Germinated Waxy Black Rice Ameliorates Hyperglycemia and Dyslipidemia in Streptozotocin-Induced Diabetic Rats

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Received March 19, 2017; accepted August 2, 2017

This study aimed to examine the anti-diabetic effect of germinated waxy black rice (GWBR) using streptozotocin (STZ)-induced diabetic rats. In the diabetic rats, GWBR supplementation for 8 weeks reduced plasma blood glucose concentrations, improved glucose clearance and prevented diabetes-induced weight loss. Rats with STZ-induced diabetes who received GWBR supplementation exhibited decreased expression of sodium-dependent glucose transporter 1 (SGLT1) and glucose transporter (GLUT2) 2 genes and proteins in the small intestine via decreases in hepatocyte nuclear factor (HNF)-1α, HNF-1β, and HNF-4α, transcriptional factors that are involved in the regulation of SGLT1 and GLUT2, compared with the rats with STZ-induced diabetes that did not receive GWBR supplements. GWBR supplementation also enhanced the expression of GLUT4 and the genes and proteins involved in GLUT4 translocation, such as insulin receptor (IR) and insulin receptor substrate 1 (IRS1), and increased the phosphorylation of phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt) proteins in skeletal muscle. GWBR further increased glycogen synthase (GS) 1 by decreasing glycogen synthase kinase (GSK)-3β in skeletal muscle. Interestingly, GWBR recovered STZ-impaired pancreatic β-cells, resulting in increased insulin synthesis and secretion. In addition, GWBR reduced serum triglyceride, total cholesterol, low-density lipoprotein cholesterol, aspartate transaminase, and alanine transaminase and increased high-density lipoprotein cholesterol concentrations. Taken together, these findings suggest that GWBR could be a candidate for improving the diabetic condition by regulating glucose uptake in the intestine and muscle and regulating the secretion of insulin from the pancreas.

Key words anti-diabetes; germinated waxy black rice; glucose uptake; streptozotocin-induced diabetic rat

Diabetes is a metabolic disease that is mainly characterized by hyperglycemia. It is caused by an impaired pancreas that does not produce enough insulin or by tissues’ resistance to insulin. People with diabetes have increased susceptibility for other metabolic problems, such as obesity, heart diseases, nephropathy, and retinopathy. To maintain normal blood glucose concentrations, diabetic patients generally take medicines including insulin secretagogues, insulin sensitizers, α-glucosidase inhibitors, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 (GLP-1) analogs, and protein kinase B (PKB, Akt) proteins in skeletal muscle. GWBR further increased glycogen synthase (GS) 1 by decreasing glycogen synthase kinase (GSK)-3β in skeletal muscle. Interestingly, GWBR recovered STZ-impaired pancreatic β-cells, resulting in increased insulin synthesis and secretion. In addition, GWBR reduced serum triglyceride, total cholesterol, low-density lipoprotein cholesterol, aspartate transaminase, and alanine transaminase and increased high-density lipoprotein cholesterol concentrations. Taken together, these findings suggest that GWBR could be a candidate for improving the diabetic condition by regulating glucose uptake in the intestine and muscle and regulating the secretion of insulin from the pancreas.

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specifically on glucose uptake regulation in the intestines and muscles.

MATERIALS AND METHODS

Sample Preparation and Reagents GWBR was prepared with germinated waxy black rice collected from Hamyanggun, Korea in October 2012. To induce germination, waxy black rice was soaked for 2–3 d in water. The GWBR was dried at 60°C and then ground into a powder. The GWBR powder was used as supplement in animal diets (Table 1).

Animals and Diets Seven-week-old male Sprague–Dawley (SD) rats (n=40) were purchased from Orient Bio, Inc. (Seongnam, Korea). They were housed (two rats/cage) in an animal facility with a 12-h light/dark cycle (lights on at 8:00 and lights off at 20:00) and were fed a standard laboratory diet (AIN-93G, Feedlab Co., Guri, Korea) with free access to water. The temperature and humidity of the animal facility were maintained at 24±1°C and 55±5%, respectively. After 1 week of environmental acclimation, the rats were fasted overnight. To induce a diabetic condition, thirty of the forty rats were intraperitoneally injected with a single dose of freshly prepared STZ solution (Sigma-Aldrich, St. Louis, MO, U.S.A., 50 mg/kg body weight in 0.1 M cold citrate buffer, pH 4.5). To avoid hypoglycemia from the STZ injection, the rats were allowed to drink 5% sucrose solution overnight. The remaining ten rats were injected with the same volume of the buffer and formed a non-diabetic control group (NC, n=10). All the rats were maintained on a standard laboratory diet (AIN-93G, Feedlab Co.) for 9 d to induce diabetes. The rats that had persistent hyperglycemia (blood glucose ≥300 mg/dL) were then used for further experimentation. The thirty rats with STZ-induced diabetes were divided into three diet groups (n=10/group): the STZ-induced diabetic control group (DC), the STZ-induced diabetic group supplemented with GWBR 2.5% (DLB), and the STZ-induced diabetic group supplemented with GWBR 5% (DHB). The rats were kept on their diet for an additional 8 weeks. The GWBR dosages (2.5, 5%) were determined based on commonly used dosages in other diet feeding studies that used crude extract-supplemented diets. 16

Table 1. Diet Composition (g/kg Diet)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>NC</th>
<th>DC</th>
<th>DLB</th>
<th>DHB</th>
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<tr>
<td>Casein</td>
<td>200.00</td>
<td>200.00</td>
<td>198.05</td>
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<tr>
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<td>132.00</td>
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<td>0.014</td>
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<tr>
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<tr>
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</table>

Abbreviations: NC, non-diabetic control group; DC, STZ-induced diabetic control group; DLB, STZ-induced diabetic group supplemented with GWBR 2.5%; DHB, STZ-induced diabetic group supplemented with GWBR 5%; TBHQ, tert-butylhydroquinone; GWBR, germinated waxy black rice. The diet composition is described in Table 1. The diet compositions (e.g. casein, cornstarch, soybean oil and cellulose) of DLB and DHB were adjusted based on the amounts of the GWBR nutrient components according to the data released by the Korea National Institute of Agricultural Sciences (http://koreanfood.rda.go.kr/kfi/ctf/ctFoodSrch/list). One hundred grams of dried GWBR consists of 7.8 g of protein, 2.56 g of fat, 0.89 g of dietary fiber and 88.5 g of carbohydrate. Based on supplementation of 25 or 50 g of GWBR per 1000 g of diet (2.5 or 5%), the nutrient values of GWBR were calculated, and then the amounts of casein, cornstarch, soybean oil and cellulose were reduced for DLB and DHB diet compositions (Table 1). Food intake and body weight were measured weekly. During the 8-week feeding program, blood glucose concentrations were monitored weekly from the tail vein using the Accu-Chek Blood Glucose Monitoring System (Roche, Inc., Basel, Switzerland) after overnight fasting. At the week 7 of the feeding period, an oral glucose tolerance test (OGTT) was performed in the afternoon, followed by the usual morning blood glucose measurement. After the OGTT, the rats were allowed to recover and maintained on the same diet for one week. Upon the completion of the 8-week feeding study, all the rats were euthanized using ether. Blood was drawn using a cardiac puncture, and the serum was separated and stored at −20°C for further biochemical analysis. The small intestines and skeletal muscles were dissected and stored at −70°C. The rats’ pancreases were immersed in 10% buffered formalin for histological analysis. All procedures were approved by the Animal Care and Use Committee of Korea University (No. KUIACUC-2015-201).

Oral Glucose Tolerance Test At the week 7 of the 8-week-feeding period, the OGTT was performed after an overnight fast. Following measurement of the basal glucose concentrations, the rats were given 2000 mg/kg body weight of a glucose solution (20% w/v) orally. The plasma glucose concentrations were measured from the tail vein 5, 15, 30, 60, 90, and 120 min after glucose administration using the Accu-Chek Blood Glucose Monitoring System (Roche, Inc.). The area under the curve (AUC) was calculated and used for comparisons among the groups.

Biochemical Analysis Triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in the serum were measured using an automatic biochemical analyzer (Cobas C-111, Roche, Inc.).

RT-PCR Analysis Total RNA was extracted from the small intestines and skeletal muscles using easy-BLUE™ total RNA extraction reagent (iNtRON Biotechnology, Seongnam, Korea) according to the manufacturer’s protocol. One microgram of total RNA was used for the cDNA synthesis. The PCR conditions were as follows: 5 min at 95°C, followed by 45 denaturing cycles for 30 s at 95°C, annealing for 30 s at 52–63°C, and extension for 30 s at 72°C. The primers for PCR are listed in the Table 2. The PCR products were separated using electrophoresis on 2.5% agarose gel. The gels were stained with 0.5 μg/mL ethidium bromide and visualized with UV light (Gel Doc 2000 and the Quantity One program; Bio-Rad, Sydney, Australia). The expression of the target gene was calculated as a percentage of relative mRNA expression versus β-actin as an internal control.
and then transferred to a 0.45-μm polyvinylidene difluoride membrane (Millipore, Bedford, MA, U.S.A.). The membranes were blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline containing 0.05% Tween-20 (TBST) at room temperature for 1 h and then incubated with primary antibodies (1:100–10000) at 4°C overnight. Polyclonal antibodies to sodium-dependent glucose transporter 1 (SGLT1), glucose transporter 2 (GLUT2), (Abcam, Cambridge, U.K.), hepatocyte nuclear factor 1-alpha; HNF-1β, hepatocyte nuclear factor 1-beta; HNF-4α, hepatocyte nuclear factor 1-alpha; HNF-1α, and insulin receptor substrate 1; PI3K, phosphatidylinositol 3-kinase; SGLT1, sodium-dependent glucose transporter 1.

**Western Blot Analysis** Small pieces of small intestine and skeletal muscle tissues were homogenized and lysed with T-PER lysis buffer (Thermo Fisher Scientific, Waltham, MA, U.S.A.). After centrifugation at 14000×g at 4°C for 15 min, the supernatant was obtained for protein samples. Protein concentrations were determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Equal amounts of each protein sample were loaded and separated with 8% SDS-PAGE. The gels were stained with Coomassie Brilliant Blue R-250 or transferred to a PVDF membrane (Millipore, Bedford, MA, U.S.A.). After being washed in distilled water, the sections were further stained with 3,3′-diaminobenzidine (DAB) according to the manufacturer's instructions (Liquid DAB Substrate-Chromogen System, EnVision+HRP; DAB; DAKO, Carpinteria, CA, U.S.A.). After being washed in distilled water, the sections were counterstained with hematoxylin and eosin (H&E) and mounted. The slides were observed using an Axioskop microscope (Zeiss, Jena, Germany) and analyzed using Image J software (NIH, Bethesda, MD, U.S.A.). The site of the target antigen was identified according to the brown color obtained with DAB as a chromogenic substrate for peroxidase activity.

**Immunohistochemistry Analysis** Insulin receptor (IR), IRS1 and insulin proteins were determined using standard immunohistochemical protocol. Briefly, paraffin-embedded pancreas tissue sections (5-μm thick) were deparaffinized in xylene for 10 min and rehydrated sequentially in 100, 95, 75, and 50% ethanol. The sections were heated in 0.01 mol/L citrate buffer (pH 6.0) in a microwave for 20 min, incubated in 3% H2O2 for 5 min, and incubated with polyclonal antibodies against IR (1:500; Abcam), IRS1 (1:50, Santa Cruz Biotechnology), and monoclonal antibody against insulin (1:400, Cell Signaling Technology, Inc.) at 4°C overnight. After being washed in TBST three times, the sections were incubated with the HRP-conjugated anti-rabbit secondary antibody for 30 min at room temperature. After being washed, the sections were further stained with 3,3′-diaminobenzidine (DAB) according to manufacturer's instructions (Liquid DAB Substrate-Chromogen System, EnVision+HRP; DAB; DAKO, Carpinteria, CA, U.S.A.). After being washed in distilled water, the sections were counterstained with hematoxylin and eosin (H&E) and mounted. The slides were observed using an Axioskop microscope (Zeiss, Jena, Germany) and analyzed using Image J software (NIH, Bethesda, MD, U.S.A.). The site of the target antigen was identified according to the brown color obtained with DAB as a chromogenic substrate for peroxidase activity.

**Statistical Analysis** All data are presented as the mean±standard deviation (S.D.). Significant differences were determined using one-way ANOVA with Dunnett’s post hoc test following the general linear model procedure described in the SAS statistical software package (SAS Institute, Cary, NC, U.S.A.). Differences were considered significant at p<0.05.

**RESULTS**

**Effects of GWBR on STZ-Induced Hyperglycemia and Body Weight** As Fig. 1A shows, the rats with STZ-induced diabetes showed gradually increased plasma glucose concentrations (from 347 to 506 mg/dL) during the first 4 weeks. Over the next 4 weeks, the DC group maintained constant glucose concentrations, but GWBR supplementation decreased the plasma glucose concentrations of rats with STZ-induced diabetes. At the end of 8 weeks of feeding, the DLB and DHB groups exhibited 16 and 30% reductions in plasma glucose concentrations, respectively, compared with that of the DC group. Compared with the diabetic rats, the NC group maintained glucose concentrations of approximately 100 mg/dL during the experimental periods. To examine whether GWBR improved the body’s glucose clearance ability, an OGTT was performed at the week 7 of the feeding study (Fig. 1B). Following glucose administration, the plasma glucose concentrations of the DC group robustly
increased during the first 15 min and reached a peak at 15 min. The peak concentration of plasma glucose was maintained for the next 15 min and then reduced in time-dependent manner. After 120 min, the plasma glucose concentrations of the DC group were still higher than the baseline. In contrast, the plasma glucose concentrations of the DLB, DHB and NC groups peaked at 5 or 15 min and then reduced to the baseline levels. This result indicated that glucose clearance was increased in the DLB and DHB groups compared with that in the DC group as evidenced by the 85 and 129% reductions in the AUC, respectively. The resulting AUC values of the DLB and DHB groups were the same as those of the NC group.

Table 3 shows the effects of GWBR on the body weight and food intake of the rats with STZ-induced diabetes. The NC group showed an increase in body weight over 8 weeks. As a diabetic symptom, the rats in the DC group lost body weight despite consuming more food than the rats in the NC group (Table 3). This result was confirmed by the reduced food efficiency ratio (FER) of the DC group. The DLB and DHB groups maintained their initial body weights or showed slightly increased weights at the end of 8 weeks.

Effects of GWBR on Biochemical Blood Parameters To determine whether GWBR affects serum lipid concentrations, TC, TG, HDL and LDL cholesterol were measured (Table 4). The DC group showed increased serum TG, TC, and LDL-cholesterol concentrations and increased HDL cholesterol compared with those of the NC group. Compared with the DC group, 2.5 and 5% GWBR supplementation caused 55 and 70% reductions in serum TG concentrations, respectively. GWBR also reduced serum TC concentrations. Although 2.5% GWBR did not improve the HDL- and LDL-cholesterol concentrations of the rats with STZ-induced diabetes, 5% GWBR supplementation reversed HDL- and LDL-cholesterol concentrations to values of NC group. To further examine diabetes-related liver conditions, serum concentrations of ALT and AST, markers of liver damage, were measured. The rats with STZ-induced diabetes showed largely increased serum ALT and AST concentrations compared with those of the NC group.
GWBR 2.5%; DHB, STZ-induced diabetic group supplemented with GWBR 5%. Different letters indicate a significant difference (p < 0.05).

Effects of GWBR on Genes and Proteins Related to Intestinal Glucose Uptake To evaluate whether GWBR affected intestinal glucose uptake, the expression of genes and proteins involved in the regulation of glucose uptake in the intestine were examined (Fig. 2). The DC group exhibited an increase in the expression of the SGLT1 and GLUT2 genes, which encode glucose transporters that mediate glucose uptake from the intestine into the blood circulation (Fig. 2A). This induction was reduced by GWBR supplementation in a dose-dependent manner. HNF-1α and HNF-1β, nuclear receptors that are involved in the regulation of transcriptional activity of SGLT1, were also increased in the intestines of the DC group (Fig. 2A). The DLB group exhibited the same HNF-1α gene expression level that was observed in the NC group. There was no additional reduction in the DHB group (Fig. 2A). Diabetes-induced HNF-1β gene expression was decreased by GWBR supplementation in a dose-dependent manner. HNF-4α gene expression was slightly upregulated in the diabetic rats, whereas 5% GWBR supplementation led to a sizeable reduction in the expression of the HNF-4α gene (Fig. 2A). As Fig. 2B shows, GWBR showed the similar effects on the expression of SGLT1, GLUT2, HNF-1α, HNF-1β, and HNF-4α proteins as shown in the genes. The DHB group exhibited lower GLUT2 protein expression than that of the NC group (Fig. 2B).

Effect of GWBR on Genes and Proteins Related to GLUT4 Translocation in Skeletal Muscle To determine whether GWBR affects glucose uptake in muscle tissue, the genes and proteins that are involved in the regulation of GLUT4 translocation were examined (Figs. 3A, B). As Fig. 3A shows, the GLUT4 gene was not changed in the muscles of the rats with STZ-induced diabetes compared with that in the NC group, whereas GWBR supplementation caused an increase in GLUT4 gene expression. Consistent with this finding, IRS1, PI3K, and Akt, genes that encode proteins involved in the regulation of GLUT4 translocation, were reduced in the muscles of the DC group. This reduction was recovered in the DLB and DHB groups. The GSI gene, which encodes a rate-limiting enzyme to regulate glycogen synthesis, was downregulated in the DC group. This change was reversed with 5% GWBR supplementation to the same level observed in the NC group. Induced expression of the GSK-3β gene, a negative regulator of GSI in diabetes, was reduced in the DLB group and was completely reversed to the normal level in the DHB group. Compared with the changes at the gene level, GLUT4 protein expression was reduced in the DC group (Fig. 3B). This reduction was recovered by supplementation with 2.5% GWBR. The DHB group exhibited higher expression of GLUT4 protein than that of the NC group. The DC group exhibited a 50% reduction in the phosphorylation of PI3K, whereas 2.5% GWBR supplementation reversed this reduction to the control level. There was no additional induction in the DHB group. The phosphorylation of Akt in the DC group was also decreased. The DHB group showed increased phosphorylation of Akt compared with that of the DC group.

Effect of GWBR on Pancreatic β-Cells Figure 4A shows that rats with STZ-induced diabetes showed low amounts of insulin in the pancreas (Fig. 4A). The insulin amount increased with GWBR supplementation in dose-dependent manner. IR and IRS1 proteins, which are involved in the regulation of insulin synthesis and secretion, were also reduced in the pancreases of the DC group. The DHB group showed slightly increased expression of IR and IRS1 proteins, but the change was not statistically significant. The reduction in IR and IRS1 proteins in the pancreases of the rats with STZ-induced diabetes was further increased with 5% GWBR supplementation. This observation in histological images was confirmed by quantification (Fig. 4B).

DISCUSSION

STZ induces diabetes by destructing pancreatic cells, which then diminishes insulin secretion and causes hyperglycemia. In the present study, supplementation with GWBR for 8 weeks alleviated hyperglycemia in rats with STZ-induced diabetes.
Compared with the DC group, the diabetic rats supplemented with GWBR showed reduced AUCs for the OGTT. Therefore, the reduction in plasma glucose concentrations may result from increased glucose clearance ability. Normal plasma glucose concentrations are maintained by modulating glucose uptake and transport through the regulation of hormones, e.g., insulin and glucagon. The first site of plasma glucose regulation is the intestine. The intestine is a barrier that determines the absorption of nutrients into the blood circulation. Intestinal glucose absorption occurs mostly in the duodenum and in the initial portion of the jejunum by SGLT1 and GLUT2, which are highly expressed in the brush border and basolateral membranes, respectively. SGLT1 modulates glucose uptake from the lumen of the intestine into enterocytes, whereas GLUT2 facilitates the transport of glucose from enterocytes into the blood circulation. However, in cases of high luminal glucose concentrations, GLUT2 is recruited to the brush boarder membrane to uptake glucose into the enterocytes as well. It has been previously determined that SGLT1 and GLUT2 expressions are upregulated in rats with STZ-induced diabetes. Consistent with this, our DC group showed increased SGLT1 and GLUT2 gene and protein expression in the intestine, which contributes to the development of hyperglycemia. SGLT1 is transcriptionally regulated by recruiting HNF-1α and HNF-1β to the cis-regulatory element of its promoter. It is worth noting that SGLT1 transcription is increased by homodimers of HNF-1α and decreased by heterodimers of HNF-1α and HNF-1β. Although our study did not show dimerization of HNF-1, the decreased intestinal SGLT1 in the GWBR-supplemented groups may have resulted from the decrease in HNF-1α and HNF-1β genes and proteins, which then caused reduced glucose uptake to the enterocytes. The GWBR-induced decrease in intestinal GLUT2 genes and proteins successively decreased glucose transfer into the blood circulation, which resulted in decreased plasma concentrations compared with those in untreated diabetic rats. HNF-1 also interacts with other transcriptional factors, such as GATA-5 and CDX2, to increase SGLT1 promoter activity. HNF-1α activates GLUT2 by recruiting co-activators, e.g., p300, in the promoter region. HNF-4α increases HNF-1α-mediated

Fig. 2. Effect of GWBR on Intestinal Glucose Uptake

Expression levels of (A) genes and (B) proteins involved in the regulation of glucose uptake in the intestine were measured (NC, normal diabetic controls; DC, STZ-induced diabetic controls; DLB, STZ-induced diabetic group fed GWBR 2.5%; DHB, STZ-induced diabetic group fed GWBR 5%; n=8/group). β-Actin was used as an internal control. All experiments were performed in triplicate. Values are the mean±S.D. Different letters indicate a significant difference (p<0.05).
transcriptional activity, and cooperation between HNF-4α and HNF-1α is further enhanced by recruiting p300. Therefore, the decreased GLUT2 observed in the intestines of the GWBR-supplemented rats may result from the decreased cooperation of both HNF-4α and HNF-1α. When GLUT2 is recruited to the brush border membrane in response to a high glucose intake, insulin internalizes GLUT2 by returning it to the intracellular pool, which eventually causes GLUT2 degradation. However, this insulin-led response was not found in mice that were resistant to insulin. Consistent with the recovery of STZ-damaged β-cells in rats fed the DHB diet, insulin synthesis and sensitivity may be improved. Thus, this change in insulin may cause a significant decrease in GLUT2 protein expression in the DHB group by the internalization and subsequent degradation of GLUT2. This possibility suggests that GWBR might regulate SGLT1 and GLUT2 by controlling the coactivity and recruitment of transcriptional factors as well as through insulin-induced GLUT2 degradation.

Although the diabetic rats showed hyperglycemia, one characteristic observed in the rats with STZ-induced diabetes was severe weight loss even with very high levels of food consumption, also known as polyphagia. The weight loss may be caused by protein wasting due to the unavailability of carbohydrates as an energy source. However, this weight loss was not found in STZ-induced diabetic rats supplemented with GWBR. This indicates that GWBR might promote glucose energy utilization by increasing glucose uptake from blood circulation into peripheral tissues, such as muscle.

Skeletal muscle, constituting approximately 40% of mammalian body mass, serves a major target organ for glucose uptake from circulation. Approximately 80% of the postprandial glucose uptake was achieved by skeletal muscle. Impaired glucose uptake in skeletal muscles is a key feature of insulin resistance and type 2 diabetes. It is well known that GLUT4, an insulin-stimulated glucose transporter, is critical to the uptake of glucose into muscles. To serve as a glucose transporter, GLUT4 must be translocated from cytosol to the plasma membrane via the insulin-signaling pathway. Insulin binds IR and recruits IRS1 to active sequence downstream signaling factors, e.g., PI3K and Akt, via phosphorylation. Phosphorylated Akt translocates GLUT4 to the plasma membrane to uptake glucose into the muscle, which contributes to

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 3.** Effect of GWBR on Glucose Uptake and Storage in Skeletal Muscle

Expression levels of (A) genes and (B) proteins involved in regulating glucose uptake and storage in skeletal muscle were measured (NC, normal diabetic controls; DC, STZ-induced diabetic controls; DLB, STZ-induced diabetic group fed with GWBR 2.5%; DHB, STZ-induced diabetic group fed with GWBR 5%; n=8/group). β-Actin was used as an internal control. All experiments were performed in triplicate. Values are the mean±S.D. Different letters indicate a significant difference (p<0.05).
decreasing plasma glucose concentrations. Furthermore, insulin-activated Akt inhibits GSK3 via phosphorylation on N-terminals Ser21 and Ser9 of GSK-3α and GSK-3β, respectively, which activates glycogen synthase, a rate-limiting enzyme for glucose deposition in muscles. In the present study, STZ-induced diabetic rats showed reduced IRS1, PI3K and Akt gene expression in muscles, followed by decreased GLUT4 gene and protein expression. This change was reversed by GWBR supplementation by increasing PI3K and Akt protein phosphorylation. Consistent with the increased insulin signaling, GLUT4 gene and protein expression was also recovered in the muscles, which might contribute to the reduced plasma glucose concentrations observed in this study. Although Akt expression and phosphorylation (Ser473) were induced by GWBR, rats fed the DHB diet exhibited a significant increase in GLUT4 protein expression. This result indicates that other proteins and pathways may be involved in this induction in addition to PI3K and Akt-insulin signaling. Moreover, with
the significant increase in IRS1 gene expression, GWBR may make rats hypersensitive to the insulin response. 

Although GLUT4 translocation is mainly mediated by the insulin signaling pathway, various polyphenols promoted GLUT4 translocation in skeletal muscles by activating S'-adenosine monophosphate-activated protein kinase (AMPK), which is a primary regulator of energy metabolism. 

Resveratrol and black tea polyphenols increased GLUT4 translocation by increasing both insulin signaling and AMPK. 

Furthermore, black tea polyphenol-induced insulin signaling was mediated by Akt substrate 160 and Akt Thr308 without affecting Akt Ser473. 

Interestingly apple polyphenol extract increased insulin sensitivity and GLUT4 translocation by PI3K, through the peroxisome proliferator-activated receptor-gamma signaling pathway. 

Therefore, polyphenols from GWBR may increase glucose uptake in skeletal muscle by increasing insulin sensitivity and GLUT4 translocation through a combination of insulin signaling, AMPK and an unknown signaling pathway.

Along with improved glucose uptake into the skeletal muscles, increased expression of the GSK-3β gene in the skeletal muscles of rats with STZ-induced diabetes was decreased by 2.5% GWBR supplementation; with 5% GWBR supplementation, GSK-3β gene expression was further reduced, to levels comparable those of non-diabetic rats. The inhibition of GSK-3β by GWBR-activated insulin signaling slightly increased GS1 gene expression. Type II diabetic patients showed increased GS1 expression and activity in the skeletal muscles, while the inhibition of GSK-3 using small molecules improved insulin sensitivity and glycogen synthesis in diabetic rats. Therefore, our data indicate that GWBR improves abnormally high plasma glucose concentrations by increasing insulin-stimulated glucose uptake and storage in the skeletal muscles.

Although glycemic control is a priority in diabetes, it is also important to prevent complications, such as dyslipidemia, which make diabetic patients susceptible to developing other diseases. Rats with STZ-induced diabetes showed higher triglycerides, total cholesterol and LDL cholesterol and lower HDL cholesterol concentrations. GWBR completely reversed the abnormal hyperlipidemic conditions that developed in diabetic rats to normal levels, which indicates that GWBR supplementation has the benefit of regulating glucose and lipid metabolism in diabetes.

It is known that STZ causes hyperglycemia and hyperlipidemia by destroying the insulin-producing β-cells from the islets of Langerhans in the pancreas, thus inducing diabetes. 

In the present study, STZ-induced β-cell death was ameliorated by GWBR supplementation, a finding confirmed by the increased area of pancreatic islets stained with H&E. Consistent with protection of β-cells, the amount of insulin in the β-cells was increased by GWBR supplementation, as were the amounts of IR and IRS1 proteins. This finding suggests that GWBR may be able to recover the function of STZ-induced damaged β-cells, which synthesize and secrete insulin. Therefore, increased insulin secretion may improve the hyperglycemia and hyperlipidemia observed in rats with STZ-induced diabetes.

Based on our finding that GWBR caused β-cell regeneration, this study exhibited some limitations. First, because increased glucose clearance by GWBR may result from increased availability of insulin from regenerated β-cells, plasma insulin concentrations during OGTT should be confirmed. Second, because STZ-induced diabetic rats are developed by destroying β-cells and thus are indicated to have type 1 diabetes, type 2 diabetic animal models (e.g. Goto–Kakizaki rats) may be a better experimental model. Although the current study showed an anti-diabetic effect of GWBR in STZ-induced diabetic rats, the effect of GWBR in non-diabetic rats was not investigated, which these latter models might provide additional understanding of the effect of GWBR. Therefore, future studies with type 2 diabetic animal models and a better experimental design that includes GWBR exposure in both normal and diabetic rats are warranted to examine insulin regulation in glucose uptake through β-cell regeneration by GWBR.

In conclusion, GWBR supplementation ameliorated STZ-diabetic hyperglycemia by decreasing and increasing glucose uptake in intestine and muscle, respectively and hyperlipidemia, which may result from increased insulin availability from GWBR-induced β-cell regeneration. Taken together, GWBR has the potential health benefit of improving the diabetic condition.

Acknowledgment This study was supported by a National Research Foundation of Korea Grant (NRF-2010-0024307).

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

10) Hu C, Zawistowski J, Ling W, Kitts DD. Black rice (Oryza sativa L. indica) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. J. Agric.


