Effects of Ginseng-Added Brown Rice Cookie on the Regulation of Hepatic Glucose Metabolism Mediated through the Insulin Signaling Pathway in *db/db* Obese Mice

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Abstract   In the present study, the effects of ginseng-added brown rice cookie (GBRC) on the regulation of hepatic glucose and lipid metabolism mediated through the insulin signaling pathway were examined in *db/db* mice. Isocaloric diets, prepared by adding the individual ingredients of rice cookie (RC) or GBRC to AIN-93G diet (10%, w/w), were fed to the animals for 10 weeks (n = 7 per group). The plasma insulin level and oral glucose tolerance test-derived area under the curve were lower in the GBRC group than in the RC group ($P < 0.05$). In the GBRC group, the insulin signaling pathway was significantly elevated through phospho-insulin receptor substrate-1 and phospho-Akt upregulation ($P < 0.05$), indicating that glucose utilization was increased. Subsequently, hepatic triacylglycerol synthesis was inhibited via the downregulation of fatty acid synthase, regulated by sterol regulatory element binding protein-1. In addition, fatty acid oxidation was increased. Protein expression levels of the lipolytic enzymes carnitine palmitoyltransferase I and acetyl-CoA oxidase 1, regulated by peroxisome proliferator-activated receptor-alpha, were increased ($P < 0.05$). Consequently, gluconeogenesis was suppressed via the downregulation of gluconeogenic enzymes, such as pyruvate carboxykinase and glucose-6-phosphatase, regulated by phospho-5′ adenosine monophosphate-activated protein kinase ($P < 0.05$). Protein expression levels of the nuclear factor kappa B-regulated cyclooxygenase-2 and inducible nitric oxide synthase were inhibited, thereby diminishing reactive oxygen species and peroxynitrite generation ($P < 0.05$). In conclusion, GBRC revealed antidiabetic effects by promoting hepatic glucose utilization mediated through the insulin signaling pathway, which enhanced lipid oxidation rather than triacylglycerol synthesis.

Keywords: brown rice, diabetes mellitus, ginseng, insulin, lipid metabolism

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1. Introduction

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance, impaired glucose tolerance, and dyslipidemia [1]. Insulin plays a critical role in controlling the plasma glucose level during the fed state by stimulating glucose uptake in the liver and muscles. The insulin resistance observed in obesity-related T2DM is caused by a decrease in insulin receptor activity. Insulin sensitivity is dependent on the insulin signaling pathway, known as the insulin receptor substrate (IRS)-phosphoinositide 3-kinase (PI3K)-Akt pathway, the activation of which stimulates glucose uptake for energy production and glycogen synthesis. Besides insulin, 5′ adenosine monophosphate-activated protein kinase (AMPK), a key enzyme for energy production, also activates the IRS-

PI3K-Akt pathway but inhibits gluconeogenesis [2], the latter of which is the opposite of glycolysis and an important regulatory process for maintaining plasma glucose levels during fasting or starvation. Pyruvate carboxykinase (PCK) and glucose-6-phosphatase (G6Pase) are the main enzymes involved in gluconeogenesis [3].

Dyslipidemia, characterized by high plasma triacylglycerol (TG) levels, is another pathological condition of T2DM [1]. Chronic elevations of free fatty acids (FFAs) in the plasma can cause insulin resistance through disturbance of the insulin signaling pathway [4]. Moreover, insulin resistance inhibits lipolysis, leading to FFA elevation. Apparently, the hepatic TG concentration might be higher in insulin-resistant individuals with obesity-related T2DM than in healthy individuals [5,6]. FFA metabolism is regulated by transcription factors including peroxisome proliferator-activated receptor α (PPARα) for FFA oxidation and sterol regulatory element binding protein-1 (SREBP-1)

for FFA synthesis [7]. Carnitine palmitoyltransferase I (CPT1) and acetyl CoA oxidase 1 (ACOX1) are regulated by PPARα, whereas fatty acid synthase (FAS) is regulated by SREBP-1. AMPK regulates lipid homeostasis through PPARα upregulation and SREBP-1 downregulation [8]. Recent market attention has focused on products that target T2DM by reducing insulin resistance and thereby ameliorating hyperglycemia and dyslipidemia [9]. Antioxidants, such as vitamins C and E, glutathione, and tempol, have been shown by *in vitro* and *in vivo* studies and clinical trials to increase insulin sensitivity by decreasing plasma insulin and glucose levels [1].

The frequency of snacking and incidence of obesity are positively associated because most snacks are calorie-dense and have high glycemic indices. Consequently, there are increasing demands for functional snacks that can alleviate public health concerns [10]. The best methods for the development of functional snacks are either to fortify them with a certain ingredient or replace an existing unhealthy ingredient with a healthier alternative [11]. The lipid-lowering effects of Korean traditional rice cookie (RC; called *dasik*), made of steamed rice flour, mung bean starch, and honey, were found to be higher than that of a western style cookie made of wheat flour, butter, eggs, and sugar [12], indicating that the ingredients used for snack preparation could make a significant difference in health benefits. In our previous study, ginseng-added brown rice cookie (GBRC) demonstrated cholesterol-lowering effects in middle age women compared to the effect of western traditional cookies [13]. GBRC was developed by replacing the white rice and honey ingredients of RC with brown rice and fructooligosaccharide (FOS), respectively, and also adding red ginseng extract. The gamma-oryzanol in brown rice has antioxidative and lipid-lowering effects [14]. Red ginseng, which contains ginsenosides, saponins, and phenolics, has demonstrated numerous biological activities, including antihyperlipidemic [15] and antioxidative effects [16]. Moreover, FOS has a sweet taste but less calories than sugar, and has revealed anti-inflammatory [17] and lipid-lowering effects [18]. Therefore, in this study, we aimed to investigate whether GBRC, with a lower glycemic index than that of RC, could improve hepatic glucose and lipid metabolism through the amelioration of insulin resistance in *db/db* mice.

2. Materials and Methods

2.1. Sample Selection and Preparation of Experimental Diets

Two *dasik* samples, the RC and GBRC are used to compare their anti-diabetic effects in *db/db* mice. In our previous studies, our team has observed lipid-lowering effects of the RC in high fat fed mice compared with the western style cookie [12]. In addition, GBRC demonstrated greater lipid lowering effects relative to the RC [19]. The ingredients of RC are 55.6% rice cake flour, 22.2% mung bean starch, and 22.2% honey. GBRC, developed functional dasik cookie, is composed of 75.6% brown rice flour, 22.0% FOS, 2.3% red ginseng extract, and 0.06% propolis [19]. The calories of RC and GBRC are 347.9 and 344.9 kcal per 100 g of cookie, respectively. The contents of carbohydrate, protein, lipid, and fiber in RC are 75.1 g, 7.9 g, 1.0 g, and 1.7 g, respectively, and those in GBRC are 77.5 g, 5.9 g, 2.1 g, and 10.7 g, respectively (CAN-pro 3.0; Korean Nutrition Society, Seoul, Korea). The experimental isocaloric diets (i.e., 3.9 kcal/g each) were prepared by adding the individual ingredients of RC or GBRC to the AIN-93G diet (Table 1). The RC or GBRC components made up 10% of the total diet.

Table 1. Composition of the experimental diets

Ingredient (g)	RC	GBRC
Cornstarch	35.77	35.77
Casein	18.00	18.00
Dextrinized cornstarch	11.88	11.88
Sucrose	9.00	9.00
Soybean oil	6.30	6.30
Fiber	4.50	4.50
Mineral mix	3.15	3.15
Vitamin mix	0.90	0.90
L-Cystine	0.27	0.27
Choline bitartrate	0.23	0.23
Tert-butylhydroquinone	0.01	0.01
Rice cake flour	5.68	-
Mung bean starch	2.05	-
Brown rice flour	-	7.21
Honey	2.27	-
Fructooligosaccharide	-	2.06
Red ginseng extract	-	0.72
Propolis	-	0.01

RC, Rice cookie; GBRC, ginseng-supplemented brown rice cookie.

2.2. Experimental Animals and Treatment

Male C57BLKS/J *db/db* mice (6 weeks old) were purchased from SLC Inc. (Hamamatsu, Japan). 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 assigned into the RC group or GBRC group (n = 7 per group) and fed the RC- or GBRC-containing diet, respectively. The animals had free access to the diet and water for 10 weeks. The food consumption and body weight of the mice were measured every week. Prior to sacrifice, the mice were fasted for 12 h and then anesthetized by an intraperitoneal administration of 30 mg/kg of Zoletil 50 (Virbac Laboratories, Carros, France) and 10 mg/kg of xylazine (Rompun; Bayer Korea, Seoul, Korea). Blood was collected by cardiac puncture into heparin tubes, and the plasma was obtained by centrifugation of the sample at $3,012 \times g$ and 4°C for 20 min. The liver was excised after perfusion with ice-cold phosphate-buffered saline (PBS) and rinsed several times with PBS. The plasma and liver samples were stored at −80°C until analysis. 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-2012-0124).

2.3. Oral Glucose Tolerance Test

An oral glucose tolerance test (OGTT) was performed after overnight fasting (16 h) of the animals on the last day of the experiment. The blood glucose concentration was determined with a glucometer (Roche Diagnostics GmbH, Mannheim, Germany), using samples drawn from the tail at 0, 30, 60, and 120 min after oral glucose administration (100 mg/100 g body weight). The area under the curve (AUC) was calculated using the method previously described [20].

2.4. Determination of Biochemical Parameters in the Plasma and Liver

The plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities were measured with a commercially available kit (AM101-K; Asan Pharm., Seoul, Korea). The plasma insulin level was determined using an ELISA kit according to the manufacturer's protocol (80-INSMS-E01; ALPCO Diagnostics, Salem, NH, USA). Lipid was extracted from the liver tissue according to the method described by Folch et al. [21]. The plasma and hepatic TG concentrations were measured using a commercial kit (AM157S-K; Asan Pharm.).

2.5. Oxidative Stress-Related Parameters in the Plasma and Liver Tissue

Thiobarbituric acid reactive substances (TBARS) in liver homogenates were determined by using malondialdehyde [22] as a standard.

The post-mitochondrial fraction, obtained by carrying out 2 numbers of centrifugation of the liver homogenate at $18,627 \times g$ at 4°C for 15 min, was used for the reactive oxygen species (ROS) and peroxynitrite assays. The ROS and peroxynitrite levels were determined using 2′,7′-dichlorofluorescein-diacetate [23] and dihydrorhodamine 123 buffer [24], respectively. Changes in the fluorescence of the ROS or peroxynitrite reaction mixtures were measured for 30 min at 480 and 535 nm, respectively, using a fluorescence plate reader (FLUOstar OPTIMA; BMG Labtech, Ortenberg, Germany).

2.6. Western Blot Analysis

The western blot assay was performed as previously described [25]. In brief, liver tissue was first homogenized in ice-cold lysis buffer (50 mM Tris, pH 8.0, 5 mM EDTA, 150 mM NaCl, and 1% nonidet-P40 containing a protease inhibitor cocktail), using a Polytron homogenizer (PT-MR 3100; Polytron, Kinematica, Lucerne, Switzerland). The homogenates were placed on ice for 1 h and then centrifuged at $18,627 \times g$ and 4°C for 20 min. The supernatant was quantified using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) followed by subjection to Sodium dodecyl sulfate polyacrylamide gel electrophoresis, after which proteins were transferred to nitrocellulose membrane (Protran BA 85; Schleicher & Schuell, Kent, UK). The primary antibodies used for the western blot assay were those for phospho-AMPKα (p-AMPK, #2535), phospho-IRS1 (p-IRS, #2385), phospho-PI3K (p-PI3K, #4228), and phospho-Akt (p-Akt, #9271), all from Cell Signaling Technology (Beverly, MA, USA). Antibodies against G6Pase (sc-25840), SREBP-1 (sc-8984), PPARα (sc-9000), CPT1 (sc-139482), ACOX1 (sc-98499), nuclear factor kappa B (NF-κB, sc-109), inhibitor of NF-κB (IκB, sc-371), cyclooxygenase-2 (COX-2, sc-1747), and inducible nitric oxide synthase (iNOS, sc-651) were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Antibodies against alpha (α)-tubulin (ab52866), FAS (ab22759), and PCK (ab70358) were from Abcam Inc. (Cambridge, UK). The secondary horseradish peroxidase-conjugated antibodies (all from Abcam Inc.) were rabbit anti-goat IgG H&L (ab6741), donkey anti-rabbit IgG H&L (ab6802), and rabbit anti-mouse IgG H&L (ab6728). 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 α-tubulin (ab52866; Abcam Inc.).

2.7. Statistical Analysis

Data are expressed as the mean ± standard deviation. The data were analyzed by Student's *t*-test, and statistical significance was considered at $P < 0.05$.

3. Results

3.1. Food Intake, Body Weight Gain, and Plasma Aminotransferase Activity

The food intakes and body weight gains were not significantly different between the RC and GBRC groups (Table 2). The plasma ALT level was significantly lower in the GBRC group than in the RC group, by 33.65% ($P < 0.05$). However, the plasma AST levels were not significantly different between the two groups. Plasma and hepatic TG levels were significantly reduced in the GBRC group, relative to the RC group.

Table 2. Food intake, body weight gain, and transaminase and triacylglycerol levels in *db/db* mice fed different Korean cookie for 10 weeks

Group	RC	GBRC
Food intake (g/day)	7.20 ± 0.71	7.50 ± 0.68[NS]
Body weight gain (g)	11.92 ± 2.77	11.48 ± 4.37[NS]
AST (Karmen unit/mL)	74.76 ± 27.90	56.11 ± 19.81[NS]
ALT (Karmen unit/mL)	189.28 ± 45.89	125.59 ± 37.82[*]
Plasma TG (mg/dL)	69.45 ± 5.26	54.96 ± 10.64[*]
Hepatic TG (mg/g tissue)	145.82 ± 10.77	122.27 ± 10.94[*]

Data are the mean ± SD (n = 7 per group). ALT, alanine transaminase; AST, aspartate transaminase; GBRC, ginseng-supplemented brown rice cookie; RC, rice cookie; TG, triacylglycerol. Significant differences between RC and GBRC groups are expressed as p-values calculated by Student's *t*-test. [*] $P < 0.05$. [NS] Not significantly different at $P < 0.05$.

3.2. Insulin Level and OGTT-Derived AUC

Compared to the levels in the RC group, the plasma insulin levels in the GBRC group were significantly decreased by 72.74% ($P < 0.001$), and the OGTT-derived AUC was 19.75% lower ($P < 0.05$) (Figure 1).
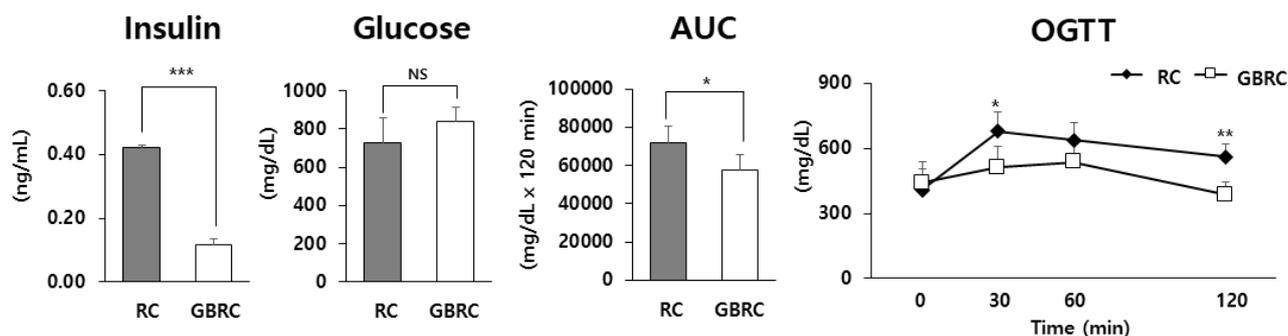
Figure 1. Plasma insulin and glucose levels, and area under the curve from the oral glucose tolerance test in *db/db* mice. See the footnote text of Table 1 for a description of the experimental groups. *Significant differences between two experimental groups are expressed as p-values calculated by Student's *t*-test (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$)

3.3. Increase in Insulin Signaling Pathway-Related Proteins in the Liver

Compared to the levels in the RC group, the protein expression levels of p-IRS and p-Akt in the GBRC group were significantly increased by 221.37% and 219.54%, respectively ($P < 0.05$) (Figure 2). On the other hand, the p-PI3K levels were not significantly different between the two groups, albeit the level was slightly higher in the GBRC group.

3.4. Regulation of Fatty Acid Metabolism in the Liver

The protein expression levels of mature forms of SREBP-1 and FAS were decreased by 39.99% ($P < 0.05$) and 52.39% ($P < 0.01$), respectively, in the GBRC group relative to the levels in the RC group (Figure 3). In

contrast, the hepatic expression levels of PPARα, CPT1, and ACOX1 were increased by 199.00% (P < 0.05), 139.98% ($P < 0.05$), and 156.37% ($P < 0.01$), respectively, in the GBRC group. The plasma and hepatic TG levels in the GBRC group were reduced by 20.87% and 16.15%, respectively, relative to those in the RC group (Table 2, $P < 0.05$), which was in line with the results for hepatic lipid metabolism.

3.5. Reduction of Gluconeogenesis in the Liver

Compared to that seen in the RC group, the GBRC group showed a higher protein expression level of p-AMPK, with a significant increase of 176.24% ($P < 0.05$) (Figure 4). In contrast, the protein expression levels of G6Pase and PCK were 74.23% and 34.16% lower, respectively, in the GBRC group ($P < 0.05$).
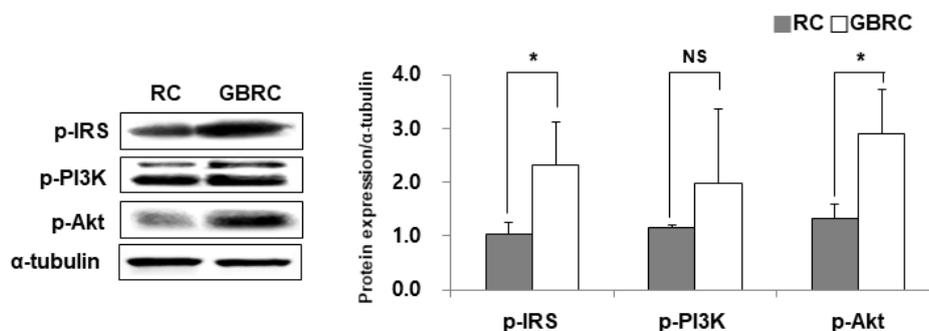

Figure 2. Hepatic expression of insulin signaling pathway-related proteins in *db/db* mice. See the footnote text of Table 1 for a description of the experimental groups. *Significant differences between two experimental groups are expressed as p-values calculated by Student's *t*-test (*$P < 0.05$)
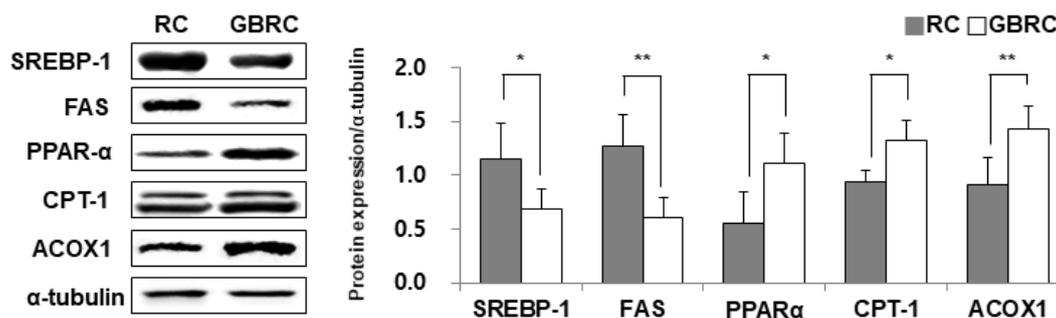

Figure 3. Hepatic expression of proteins related to fatty acid synthesis and metabolism in *db/db* mice. See the footnote text of Table 1 for a description of the experimental groups. *Significant differences between two experimental groups are expressed as p-values calculated by Student's *t*-test (*$P < 0.05$ and **$P < 0.01$)
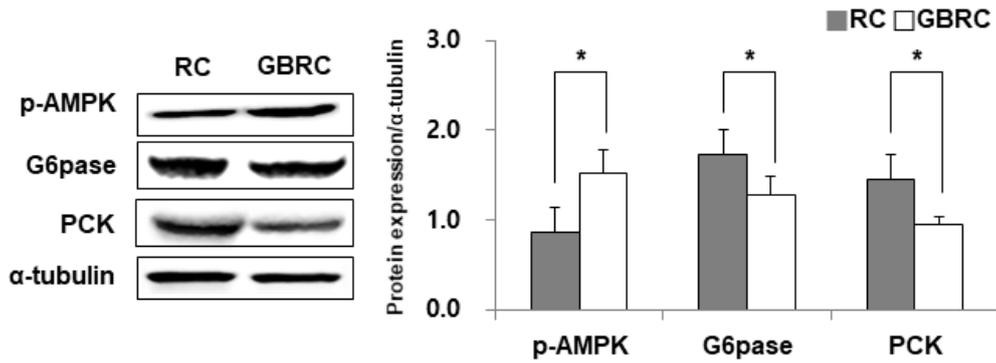
Figure 4. Hepatic protein expression of p-AMPK and gluconeogenic enzymes in *db/db* mice. See the footnote text of Table 1 for a description of the experimental groups. *Significant differences between two experimental groups are expressed as p-values calculated by Student's *t*-test (*P < 0.05). p-AMPK, phospho-5′-adenosine monophosphate-activated protein kinase
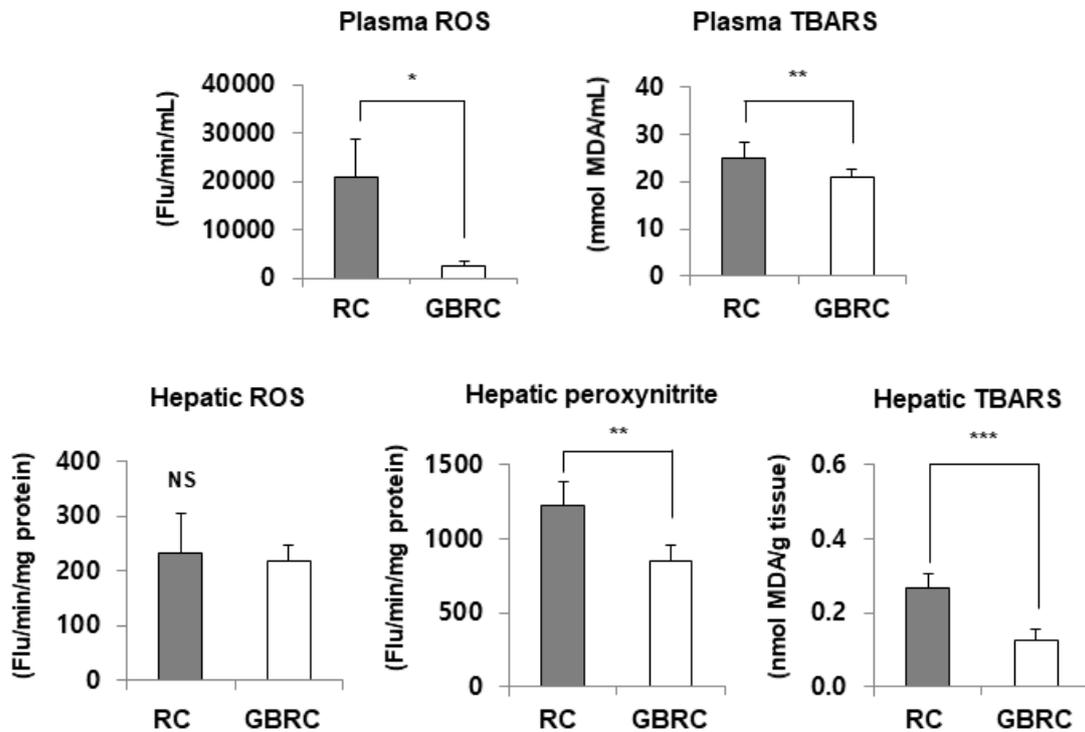

Figure 5. Oxidative stress levels in the plasma and liver of *db/db* mice. See the footnote text of Table 1 for a description of the experimental groups. *Significant differences between two experimental groups are expressed as p-values calculated by Student's *t*-test (*P < 0.05, **P < 0.01, and ***P < 0.001).
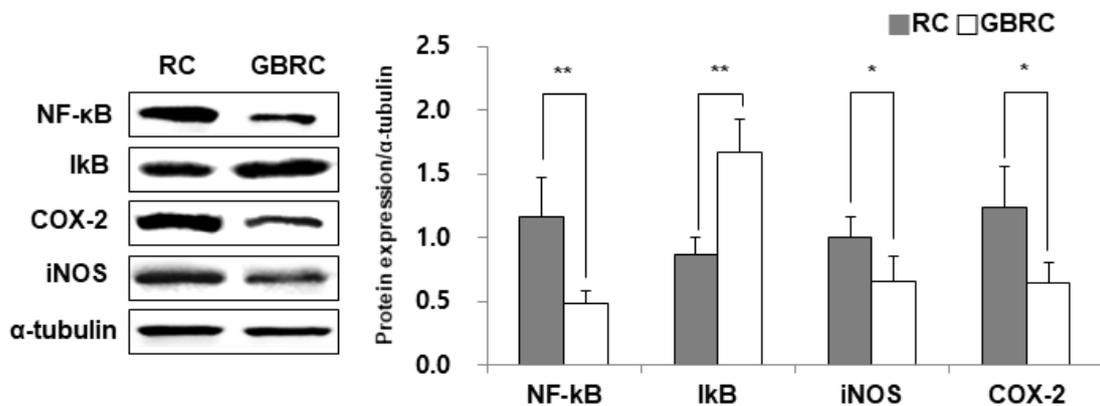

Figure 6. Hepatic expression of inflammation-related proteins in *db/db* mice. See the footnote text of Table 1 for a description of the experimental groups. *Significant differences between two experimental groups are expressed as p-values calculated by Student's *t*-test (*P < 0.05 and **P < 0.01).

3.6. Suppression of Oxidative Stress in the Plasma and Liver

The plasma ROS and TBARS levels in the GBRC group were 88.62% and 17.10% lower, respectively, than those in the RC group ($P < 0.05$) (Figure 5). Likewise, the hepatic peroxynitrite and TBARS levels were lower in the GBRC group, by 30.73% and 52.39%, respectively ($P < 0.05$). No significant differences were found in hepatic ROS levels between the two groups.

3.7. Inhibition of Inflammation in the Liver

Compared to the levels in the RC group, the protein expression level of NF-κB in the GBRC group was decreased by 58.37%, whereas that of IκB was increased by 192.27% ($P < 0.01$) (Figure 6). Consequently, the expression levels of NF-κB-regulated COX-2 and iNOS were also decreased in the GBRC group, by 47.55% and 34.33%, respectively ($P < 0.05$).

4. Discussion

Snacking is considered to be a major contributor to obesity because most snacks are energy-dense, with high sugar and fat contents [26]. There are increasing demands for healthier snacks because the calories from snacking are approximately 25% that of daily energy consumption [27]. Calorie-dense snacks might worsen the plasma glucose and lipid conditions, especially in individuals with T2DM. Insulin action, the most critical factor for controlling diabetes, is regulated mainly in the liver through the insulin signaling pathway. The insulin-mediated phosphorylation of IRS subsequently activates PI3K and Akt, which enhance glucose transport [28]. IRS dysfunction will therefore cause insulin resistance, leading to postprandial hyperglycemia. Thus, the amelioration of insulin resistance mediated through regulation of the insulin signaling pathway is considered a practical T2DM treatment [29]. In the present study, the plasma insulin level and AUC in the GBRC group were decreased, indicating that the insulin resistance was lowered. These results might be due to an elevation of insulin sensitivity mediated through activation of the insulin signaling pathway, especially in the liver, given that hepatic p-IRS and p-Akt were significantly upregulated in the GBRC group. Previous studies have demonstrated that red ginseng ameliorates insulin resistance in *db/db* mice [20,30] by lowering the fasting blood glucose and insulin levels and OGTT-derived AUC. The bioactive compounds in red ginseng, such as ginsenosides, might augment the insulin sensitivity, given that Ginsenoside Re was shown to exhibit plasma glucose-lowering effects in diabetic rats [15] and Ginsenoside Rh2 improved insulin sensitivity in fructose-rich chow-fed rats [31]. Brown rice decreased the postprandial blood glucose and insulin levels and body weights of mice with the metabolic syndrome, compared to the levels in subjects that had been fed white rice, indicating that insulin sensitivity and body weight are closely related [32]. Furthermore, FOS has demonstrated a serum insulin-lowering effect in obese rats [33]. Taking these previous results together, brown rice and FOS substitution for the original

ingredients of RC, as well as red ginseng supplementation, might contribute to the antidiabetic activities of GBRC. The plasma glucose level is also controlled by hepatic gluconeogenesis. In the present study, the protein expression levels of PCK and G6Pase for gluconeogenesis were lower in the GBRC group than in the RC group, indicating that insulin sensitivity was increased in the GBRC-fed group. Black ginseng extract suppressed the hepatic expression of PCK and G6Pase in streptozotocin-induced diabetic mice [34]. Moreover, gamma-oryzanol reduced the levels of PCK and G6Pase in high-fat diet-fed mice [35].

As a result of improper lipid metabolism, patients with T2DM have a higher risk of cardiovascular disease than do healthy individuals [4]. The condition of elevated insulin resistance due to IRS dysfunction leads to hepatic fatty acid synthesis via the upregulation of FAS mediated through SREBP-1, which was confirmed in this present study. Moreover, GBRC demonstrated TG-lowering effects that might possibly be due to the amelioration of insulin resistance, as mentioned earlier in this discussion. In the GBRC group, the SREBP-1-regulated FAS was downregulated, whereas the PPARα-regulated ACOX1 and CPT1 were upregulated, indicating that hepatic lipid synthesis was inhibited, which subsequently influenced the plasma TG level. These TG-lowering effects of GBRC might be from the red ginseng, as discussed in another study, red ginseng-fed *db/db* mice also had lower plasma TG and FFA levels due to inhibition of the FAS activity regulated by SREBP-1 [30]. In addition, black ginseng extract increased the expression of ACOX1 and CPT1 in C2C12 cells [34]. Ginsenosides Re [15] and Rb$_1$ [36] exerted inhibitory effects on hepatic fat accumulation by decreasing the FFA level in diabetic obese mice. Moreover, the gamma-oryzanol in brown rice [14] and FOS [33] have also shown TG-lowering effects. Our results imply that GBRC attenuates diabetic symptoms by ameliorating hepatic lipid metabolism in *db/db* mice. Lipid peroxidation and inflammation are signature features of diabetic complications [37]. In the present study, the ROS, peroxynitrite, and TBARS levels in the plasma and liver of mice in the GBRC group were decreased, as was NF-κB-regulated COX-2 and iNOS expression, suggesting that the inflammatory responses induced by oxidative stress in obesity-related diabetes could be decreased when insulin resistance is ameliorated.

In summary, GBRC increased both glucose metabolism, as mediated through the insulin signaling pathway, and lipid metabolism. Furthermore, the increase of glucose metabolism reduced oxidative stress and inflammation. It is apparent that the ingredients ginseng, brown rice, and/or FOS might have contributed to the antidiabetic effects of GBRC. Our findings suggest that a functional cookie like GBRC could be a suitable snack for individuals with diabetes.

Acknowledgments

Conflict of Interest

The authors declared that they have no conflict of interest.

References

[1] Tangvarasittichai, S., "Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus." *World Journal of Diabetes* 6(3). 456-480. 2015.

[2] Srivastava, R. A. K., Pinkosky, S. L., Filippov, S., Hanselman, J. C., Cramer, C. T., and Newton, R. S., "AMP-activated protein kinase: an emerging drug target to regulate imbalances in lipid and carbohydrate metabolism to treat cardio-metabolic diseases Thematic Review Series: New Lipid and Lipoprotein Targets for the Treatment of Cardiometabolic Diseases." *Journal of Lipid Research* 53(12): 2490-2514. 2012.

[3] Kim, O. K., Nam, D. E., Jun, W., and Lee, J., "Cudrania tricuspidata water extract improved obesity-induced hepatic insulin resistance in db/db mice by suppressing ER stress and inflammation." *Food & Nutrition Research* 59(1). 29165. 2015.

[4] Carmena, R., "Type 2 diabetes, dyslipidemia, and vascular risk: rationale and evidence for correcting the lipid imbalance." *American Heart Journal* 150(5). 859-870. 2005.

[5] Fabbrini, E., Magkos, F., Mohammed, B. S., Pietka, T., Abumrad, N. A., Patterson, B. W., Okunade, A. and Klein, S., "Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity." *Proceedings of the National Academy of Sciences* 106(36). 15430-15435. 2009.

[6] Samuel, V. T., Petersen, K. F., and Shulman, G. I., "Lipid-induced insulin resistance: unravelling the mechanism." *The Lancet* 375(9733). 2267-2277. 2010.

[7] Strable, M. S., and Ntambi, J. M., "Genetic control of de novo lipogenesis: role in diet-induced obesity." *Critical Reviews in Biochemistry and Molecular Biology* 45(3). 199-214. 2010.

[8] Hardie, D., "AMPK: a key regulator of energy balance in the single cell and the whole organism." *International Journal of Obesity* 32. S7-S12. 2008.

[9] Goldberg, R. B., Kendall, D. M., Deeg, M. A., Buse, J. B., Zagar, A. J., Pinaire, J. A., Tan, M. H., Khan, M. A., Perez, A. T., and Jacober, S. J., "A comparison of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia." *Diabetes Care* 28(7). 1547-1554. 2005.

[10] Ndife, J., Abdulraheem, L., and Zakari, U., "Evaluation of the nutritional and sensory quality of functional breads produced from whole wheat and soya bean flour blends." *African Journal of Food Science* 5(8). 466-472. 2011.

[11] Kārkliņa, D., Gedrovica, I., Reca, M., and Kronberga, M., Production of biscuits with higher nutritional value. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences*. 3. 113-116. 2012.

[12] Hong, S. H., Kim, M. J., Woo, M. J., and Song, Y. O., "Rice Cookie Decreases Plasma and Hepatic Lipid Levels in High-Fat Diet-fed Mice: A Comparison Study with Traditional Western Style Cookies." Journal of Food and Nutrition Research 5(7). 451-457. 2017.

[13] Hong, S. H., Kim, M., Woo, M., Noh, J. S., Lee, J., Chung, L., and Song, Y. O., "The amelioration of plasma lipids by Korean traditional confectionery in middle-aged women: A cross-over study with western cookie." *Nutrition Research and Practice* 10(6). 590-596. 2016.

[14] Son, M. J., Rico, C. W., Nam, S. H., and Kang, M. Y., "Influence of oryzanol and ferulic acid on the lipid metabolism and antioxidative status in high fat-fed mice." *Journal of Clinical Biochemistry and Nutrition* 46(2). 150-156. 2010.

[15] Cho, W. C., Chung, W.-S., Lee, S. K., Leung, A. W., Cheng, C. H., and Yue, K. K., "Ginsenoside Re of Panax ginseng possesses significant antioxidant and antihyperlipidemic efficacies in streptozotocin-induced diabetic rats." *European Journal of Pharmacology* 550(1). 173-179. 2006.

[16] Kim, H. J., Lee, S. G., Chae, I. G., Kim, M. J., Im, N. K., Yu, M. H., Lee, E. J., and Lee, I. S., "Antioxidant effects of fermented red ginseng extracts in streptozotocin-induced diabetic rats." *Journal of Ginseng Research* 35(2). 129. 2011.

[17] Merino-Aguilar, H., Arrieta-Baez, D., Jiménez-Estrada, M., Magos-Guerrero, G., Hernández-Bautista, R. J., Susunaga-Notario, A. d. C., Almanza-Pérez, J. C., Blancas-Flores, G., Román-Ramos, R., and Alarcón-Aguilar, F. J., "Effect of fructooligosaccharides fraction from Psacalium decompositum on inflammation and dyslipidemia in rats with fructose-induced obesity." *Nutrients* 6(2). 591-604. 2014.

[18] Nakamura, Y., Natsume, M., Yasuda, A., Ishizaka, M., Kawahata, K., and Koga, J., "Fructooligosaccharides suppress high-fat diet-induced fat accumulation in C57BL/6J mice." *Biofactors* 43(2). 145-151. 2017.

[19] Hong, S. H., Kim, M., Woo, M., and Song, Y. O. "Effects of ingredients of Korean brown rice cookies on attenuation of cholesterol level and oxidative stress in high-fat diet-fed mice." *Nutrition Research and Practice*, 11(5), 365-372. 2017.

[20] Jeon, W. J., Oh, J. S., Park, M. S., and Ji, G. E., "Anti‐Hyperglycemic Effect of Fermented Ginseng in Type 2 Diabetes Mellitus Mouse Model." *Phytotherapy Research* 27(2). 166-172. 2013.

[21] Folch, J., Lees M., and Sloane-Stanley, G., "A simple method for the isolation and purification of total lipids from animal tissues." *Journal of Biological Chemistry* 226(1). 497-509. 1957.

[22] Ohkawa, H., Ohishi, N., and Yagi, K., "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction." *Analytical Biochemistry* 95(2). 351-358. 1979.

[23] Ali, S., LeBel, C., and Bondy, S., "Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity." *Neurotoxicology* 13(3). 637-648. 1991.

[24] Kooy, N. W., Royall, J. A., Ischiropoulos, H., and Beckman, J. S., "Peroxynitrite-mediated oxidation of dihydrorhodamine 123." *Free Radical Biology and Medicine* 16(2). 149-156. 1994.

[25] Jung, K., Hong, S. H., Kim, M., Han, J.-S., Jang, M.-S., and Song, Y. O., "Antiatherogenic effects of Korean cabbage kimchi with added short arm octopus." *Food Science and Biotechnology* 24(1). 249-255. 2015.

[26] Field, A. E., Austin, S. B., Gillman, M. W., Rosner, B., Rockett, H. R., and Colditz, G. A., "Snack food intake does not predict weight change among children and adolescents." International journal of obesity 28(10): 1210-1216.

[27] Farley, T. A., Baker, E. T., Futrell, L., and Rice, J. C., "The ubiquity of energy-dense snack foods: a national multicity study." *American Journal of Public Health* 100(2). 306-311. 2010.

[28] Choi, K., and Kim, Y. B., "Molecular mechanism of insulin resistance in obesity and type 2 diabetes." *The Korean Journal of Internal Medicine* 25(2). 119-129. 2010.

[29] Mokdad, A. H., Ford, E. S., Bowman, B. A., Dietz, W. H., Vinicor, F., Bales, V. S., and Marks, J. S., "Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001." *Jama* 289(1). 76-79. 2003.

[30] Yuan, H. D., Shin, E. J., and Chung, S. H., "Anti-diabetic effect and mechanism of Korean red ginseng in C57BL/KsJ db/db mice." *Journal of Ginseng Research* 32(3). 187-193. 2008.

[31] Lee, W. K., Kao, S. T., Liu, I. M., and Cheng, J. T., "Ginsenoside Rh2 is one of the active principles of Panax ginseng root to improve insulin sensitivity in fructose-rich chow-fed rats." *Hormone and Metabolic Research* 39(05). 347-354. 2007.

[32] Kozuka, C., Yabiku, K., Sunagawa, S., Ueda, R., Taira, S. I., Ohshiro, H., Ikema, T., Yamakawa, K., Higa, M. and Tanaka, H., "Brown rice and its component, γ-oryzanol, attenuate the preference for high-fat diet by decreasing hypothalamic endoplasmic reticulum stress in mice." *Diabetes* 61(12). 3084-3093. 2012.

[33] Kaume, L., Gilbert, W., Gadang, V., and Devareddy, L., "Dietary supplementation of fructooligosaccharides reduces hepatic steatosis associated with insulin resistance in obese Zucker rats." *Functional Foods in Health and Disease* 1(5). 199-213. 2011.

[34] Seo, Y. S., Shon, M. Y., Kong, R., Kang, O. H., Zhou, T., Kim, D. Y., and Kwon, D. Y., "Black ginseng extract exerts anti-hyperglycemic effect via modulation of glucose metabolism in liver and muscle." *Journal of Ethnopharmacology* 190. 231-240. 2016.

[35] Son, M. J., Rico, C. W., Nam, S. H., & Kang, M. Y., "Effect of Oryzanol and Ferulic Acid on the Glucose Metabolism of Mice Fed with a High‐Fat Diet." *Journal of Food Science* 76(1). 2011.

[36] Yu, X., Ye, L., Zhang, H., Zhao, J., Wang, G., Guo, C., and Shang, W., "Ginsenoside Rb1 ameliorates liver fat accumulation by upregulating perilipin expression in adipose tissue of db/db obese mice." *Journal of Ginseng Research* 39(3): 199-205. 2015.

[37] Kaul, K., Hodgkinson, A., M Tarr, J., M Kohner, E., and Chibber, R., "Is inflammation a common retinal-renal-nerve pathogenic link in diabetes?" Current diabetes reviews 6(5). 294-303. 2010.