INTRODUCTION

The incidence of obesity and type 2 diabetes mellitus (T2DM) is a major worldwide health concern. Epidemics of obesity, T2DM and dyslipidemia are highly correlated with an increase in food intake and a prolonged energy imbalance (American Diabetes, 2009). Moreover, modern lifestyle is represented by high calorie intake and low physical activity resulting in an increase in metabolic disorders. Hence, it is important to control insulin resistance and obesity by the modification of lifestyle factors such as diet.

Epidemiological studies suggest that cereal fibers and whole grain products can prevent obesity and weight gain, as well as
contribute to the reduction of the risk for the development of T2DM (Cho, Qi, Fahey, & Klurfeld, 2013). Dietary fiber could reduce the intestinal uptake of dietary fat and cholesterol, and the intake of high amylose, a type of dietary fibers, is known to decrease lipogenesis and steroidogenesis (Liu et al., 2003). The intake of dietary fibers is known to modulate intestinal microbiota, which can affect the ingestion of food and the adiposity of the individual. Recent intervention studies have shown that T2DM can be prevented by modulating diet increasing fiber intake (Bruttomesso & Tessari, 2019).

Dietary fibers include several starch and non-starch polysaccharides including amylose, cellulose, hemicellulose, and β-glucans (Burton-Freeman, 2000). Dietary fibers can be classified as prebiotic (Slavin, 2013) since these fibers are substrate for short-chain fatty acid (SCFA) production through bacterial fermentation by gut microbiota (den Besten et al., 2013), which could in turn demonstrate several biological activities including reduction of plasma cholesterol and triglyceride concentrations.

Rice (Oryza sativa L.) is the most important cultured crop in Asia and has been used as a staple food for more than half of the population in the world (Huang & Lai, 2016). Several rice varieties have been developed for a specific nutritional purpose. Specially bred rice variety enriched with dietary fibers showed improved biological effects. Oryza sativa L., Goami-3 is a rice variety with high levels of fibers, resulted in a significant reduction in body fat (~23%), total cholesterol (~20%), and triglyceride concentrations (~30%). Goami-3 rice also improved dyslipidemia and adiposity in diet-induced obese mice (Kim et al., 2013). Thus, different rice strains may have unique health effects.

Oryza sativa L. Dodamssal (DO) is a high amylose, high dietary fiber-containing variety developed by the Rural Development Administration (RDA) of Korea (Republic of Korea). DO contains more than 38% amylose, whereas normal rice contains only 18% of amylose content (Sim et al., 2015). High amylose rice has been shown to be more resistant to digestion than low amylose varieties (Panlasigui & Thompson, 2006). The dietary fiber content of DO is 4.2%, which is more than twice that of normal rice (Sim et al., 2015). Following these reports, the present article aims to elucidate the role of a newly developed rice strain, DO, a high content of dietary fiber in relation to glucose and insulin metabolism and its link to the pathogenesis of obesity and T2DM as well as the metabolic disorders that usually precede their onset, including metabolic syndrome.

2 | MATERIALS AND METHODS

2.1 | Preparation of DO rice samples

DO rice samples were obtained from the RDA, Suwon, Korea. DO was bred for food processing. Rice samples for the oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were finely ground with a 100-mesh sieve and dissolved in sterilized double-distilled water. Rice powder (1.6 g/kg body weight) was administered orally for the glucose tolerance test.

2.2 | Animal feeding studies and diet

We performed two independent animal feeding studies. The animals were housed with ad libitum access to rodent chow and water for a 1-week acclimation period before experimental manipulations began and maintained in a temperature-controlled (23°C ± 1°C) facility with a 12-hr light/dark cycle. db/db and C57BL/6J mice were fed a 45% high-fat diet (HFD) based on AIN-93 ad libitum. The protocol of two animal studies are described below (Figure S1).

2.2.1 | Animal experiments #1

Six-week-old C57BL/6J male mice (20–23 g, Samtako Co., Gyenggi-Do, Korea) were maintained in a temperature-controlled (21°C–25°C) environment on a 12-hr light/dark cycle. Mice were fed a 45% HFD for 4 weeks to induce hyperglycemia. Then, mice were randomly assigned into two groups. One group received orally with 1.6 g day⁻¹ kg⁻¹ of body weight of either corn starch (HF) as negative control or DO white rice for 1 week under HFD (Figure S1a).

2.2.2 | Animal experiment #2

Six-week-old db/db male mice (25–27 g, Samtako Co., Gyenggi-Do, Korea) were maintained in a temperature-controlled (21°C–25°C) environment on a 12-hr light/dark cycle. The db/db mice were fed a 45% HFD for 2 weeks to induce hyperglycemia. Then, db/db (5 weeks) were fed either with HFD containing 45% of calories from fat (HF group) or HFD supplemented with 30% (w/w) of isocaloric DO (DO group, Figure S1b). DO-containing isocaloric diets were custom designed and manufactured in dry pellet form by Du-Yeol Biotech (Gyenggi-Do, Korea). The components and energy density of these isocaloric diets are listed in Table 1.

In both mouse feeding experiments; food intake and body weight were assessed weekly. At the end of the both animal experimental, mice were sacrificed after 12-hr fasting and blood was collected from the heart puncture into BD Vacutainer blood collection EDTA tubes (Becton, Dickinson and Company, NJ, USA). Blood samples were centrifuged for 15 min at 12,000 rpm at 4°C to collect plasma samples. Then, stools and the organs containing the liver, ileum, muscle, and other tissues were removed and immediately frozen in liquid nitrogen. The tissues and plasma samples were stored at ~80°C for further analysis. All animal experiments were performed according to a protocol approved by the Animal Experiment Committee of Korea University (protocol No. KUIACUC-2016-97).

2.3 | Blood chemistry and hormone analysis

The plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glucose
levels were measured by an automated clinical chemistry analyzer (Cobas111, Roche, Basel, Switzerland) according to the manufacturer’s instructions. The homeostatic model assessment-insulin resistance (HOMA-IR) index was calculated according to this formula:

\[ \text{HOMA-IR} = \frac{\text{Glucose in mass unit of mg/dL}}{\text{Insulin level in U/mL}} \times \frac{405}{\text{body weight in kg}} \]

The plasma insulin level was determined by an ultra-sensitive mouse/rat insulin enzyme-linked immunosorbent assay (ELISA) kit from Merck Inc (Cat # EZRMI-13K, Darmstadt, Germany). Plasma adiponectin was determined using a mouse adiponectin ELISA kit (Cat # ab108785, abcam, Cambridge, UK). Plasma PYY was determined by a mouse PYY (Peptide) ELISA kit from Elabscience (Cat # E-EL-M2375, Houston, TX, USA), and plasma free fatty acid (FFA) was measured using an FFA quantification colorimetric and fluorometric kit (K612-100, BioVision, CA, USA) according to the manufacturer’s instructions.

### 2.4 | Histological analysis of liver

Livers from db/db mice were fixed in 4% paraformaldehyde and then stained with hematoxylin and eosin in the Histopathology Department of Anam Korea University Hospital (Seoul, Korea). For microscopic analysis, images were obtained using an inverted microscope (Eclipse Ti-s; Nikon).

### 2.5 | Fecal total bile acids

Stool samples were collected weekly after hyperglycemia induction. One milliliter of 75% ethanol was added to 50 mg of dried pulverized feces (two different time points; n = 3 or 4 per group), incubated at 50°C for 2 hr, and then centrifuged at 1,050g for 10 min. One hundred microliters of supernatant were added to 500 µl of phosphate-buffered saline, vortexed vigorously, and assayed using CrystalChem mouse total bile acids kit (Downers, Grove, IL) according to the manufacturer’s instructions.

### 2.6 | Oral glucose tolerance test (OGTT)

After daily oral feeding of DO for 1 week, C57BL/6J mice were fasted overnight. Glucose levels were determined from the tail vein before the oral administration of glucose (1.6 g/kg body weight). Blood samples were measured at regular intervals (15, 30, 60, 90, and 120 min), and glucose levels were quantified using a portable blood glucose monitoring system (Accu-Check Go, Roche).

### 2.7 | Insulin tolerance test (ITT)

After 1 week of daily oral administration. C57BL/6J mice were fasted (6 hr) and insulin (0.35 U/kg insulin, Sigma) was injected intraperitoneally. Blood samples were measured at regular intervals (15, 30, 60, 90, and 120 min) and glucose levels were quantified using a portable blood glucose monitoring system (Accu-Check Go, Roche).

### 2.8 | Quantitative polymerase chain reaction (qPCR)

Total RNA of liver and ileum tissues was isolated using RNeasy Plus (Takara Bio Inc., Shiga, Japan) according to the manufacturer’s protocol. First-strand cDNA was synthesized from 1 µg of total RNA of each sample using ReverTra Ace qPCR-RT Master Mix with g-DNA remover (Toyobo, Osaka, Japan). Extraction of total and synthesis of cDNA were performed as described previously (Jia et al., 2013) using ReverTra Ace qPCR-RT Master Mix with g-DNA remover (Toyobo, Osaka, Japan). The cDNA samples were then used as templates for PCR reactions. Specific primers were designed by the OligoPerfectTM Designer Program (Invitrogen, CA, USA). Primer sequences are shown in Table 2. Real-time qPCR was performed with the SYBR Green PCR system (Toyobo, Osaka, Japan) and with the Bio-Rad IQ5 cycler system (Bio-Rad, Hercules, CA, USA). The PCR reaction conditions were 95°C for 3 min followed by 50 cycles of 95°C for 20 s, 60°C for 20 s, 60°C for 20 s, and 72°C for 60 s. A melting curve of 71 cycles, starting at

### TABLE 1 | Isocaloric composition of the group-specific diets and Dodamssal (DO) composition analysis

<table>
<thead>
<tr>
<th></th>
<th>HFD (HF)</th>
<th>DO</th>
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<tr>
<td></td>
<td>g</td>
<td></td>
<td>K_cal</td>
</tr>
<tr>
<td>Casein, lactic</td>
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<td>1840</td>
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<tr>
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<tr>
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<tr>
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<td>195</td>
<td>1755</td>
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<tr>
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<tr>
<td>Mineral mix</td>
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<tr>
<td>Vitamin mix</td>
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<td>72</td>
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<tr>
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<td>Water soluble fibers</td>
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<tr>
<td>Total calories</td>
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*Recommended for the standard diet (AIN-93).*
55°C and increasing by 0.5°C every 10 s, was performed to determine primer specificity. The ribosomal protein L32 (Rpl32) was used for normalizing data, and gene expression levels were calculated according to IQ5 Optical System Software (version 2; Bio-Rad, Hercules, CA, USA).

### 2.9 Immunoblot analysis

Liver samples were homogenized in 200 µl of RIPA lysis buffer (10 mM Tris–HCL, pH 7.5, 1% NP-40, 0.1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, and 1 mM EDTA) containing 1% protease and phosphatase inhibitor cocktail (Thermo Scientific, MA, USA) and centrifuged at 14,000 rpm for 10 min at 4°C to collect the supernatant as described previously (Jia et al., 2013). The concentration was determined with a Bio-Rad reagent (Bio-Rad, PA, USA). The protein was denatured by heating and then loaded on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted. Briefly, the protein samples (40 µg) were resolved on 10% SDS-PAGE gels and then transferred onto nitrocellulose membranes (Daeillab Service Co. Ltd., Seoul, Korea). Nonspecific binding was blocked with 5% nonfat milk in Tris-buffered saline with 0.1% Tween-20 (TBS-T, pH 8.0, 25 mM Tris, 137 mM NaCl, 2.7 mM KCl, and 0.1% Tween-20) at room temperature for 1 hr, followed by overnight incubation with primary antibodies at 4°C. Antibodies specific for PCK1, G6PC, AKT, phosphorylated AKT, and α-tubulin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-microtubule-associated protein 1A/1B-light chain 3 (LC3) antibody was purchased from Novus Biologicals (Littleton, CO, USA). After incubation, the membranes were washed with TBS-T for 40 min. Then, the blots were hybridized with secondary antibody in 5% nonfat milk dissolved in TBS-T at room temperature for 1 hr after they were washed with TBS-T 4 times. Immunoreactive protein bands were detected with an enhanced chemiluminescence system (Thermo Scientific, MA, USA), visualized using ChemiDoc (Bio-Rad, CA, USA), and quantified with Image Lab software (Bio-Rad, CA, USA).

### 2.10 Statistical analysis

All experiments were conducted in triplicate; the results are expressed as mean ± standard error of the mean (SEM). Data analyses between the treated groups and the control group were performed using GraphPad 5 software. Unpaired t test and one-way analysis of variance were adopted for general data analysis. Significant differences between the two groups of data were determined with a t test (p < .05).

## 3 RESULTS AND DISCUSSION

### 3.1 Acute oral administration of DO improves glucose and insulin tolerance

After 1 week of DO administration, body weight and food intake were not different between control and DO mice (data not shown). In OGTT, glucose concentrations were reduced in the DO mice at
all time points compared with the levels in controls (Figure 1a). The glucose response area (area under the curve [AUC]) was reduced by 30.8% in the DO mice compared with those of the controls (Figure 1b). In ITT, the DO group showed a significant reduction in insulin response area (AUC) compared with those in the HFD group by 25.1% (Figure 1c,d). These results suggest that acute oral administration of DO improves glucose and insulin tolerance. Consumption of foods rich in dietary fibers (or a mixture of whole grains and bran) is modestly associated with a reduced risk of obesity and T2DM (Cho et al., 2013). Furthermore, fiber can regulate blood glucose levels and prevent lipid accumulation (Venn & Mann, 2004).

3.2 | DO decreased LDL cholesterol and triglyceride concentrations in mice

After 1 week of DO administration, mice showed a significant reduction in fasting plasma glucose (Figure 2a). DO also reduced total (−13.8%) and LDL cholesterol (−27.8%), plasma triglyceride levels (−46.4%) and FFAs (−54.3%) compared with control (Figure 2b,c,g). LDL-to-HDL ratio was significantly reduced, while HDL cholesterol levels were slightly increased, although not significantly (Figure 2d,e). ALT and AST levels were marginally altered in DO mice (Figure 2h,i). These results reveal that DO ameliorates HFD-induced dyslipidemia in mice. DO feeding significantly reduced plasma and LDL cholesterol, FFAs, and triglyceride concentrations in C57BL/6J which is in line with the statement that Dietary fiber can regulate glucose and lipid metabolism (Lattimer & Haub, 2010; Zawistowski, Kopec, & Kitts, 2009). Therefore, increasing the intake of dietary fibers could prevent and improve obesity and T2DM.

3.3 | Effects of DO on insulin, adiponectin, GLP-1, and PYY levels

DO administration showed that fasting insulin levels were significantly suppressed by 79.1% (p < .01, Figure 3a), while plasma adiponectin and GLP-1 were increased by 180.3% (p < .01) and 64.2% (p < .01), respectively, in DO mice compared to the controls (Figure 3b,d). Likewise, circulating concentrations of the anorexigenic peptide PYY were also significantly increased by 50.1% (p < .05) in the DO group (Figure 3c). The observed effects may be due to improved glucose and insulin tolerance which lead to improved plasma lipid and glucose metabolism by modulating adiponectin, GLP-1, and PYY secretion in mice. GLP-1, an incretin hormone, plays a pivotal role in glucose metabolism by binding the GLP-1 receptor on the surface of pancreatic β-cells to stimulate insulin secretion and block glucagon secretion for regulation of glucose homeostasis maintenance (Diez & Iglesias, 2003). Collectively, our results showed that

FIGURE 1 DO administration improves glucose and insulin tolerance in C57BL/6J mouse. Mice were fed HFD containing 45% fat calories and orally administered 1.6 g/kg body weight of Dodamssal (DO) for 1 week. Mice were orally administrated with high amyllose-rice, DO or corn starch as control (HF). (a) Oral glucose tolerance test. Blood glucose levels were checked at 0, 15, 30, 60, 90, 120 min after the oral administration of each rice sample. (b) Area under curve (AUC) was calculated for plasma glucose levels. (c) Insulin tolerance test (ITT). Blood insulin level were checked at 15, 30, 60, 90, 120 min after the oral administration of each rice sample (D) Area under curve (AUC) was calculated for plasma insulin levels. The values are expressed as mean ± SEM. Tukey’s test was performed for multiple group comparisons. *, p < .05; **, p < .01; ***, p < .001; HF, High-fat diet; DO, Dodamssal
GLP-1 concentrations were significantly increased in the DO group of C57BL/6J and db/db mice, suggesting that DO may have beneficial effects on glucose homeostasis. Equivalently, adiponectin is an adipokine that mediates several metabolic processes such as fatty acid oxidation and glucose regulation (Diez & Iglesias, 2003), and thus, the plasma concentrations of adipokines are tightly associated with both lipid and glucose metabolisms.

### 3.4 DO induce AKT phosphorylation and suppressing gluconeogenesis gene expressions

To investigate the mechanism of DO in the regulation of glucose metabolism, we assessed the gene and protein expressions involved in gluconeogenesis and insulin signaling pathway. Two key genes in gluconeogenesis (G6pc and Pck1) were measured by qPCR.
Hepatic mRNA and protein levels of G6pc were reduced in DO group (Figure 4a,c), whereas Pck1 expression was not significantly different in DO compared to the controls (Figure 4b,c). Hepatic AKT activity was assessed by immunoblotting analysis. Phosphorylated AKT was increased by 1-fold ($p = .0457$) in DO group (Figure 4d), suggesting marginal improved insulin signaling pathways following DO administration. Autophagy is reported to protect against insulin resistance (Yang, Li, Fu, Calay, & Hotamisligil, 2010). DO tended to increase LC3-II levels, a marker for autolysosome formation, and the LC3-II-to-LC3-I ratio, but not significantly (Figure S2). These results suggest that DO feeding may improve glucose and insulin tolerance by enhancing insulin signaling pathways that suppress hepatic gluconeogenesis. Suppression of gluconeogenesis is a major mechanism to improve insulin sensitivity and hyperglycemia. PEPCK and G6Pase are two key enzymes in gluconeogenesis and inhibition of these enzymes or the expression of these genes could suppress the rate of gluconeogenesis. In our results, the expression of G6pc, which encode G6Pase, was consistently reduced in both C57BL/6J and db/db mice. Thus, inhibition of hepatic gluconeogenesis may be the major mechanism to improve hyperglycemia and glucose/insulin tolerance along with elevation of adiponectin and GLP-1 levels. AKT is a major effector of the IR-IRS-1-PI3K pathway and is activated by its phosphorylation (Hong et al., 2014), which is a hallmark for improved insulin sensitivity. Our results showed that DO supplementation restored AKT activity in the insulin signaling pathway, especially in C57BL/6J mice.

### 3.5 DO regulates the expression of bile acid metabolism genes

Plasma cholesterol levels were reduced significantly in the DO group; thus, we investigated the expression of key genes involved in cholesterol and bile acid metabolism in the liver and ileum (Figure 5). Bile acids are efficiently reabsorbed (>95%) from the intestine, mainly by active transport mediated by the ileal bile acid transporter (also known as Slc10a2 or Asbt; Li & Chiang, 2015). Bile acids are synthesized from cholesterol by a process that requires the concerted actions of at least 14 liver enzymes (Russell, 2003). Cyp7a1, which encodes hepatic cholesterol 7α-hydroxylase, is the first and rate-limiting enzyme in the classic pathway for bile acid synthesis in the liver, and Cyp27a1 gene encodes sterol 27-hydroxylase and initiates bile acid metabolism in the alternative pathway (Russell, 2009).

Farnesoid X receptor (FXR; Fxr, also known as Nrhl4) activation in the intestine, specifically the ileum, has a major role in bile acid homeostasis (Ferrebee & Dawson, 2015). It has been suggested that
intestinal FXR regulates hepatic Cyp7a1 by the inducing ileal fibroblast growth factor-15 (Fgf-15) expression (Inagaki et al., 2005; Sayin et al., 2013; Zimmer et al., 2012) and regulates expression of the cytosolic ileal bile acid-binding protein, Ibabp (also known as Fabp6); And the polytopic transmembrane protein, Niemann-Pick C1-Like 1 (Npc1l1), a cholesterol transporter in the intestine, which facilitates the transfer of secreted biliary cholesterol back into hepatocytes (Betters & Yu, 2010; Howles & Hui, 2012).

Results showed that the expression of Asbt/Slc10a2 in the ileum was not changed (Figure 5a). In contrast, ileal Fxr/Nr1h4 (Figure 5b) and Fabp6 (Figure 5c) were significantly upregulated in the DO group (0.45-fold and 1.2-fold), respectively, compared with the control group. Also, the expression of Fgf-15 in the
intestine did not change (Figure 5d). DO significantly suppressed the expression of ileal Npc1l1 (~29.0%, \( p < .05 \)) but not the hepatic Npc1l1 (Figure 5e,f). HFD intake is correlated with impaired insulin action which increases delivery of FFA to the liver and consequently increasing production of hepatic triglyceride (Seo et al., 2008; Shimada et al., 1995). FXR negatively regulates bile acid synthesis directly in the liver leading to reduced transcription of Cyp7a1 (Chiang, 2009; Xu, 2011). In DO, the expression of hepatic Cyp7a1 was also significantly reduced but not the Cyp27a1 (Figure 5g,h). HMG-CoA reductase (Hmgcr) is considered a major rate-limiting enzyme in cholesterol synthesis (Sato & Takano, 1995). Hepatic Hmgcr expression (Figure 5i) was significantly reduced, however, the expression of Fxr/Nrh14 and Ldlr (Figure 5j,k respectively) were not changed in DO group compared with controls possibly due to the existence of a cholesterol secretion pathway that is independent of this hepatobiliary tract (Theuwissen & Mensink, 2008; Vrins, 2010). However, this should be study more in detail in further research.

DO group showed a significant increase in fecal bile acids excretion (+39.2%, \( p < .05 \)) compared with the control group (Figure 5l) after 1 week of oral DO administration. Suggesting that the hypocholesterolemic effects of DO rice may be through the induction of bile acid excretion and the suppression of hepatic cholesterol biosynthesis. Dietary fibers have the ability to increase intraluminal viscosity thereby affecting the enterohepatic recirculation of bile acids (Kristensen et al., 2012) which could also be linked to the effects that DO exerts. By comparing expression of direct FXR target genes in the liver and ileum, it seems that the gut microbiota primarily affects FXR targets in the ileum and not the liver. A schematic representation of DO regulatory mechanism was proposed (Figure 5m). Moreover, DO appears to induce Ppara gene expression and may regulate its responsive genes involved in hepatic lipid metabolism. Gene expression of Pparαgc1, Cpt1α, Acox1, which drives fatty acid β-oxidation, were upregulated significant compared with those of HF-fed mice. The gene expressions of Srebp1 as well as its responsive genes Fas and Scd1, which regulate lipogenesis (fatty acid and triglyceride synthesis) in the liver were downregulated by DO oral administration (Figure 5n).

Ppara mediates the gene transcription involved in fatty acid uptake and oxidation of fatty acids in hepatic lipid metabolism. In the liver, fatty acids are esterified to fatty acyl-CoAs in a Ppara-dependent manner and then transferred to the mitochondrial for β-oxidation catalyzed by Pparαgc1, Acox1, and Cpt1, which are directly activated by Ppara (Fan et al., 1998; Jia et al., 2013). Furthermore, Ppara enhance the location of Srebp1 on ER preventing the following activation Fas and Scd1 which are involved in hepatic lipogenesis synthesis (Yecies et al., 2011). Collectively, our results suggest that DO may, at least in part, act throughout Fxr to suppress the expression of Asbt, this along with a reduced expression of Npc1l1 may contribute to a reduction in total plasma and LDL in mice via a significant increase in fecal bile acid excretion and the hypolipidemic effects of DO may be achieved by direct activation of hepatic Ppara expression and its responsive genes regulating hepatic fatty acid uptake and β-oxidation, while downregulating the hepatic fatty acid synthesis gene expression to decrease triglyceride concentrations in circulatory system and liver lipid accumulation.

### 3.6 Administration of DO for 5 weeks reduces fasting glucose concentrations in db/db mice

We next assessed the long-term administration effects of DO in db/db mice. In mice fed isocaloric, high-fat containing, DO diet (30%, w/w of DO, 45% calories from fat) for 5 weeks, fasting plasma glucose concentrations were modestly decreased at weeks 3 to 5, respectively, during supplemented rice-diet intake (Figure 6a) compared to the control group, although body weight (Figure 6b), and tissue weights were not different between the DO and the control groups (data not shown). Food intake remained unchanged (data not shown). Hematoxylin and eosin staining of mouse livers revealed that DO reduced the ballooning in hepatocytes, a marker of hepatic steatosis (Figure 6c). DO feeding significantly reduced, plasma total cholesterol, LDL cholesterol, triglyceride, and FFA levels compared to the control group (Figure 6d–e,h–i). LDL-to-HDL ratio tended to be reduced, while HDL cholesterol levels were not changed (Figure 6f,g). ALT, and AST concentrations were marginally altered in DO mice (Figure 6j,k). In addition, DO reduces plasma glucose and slightly improves glucose tolerance and insulin sensitivity (Figure 6l,m). These results suggest that long-term administration of DO showed marginally hypoglycemic and hypolipidemic effect with amelioration of hepatic steatosis in db/db mice. For example, Wu, Yang, So, Lee, and Kim (2016) showed that mice fed with lipum brown rice showed significantly reduced plasma total cholesterol levels and did not result in obesity. In line with our short-term results, it is suggested that DO rice has similar biological effects comparable to the intake of dietary fibers thus, show regulation of key parameters in the lipid and glucose metabolism (He & Shi, 2017; Kovatcheva-Datchary et al., 2015).

### 3.7 Effects of 5-week administration of DO on hormone levels, AKT activity, and the expression of gluconeogenesis genes in db/db mice

We quantified plasma hormones in db/db mice. Insulin levels were suppressed by 63.8% (\( p < .01 \); Figure 7a), while adiponectin, PYY, and GLP-1 concentrations were significantly increased by 20.8% (\( p < .05 \)), 49.7% (\( p < .01 \)), and 104.7% (\( p < .05 \)), respectively, in the DO group compared to the controls (Figure 7b–d). These findings are in line with the results from the C57BL/6J mice after 1 week of DO administration. The gene and protein expression analysis revealed that the mRNA expression of G6pc and Pck1 (Figure 7e–g) was significantly suppressed in DO livers. In immunoblot analysis, phosphorylation of AKT and the p-AKT-to-AKT ratio tended to be increased, although not significantly (\( p = .6179 \); Figure S3a). Protein expression of LC3-II, a marker for autophagosome formation, tended
to be increased in DO livers (p = .1964; Figure S3b). These results suggest that long-term administration of DO in db/db mice has a hypoglycemic effect, primarily because of the suppression of hepatic gluconeogenic gene expressions and induction of GLP-1, as well as PYY, and adiponectin concentrations. Based on these remarks, we suggest that DO intake might be beneficial in improving insulin resistance and glucose homeostasis in both mice strains, which appeared to be mediated through elevation of plasma insulin level that caused activation of glycolysis and inhibition of gluconeogenic and lipid metabolic enzymes in liver although further detailed mechanism seems to be elucidated.

3.8 | DO effects on diet-related diseases

High-fat diet induced inflammation and increased circulating plasma concentrations of lipopolysaccharide (LPS), which subsequently promotes inflammation, insulin resistance, obesity, and T2DM in humans and rodents (Cani et al., 2007; Lyu et al., 2017; Sánchez-Tapia et al., 2017). High lipopolysaccharide (LPS) content is linked to excess dietary fat (Cani et al., 2007). Studies (Cani et al., 2007; Everard et al., 2011; Kobyliak et al., 2016; Koren et al., 2011; Savcheniuk et al., 2014) have indicated that modulation of the gut microbiota reduced metabolic endotoxemia and the fecal content of LPS as well as...
**FIGURE 7** Effects of DO on glucose metabolism in db/db mice. GI hormones and gene and protein expressions were measured in mice after 5 weeks of DO diet in db/db mice. (a) Plasma insulin, (b) Adiponectin, (c) PYY, and (d) GLP-1 concentrations. (e) The expression of mRNA expression of (e) G6pc and (f) Pck1 in livers. Immunoblot analysis of hepatic (g) PCK1 and G6PC. The values are expressed as mean ± SEM. Tukey's test was performed for multiple group comparisons. *, p < .05; **, p < .01; HF, High-fat diet; DO, Dodamssal

**FIGURE 8** DO diet reduces LPS and TMAO levels in C57BL6/J and db/db mice. Plasma LPS and TMAO concentrations in (a) db/db mice and (b) C57BL/6J mice fed HFD. Blood samples were collected from the heart upon sacrifice after 5-week and 1-week treatment diets, respectively. Corn starch (CS) was used as control for the oral feeding control group of C57BL/6J mice. Plasma was obtained after centrifugation at 3,000 rpm for 20 min. The values are expressed as mean ± SEM. Tukey's test was performed for multiple group comparisons. *, p < .05; **, p < .01; HF, High-fat diet; DO, Dodamssal
improved low-grade inflammation, steatosis, glucose intolerance, and insulin sensitivity. Hence, to causally link changes in gut microbiota to HFD-induced vascular inflammation and cardiovascular diseases (CVDs) disorders, we sought to analyze the levels of plasma LPS in vivo. We found that DO intake suppressed metabolic endotoxemia levels of LPS in both HFD-fed C57BL/6J and db/db mice (Figure 8a, b, respectively).

Additionally, literature suggests a correlation on low-fiber intake and CVDs, since elevated concentrations of Trimethylamine N-oxide (TMAO) and its precursor TMA were associated with increased risks of major adverse cardiovascular events (Heianza, Ma, Manson, Rexrode, & Qi, 2017). Since TMAO is a plasma biomarker in the diagnosis and prevention of CVDs (Lyu et al., 2017) and accelerated vascular inflammation (Chen et al., 2016), we assessed the TMAO concentration in C57BL6J and db/db mice plasma after short-term and long-term intake of DO, respectively. Results showed TMAO concentrations were markedly reduced by DO intake in both high-fat-fed C57BL/6J and db/db mice (Figure 8a, b correspondingly). This was correlated with improved glucose intolerance, lower plasma LPS concentrations, lipid and bile acid markers (mRNA expression) in hepatic and ileal tissues. These results suggest that DO slightly counteracts the effect of HFD intake related to metabolic inflammation and vascular inflammation. Thus, suggesting that DO modulates microbiota to suppress inflammation and ameliorate the risk of CVDs due to a reduction in LPS endotoxin and plasma TMAO circulating levels.

4 | CONCLUSION

Obesity and T2DM are lifestyle metabolic diseases that can lead to many complications including CVDs and stroke. A balanced nutrition and energy intake are important to maintain health and metabolic homeostasis. Therefore, in the present study, we investigated a novel rice strain, Oryza sativa L. DO, with a high content of dietary fiber-like amyllose. Our results demonstrate that DO reduces fasting plasma glucose in the short-term (C57BL/6J) and confirm long-term experiments (db/db) in mice compared with the control diets. DO supplementation tends to counteract the deleterious effects characterized during the intake of high-fat diet, related to plasma TG, ALT, and AST and elevate HDL-C. DO also significantly suppressed the expression of Pck1 and G6pc, key genes in hepatic...
gluconeogenesis and induced phosphorylation activation of AKT, especially in C57BL/6J mice. DO supplementation may stimulate the secretion of the appetite control hormones such as GLP-1, PYY, insulin, and adiponectin leading to a decrease in blood glucose, while increasing the total bile acid excretion in stools and reducing plasma TMAO and LP5 plasma concentrations (Figure 9). Collectively, the results from the present study suggest that newly developed DO high amylose rice strain showed hypolipidemic effects and may improve glucose tolerance in mice. And therefore, may be a potential adjuvant-treatment agent to prevent disease development by improving glucose metabolism and lipid metabolism.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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REFERENCES


**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the Supporting Information section.

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