD group, and by 154.32%, 130.18%, and 154.64%, respectively, compared with the HFD-WSC group. Since cooked rice has been reported to reduce the expression of lipogenic genes (e.g., SREBP-1, ACC, and FAS), while increasing the β-oxidation-related genes (including PPARα and its target enzymes CPT1 and ACOX1) in HFD-fed hamster [4], the lipid-lowering effects of the KRC in our study are likely due to its major ingredient, rice cake flour.

3.5. Effects of Korean Rice Cookie on the Regulation of Hepatic Cholesterol Metabolism

SREBP-2 regulates the TC level in the body by increasing cholesterol biosynthetic enzymes, including HMGCR [10], whereas CYP7A1 catalyzes the excretion of cholesterol via the digestive tract [12]. In this study, the mature forms of SREBP-2 and HMGCR were increased and CYP7A1 was decreased in the HFD group relative to the levels in the NOR group ($p<0.05$, Figure 2). HFD-KRC significantly decreased the expression of SREBP-2 and HMGCR and increased that of CYP7A1 relative to the levels in the HFD group ($p<0.05$). In addition, SREBP-2 and HMGCR expressions were 20.69% and 21.32% lower, respectively, in the HFD-KRC group than in the HFD-WSC group ($p<0.05$). In the same way that cooked rice lowered cholesterol levels via the regulation of cholesterol metabolism, downregulation of HMGCR mRNA and protein levels, and upregulation of CYP7A1 expression in HFD-fed hamsters [4], so too had the rice flour in the KRC executed similar effects in HFD-fed mice.

Table 3. Biochemical parameters in the plasma and liver of mice fed a cookie-supplemented high-fat diet for 9 weeks

Group[1)]	NOR	HFD	HFD-KRC	HFD-WSC
Plasma				
AST	57.30 ± 5.77[b]	74.50 ± 8.85[a]	60.80 ± 2.87[b]	59.19 ± 9.66[b]
ALT	49.47 ± 4.68[b]	63.38 ± 7.14[a]	52.31 ± 2.33[b]	51.00 ± 7.82[b]
TG	28.52 ± 1.30[b]	43.34 ± 4.45[a]	35.33 ± 4.67[b]	46.09 ± 6.83[a]
TC	102.53 ± 29.21[b]	155.22 ± 16.58[a]	132.23 ± 12.94[a,*]	136.23 ± 22.45[a]
Liver				
TG	21.06 ± 4.81[d]	99.82 ± 13.95[a]	61.66 ± 14.58[c]	78.41 ± 9.48[b]
TC	2.42 ± 0.33[c]	14.73 ± 3.20[a]	10.82 ± 1.49[b]	12.60 ± 0.74[ab]
ROS	1.00 ± 0.12[c]	2.29 ± 0.88[ab]	1.68 ± 0.36[bc]	2.54 ± 0.76[a]
$ONOO^-$	1.00 ± 0.44[c]	2.06 ± 0.72[ab]	1.34 ± 0.41[bc]	2.88 ± 0.91[a]
TBARS	6.20 ± 0.10[b]	10.59 ± 2.23[a]	6.34 ± 1.18[b]	8.14 ± 2.48[ab]
GSH	0.090 ± 0.066[a]	0.030 ± 0.016[b]	0.069 ± 0.030[ab,*,†]	0.028 ± 0.014[b]

Data are the mean ± SD (n = 7 per group).
ALT, alanine transaminase; AST, aspartic acid transaminase; GSH, glutathione; $ONOO^-$, peroxynitrite; ROS, reactive oxygen species; TBARS, thiobarbituric acid-related substances; TC, total cholesterol; TG, triglycerides.
[1)]NOR, mice fed the AIN-76 diet; HFD, mice fed the high-fat diet only; HFD-KRC, mice fed the HFD supplemented with the Korean rice cookie (KRC); HFD-WSC, mice fed the HFD supplemented with the Western style cookie (WSC).
[a-d]Data with different letters in the row are significantly different according to one-way ANOVA followed by Duncan's multiple range test at $p<0.05$.
[*]Significantly different according to Student's *t*-test at $p<0.05$ compared with the HFD group.
[†]Significantly different according to Student's *t*-test at $p<0.05$ compared with the HFD-WSC group.

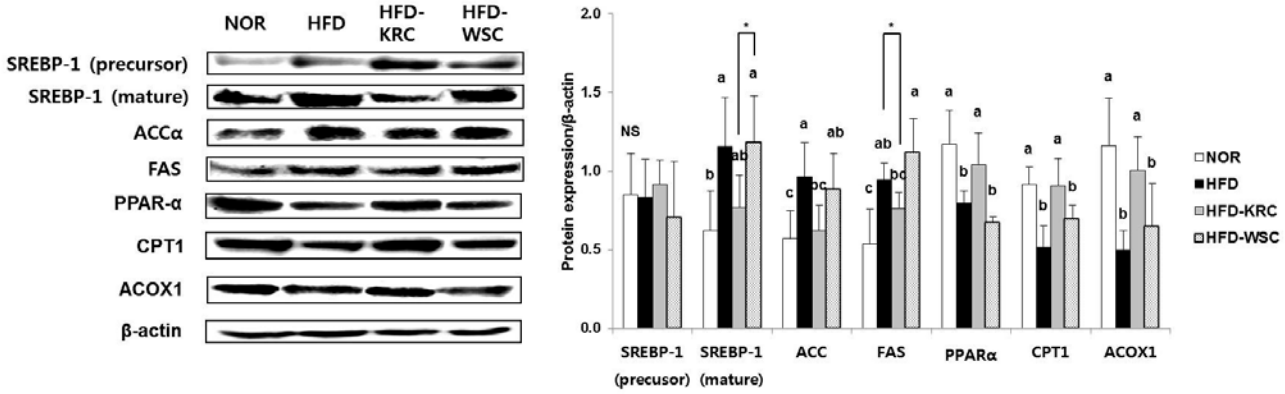

Figure 1. Protein expression of transcription factors and enzymes involved in fatty acid metabolism in the liver of mice fed a cookie-supplemented high-fat diet for 9 weeks. NOR, mice fed the AIN-76 diet; HFD, mice fed the high fat diet only; HFD-KRC, mice fed the HFD supplemented with the Korean rice cookie; HFD-WSC, mice fed the HFD supplemented with the Western style cookie. [a-c]Data with different letters are significantly different according to one-way ANOVA followed by Duncan's multiple range test at $p<0.05$. [*]Significantly different according to Student's *t*-test at $p<0.05$ between two groups. [NS]Not significantly different at $p<0.05$

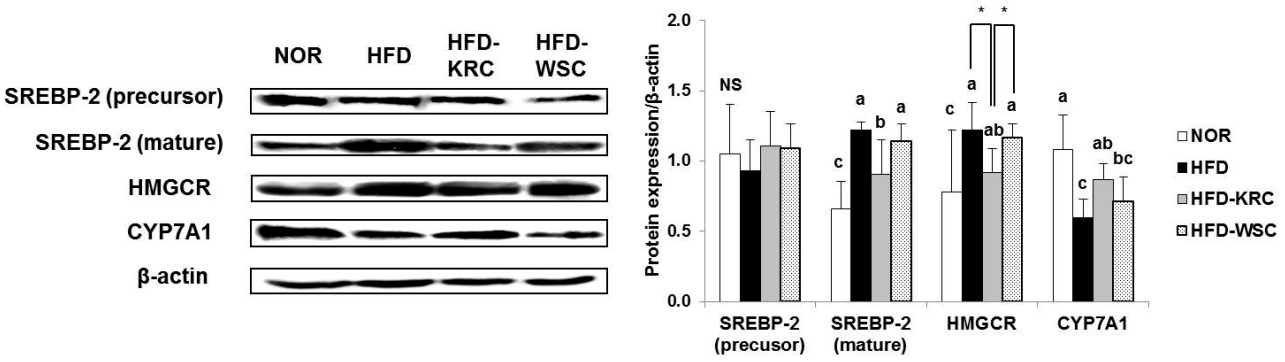

Figure 2. Protein expression of transcription factors and enzymes involved in cholesterol metabolism in the liver of mice fed a cookie-supplemented high-fat diet for 9 weeks. NOR, mice fed the AIN-76 diet; HFD, mice fed the high-fat diet only; HFD-KRC, mice fed the HFD supplemented with the Korean rice cookie; HFD-WSC, mice fed the HFD supplemented with the Western style cookie. [a-c]Data with different letters are significantly different according to one-way ANOVA followed by Duncan's multiple range test at $p<0.05$. [*]Significantly different according to Student's *t*-test at $p<0.05$ between two groups. [NS]Not significantly different at $p<0.05$

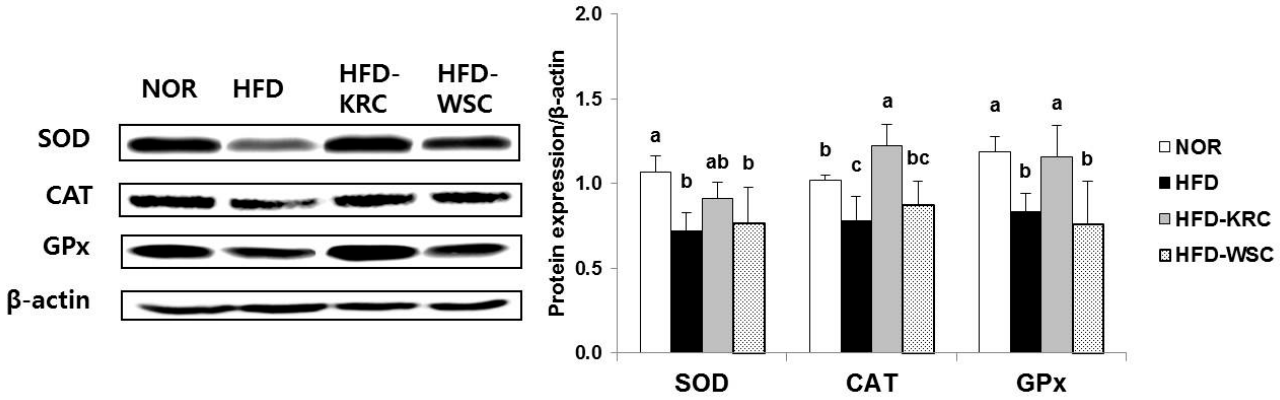

Figure 3. Protein expression of antioxidant enzymes in the liver of mice fed a cookie-supplemented high-fat diet for 9 weeks. NOR, mice fed the AIN-76 diet; HFD, mice fed the high-fat diet only; HFD-KRC, mice fed the HFD supplemented with the Korean rice cookie; HFD-WSC; mice fed the HFD supplemented with the Western style cookie. [a-c]Data with different letters are significantly different according to one-way ANOVA followed by Duncan's multiple range test at $p<0.05$. CAT, catalase; GPx, glutathione peroxidase; SOD, superoxide dismutase

3.6. Inhibition of Oxidative Stress by Korean Rice Cookie

Hyperlipidemia is associated with increased oxidative damage [16] due to enhanced mitochondrial fatty acid oxidation [29]. In this study, the hepatic ROS, $ONOO^-$, and TBARS levels were higher, and the GSH concentration was lower, in the HFD-fed group than in the NOR group ($p<0.05$, Table 3). The concentrations of these oxidative stress-related markers in the HFD-WSC group were comparably high or even higher than those in the HFD group. However, the hepatic ROS and $ONOO^-$ concentrations of the HFD-KRC group were significantly decreased by 33.86% and 53.47%, respectively, compared with the levels in the HFD-WSC group ($p<0.05$). The hepatic TBARS concentration of the HFD-KRC group was reduced by 40.13% compared with that of the HFD group ($p<0.05$). On the other hand, the hepatic GSH level was higher in the HFD-KRC group than in the HFD and HFD-WSC groups (by 230% and 246.43%, respectively; $p<0.05$). Hyperlipidemia or hepatic lipid accumulation elevates oxidative stress. It has been reported that honey supplementation significantly reduced the MDA level and increases the GSH content in rat liver [30], the oxidative stress lowering effect of the KRC might in part be attributed to the antioxidant property of honey component.

3.7. Elevation of the Antioxidant Status by Korean Rice Cookie

Antioxidant enzymes constitute an organism's native defense system to eliminate free radicals, thereby preventing oxidative damage [31]. In the HFD group, the expression levels of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were significantly decreased by 32.71%, 23.53%, and 30.25%, respectively, compared with those in the NOR group ($p<0.05$, Figure 3). The levels of antioxidant enzyme expression in the HFD and HFD-WSC group were similar whereas those of CAT and GPx in the HFD-KRC group were significantly higher. Compare to the HFD-WSC group, CAT and GPx in the HFD-KRC were higher by 140.1% ($p<0.05$) and 152.24%, respectively ($p<0.05$). It has been reported that honey supplementation restores the activities of CAT and GPx in the liver of young and middle-aged rats [32]. Moreover, oral supplementation of trichlorfon with pine tree honey, the activities of hepatic SOD, CAT, and GPx were increased in male BALB/c mice [33]. Therefore, the antioxidant effects of the KRC are very likely due to its honey component.

4. Conclusion

In this study, lipid-lowering and antioxidant effects were revealed in mice fed a HFD supplemented with KRC, *dasik*. These effects were greater than those for a traditional WSC. The rice cake flour, mung bean starch, and honey ingredients of the KRC were likely instrumental in decreasing the plasma and hepatic TG concentrations through the downregulation of TG synthesis and the upregulation of fatty acid oxidation. Moreover, the KRC augmented antioxidant enzyme expression in the liver of HFD-fed mice.

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Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

ACC, acetyl-CoA carboxylase; ACOX1, acyl-coenzyme A oxidase 1; ALT, alanine transaminase; AST, aspartate transaminase; CAT, catalase; CPT1, carnitine palmitoyltransferase 1; CYP7A1, microsomal cytochrome

P450 family 7 subfamily A member 1; FAS, fatty acid synthase; GI, glycemic index; GPx, glutathione peroxidase; GSH, glutathione; HFD, high-fat diet; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; KRC, Korean traditional rice cookie; MDA, malondialdehyde; PBS, phosphate-buffered saline; PPAR-α, peroxisome proliferator-activated receptor-alpha; ROS, reactive oxygen species; SOD, superoxide dismutase; SREBPs, sterol regulatory element-binding proteins; TBARS, thiobarbituric acid-related substances; TC, total cholesterol; TG, triglyceride; WSCs, western style cookies

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