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Coating rice with mulberry leaves rich in deoxynojirimycin ameliorates hyperglycemia and dyslipidemia in C57BL/KsJ *db/db* mice

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BACKGROUND/OBJECTIVES: Mulberry leaf (ML) has been shown to have an inhibitory effect on α-glucosidase, and suppresses postprandial hyperglycemia, which may be related to its deoxynojirimycin (DNJ) content. This study was conducted to investigate the hypoglycemic and dyslipidemic effects of rice coated with ML rich in DNJ in a type 2 diabetes mouse model.
MATERIALS/METHODS: The mice were divided into four groups (n = 8 each): non-diabetic normal control (NC); diabetic control (DM-C), fed with 10% polished rice powder (DM-R); and fed with 10% polished rice powder coated with DNJ-rich ML (DM-DNJR).
RESULTS: Supplementation with DNJR for six weeks decreased levels of fasting blood glucose, plasma insulin, triglyceride, total cholesterol, and blood glycosylated hemoglobin; conversely, levels of glucagon-like peptide-1 and high-density lipoprotein-cholesterol showed an increase in the same treatment. In addition, weights of mesenteric, epididymal, and total adipose tissues decreased with DNJR supplementation, when compared with diabetic control *db/db* mice, while maltase, lactase, and sucrase activity in the small intestine were inhibited. The anti-diabetic effects were marginally greater in the DM-DNJR group than in the DM-R group.
CONCLUSIONS: These results suggest that rice coated with ML rich in DNJ can reduce hyperglycemia and hyperlipidemia in *db/db* mice, and may prove useful for individuals with diabetes.

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INTRODUCTION

Diabetes is a chronic metabolic disorder resulting from impaired regulation of blood glucose due to abnormal insulin production or insulin resistance in peripheral tissues [1]. It can also lead to abnormal lipid metabolism by increasing the blood levels of triglyceride (TG) and total cholesterol (TC), as well as by lowering the blood levels of high-density lipoprotein (HDL)-cholesterol [2]. By treating hyperglycemia and abnormal lipid metabolism in patients with diabetes, multiple complications can be prevented. To treat diabetes, patients are administered oral hypoglycemic agents in conjunction with diet therapy. However, hypoglycemic agents are reported to be associated with a risk of lactic acid build-up, and exacerbation of symptoms such as onset of renal failure, hepatotoxicity, and abdominal distension [3]; in addition, patients with diabetes may develop tolerance to these drugs. Accordingly, research has focused on the identification of bioactive natural compounds that can prevent and suppress diabetes [4-8]; moreover, natural substances that present in traditional diabetes remedies, or have hypoglycemic effects, have also recently gained attention.

The mulberry (*Morus alba* L.) leaf (ML), traditionally used in folk medicine in Korea, Japan, China, and others Asian countries, contains various functional components with hypoglycemic-like properties [4-11]. The bioactive compounds of ML are polyphenol, flavonoids, coumarins, and plant sterols including campesterol, β-sitosterol glucoside, β-ecdysone, and inosterol. Also present among the compounds found in ML is deoxynojirimycin (DNJ), an N-containing sugar that can be isolated from mulberry leaf and root. DNJ is a polyhydroxylated alkaloid belonging to a class of monocyclic piperidines and is known to inhibit the activity of α-glucosidase, an enzyme that breaks down carbohydrates, through a structural similarity to glucose [4-11], thus providing it with excellent anti-hyperglycemic properties.

Because diseases like diabetes and obesity have been reported to be associated with dietary patterns in the US, there is increasing interest in rice-based diets. Functional compounds in rice contain large concentrations of plant sterols such as β-sitosterol and stigmasterol, as well as squalenes, and high amounts of tocopherols, γ-oryzanol, and γ-aminobutyrate, which are involved in regulating blood pressure, as well as blood levels of TG and TC [12-14]. A study of changes in blood glucose levels and reactions to insulin following the consumption of rice, corn, potatoes, and bread revealed a drastic increase in blood glucose

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levels following the consumption of corn, potatoes, and bread; rice, however, was found to increase blood glucose levels at the slowest rate [15,16]. In addition, rice was found to increase blood glucose levels at different rates, depending on the method of preparation. For instance, steamed rice was found to inhibit increases in blood glucose levels and insulin secretion to a greater extent than porridge or rice cake [17].

Although functional rice is known to improve blood glucose levels [5,15-17], to date no studies have examined the anti-diabetic effects of rice coated with ML, which is rich in DNJ. Therefore, this study was conducted to investigate the anti-diabetic effects of rice coated with ML rich in DNJ in *db/db* mice, to find out a potential candidate for developing such functional food.

MATERIALS AND METHODS

Materials

The DNJ standard was purchased from Wako Pure Chemical Industrials Ltd. (Osaka, Japan). TG, TC, HDL-cholesterol, glucose, total protein, and albumin commercial kits were obtained from Fujifilm Photo Manufacturing Co., Ltd. (Kangawa, Japan); insulin assay kits were obtained from Eiken Chemical Co., Ltd. (Tokyo, Japan); and GLP-1 ELISA kits were obtained from Shibayagi (Gunma, Japan). All other reagents and solvents were guaranteed analytical grade and purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Production of rice coated with ML rich in DNJ

The rice coated with ML rich in DNJ used in this study was produced from BioResource, Inc. (Suncheon, Korea). MLs were cleaned of any debris, washed, and dried using a salad spinner. The leaves were frozen in a deep freezer and dried by lyophilization. The dried sample was ground into powder using a 100-mesh grinder. *Lactobacillus*, a genus of microorganisms that are highly resistant to α-glucosidase activity, were isolated from kimchi. A total of 100 g kimchi was pulverized in a homogenizer, and the juice was filtered and diluted 5-10 times. Colonies were isolated from the juice, and *Lactobacillus reuteri* BR-KC, which shows the highest resistance to α-glucosidase, was isolated and identified. *L. reuteri* BR-KC was used to ferment the ML powder. For the liquid culture of *L. reuteri* BR-KC, the bacterial colony was inoculated in 100 mL MRS broth, cultured at 37°C for 24 h, and used as a seed culture. Next, 20 mL *L. reuteri* BR-KC culture medium was evenly sprayed onto 100 g ML, and the powder was fermented at 37°C for 24 h. Following fermentation, the MLs were dried at 45°C for 24 h and used as a DNJ extract. Ethanol (30 mL) was added to 100 g lactic acid bacteria (LAB)-fermented ML. ML was stored at room temperature (25°C) until the DNJ was isolated. Next, a 3M filter paper was placed in the sample to obtain an ethanol extract of fermented ML. The extract filtrate was added to the rice three times (30 mL/100 g) by spraying, and the rice was dried at 45°C for 24 h to produce rice coated with ML rich in DNJ.

Determination of DNJ content

A modified version of the method described by Stead and Richards [18] was used to analyze the DNJ content in non-fermented and LAB-fermented ML. Water (10 mL) was added to 1 g of each sample. The samples were incubated on ice, sonicated, and then vortexed. Samples were extracted again at 60°C for 1 h and centrifuged at 15,000 × g for 15 min to obtain the supernatant. This process was repeated twice, and the supernatants obtained from each process were combined and diluted in 100 mL water. Each sample (10 μL) was added to an Eppendorf tube with 10 μL 0.4 M potassium borate buffer (pH 8.5) and 20 μL 5 mM 9-flurenylmethyl chloroformate, and incubated at 20°C for 20 min. The reaction was stopped by adding 10 μL 0.1 M glycine. Next, 950 μL 0.1% acetic acid was added to the reaction liquid to adjust the volume to 1 mL, and the solution was filtered through a 0.2 μL syringe nylon filter. A 10 μL sample of the filtrate obtained from this process was analyzed by high-performance liquid chromatography (HPLC; Shimadzu, Tokyo, Japan). A C18 Capcell Pak column (C18 Capcell Pak MG, 4.60 × 250 mm, Diameter 5 μm) was used. Acetonitrile-acetic acid (0.1%; 1:1, *v/v*) was used as the solvent and the filtrate was eluted at 1 mL/min. The fluorescence intensity of the extract was measured using a FL3000 fluorescence detector (excitation wavelength: 254 nm, emission wavelength: 322 nm) and analyzed using SMC21 Quick.

Determination of total polyphenol and total flavonoide content

The total polyphenol content of non-fermented and LAB-fermented ML extract were determined by the Folin-Cicocalteu method [19]. The total polyphenol content was calculated from the calibration curve, and the results were expressed as mg of tannic acid equivalent (TAE) per g dry weight (mg/g). The total flavonoid content of the extracts was measured by the aluminium chloride colorimetric method [20]. The total flavonoid content was calculated from the calibration curve, and the results were expressed as mg rutin equivalent (RE) per g dry weight (mg/g). The results represent the mean data of three parallel determinations.

Animal models and diet

Six-week-old male C57BLKS/J Iar + $Lepr^{db}$/ + $Lepr^{db}$ (*db/db*) mice and C57BLKS/J Iar-m + /m + (lean) mice were obtained from Central Lab Animal, Inc. (Seoul, Korea). The mice were adapted to a commercial pellet diet at the laboratory animal center of Chosun University for 1 week. The lean mice were used as the non-diabetic normal control. After confirming that diabetes was successfully induced in the animals by measuring weight and blood glucose levels, twenty-four mice meeting the conditions for the next experiment and showing no general symptoms were selected. The mice were divided into four groups: non-diabetic normal control group fed with a normal diet (NC), diabetic control group fed with a normal diet (DM-C), diabetic group fed with a diet containing 10% rice (DM-R), and diabetic group fed with a diet containing 10% rice coated with ML rich in DNJ (DM-DNJR). Experimental diets were prepared by using a modified AIN-93 rodent diet as shown in Table 1 [21]. The crude DNJ compounds have been reported to be non-toxic, to substantially lower the blood glucose levels in fasting glucose and oral glucose tolerance tests, and to have anti-diabetic effects in laboratory animals when administered at 40-150 mg/kg body weight/day [6-8]. The DNJ content of

Table 1. Composition of experimental diets for the diabetes mouse model

Diet composition (g/kg)	NC	DM-C	DM-R	DM-DNJR
Corn starch	397.486	397.486	303.886	303.886
Sucrose	100.000	100.000	100.000	100.000
Maltodextrin	132.000	132.000	132.000	132.000
Polished rice	0.000	0.000	100.000	0.000
DNJ-rich-ML-coated polished rice	0.000	0.000	0.000	100.000
Casein	200.000	200.000	193.600	193.600
L-Cysteine	3.000	3.000	3.000	3.000
Soybean oil	70.000	70.000	200.000	200.000
α-Cellulose	50.000	50.000	50.000	50.000
Mineral mix[1)]	35.000	35.000	35.000	35.000
Vitamin mix[1)]	10.000	10.000	10.000	10.000
Choline bitartrate	2.500	2.500	2.500	2.500
t-Butylhydroquinone	0.014	0.014	0.014	0.014

DNJ, deoxynojirimycin; ML, mulberry leaf; NC, non-diabetic normal control group; DM-C, diabetic control group; DM-R, 10% polished rice-supplemented diabetic group; DM-DNJR, 10% polished rice coated with ML rich in DNJ- supplemented diabetic group.
[1)] Based on AIN-93-MX mineral mixture and AIN-93-VX vitamin mixture [21].

the diets used in the experimental groups, for which the diets contained rice coated with ML rich in DNJ, was found to be 40 mg/kg body weight/day. Water and food were supplied *ad libitum*. The breeding room temperature was maintained at 18 ± 2℃, and a 12 h light/dark cycle was used. During the six-week experiment, body weight and food intake were measured once every week. The animal experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee of Chosun University (CIACUC2015-A0013).

Blood and organ collection

After completion of the feeding experiments, the mice were made to fast for 12 h, and were then euthanized by CO_2 inhalation, followed by thoracotomy. The collected blood was immediately incubated with ethylenediaminetetraacetic acid and stirred to prevent coagulation. Some of the blood samples were used to measure blood glucose levels and glycosylated hemoglobin (HbA1c) content. The remaining samples were centrifuged at 1,900 × g for 20 min to isolate the plasma. The plasma was used to measure the glucose, insulin, glucagon-like peptide (GLP-1), and lipid content. The liver and white fat pads (i.e., epididymal, mesenteric retroperitoneal, and perirenal fat pads) were extracted and cleaned of any blood or extraneous matter using a 0.9% saline solution. Their weight was then measured.

Blood glucose levels, oral glucose tolerance test (OGTT), and area under the curve (AUC)

Following a 12 h-fast after 0, 2, 4, and 6 weeks of feeding, blood was collected from the tail veins of the mice, and whole blood glucose levels were measured using a glucose analyzer (Accu-Chek Active, Roche, Basel, Switzerland). To evaluate the glucose tolerance of each experimental group, the OGTT was performed at the end of the experiment. Glucose was orally administered to mice, after fasting for 12 h, at 2 g/kg body weight. Blood samples were collected from the tail vein after 0, 30, 60, 90, and 120 min to measure the blood glucose levels. To determine the AUC according to the OGTT, the following equation was used [22]:

$$AUC = [(M2 + M1)/2] + [(M3 + M2)/2] + [(M4 + M3)/2] + [(M5 + M4)/2]$$

where M1-M5 indicate the blood glucose levels at 0 min, 30 min, 60 min, 90 min, and 120 min, respectively.

Blood and plasma biomarkers

Plasma insulin levels were measured using an insulin radioimmunoassay kit (Eiken Chemical Co., Ltd., Tokyo, Japan). Plasma GLP-1 levels were measured using a GLP-1 enzyme-linked immunosorbent assay (ELISA) kit (Shibayagiz, Gunma, Japan). The blood levels of HbA1c were measured by analyzing whole blood samples, using the MicroMat II Hemoglobin analyzer (Bio-Rad Laboratories, Hercules, CA, USA).

Measurement of disaccharidase activity in the intestinal mucosa

Disaccharidase activity (lactase, maltase, and sucrase) in the intestinal mucosa was measured as described by Dahlqvist [23]. Immediately after sacrificing the mice, the small intestines were dissected and washed with a saline solution on ice. After removing the duodenum, the small intestines were sectioned into three parts (proximal, middle, and distal) and washed with a cold saline solution. The intestinal mucosa was placed on a cooling plate on ice, scratched with a microscopic glass, weighed, homogenized with a 4-fold volume of distilled water (IKA MT-25 Janke & Kunkel, Baden-Württemberg, Germany), and centrifuged at 7,000 × g for 10 min at 4℃. The obtained supernatant was used to measure enzyme activity. A 0.1 mL diluted enzyme sample and 0.1 mL buffer solution (0.056 M disaccharide solution and 0.1 M sodium malate buffer, pH 6.0) were added to a test tube, mixed, and incubated at 37℃ in a water bath for 60 min. Next, 0.8 mL distilled water was added, and the solution was placed in boiling water for 2 min and cooled to room temperature. Subsequently, 0.5 mL of the sample solution was transferred to another test tube, to which 3 mL glucose oxidase solution was added. The solution was incubated in a water bath at 37℃ for 1 h, and its optical density was measured at 420 nm. Saccharide activity was measured in terms of specific activity (units of activity/g protein). For the protein level in the intestinal mucosa, bovine serum albumin was measured as the reference protein, using the Lowry method [24].

Plasma lipid profiles

Plasma TG, TC, and HDL-cholesterol levels were measured using an automatic dry-chemistry analyzer (Fuji Dri-Chem 3500, Fujifilm, Tokyo, Japan). The plasma low-density lipoprotein (LDL)-cholesterol levels were calculated using Friedwald's equation [25].

Statistical analysis

Data were analyzed using SPSS software (Version 19.0, IBM, Armonk, NY, USA). Experimental results for each group were expressed as the Mean±standard error (SE). To detect statistically

significant differences between groups, one-way analysis of variance (ANOVA) was performed, followed by a Tukey's post-hoc test with the level of statistical significance set at $P < 0.05$.

RESULTS

DNJ, total polyphenol, and total flavonoid content in non-fermented and LAB-fermented ML

Fig. 1 shows the functional components in non-fermented and LAB-fermented ML. The DNJ content in the non-fermented and LAB-fermented ML was 0.50 and 2.69 mg/g, respectively; the LAB-fermented ML had a higher DNJ content than the non-fermented ML (Fig. 1A,B). Total polyphenol (Fig. 1C) and total flavonoid (Fig. 1D) contents of the LAB-fermented ML were also marginally higher than those of the non-fermented ML.

Weight gain, food intake, and water consumption

Table 2 shows a comparison of the weight gain, food intake, and water consumption between the mice after six weeks. The *db/db* groups (DM-C, DM-R, and DM-DNJR) showed considerable weight gain and increases in food intake when compared with the NC group. Although there was no significant difference in weight gain and food intake among the *db/db* groups, the DM-DNJR group exhibited marginally lower weight gain and

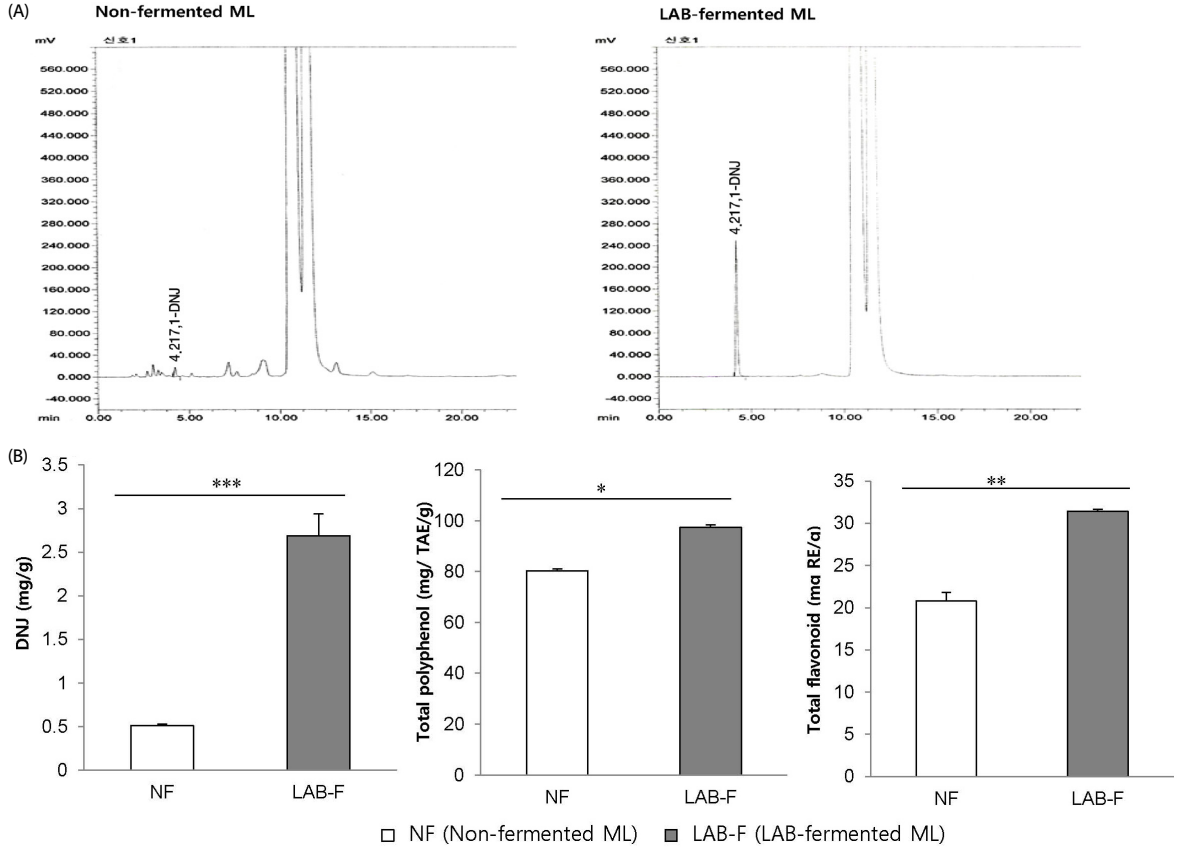

Fig. 1. Comparison of HPLC profiles of DNJ from non-fermented and LAB-fermented ML (A), and the DNJ (B), total polyphenol (C), and total flavonoid (D) contents of ethanol extracts of non-fermented and fermented ML. Values are Means±SE of triplicate experiments. Significant differences between non-fermented and LAB-fermented ML assessed by Student's *t*-test (* $P < 0.05$). HPLC, high-performance liquid chromatography; DNJ, deoxynojirimycin; ML, mulberry leaf

Table 2. Changes in body weight gain, food intake, and water consumption of non-diabetic and *db/db* mice fed experimental diets

Group	Body weight (g)				Food intake (g/day)	Water consumption (mL/day)
	Initial weight	Final weight	Total weight gain	Weight gain (g/day)		
NC	23.16 ± 1.23[1)2)a]	27.25 ± 2.75[a]	4.31 ± 2.15[a]	0.24 ± 0.001[a]	4.02 ± 0.23[a]	6.31 ± 0.83[c]
DM-C	35.21 ± 2.29[b]	52.52 ± 2.42[b]	17.31 ± 3.40[b]	0.46 ± 0.002[b]	7.68 ± 0.51[b]	36.23 ± 3.29[a]
DM-R	35.19 ± 2.32[b]	50.73 ± 2.45[b]	14.54 ± 2.82[b]	0.44 ± 0.001[b]	7.13 ± 0.24[b]	33.67 ± 2.77[b]
DM-DNJR	35.21 ± 2.35[b]	49.31 ± 3.36[b]	13.26 ± 0.12[b]	0.43 ± 0.001[b]	6.67 ± 0.19[b]	33.08 ± 2.84[b]

NC, non-diabetic normal control group; DM-C, diabetic control group; DM-R, 10% polished rice-supplemented diabetic group; DM-DNJR, 10% polished rice coated with ML rich in DNJ-supplemented diabetic group.
[1)] Data are shown as the Mean±SE (n = 8 mice per group).
[2)] Values with different superscripts in the same column are significantly different ($P < 0.05$) between groups

Table 3. Changes in the weight of the liver, subcutaneous, mesenteric, epididymal, retroperitoneal, and total adipose tissues in non-diabetic and *db/db* mice fed experimental diets

Group	Liver	Subcutaneous fat pads	Mesenteric fat pads	Retroperitoneal fat pads	Epididymal fat pads	Total white fat pads
	(g/100g body weight)					
NC	1.34 ± 0.25[b]	0.46 ± 0.02[1)2)b]	0.73 ± 0.05[c]	0.34 ± 0.04[b]	1.35 ± 0.15[c]	2.88 ± 0.09[c]
DM-C	5.01 ± 0.24[a]	1.61 ± 0.12[a]	1.18 ± 0.15[a]	0.64 ± 0.07[a]	1.78 ± 0.23[a]	5.21 ± 0.12[a]
DM-R	4.96 ± 0.58[a]	1.56 ± 0.12[a]	1.12 ± 0.08[a]	0.59 ± 0.05[a]	1.72 ± 0.25[a]	4.99 ± 0.19[ab]
DM-DNJR	4.80 ± 0.72[a]	1.54 ± 0.13[a]	0.91 ± 0.09[b]	0.50 ± 0.03[a]	1.66 ± 0.14[b]	4.61 ± 0.10[b]

NC, non-diabetic normal control group; DM-C, diabetic control group; DM-R, 10% polished rice-supplemented diabetic group; DM-DNJR, 10% polished rice coated with ML rich in DNJ-supplemented diabetic group.
[1)] Data are shown as the Mean±SE (n = 8 mice per group).
[2)] Values with different superscripts in the same column are significantly different (P< 0.05) between groups.

food intake than the DM-C group. Water consumption was higher in the *db/db* groups than in the NC group. The DM-R and DM-DNJR groups, which were fed rice, showed a substantially reduced level of water consumption when compared with the DM-C group.

Liver and adipose tissue weights

Table 3 shows the weights of liver and adipose tissue of the mice after six weeks. Liver weight increased significantly in the *db/db* groups when compared with the NC group. Although there was no significant difference in liver weight among the *db/db* groups, the DM-DNJR group had marginally reduced liver weights when compared with the DM-C group. The weights of subcutaneous and retroperitoneal adipose tissues increased significantly in the DM-C group, relative to the NC group. Although the values were lower in the DM-DNJR than in the DM-C group, the difference was not significant. The weights of mesenteric and epidydimal tissues also showed significant increases in the DM-C group compared with that in the NC group. Furthermore, these weights presented significantly lower values in the DM-DNJR group relative to the DM-C group. The weight of total adipose tissue also increased significantly in the DM-C group relative to the NC group, and decreased significantly in the DM-DNJR group when compared with the DM-C group.

OGTT and blood glucose levels

To investigate the effects of DNJR on OGTT, an OGTT was performed, and the AUC of blood glucose levels measured, one day before autopsy during the sixth week of the experiment. The results are shown in Fig. 2. The OGTT showed that maximum blood glucose levels were reached at 30 min after glucose administration in the all mice, and the levels then gradually decreased starting at 60 min. The DM-DNJR group showed reduced levels of blood glucose at 30, 60, 90, and 120 min when compared with the DM-C group, although a significant decrease was observed in blood glucose levels only after 120 min. Although the DM-R group showed a marginal decrease in blood glucose levels compared with that in the DM-C group starting at 90 min, the difference was not significant.

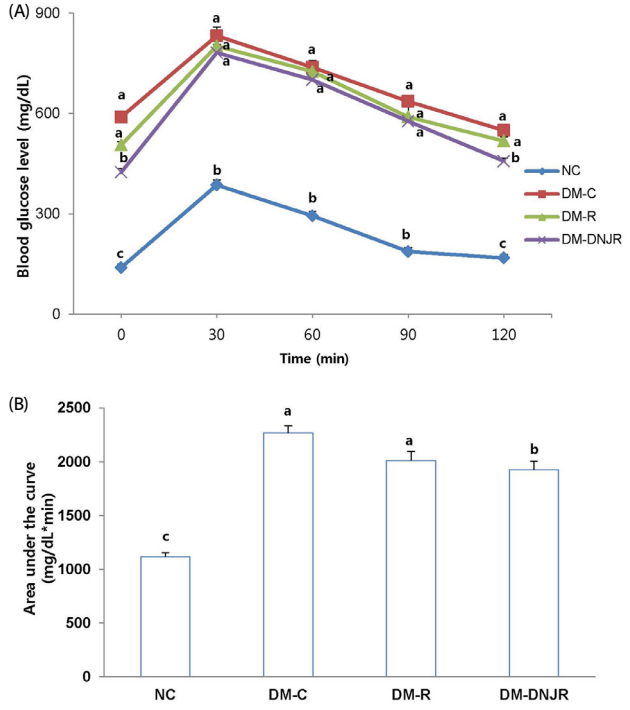

Fig. 2. Oral glucose tolerance test in non-diabetic and *db/db* mice fed experimental diets. Results represent oral glucose tolerance test (OGTT) (A) and corresponding calculated relative area under the curve (AUC) for glucose concentration (B). NC, non-diabetic normal control group; DM-C, diabetic control group; DM-R, 10% polished rice-supplemented diabetic group; DM-DNJR, 10% polished rice coated with ML rich in DNJ-supplemented diabetic group; AUC_{OGTT}: area under the curve for oral glucose tolerance test. Data are shown as the Mean±SE (n = 8 mice per group). Values with different superscripts in the same column are significantly different (P< 0.05) between groups.

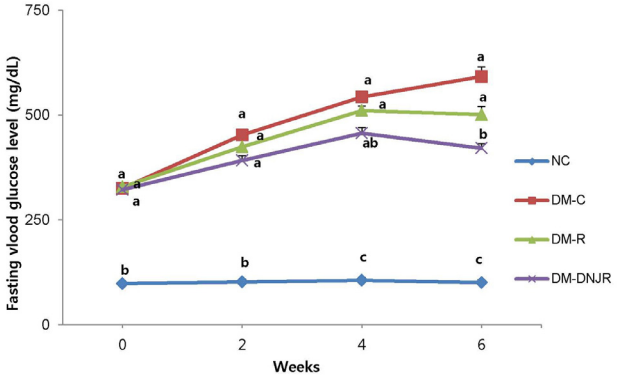

Fig. 3. Changes in fasting blood glucose levels in non-diabetic and *db/db* mice fed experimental diets. NC, non-diabetic normal control group; DM-C, diabetic control group; DM-R, 10% polished rice-supplemented diabetic group; DM-DNJR, 10% polished rice coated with ML powder rich in DNJ-supplemented diabetic group. Data are shown as the Mean±SE (n = 8 mice per group). Values with different superscripts in the same column are significantly different (P< 0.05) between groups.

Table 4. Plasma glucose, albumin, total protein, insulin, glucagon-like peptide 1 (GLP-1), and blood glycosylated hemoglobin (HbA1c) levels in non-diabetic and *db/db* mice fed experimental diets

Group	Glucose (mg/dL)	Albumin (g/dL)	Total protein (g/dL)	Insulin (μM/mL)	GLP-1 (pg/mL)	Blood HbA1c (%)
NC	147.81 ± 11.31[1)2)c]	2.60 ± 0.11[b]	5.82 ± 1.34[NS3)]	12.69 ± 156[c]	12.32 ± 1.65[a]	6.97 ± 2.09[c]
DM-C	411.54 ± 17.17[a]	3.08 ± 0.23[a]	6.34 ± 0.92	57.98 ± 3.25[a]	6.43 ± 1.08[c]	19.51 ± 2.15[a]
DM-R	396.63 ± 20.48[a]	3.95 ± 0.13[a]	6.09 ± 1.09	55.37 ± 4.83[a]	6.92 ± 0.87[c]	17.12 ± 3.13[a]
DM-DNJR	319.47 ± 19.11[b]	3.58 ± 0.19[a]	6.21 ± 1.45	45.98 ± 2.89[b]	8.33 ± 1.28[b]	12.65 ± 0.17[b]

NC, non-diabetic normal control group; DM-C, diabetic control group; DM-R, 10% polished rice supplemented diabetic group; DM-DNJR, 10% polished rice coated with ML rich in DNJ-supplemented diabetic group.
[1)] Data are shown as the Mean±SE (n = 8 mice per group).
[2)] Values with different superscripts in the same column are significantly different (P< 0.05) between groups.
[3)] NS, not significantly different between groups.

Table 5. Lactase, maltase, and sucrase activity of small intestinal segments in non-diabetic and *db/db* mice fed experimental diets

Disaccharidase	Intestinal segment	NC	DM-C	DM-R	DM-DNJR
Lactase (U/g protein)	Proximal	2.97 ± 0.09[1)b2)]	4.43 ± 0.27[a]	3.98 ± 0.13[a]	3.16 ± 0.08[b]
	Middle	4.02 ± 0.21[NS3)]	4.69 ± 0.19	4.21 ± 0.18	4.39 ± 1.20
	Distal	1.57 ± 0.18[b]	5.29 ± 0.13[a]	5.98 ± 0.27[a]	5.24 ± 0.11[a]
Maltase (U/g protein)	Proximal	90.31 ± 9.57[b]	150.11 ± 17.87[a]	146.43 ± 8.41[a]	96.23 ± 4.16[b]
	Middle	103.26 ± 8.98[c]	146.22 ± 10.33[a]	150.23 ± 9.78[a]	122.13 ± 7.12[b]
	Distal	41.23 ± 2.87[b]	61.78 ± 4.01[a]	57.33 ± 5.01[a]	56.27 ± 3.28[a]
Sucrase (U/g protein)	Proximal	12.06 ± 1.34[c]	32.47 ± 3.02[a]	31.46 ± 2.97[a]	24.32 ± 2.03[b]
	Middle	8.26 ± 1.06[b]	26.08 ± 1.87[a]	24.31 ± 1.85[a]	28.26 ± 1.96[a]
	Distal	7.08 ± 0.96[NS]	8.32 ± 1.92	7.99 ± 1.20	7.49 ± 0.97

NC, non-diabetic normal control group; DM-C, diabetic control group; DM-R, 10% polished rice-supplemented diabetic group; DM-DNJR, 10% polished rice coated with ML rich in DNJ-supplemented diabetic group.
[1)] Data are shown as the Mean±SE (n = 8 mice per group).
[2)] Values with different superscripts in the same row are significantly different (P< 0.05) between groups.
[3)] NS, not significantly different between groups.

The AUC of the blood glucose levels over time significantly increased in the *db/db* groups relative to the NC group. However, the DM-DNJR group showed a significant decrease in the AUC when compared with the DM-C group.

Changes in whole blood glucose levels

Fig. 3 shows the changes in whole blood glucose levels in the mice after six weeks. Before performing measurements, blood was collected from the tail vein after an 8-h fast. A total of four measurements were performed every two weeks over a six-week period, starting on the first day of the experiment. Blood glucose level was lower in the DM-DNJR group than in the DM-C group starting at four weeks and showing a significant decrease after six weeks. The *db/db* groups exhibited significantly higher blood glucose levels than the NC group prior to the experiment. The final blood glucose levels were elevated relative to the initial level in all *db/db* groups, in contrast to that in the NC group. However, the rate of increase in the level of blood glucose decreased in mice fed rice coated with ML powder rich in DNJ. In addition, the hypoglycemic effects of rice coated with ML powder containing DNJ were found to increase over time.

Levels of plasma glucose, albumin, total protein, insulin, GLP-1, and blood HbA1c

Table 4 shows the plasma glucose, albumin, total protein, insulin, GLP-1, and blood HbA1c contents in the mice after six weeks. The fasting plasma glucose level increased significantly in the DM-C group when compared with the NC group. The plasma glucose level showed a significant reduction in the DM-DNJR group relative to the DM-C group, and an increase relative to the NC group. Although the level of plasma albumin was higher in the *db/db* groups than in the NC group, there was no difference in levels among the diabetic groups. No significant differences in total plasma protein levels were observed among the experimental groups. The plasma insulin level was 4.6-fold higher in the DM-C group than in the NC group. In the DM-R and DM-DNJR groups, the plasma insulin levels showed reductions of 4.50% and 20.70%, respectively, relative to the DM-C group. The level of plasma GLP-1 showed a significant decrease of 47.81% in the DM-C group when compared with the NC group, and increases of 7.62% and 29.55% in the DM-R and DM-DNJR groups, respectively, relative to the DM-C group. The blood HbA1c level was 2.8-fold higher in the DM-C group than in the NC group and exhibited significant reductions of 12.25% and 35.32% in the DM-R and DM-DNJR groups, respectively, relative to the DM-C group.

Disaccharidase activity in the intestinal mucosa

To investigate whether DNJR inhibits glucose absorption in the small intestine, the effects of the rice on disaccharidase activity in the proximal, middle, and distal regions of the small intestine were measured (Table 5). Lactase, maltase, and sucrase activity presented significant reductions in all three regions of the small intestine in the DM-C group when compared with the NC group. In the DM-DNJR group, lactase and sucrase

Table 6. Plasma triglyceride, total cholesterol, high-density lipoprotein (HDL)-cholesterol, and low-density lipoprotein (LDL)-cholesterol contents in non-diabetic and *db/db* mice fed experimental diets

Group	Triglyceride	Total cholesterol	HDL- cholesterol	LDL- cholesterol
	(mg/dL)			
NC	139.00 ± 12.21[1)c2)]	154.50 ± 17.93[b]	81.00 ± 2.97[a]	82.20 ± 8.41[c]
DM-C	215.25 ± 9.25[a]	408.00 ± 19.19[a]	66.52 ± 5.95[b]	202.35 ± 6.25[a]
DM-R	189.75 ± 10.48[a]	382.75 ± 28.69[a]	68.09 ± 6.23[b]	192.92 ± 10.24[a]
DM-DNJR	153.25 ± 7.26[b]	352.75 ± 15.69[a]	70.77 ± 7.25[a]	150.46 ± 11.04[b]

NC, non-diabetic normal control group; DM-C, diabetic control group; DM-R, 10% polished rice-supplemented diabetic group; DM-DNJR, 10% polished rice coated with ML rich in DNJ-supplemented diabetic group.
[1)] Data are shown as the Mean±SE (n = 8 mice per group).
[2)] Values with different superscripts in the same column are significantly different ($P < 0.05$) between groups.

activity was significantly reduced in the proximal region relative to the DM-C group. However, no significant differences in lactase and sucrase activity in the middle and distal regions were observed among the *db/db* groups. Lactase activity in the proximal and middle regions was significantly reduced in the DM-DNJR group when compared with the DM-C group. Although the DM-R group showed reduced disaccharidase activity in the proximal, middle, and distal regions relative to the DM-C group, the difference was not significant.

Plasma TG, TC, HDL-cholesterol, and LDL-cholesterol content

Table 6 shows the plasma levels of TG, TC, HDL-cholesterol, and LDL-cholesterol in the mice after six weeks. The plasma TG level increased significantly in the DM-C group relative to the NC group. The serum TG level was significantly lower in the DM-DNJR group when compared with the DM-C group, and higher when compared with the NC group. Although plasma TC levels increased significantly in the DM-C group relative to the NC group, no difference was observed among the diabetic groups. The plasma HDL-cholesterol level decreased significantly in the DM-C group compared with that in the NC group, as well as in the DM-DNJR group, relative to the DM-C group. The plasma LDL-cholesterol level increased significantly in the DM-C group, compared with that in the NC group, and exhibited a significant reduction in the DM-DNJR group relative to the DM-C group. No significant difference in the level of plasma LDL-cholesterol was found between the DM-R and the DM-C groups.

DISCUSSION

Fermentation has recently been highlighted as an important technique for increasing the bioactivity of natural products. To effectively extract active compounds from plants, microorganisms are frequently used; according to Katina *et al*. [26], the fermentation of natural products using microorganisms increases bioavailability, as various enzymes released by the microorganisms, as well as bioactive compounds within the cells and tissues, become separated. ML has large concentrations of DNJ, total polyphenol, and flavonoid compounds, which have been shown to exhibit anti-diabetic and anti-oxidative activity [11,27]. Compared with the ML extracts obtained by sugar-leaching fermentation, ML extracts obtained by lactic acid fermentation were found to contain higher levels of polyphenol and anthocyanin, as well as piperidine alkaloid, which inhibits glucose metabolism [11]. DNJ is an alkaloid compound with a piperidine structure; it is a functional compound that can be eluted into slightly acidic or ethanolic solutions. Thus, the fermented extract has a higher DNJ content because of the effective elution of alkaloid compounds resulting from the increase in acidity caused by the microorganisms. Similarly, in this study, ML extracts obtained by lactic acid fermentation showed a higher content of DNJ, total polyphenol, and flavonoid compounds than the non-fermented extracts. Consequently, the present study was conducted to investigate whether functional rice produced by coating rice with ML with a high DNJ content, and fermented with *L. reuteri* BR-KC, would have anti-diabetic effects in animal models for type 2 diabetes.

Db/db mice, an animal model for type 2 diabetes, exhibit clinical characteristics similar to patients with type 2 diabetes such as hyperorexia, obesity, hypertension, insulin resistance, hyperleptinemia, and hyperinsulinemia [28]. In a previous study, *db/db* mice showed hyperinsulinemia and weight gain until 12 weeks, followed by a gradual decrease in blood insulin levels and weight [29]. In the diabetic state, glucose cannot enter cells efficiently, leading to increased blood glucose levels and excretion of glucose through urine, which can lead to polyuria, polydipsia, and polyphagia [30]. In this study, body weight, food intake, and water consumption of mice in the *db/db* groups were significantly higher than in the NC group. However, a diet consisting of rice coated with ML rich in DNJ led to weight loss during the six-week experimental period that, although not significant, may have positive effects on weight loss if ingested for a longer period of time. Park *et al*. [31] reported that diabetic mice gained weight because of polyphagia, which is a symptom of diabetes.

Liver size and weight are reported to increase in the presence of toxic substances due to frequent detoxication [32]. According to Goldberg [33], body fat breakdown increases under conditions of insulin deficiency in patients with diabetes, and the resulting free fatty acids are used in the synthesis of TG. This increases lipid accumulation in the liver, resulting in increased liver weight. Our results showed that liver weight increased significantly in the *db/db* groups when compared with the NC group. Although liver weight was lower in the DM-DNJR group than in the DM-C group, the difference was not significant. And while the weights of subcutaneous and retroperitoneal tissues increased significantly in the DM-C group relative to the NC group, there was no significant difference in the weights of these tissues among the *db/db* groups. However, the weights of epididymal, mesenteric, and total adipose tissues showed a

significant reduction in the DM-DNJR group when compared with the DM-C group. Considering that the weight of adipose tissue decreased following ingestion of rice coated with ML rich in DNJ, this suggests that DNJ-coated rice may have anti-obesity effects. However, no significant difference in the weight of adipose tissue was observed between the DM-R and the DM-C groups.

Fasting and postprandial glucose levels, as well as insulin, GLP-1, and HbA1c levels, are major markers of blood glucose regulation. In this report, the level of whole blood glucose was observed to gradually decrease, starting at four weeks, in the DM-DNJR group relative to the DM-C group, and significantly decrease after six weeks. Plant phenols inhibit the activity of α-amylase, sucrase, and α-glucosidase, which are enzymes that break down glucose in the small intestine, thereby lowering blood glucose and insulin levels [34]. This implies that the decrease in blood glucose levels observed was due not only to DNJ, but also to phenolic compounds in the fermented ML [4,25]. Similarly, the level of blood glucose decreased significantly in a group fed with an ML diet when compared with a diabetic group in a study by Han *et al*. [5]. In the present study, the level of fasting blood glucose showed a significantly decrease when mice were fed rice coated with ML rich in DNJ. Similar results were observed in experiments by Kim *et al*. [35] and Yang and Han [36], in which a significant decrease in the level of blood glucose was observed for mice fed a diet containing anaerobically treated ML. The risk of epilepsy and ischemic heart diseases decreased by 21% and 23%, respectively, for every 1 mM decrease in the blood glucose level [37]. Therefore, maintaining a normal blood glucose level is important for the treatment of diabetes and the prevention of complications in patients with diabetes [38]. In a prospective study on patients with diabetes, the incidence of complications including retinopathy, nephropathy, and neuropathy decreased when the blood glucose level was maintained at close to normal levels [39]. Studies have also reported that oxidative stress caused by hyperglycemia plays an important role in the pathogenesis of complications of diabetes, and that it impairs the function of vascular epithelial cells [40]. Based on these findings, the hypoglycemic effects of rice coated with ML rich in DNJ may originate not only from DNJ, but also from polyphenolic compounds and flavonoids involved in blood glucose regulation.

Most patients with type 2 diabetes or insulin-independent diabetes develop hyperinsulinemia owing to insulin resistance. Hyperinsulinemia is a mutual risk factor for impaired lipid metabolism, hypertension, and atherosclerosis. It causes obesity and directly affects the renin-angiotensin system to increase blood pressure [41]. Hyperinsulinemia impairs serum lipid metabolism to induce atherosclerosis and exacerbates cardiovascular disease and retinopathy [42]. Chung *et al*. [43] reported that the hypoglycemic effects of ML competitively inhibit the activity of the intestinal carbohydrase enzymes α-glucosidase, α-mannosidase, and β-galactosidase, without affecting insulin levels. Based on the results of the present study, the decrease in plasma insulin levels following the ingestion of DNJ-coated rice may be attributed to the reduced stimulation of insulin secretion because of reduced blood glucose levels, similar to the results obtained by Han *et al*. [5].

GLP-1, secreted from L-cells in the gastrointestinal tract, has been reported to reduce fasting blood glucose levels and improve insulin resistance in peripheral tissues. When administered to patients with type 2 diabetes, GLP-1 ameliorates hyperglycemia and is known to be a major factor in the improvement of hyperglycemia in patients with diabetes [44]. In this study, the level of plasma GLP-1 was observed to increase following the ingestion of rice coated with ML rich in DNJ. This result suggests that rice may play a positive role in the prevention and improvement of type 2 diabetes by decreasing insulin resistance and inducing a reduction in blood glucose by increasing the secretion of GLP-1.

The level of HbA1c is an important marker for diabetes [45], being a more stable marker than fasting blood glucose as it reflects long-term blood glucose levels. When the level of HbA1c increases, oxygen breakdown by red blood cells decreases, as does the movement of oxygen into tissues, which can result in complications. In the present study, the level of blood HbA1c increased significantly in the DM-C group when compared with the NC group, and decreased significantly in the DM-DNJR group when compared with the DM-C group. In patients with diabetes, the fasting blood glucose level is affected by various factors [46]. In addition, HbA1c accounts for 3-6% of normal hemoglobin and HbA1c levels show 2-3 fold increase in patients with diabetes [47]. As rice coated with ML rich in DNJ significantly decreased blood glucose and HbA1c levels, it is suggested that it may be useful for treating diabetes. A negligible decrease in the blood glucose level was observed in the DM-R group. Unpolished rice has higher fiber content than polished rice and has a low glycemic index. Thus, unpolished rice has been reported to have hypoglycemic effects [48]. These hypoglycemic effects were not observed in mice fed with rice, as polished rice was used.

Maltase, sucrase, and lactase activity in the proximal region of the small intestine are important factors affecting the rate of glucose absorption that occurs upon carbohydrate intake and blood glucose elevation. DNJ inhibits the breakdown of disaccharides into monosaccharides in the chorion of the small intestine, thereby delaying carbohydrate digestion and glucose absorption within the intestines. This prevents a rapid increase in blood glucose levels, reduces excessive insulin secretion, and inhibits increases in blood glucose levels [7,49]. It has been reported that DNJ does not stimulate insulin secretion in the pancreas and does not induce hyperinsulinemia, as insulin secretion is maintained in patients with insulin-independent diabetes [49]. In this study, the ingestion of rice coated with ML rich in DNJ led to reduced maltase, sucrase, and lactase activity. This result suggests that DNJ inhibited disaccharide degradation in the small intestine and interfered with glucose absorption, thereby preventing a rapid increase in the level of blood glucose. Through these processes, DNJ may inhibit increases in blood insulin levels, resulting in an improvement to harmful changes in the metabolism of nutrients induced by diabetes [50]. Although maltase, sucrase, and lactase activity showed an increase in the DM-C group relative to the NC group, activity was reduced in the DM-DNJR group relative to the DM-C group and increased relative to the NC group. Consequently, rice coated with ML rich in DNJ may inhibit saccharide

degradation in the small intestine and delay glucose absorption, thereby inhibiting drastic increases in blood glucose levels. In Asian populations, whose staple food is polished rice, blood glucose levels may be more effectively controlled by coating polished rice with ML rich in DNJ as part of dietary therapies.

Hyperlipidemia is common in patients with type 2 diabetes, with hypertriglyceridemia occurring with the highest frequency. Hyperlipidemia is a major risk factor for atherosclerosis and, ultimately, diabetes is reported to affect the incidence of cardiovascular complications. Abnormal lipid metabolism is often observed together with increased plasma levels of TG and TC, reduced HDL-cholesterol levels, and reduced movement of LDL-cholesterol into tissues [51]. In addition, although increased plasma HDL-cholesterol levels inhibit or delay progression to atherosclerosis, lower plasma HDL-cholesterol levels have been reported in patients with insulin-independent diabetes not administered oral hypoglycemic agents [52]. In this study, higher plasma levels of TG, TC, and LDL-cholesterol were observed in the DM-C group than in the NC group, and the HDL-cholesterol level was similarly significantly reduced. However, the plasma levels of TG, TC, and LDL-cholesterol were lower in the DM-DNJR group than in the DM-C group. The present study suggests that coating rice with ML rich in DNJ may improve lipid metabolism and this DNJ-coated rice may be useful as a functional or medicinal food for the management and prevention of type 2 diabetes.

Based on the above results, coating of rice with ML rich in DNJ decreased fasting blood glucose, plasma insulin, and blood HbA1c levels. In addition, it increased GLP-1 blood levels, improved insulin resistance, and inhibited disaccharidase activity in mice intestinal mucosa, when compared with diabetic control *db*/*db* mice, without showing any adverse effects. Moreover, it reduced the weight of mesenteric, epididymal, and total adipose tissue, and improved the plasma lipid profiles. These effects were attributed to the various constituents of the complex mixture, such as total polyphenol, total flavonoids, dietary fiber, and iminosugars, contained in rice coated with ML rich in DNJ. These results show that rice coated with ML rich in DNJ is a strong candidate for use as a functional food to regulate blood glucose and control hyperlipidemia.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interests.

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REFERENCES

1. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37:1595-607.
2. Hayden JM, Reaven PD. Cardiovascular disease in diabetes mellitus type 2: a potential role for novel cardiovascular risk factors. Curr Opin Lipidol 2000;11:519-28.
3. Bailey CJ. Insulin resistance and antidiabetic drugs. Biochem Pharmacol 1999;58:1511-20.
4. Hu XQ, Thakur K, Chen GH, Hu F, Zhang JG, Zhang HB, Wei ZJ. Metabolic effect of 1-deoxynojirimycin from mulberry leaves on db/db diabetic mice using liquid chromatography-mass spectrometry based metabolomics. J Agric Food Chem 2017;65:4658-67.
5. Han CK, Kim SS, Choi SY, Park JH, Lee BH. Effects of rice added with mulberry leaves and fruit on blood glucose, body fat and serum lipid levels in rats. J Korean Soc Food Sci Nutr 2009;38:1336-41.
6. Li YG, Ji DF, Zhong S, Lv ZQ, Lin TB, Chen S, Hu GY. Hybrid of 1-deoxynojirimycin and polysaccharide from mulberry leaves treat diabetes mellitus by activating PDX-1/insulin-1 signaling pathway and regulating the expression of glucokinase, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase in alloxan-induced diabetic mice. J Ethnopharmacol 2011;134:961-70.
7. Li YG, Ji DF, Zhong S, Lv ZQ, Lin TB. Cooperative anti-diabetic effects of deoxynojirimycin-polysaccharide by inhibiting glucose absorption and modulating glucose metabolism in streptozotocin-induced diabetic mice. PLoS One 2013;8:e65892.
8. Liu Q, Li X, Li C, Zheng Y, Wang F, Li H, Peng G. 1-Deoxynojirimycin alleviates liver injury and improves hepatic glucose metabolism in db/db mice. Molecules 2016;21:279.
9. Riche DM, Riche KD, East HE, Barrett EK, May WL. Impact of mulberry leaf extract on type 2 diabetes (Mul-DM): a randomized, placebo-controlled pilot study. Complement Ther Med 2017;32:105-8.
10. Józefczuk J, Malikowska K, Glapa A, Stawińska-Witoszyńska B, Nowak JK, Bajerska J, Lisowska A, Walkowiak J. Mulberry leaf extract decreases digestion and absorption of starch in healthy subjects-A randomized, placebo-controlled, crossover study. Adv Med Sci 2017;62:302-6.
11. Ryu IH, Kwon TO. Functional quality characteristics of extracts by sugar-leaching and lactic acid fermentation of mulberry leaves (*Morus alba* L.). J Sericult Entomol Sci 2013;51:164-72.
12. Sharma RD, Rukmini C. Rice bran oil and hypocholesterolemia in rats. Lipids 1986;21:715-7.
13. Ha TY, Ko SN, Lee SM, Kim HR, Chung SH, Kim SR, Yoon HH, Kim IH. Changes in nutraceutical lipid components of rice at different degree of milling. Eur J Lipid Sci Technol 2006;108:175-81.
14. Kahlon TS, Saunders RM, Sayre RN, Chow FI, Chiu MM, Betschart AA. Cholesterol-lowering effects of rice bran and rice bran oil fractions in hypercholesterolemic hamsters. Cereal Chem 1992; 69:485-9.
15. Crapo PA, Insel J, Sperling M, Kolterman OG. Comparison of serum glucose, insulin, and glucagon responses to different types of complex carbohydrate in noninsulin-dependent diabetic patients. Am J Clin Nutr 1981;34:184-90.
16. Jiraratsatit J, Mangklabruks A, Keoplung M, Matayabun S, Chumsilp L. Glycemic effects of rice and glutinous rice on treated-type II diabetic subjects. J Med Assoc Thai 1987;70:401-9.
17. Ha TY. Health functional properties of rice. Food Ind Nutr 2008;13:22-6.
18. Stead DA, Richards RM. Sensitive fluorimetric determination of gentamicin sulfate in biological matrices using solid-phase extraction, pre-column derivatization with 9-fluorenylmethyl chloroformate and reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Appl 1996;675:295-302.
19. Kaur C, Kapoor HC. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int J Food Sci Technol 2002;37:153-61.

20. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002;10:178-82.
21. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939-51.
22. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 2003;28:916-31.
23. Dahlqvist A. Assay of intestinal disaccharidases. Anal Biochem 1968;22:99-107.
24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
25. Rosenfeld L. Lipoprotein analysis. Early methods in the diagnosis of atherosclerosis. Arch Pathol Lab Med 1989;113:1101-10.
26. Katina K, Liukkonen KH, Kaukovirta-Norja A, Adlercreutz H, Heinonen SM, Lampi AM, Pihlava JM, Poutanen K. Fermentation-induced changes in the nutritional value of native or germinated rye. J Cereal Sci 2007;46:348-55.
27. Chae JY, Lee JY, Hoang IS, Whangbo D, Choi PW, Lee WC, Kim JW, Kim SY, Choi SW, Rhee SJ. Analysis of functional components of leaf of different mulberry cultivars. J Korean Soc Food Sci Nutr 2003;32:15-21.
28. Lee SM, Bustamante S, Flores C, Bezerra J, Goda T, Koldovský O. Chronic effects of an alpha-glucosidase inhibitor (Bay o 1248) on intestinal disaccharidase activity in normal and diabetic mice. J Pharmacol Exp Ther 1987;240:132-7.
29. Orland MJ, Permutt MA. Quantitative analysis of pancreatic proinsulin mRNA in genetically diabetic (db/db) mice. Diabetes 1987;36:341-7.
30. Park KS, Lee DE, Sung JH, Chung SH. Comparisons of antidiabetic effect of Panax ginseng on MLD STZ-induced diabetic rats in terms of time of administration. J Ginseng Res 2002;26:191-5.
31. Park BH, Shin JW, Lee SI, Kim SD. The effects of *Cudrania tricupidata* tea leaves on the blood glucose and serum lipids profiles of streptozotocin-induced hyperglycemic rats. J East Asian Soc Diet Life 2008;18:516-23.
32. Lee SG, Han KS, Jeong SG, Oh MH, Jang AR, Kim DH, Bae IH, Ham JS. A study on the sensory characteristic of yogurt and antimicrobial activity of *Lactobacillus plantarum* LHC52 isolated from kimchi. J Korean Soc Food Sci Anim Resour 2010;30:328-35.
33. Goldberg RB. Lipid disorders in diabetes. Diabetes Care 1981;4: 561-72.
34. Matsumoto N, Ishigaki F, Ishigaki A, Iwashina H, Hara Y. Reduction of blood glucose levels by tea catechin. Biosci Biotechnol Biochem 1993;57:525-7.
35. Kim SY, Ryu KS, Lee WC, Ku HO, Lee HS, Lee KR. Hypoglycemic effect of mulberry leaves with anaerobic treatment in alloxan-induced diabetic mice. Korean J Pharmacogn 1999;30:123-9.
36. Yang JH, Han JS. Effect of mulberry leaf extract supplement on blood glucose, glycated hemoglobin and serum lipids in type II diabetic patients. J Korean Soc Food Sci Nutr 2006;35:549-56.
37. Asia Pacific Cohort Studies Collaboration. Blood glucose and risk of cardiovascular disease in the Asia Pacific region. Diabetes Care 2004;27:2836-42.
38. Diabetes Control and Complications Trial Research Group, Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, Rand L, Siebert C. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-86.
39. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837-53.
40. Janle EM, Portocarrero C, Zhu Y, Zhou Q. Effect of long-term oral administration of green tea extract on weight gain and glucose tolerance in Zucker diabetic (ZDF) rats. J Herb Pharmacother 2005;5:55-65.
41. Kannel WB, Wilson PW, Zhang TJ. The epidemiology of impaired glucose tolerance and hypertension. Am Heart J 1991;121:1268-73.
42. DeFronzo RA. The effect of insulin on renal sodium metabolism. A review with clinical implications. Diabetologia 1981;21:165-71.
43. Chung SH, Yu JH, Kim EJ, Ryu KS. Blood glucose lowering effect of silkworm. Bull K H Pharma Sci 1996;24:95-100.
44. Trümper K, Trümper A, Trusheim H, Arnold R, Göke B, Hörsch D. Integrative mitogenic role of protein kinase B/Akt in beta-cells. Ann N Y Acad Sci 2000;921:242-50.
45. Rahbar S. An abnormal hemoglobin in red cells of diabetics. Clin Chim Acta 1968;22:296-8.
46. Park HR, Cho JS. Effects of a natural medicinal multi-plant extract on blood glucose, insulin levels, and serum malondialdehyde concentrations in streptozotocin-induced diabetic rats. J East Asian Soc Diet Life 2007;17:205-12.
47. Khalafallah A, Phuah E, Al-Barazan AM, Nikakis I, Radford A, Clarkson W, Trevett C, Brain T, Gebski V, Corbould A. Glycosylated haemoglobin for screening and diagnosis of gestational diabetes mellitus. BMJ Open 2016;6:e011059.
48. Lee JS, Lee JS, Yang CB, Shin HK. Blood glucose response to some cereals and determination of their glycemic index to rice as the standard food. Korean J Nutr 1997;30:1170-9.
49. Kimura T, Nakagawa K, Kubota H, Kojima Y, Goto Y, Yamagishi K, Oita S, Oikawa S, Miyazawa T. Food-grade mulberry powder enriched with 1-deoxynojirimycin suppresses the elevation of postprandial blood glucose in humans. J Agric Food Chem 2007;55:5869-74.
50. Kennedy DL, Piper JM, Baum C. Trends in use of oral hypoglycemic agents 1964-1986. Diabetes Care 1988;11:558-62.
51. Fuliang HU, Hepburn HR, Xuan H, Chen M, Daya S, Radloff SE. Effects of propolis on blood glucose, blood lipid and free radicals in rats with diabetes mellitus. Pharmacol Res 2005;51:147-52.
52. Park CH, Lee GD, Kim JO, Kim KS, Lee WY, Hong JH. Effect of *Bulnesia sarmienti* water extracts on lipid metabolism in neonatally streptozotocin-induced diabetic rats. J Life Sci 2008;8:999-1004.