

## ORIGINAL RESEARCH

# Effects of a ready-to-eat cereal formula powder on glucose metabolism, inflammation, and gut microbiota in diabetic db/db mice

Caina Li | Xing Wang | Sujuan Sun | Shuainan Liu | Yi Huan | Rongcui Li |  
Quan Liu | Hui Cao | Tian Zhou | Lei Lei | Minzhi Liu | Zhufang Shen 

State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Key Laboratory of Polymorphic Drugs of Beijing, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

**Correspondence**

Zhufang Shen, No.1 Xiannongtan Street, Beijing 100050, China.  
Email: shenzhf@imm.ac.cn

**Funding information**

CAMS Initiative for Innovative Medicine, Grant/Award Number: 2016-I2M-2-006; Drug Innovation Major Project, Grant/Award Number: 2018ZX09711001-003-009

**Abstract**

The cereal formula powder, Zhengda Jingshan (ZDJS), comprises dietary fiber, multivitamins, fine protein, and various cereal ingredients. The present study evaluated the effects of ZDJS on glucose metabolism and explored the corresponding mechanisms in terms of modulating gut microbiota and the fecal metabolome. Type 2 diabetic db/db mice were given ZDJS (1 g/kg) orally twice daily for 55 days, after which glucose metabolism, inflammation, gut microbiota, and fecal metabolomics were assayed. Repeated administration of ZDJS was associated with a trend toward decreasing fasting blood glucose and a 0.12% decrease in hemoglobin A1c (HbA1c), as well as statistically significant increases in the insulin sensitivity index and decreases in serum levels of tumor necrosis factor (TNF- $\alpha$ ) and ileum expression of mucin-2. ZDJS also ameliorated the compensatory enlargement of islets and decreased the ratio of the  $\alpha$ -cell area to total islet area; however, this amelioration of impaired oral glucose tolerance became less pronounced as treatment continued. In addition, ZDJS remarkably decreased the abundance of phylum Proteobacteria and the phylum ratio of Firmicutes to Bacteroidetes, as well as altered the fecal metabolic profile. Taken together, our findings demonstrate that ZDJS improved glucose metabolism and reduced inflammation in type 2 diabetic db/db mice, which may be associated with a reshaping of the gut microbiome and fecal metabolome in db/db mice. Thus, our study suggests that ZDJS may represent a complementary therapy for patients with type 2 diabetes.

**KEYWORDS**

cereal formula powder, fecal metabolome, glucose metabolism, gut microbiota, inflammation

## 1 | INTRODUCTION

Type 2 diabetes mellitus (T2DM) is threatening the health of more and more individuals over time. In addition, many others are pre-diabetic—characterized by obesity, insulin resistance, and impaired

glucose tolerance—which can easily develop into overt diabetes. Although there has been an increasing number of antidiabetic drugs in the clinic, dietary and lifestyle modifications are still the most promising, safe, and economically efficient methods (Russell et al., 2016) for ameliorating symptoms, especially for individuals

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *Food Science & Nutrition* published by Wiley Periodicals LLC.

with prediabetes. Dietary fibers are defined as edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine, and with complete or partial fermentation in the large intestine (Howlett et al., 2010). Clinical studies have shown that adequate intake of dietary fiber is associated with better glycemic control, increased first-phase insulin secretion, and improved insulin sensitivity (Bodinham, Smith, Wright, Frost, & Robertson, 2012; Fujii et al., 2013; Wolever, Campbell, Geleva, & Anderson, 2004).

Gut microbiota inhabit the distal gut and possess more carbohydrate-active enzymes than those of eukaryotic human cells, which enable gut microbiota to depolymerize and ferment ingested food that cannot be digested in the stomach or absorbed in the intestine. Intake of dietary fiber not only influences the composition of gut microbiota (Candela et al., 2016) but also provides an essential substrate for microbiota, whereas fiber deprivation exacerbates pathogen susceptibility (Desai et al., 2016). In addition, gut microbiota ferment dietary fiber into short-chain fatty acids (SCFAs)—such as butyrate, propionate, and acetate—which are important signaling molecules for transducing the functions of gut microbiota to the host, thus influencing appetite regulation, immune function, epithelial cell integrity, and homeostasis of glucose and lipids (Holscher, 2017; Koh, De Vadder, Kovatcheva-Datchary, & Bäckhed, 2016).

Zhengda Jingshan (ZDJS) is a type of ready-to-eat cereal formula powder consisting of dietary fiber, multivitamins, fine protein, and various cereal ingredients, and the total energy of 30 g ZDJS is 421 KJ. The proportions of nutrient reference values for dietary fiber, protein, fat, and carbohydrates in ZDJS are 48%, 13%, 5%, and 2%, respectively. The detailed ingredients and contents of ZDJS are listed in Table S1. The present study aimed to evaluate the effects of ZDJS on glucose metabolism and to explore its functional mechanisms in terms of modulation of gut microbiota.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals

Male BKS-Lepr<sup>em2Cd479</sup>/Nju (Lepr KO/KO, db/db) mice and wild-type control mice (Lepr wt/wt, db/m), aged 4–8 weeks, were obtained from the Nanjing Biochemical Research Institute of Nanjing University (Nanjing, China) and were raised with special feed (XieTong Organism, Nanjing, China) in a temperature- and humidity-controlled environment with a 12-hr light/dark cycle and free access to water. Diabetic db/db mice were divided into a vehicle-treated group (Con) and a ZDJS-treated group (ZDJS, 1 g/kg;  $n = 11$ ). Age-matched wild-type mice were used as healthy controls (Nor,  $n = 12$ ). ZDJS-treated mice were given ZDJS in water (1 g/kg, 0.1 ml/10 g BW) twice daily for 55 days. Vehicle-treated mice and healthy control mice were given equal volumes of water. The present study was carried out with another study; these two studies used the same diabetic control mice and healthy control mice.

### 2.2 | Assays measuring nonfasting blood glucose, fasting blood glucose, and HbA1c

After 10, 25, 42, and 45 days of treatments, blood was collected from tail tips to determine nonfasting blood glucose (NFBG) levels via the glucose oxidase method (Biosino Bio-Technology & Science Inc., Beijing, China). After 10, 22, 30, and 37 days of treatments, all of the mice were fasted for 4 hr with free access to water, and then, fasting blood glucose (FBG) levels were determined via the aforementioned glucose oxidase method. After 45 days of treatments, hemoglobin A1c (HbA1c) levels were measured with a commercial kit (Homa Biological, Beijing, China).

### 2.3 | Assays measuring oral glucose tolerance and insulin sensitivity

All of the mice were fasted for 4 hr, with water provided ad libitum, before the start of experiments. After 16 and 37 days of treatments, the oral glucose tolerance test (OGTT) was separately performed; the insulin tolerance test (ITT) was carried out after 30 days of treatment, as previously described (Peng et al., 2014). The area under the blood glucose–time curve (AUC) and the decreasing ratio of blood glucose at 40 min after injection of insulin were calculated in the ITT. In addition, fasting blood insulin (FBI) levels were determined (ALPCO, Salem, NH, USA) after 42 days of treatment, as was FBG. Then, the insulin sensitivity index (ISI) was calculated as follows:  $1 / (\text{FBG} \times \text{FBI})$ .

### 2.4 | Assays measuring serum TNF- $\alpha$ , IL-1 $\beta$ , and glucagon levels

After 54 days of treatments, all of the mice were fasted for 4 hr, with water provided ad libitum, followed by collection of whole blood from the orbital cavity for determination of glucagon (R&D, Minneapolis, MN, USA), tumor necrosis factor (TNF- $\alpha$ ), and interleukin-1 $\beta$  (IL-1 $\beta$ ) (R&D, Minneapolis, MN, USA).

### 2.5 | Immunofluorescent staining of insulin and glucagon, and immunohistochemical staining of mucin-2 and occludin

After 55 days of treatments, all of the mice were fasted (as described above) and were sacrificed via cervical dislocation. Subsequently, the pancreas and ileum were isolated, separately fixed in Bouin and paraformaldehyde solution, and then, both were embedded in paraffin. Serial 5- $\mu\text{m}$  sections of each collected pancreas were prepared for staining of insulin and glucagon ( $n = 6$ ), and sections of ileum were stained for mucin-2 and occludin ( $n = 4$ ) (Li et al., 2019; Li et al., 2017).

## 2.6 | Gut microbial sequencing and fecal metabolomic analysis

After 44 days of treatments, feces were collected and subjected to gut microbial sequencing and metabolomic analysis, which were performed and analyzed as previously described (Li et al., 2019).

## 2.7 | Statistical analysis

Data are expressed as the mean  $\pm$  standard error of the mean (SEM) and were analyzed using either two-tailed Student's *t* tests or one-way analyses of variance (ANOVAs) with Bonferroni corrections via GraphPad Prism 8.01 (GraphPad Software, San Diego, USA). The OGTT and ITT data were analyzed with two-way ANOVAs with Bonferroni corrections. A  $p < .05$  was considered to be statistically significant.

## 3 | RESULTS

### 3.1 | Effects of ZDJS on glycaemic control and insulin sensitivity

In comparison with those of the Con group, repeated treatments with ZDJS (1 g/kg) had no significant influences on FBG or NFBG (Figure 1a and b), but it decreased FBG by 6.71% ( $p > .05$ ), 13.83% ( $p < .05$ ), 8.52% ( $p > .05$ ), and 11.60% ( $p > .05$ ) after 10, 22, 30, and 37 days of treatment, respectively. These values were calculated using the average blood glucose levels. In addition, repeated treatments with ZDJS reduced the HbA1c level by 0.12% but the

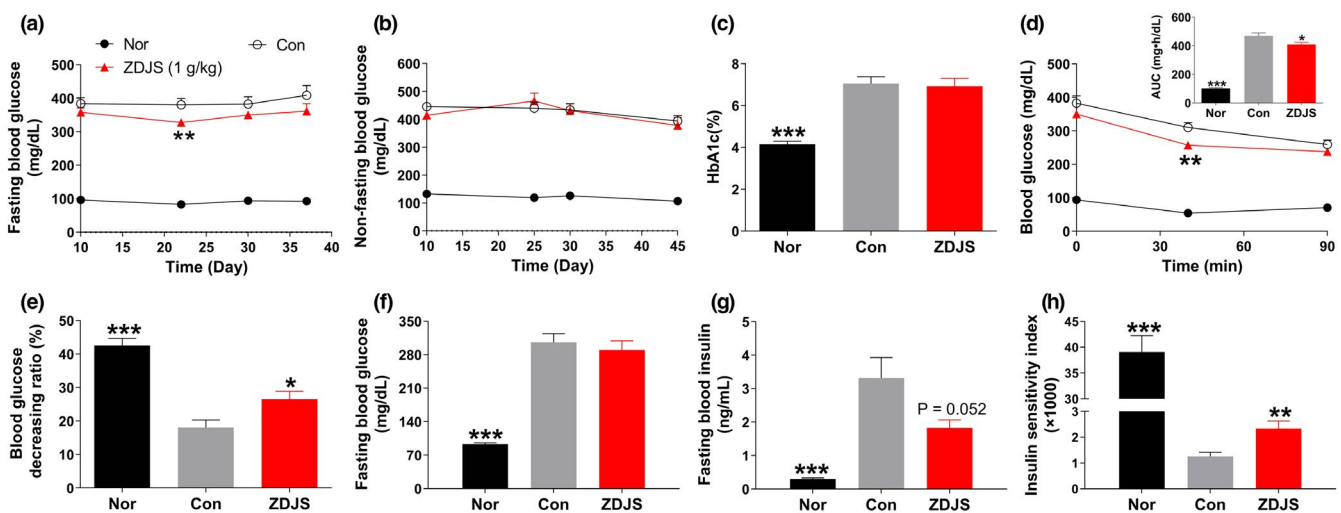
difference was not statistically significant (Figure 1c). Repeated treatments with ZDJS for 30 days significantly decreased the AUC and blood glucose decreasing ratio after 40 min of insulin injection (vs. the blood glucose level at 0 min) in the ITT (Figure 1d and e). Furthermore, repeated treatments with ZDJS for 42 days did not significantly alter FBI levels ( $p = .052$ , Figure 1g) but did elevate the ISI ( $p < .01$ , Figure 1h).

### 3.2 | Effects of ZDJS on $\beta$ -cell function and the $\alpha$ - and $\beta$ -cell ratio in islets

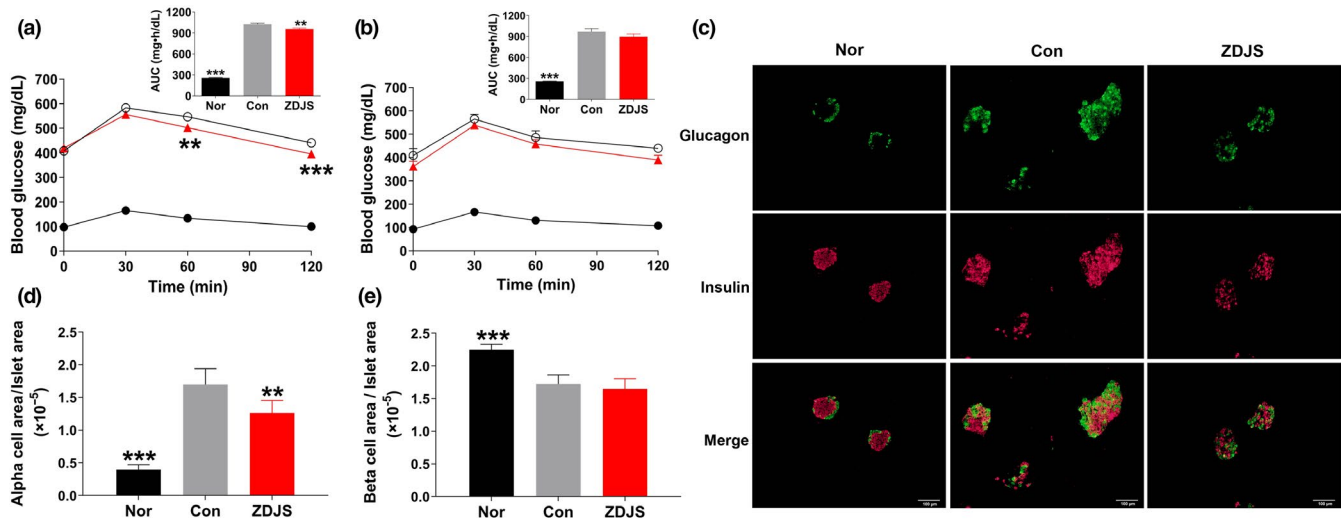
In comparison with those of the Con group, repeated treatments with ZDJS (1 g/kg) decreased blood glucose levels and AUC following oral glucose loading after treatment for 16 days ( $p < .05$ , Figure 2a), but this was attenuated after 37 days of treatment (Figure 2b). In addition, immunofluorescent staining showed that repeated treatments with ZDJS ameliorated the compensatory increase of islets in the Con group (Figure 2c) and reduced the ratio of the  $\alpha$ -cell area to total islet area ( $p < .01$ , Figure 2d), which was consistent with the reduction of fasting blood glucagon ( $31.7 \pm 2.9$  for ZDJS vs.  $65.0 \pm 11.3$  for Con), but did not influence the distributions of  $\alpha$  cells or  $\beta$  cells (Figure 2c) or the ratio of the  $\beta$ -cell area to total islet area (Figure 2e).

### 3.3 | Effects of ZDJS on intestinal mucin-2 expression and serum TNF- $\alpha$ and IL-1 $\beta$ levels

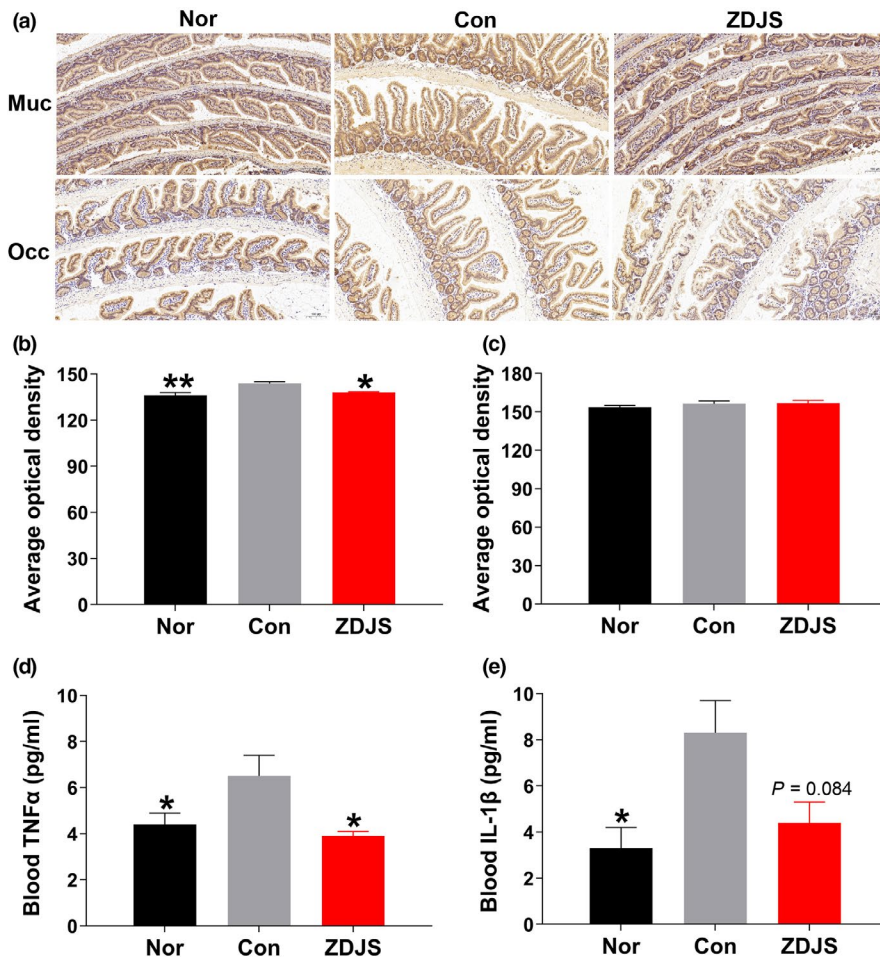
In comparison with those of Nor, intestinal mucin-2 expression and serum TNF- $\alpha$  and IL-1 $\beta$  levels were significantly increased in diabetic db/db mice (Con), and repeated treatments with ZDJS



**FIGURE 1** Effects of ZDJS on glycaemia and insulin sensitivity in diabetic db/db mice. (a) Fasting blood glucose, (b) nonfasting blood glucose, and (c) HbA1c are shown. (d) Insulin tolerance test was performed after treatments for 30 days, and the blood glucose variation and area under the blood glucose–time curve (AUC) are shown. (e) The decreasing ratio of blood glucose at 40 min after subcutaneous injection of insulin is shown. (f–i) Fasting blood glucose (f), fasting blood insulin (g), and the insulin sensitivity index (h) after treatment for 42 days are shown. Data are expressed as the mean  $\pm$  SEM ( $n = 9$ –12,  $***p < .001$ ,  $**p < .01$  vs. Con). Nor denotes the healthy control mice; Con denotes diabetic mice; and ZDJS denotes Zhengda-Jingshan-treated mice



**FIGURE 2** Effects of ZDJS on  $\beta$ -cell function and the  $\alpha$ - and  $\beta$ -cell ratio in islets. (a–b) The oral glucose tolerance test was performed after treatments for 16 days (a) and 37 days (b), and the blood glucose variation and area under the blood glucose–time curve (AUC) are shown. (c) Immunofluorescent staining of glucagon and insulin is shown. Images were obtained at 200× magnification. (d–e) Analysis of the ratios of the  $\alpha$ -cell area to total islet area (d) and the  $\beta$ -cell area to total islet area (e). Data are expressed as the mean  $\pm$  SEM ( $n = 10$ –12 for a and b,  $n = 6$  for c–e). \*\*\* $p < .001$ , \*\* $p < .01$  vs. Con). Nor denotes the healthy control mice; Con denotes diabetic mice; and ZDJS denotes Zhengda-Jingshan-treated mice



**FIGURE 3** Effects of ZDJS on intestinal mucin-2 expression and serum TNF- $\alpha$  and IL-1 $\beta$  levels (a) Immunohistochemical staining of mucin-2 and occludin is shown. Images were obtained at 200× magnification. (b–c) Average optical-density analysis of mucin-2 (b) and occludin (c) is shown. (d) Blood TNF- $\alpha$  is shown. (e) Blood IL-1 $\beta$  is shown. Data are expressed as the mean  $\pm$  SEM ( $n = 4$  for a–c, 8–12 for d and  $n = 6$ –10 for e). \*\* $p < .01$ , \* $p < .05$  vs. Con). Nor denotes the healthy control mice; Con denotes diabetic mice; and ZDJS denotes Zhengda-Jingshan-treated mice



remarkably decreased the expression of mucin-2 (Figure 3a and b) and lowered serum TNF- $\alpha$  ( $p < .05$ ), but they had no significant influence on IL-1 $\beta$  ( $p = .064$ ) levels (Figure 3d and e). There were no differences in the expression levels of occludin among the three groups (Figure 3a and c).

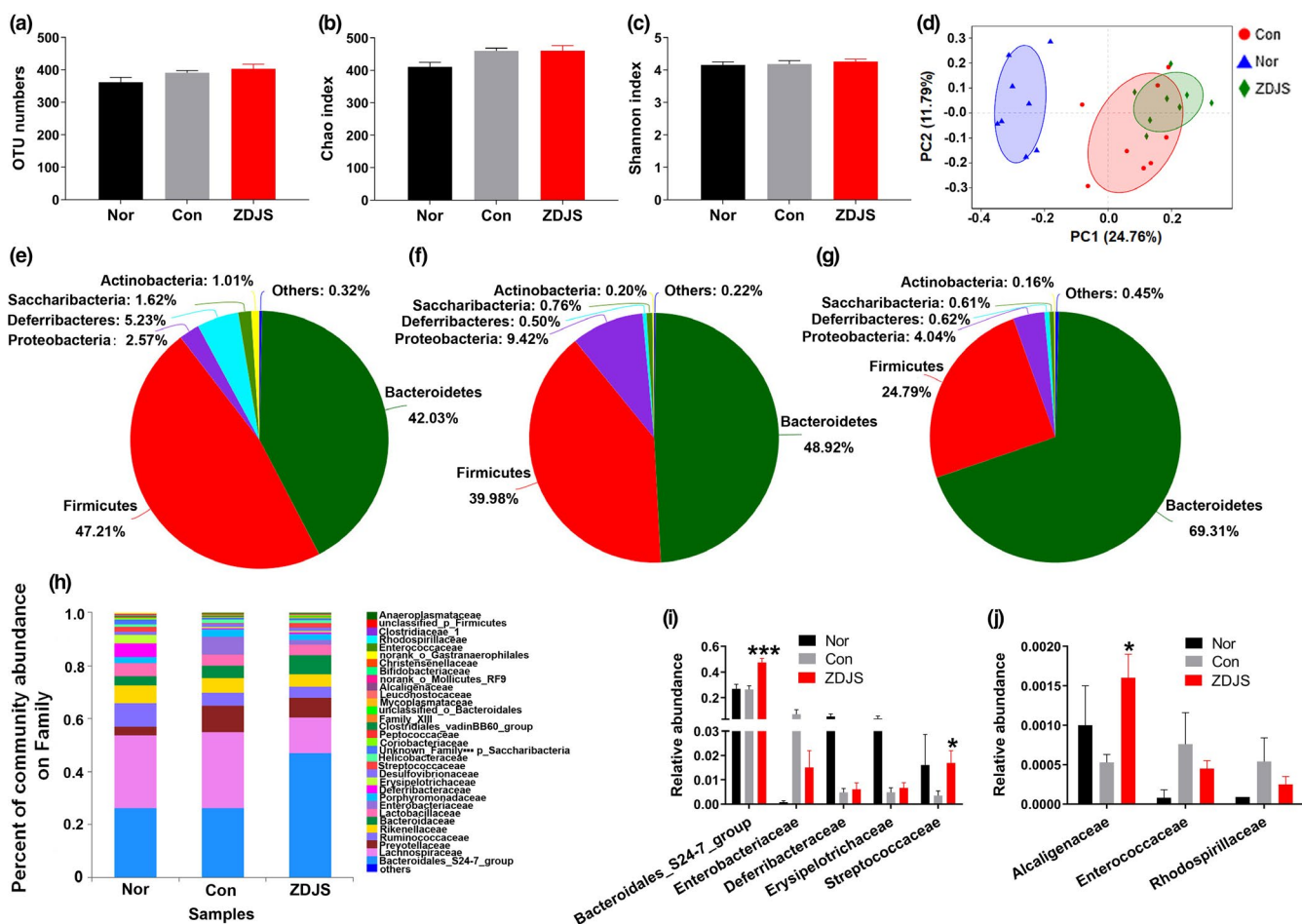
### 3.4 | Effects of ZDJS on gut microbiota profiles

Illumina sequencing produced a total of 704,904 sequences from 24 samples after normalization based on the minimum sample-sequence number, and these sequences were then clustered into 665 operational taxonomic units (OTUs, Table S2). Based on the analysis of OTUs, the OTU numbers and the Chao index or Shannon index were not significantly different among the three groups (Figure 4a–c). However, principal coordinates analysis (PCoA) based on the Bray–Curtis showed significant variation between the Nor and Con groups, whereas ZDJS diverged from the Con group along the first principal coordinate (PC1) and the second principal coordinate (PC2) (Figure 4d).

In comparison with that of Nor, diabetic db/db mice displayed disparate community abundance, but all groups possessed dominant bacterial phyla in terms of Firmicutes and Bacteroidetes (Figure 4e–g). In comparison with the results of the Con group, the ZDJS-treated group exhibited a decreased ratio of Firmicutes to Bacteroidetes and a reduced abundance of Proteobacteria (Figure 4f and g). In addition, the ZDJS-treated group also exhibited a different bacterial community from that of the Con group at the family level (Figure 4h) and also had a significantly increased relative abundance of *Bacteroidales\_S24-7\_group* ( $p < .001$ ), *Streptococcaceae* ( $p < .05$ ), and *Alcaligenaceae* ( $p < .05$ ) (Figure 4i and j).

### 3.5 | Effects of ZDJS on the fecal metabolome

Principal component analysis (PCA) revealed that there were no outlier data from any samples and comparisons between the Nor and Con groups (Figure 5a and b), but one outlier was found following a comparison of the Con group and ZDJS group (Figure 5c). In addition, orthogonal partial least-squares discriminant analysis



**FIGURE 4** Effects of ZDJS on gut microbiota profiles. (a) Total OTU numbers, (b) Chao index, (c) Shannon index, and (d) PCoA based on Bray–Curtis are shown. e–g) Pie charts of the relative abundances of bacteria at the phylum level in the Nor (e), Con (f), and ZDJS (g) groups are shown. (h) Column graphs of the relative abundances of bacteria at the family level are shown. (i–j) The relative abundances of specific bacteria at the family level are shown. Data for a–c are expressed as the mean  $\pm$  SEM ( $n = 8$ ). Nor denotes the healthy control mice; Con denotes diabetic mice; and ZDJS denotes Zhengda–Jingshan-treated mice

(OPLS-DA) was verified by the permutation test, which showed that it was not random or overfitted in the comparisons of Nor versus Con groups or the Con versus ZDJS groups (Figure 5d and e). The OPLS-DA results revealed that there were significant differences in the comparisons of Nor versus Con groups or the Con versus ZDJS groups (Figure 5f and g).

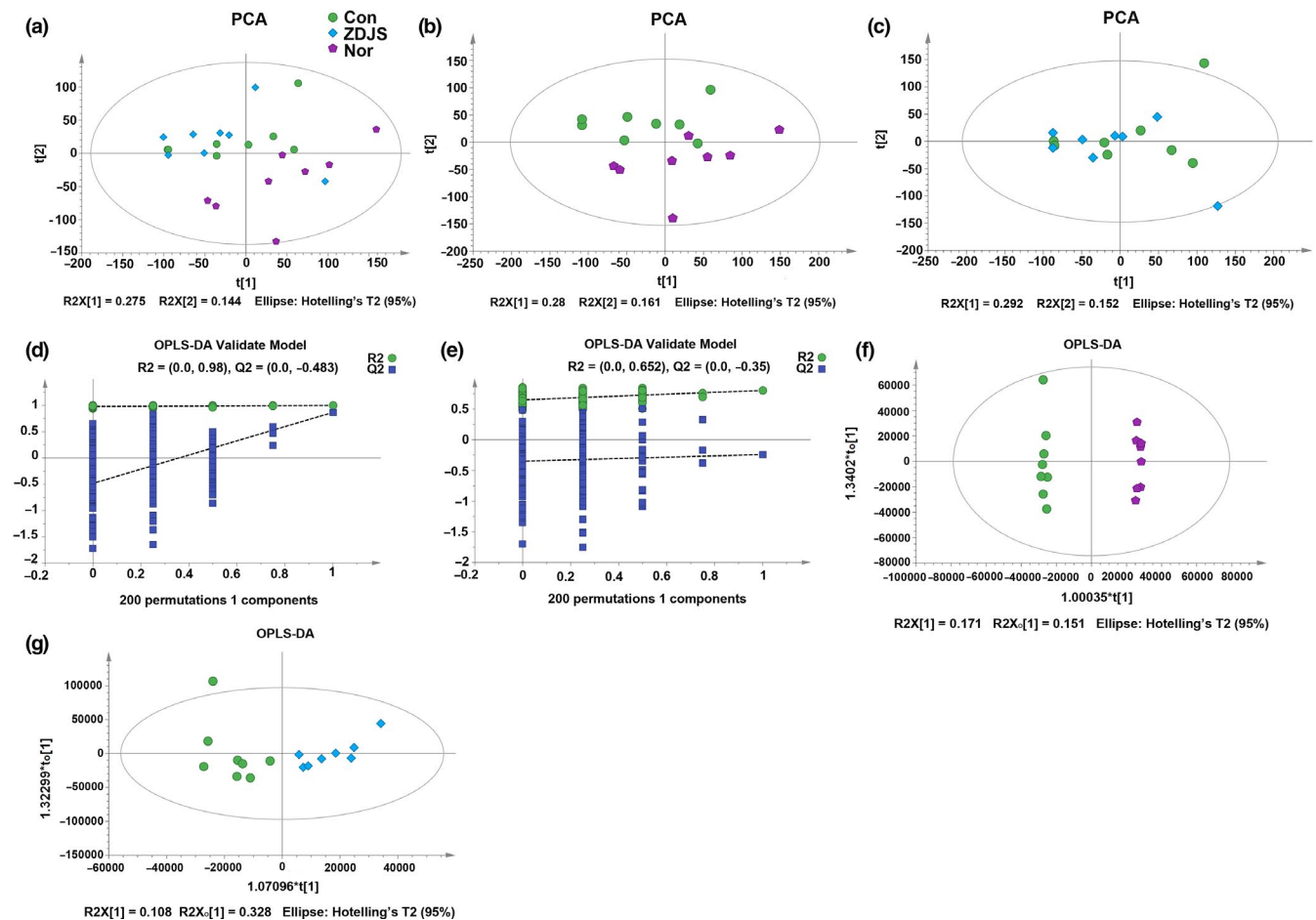
A total of 60 differential metabolites were screened between the Con group and the ZDJS-treated group, and ten of the differential metabolites—including 16-hydroxyestrone, 3-hydroxyanthranilic acid, 4 $\alpha$ -carboxy-4 $\beta$ -methyl-zymosterol, 5 $\alpha$ -pregnane-3,20-dione, 7-dehydrocholesterol, chenodeoxycholic acid glycine conjugate, D-maltose, eicosadienoic acid, eugenol, and N-acetylneuraminic acid—were classified into 21 pathways (Table S3, Table 1). In comparison with those of the Con group, repeated treatments with ZDJS significantly increased the metabolites, namely 4 $\alpha$ -carboxy-4 $\beta$ -methyl-zymosterol, eugenol, 7-dehydrocholesterol, chenodeoxycholic acid glycine, D-maltose, and 16 $\alpha$ -hydroxyestrone, and decreased the metabolites, namely 5 $\alpha$ -pregnane-3,20-dione, eicosadienoic acid, N-acetylneuraminic acid, and 3-hydroxyanthranilic acid (Figure 6a).

### 3.6 | Network analysis

Correlations among the ten differential metabolites (Figure 6a) and the eight selected bacterial families were analyzed (Figure 4i and j). In the comparison of the Con versus ZDJS groups, the bacterial *Bacteroidales\_S24-7\_group* was positively correlated with the metabolites, namely 4 $\alpha$ -carboxy-4 $\beta$ -methyl-zymosterol, eugenol, and 7-dehydrocholesterol, and was also negatively correlated with the metabolites, namely 5 $\alpha$ -pregnane-3,20-dione and eicosadienoic acid; the bacterial *Streptococcaceae* was positively correlated with the metabolites, namely chenodeoxycholic acid glycine and 7-dehydrocholesterol; the bacterial *Alcaligenaceae* was positively correlated with D-maltose and was negatively correlated with eicosadienoic acid (Figure 6b and c).

## 4 | DISCUSSION

Intake of dietary fiber is efficacious in reducing the risk of T2DM and in improving insulin resistance (Weickert & Pfeiffer, 2018),



**FIGURE 5** Metabolomic characteristics in feces. (a) PCA from all samples is shown. (b–c) PCA for the comparisons of the Nor versus Con groups (b) and for those of the Con versus ZDJS groups (c) are shown. (d–e) Permutation tests for the comparisons of the Nor versus Con groups (d) and the Con versus ZDJS groups (e) are shown. (f–g) OPLS-DA for the comparisons of the Nor versus Con groups (f) and Con versus ZDJS groups (g) are shown ( $n = 8$ ). Nor denotes the healthy control mice; Con denotes diabetic mice; and ZDJS denotes Zhengdajingshan-treated mice

**TABLE 1** Ten differential metabolites in the comparison of Con versus ZDJS

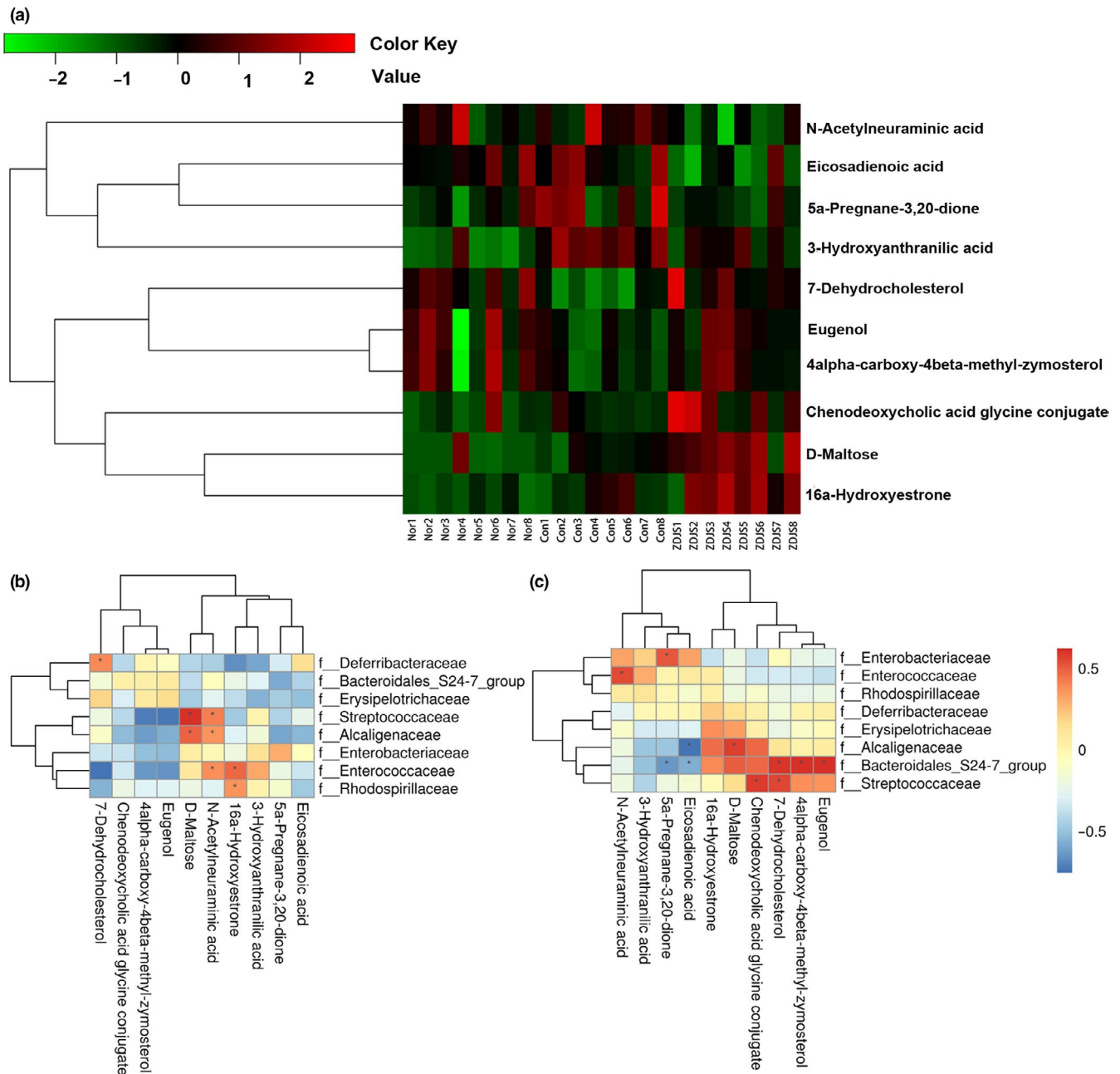
KEGG/HMDB/LMID	Metabolites	Class	Pathway
C05300 HMDB00335	16 $\alpha$ -Hydroxyestrone	Steroids and steroid derivatives	Steroid hormone biosynthesis
C00632 HMDB01476	3-Hydroxyanthranilic acid	Benzene and substituted derivatives	Aminobenzoate degradation Tryptophan metabolism
C15808 LMST01010150	4 $\alpha$ -carboxy-4 $\beta$ -methyl-zymosterol	Sterol lipids	Biosynthesis of antibiotics Steroid biosynthesis
C03681 HMDB03759	5 $\alpha$ -Pregnane-3,20-dione	Steroids and steroid derivatives	Steroid hormone biosynthesis
C01164 C05443 HMDB00032	7-Dehydrocholesterol	Steroids and steroid derivatives	Rheumatoid arthritis Steroid biosynthesis Vitamin digestion and absorption
C05466 HMDB00637	Chenodeoxycholic acid glycine conjugate	Steroids and steroid derivatives	Bile secretion Cholesterol metabolism Primary bile acid biosynthesis Secondary bile acid biosynthesis
C00208 HMDB00163	D-Maltose	Organooxygen compounds	ABC transporters Bacterial chemotaxis Carbohydrate digestion and absorption Phosphotransferase system (PTS) Starch and sucrose metabolism Taste transduction
C16525 HMDB05060	Eicosadienoic acid	Fatty acyls	Biosynthesis of unsaturated fatty acids
C10453 HMDB05809	Eugenol	Phenols	Phenylpropanoid biosynthesis
C00270 HMDB00230	N-Acetylneuraminic acid	Organooxygen compounds	Amino sugar and nucleotide sugar metabolism

especially when taken in the form of insoluble cereal fibers that are nonviscous and fermented by gut microbiota in the colon; in contrast, soluble and viscous fibers from fruit and certain vegetables are not as efficacious (Davison & Temple, 2018; Weickert & Pfeiffer, 2018). Thus, a dietary regimen along with increased physical activity is considered to be beneficial for T2DM treatment (Russell et al., 2016). However, patients with T2DM should always consult physicians about dietary supplementations if they are already taking drug treatments, because one previous report showed a high possible risk of interactions between dietary supplementation and antidiabetic drugs (Zabłocka-Słowińska, Dzielska, Gryszkin, & Grajeta, 2014). ZDJS is a type of ready-to-eat cereal formula powder comprising various cereal ingredients, as well as dietary fiber, multivitamins, and fine protein. Our present study showed that ZDJS significantly increased insulin sensitivity and decreased inflammation in diabetic db/db mice.

Islet  $\beta$ -cell function is a crucial determinant for the development of overt hyperglycemia, and it adapts to metabolic burden in the face of insulin resistance through increasing insulin secretion to maintain euglycemia (Boland, Rhodes, & Grimsby, 2017). However, sustained insulin demand eventually results in  $\beta$ -cell loss and dysfunction, which leads to the progression of T2DM. Thus, ameliorating the burden of insulin secretion represents a means of protecting  $\beta$ -cell function. Our present study found that ZDJS treatment significantly

relieved the compensatory hypertrophy of islets and decreased the ratio of  $\alpha$ -cells to total islets, whereas it had no influence on the ratio of  $\beta$  cells to total islets. In addition, the OGTT is a commonly used method for evaluating  $\beta$ -cell function. In our present study, although ZDJS treatment improved impaired oral glucose tolerance, this effect was attenuated over time. In contrast, ZDJS significantly increased insulin sensitivity, which suggested that ZDJS treatment ameliorated the compensatory hypertrophy of islets through improving insulin sensitivity.

T2DM is also closely associated with chronic inflammation, which contributes to insulin resistance (Lontchi-Yimagou, Sobngwi, Matsha, & Kengne, 2013), and many studies have shown that intake of dietary fiber significantly alleviates inflammation (Awika, Rose, & Simsek, 2018; Vieira et al., 2017; Zhang et al., 2016). Our present study showed that ZDJS decreased the inflammatory cytokine TNF- $\alpha$  in diabetic db/db mice. Furthermore, mucin-2 is a large glycoprotein secreted from goblet cells, which play a crucial role in maintaining intestinal balance (Kim & Ho, 2010). One report indicated that the expression of mucin-2 was increased under a chronic inflammatory state and that high expression of mucin-2 induced endoplasmic reticulum stress (ERS) and apoptosis of goblet cells (Mejías-Luque et al., 2010; Tawiah et al., 2018). Our present study demonstrated that ZDJS decreased the diabetic-induced increased expression of mucin-2 in the ileum of diabetic db/db mice,



**FIGURE 6** Differential metabolites and correlation analysis of gut microbiota. (a) Heat maps of the ten differential metabolites between diabetic db/db mice treated with ZDJS and vehicle are shown. (b–c) Correlation analysis for the comparisons of Nor versus Con groups (b) and Con versus ZDJS groups (c) ( $n = 8$ ). Nor denotes the healthy control mice; Con denotes diabetic mice; and ZDJS denotes Zhengda-Jingshan-treated mice

which was consistent with the amelioration of inflammation and suggested relief of ERS. However, a previous report showed that dietary pea fiber supplementation significantly increased the gene expression of mucin-2 in high-fat diet-induced glucose-intolerant rats (Hashemi, Fohse, Im, Chan, & Willing, 2017), which may have been caused by the different glycometabolic and inflammatory states between animal models.

Gut microbiota are a complex community of more than 1,000 species and have been demonstrated to be indispensable for the maintenance of host health (Sommer & Bäckhed, 2013). Compositional

abnormalities of gut microbiota have been shown to be strongly associated with metabolic disorders (Cox, West, & Cripps, 2015; Qin et al., 2012), and modulation of microbiota may mitigate or even reverse such disorders (Marchesi et al., 2016). A systematic review showed that increasing cereal fiber intake should be encouraged for promoting gut microbiota diversity and overall health (Houghton et al., 2018; Jefferson & Adolphus, 2019). Although the relationship between the ratio of Firmicutes to Bacteroidetes and obesity has remained controversial (Collado, Isolauri, Laitinen, & Salminen, 2008; Koliada et al., 2017), one report showed that the ratio of Firmicutes



to Bacteroidetes was higher in obese men than in obese women and was inversely correlated with peripheral insulin sensitivity in men, but not in women (Most et al., 2017). In our present study, we did not find any change in the ratio of Firmicutes to Bacteroidetes in diabetic obese db/db mice compared to that in nondiabetic lean control mice, but ZDJS decreased this ratio compared to that in untreated db/db mice, which may explain the concomitantly improved insulin sensitivity.

The phylum Proteobacteria is less abundant than other phyla, such as Firmicutes and Bacteroidetes. Some reports have shown that intake of artificial sweeteners and emulsifiers that impair glucose metabolism simultaneously increases the abundance of Proteobacteria (Chassaing et al., 2015; Suez et al., 2014), suggesting a correlation between glucose metabolism and Proteobacteria. Furthermore, a bloom of Proteobacteria is considered to be a microbial signature of gut dysbiosis, which has been observed in inflammatory diseases, such as inflammatory bowel disease, colorectal cancer, and necrotizing enterocolitis (Morgan et al., 2012; Normann, Fahlén, Engstrand, & Lilja, 2013; Wang et al., 2012), as well as in diseases with low-level inflammation (such as irritable syndrome and metabolic syndrome) (Carroll, Ringel-Kulka, Siddle, & Ringel, 2012; Fei & Zhao, 2013). Thus, an increased abundance of Proteobacteria is proposed to represent a potential diagnostic indicator of disease (Shin, Whon, & Bae, 2015). In the present study, an increase of Proteobacteria was found in diabetic db/db mice in comparison with that in nondiabetic control mice but was significantly decreased via ZDJS, which may have contributed to the ZDJS-induced amelioration of inflammation and insulin sensitivity.

The gut microbial profile is closely associated with the metabolome. In the present study, we also determined the variation of the fecal metabolome and found ten differential metabolites between the Con and ZDJS groups. Two of these differential metabolites, eugenol and eicosadienoic acid, are both natural compounds, and eugenol possesses significant anti-inflammatory and antioxidant properties, whereas eicosadienoic acid has been reported to modulate the metabolism of polyunsaturated fatty acids and alter the responses of macrophages to inflammatory stimulation (Barboza, da Silva Maia Bezerra Filho, Silva, Medeiros, & de Sousa, 2018; Huang, Huang, Li, & Chuang, 2011). Another of these metabolites, N-acetylneuraminic acid, has been reported to exacerbate inflammation through promoting the outgrowth of *Escherichia coli* during inflammation (Huang, Chassard, Hausmann, von Itzstein, & Hennet, 2015). In addition, another one of these metabolites, chenodeoxycholic acid glycine, is the physiological ligand for the farnesoid X receptor and has been approved for the treatment of patients with cerebrotendinous xanthomatosis, who develop abnormal lipid storage with increased plasma and tissue levels of cholesterol (Fiorucci & Distrutti, 2019). The differential metabolite, 3-hydroxyanthranilic acid, is a metabolite of kynurenine and has been reported to be positively correlated to impaired glucose tolerance in patients with obesity and is significantly decreased following bariatric surgery (Christensen et al., 2018). All of these metabolites provide a basis for ZDJS-mediated amelioration of inflammation and improvement

of glucose metabolism in diabetic db/db mice. However, there have been no reports regarding the influences of the differential metabolites, 16 $\alpha$ -hydroxyestrone and 5 $\alpha$ -pregnane-3,20-dione, which are metabolites of estradiol and progesterone, respectively, as well as another three differential metabolites, 4 $\alpha$ -carboxy-4 $\beta$ -methyl-zymosterol, 7-dehydrocholesterol, and D-maltose, on glycolipid metabolism.

In addition, we found a significant correlation between the differential metabolites and species of bacteria at the family level, suggesting that ZDJS may change the fecal metabolome through modulating the gut microbial profile and subsequently improving glucose metabolism and inflammatory status in diabetic db/db mice.

In summary, intake of the cereal formula powder, ZDJS, improved insulin sensitivity, ameliorated the compensatory increase of islets, and relieved inflammation in diabetic mice. In addition, ZDJS reshaped the gut microbiota profile through decreasing the ratio of the Firmicutes to Bacteroidetes phyla and the abundance of phylum Proteobacteria, as well by altering the fecal metabolome, which may shed light on mechanisms for improving glucose metabolism and ameliorating inflammation. Taken together, our findings suggest that ZDJS may represent a complementary therapy for patients with T2DM.

## 5 | INFORMED CONSENT

The written informed consent was obtained from all study participants.

## ACKNOWLEDGMENTS

This work was supported by the CAMS Initiative for Innovative Medicine (CAMS-I2M) (No. 2016-I2M-2-006) and the Drug Innovation Major Project (No. 2018ZX09711001-003-009). We thank Majorbio for analyzing the fecal microbiota data of the metabolome and LetPub ([www.letpub.com](http://www.letpub.com)) for providing linguistic assistance during the preparation of this manuscript.

## CONFLICTS OF INTEREST

All of the authors declare that there are no conflicts of interest.

## AUTHOR CONTRIBUTIONS

C.L. designed the study, performed the experiments, analyzed the data, and prepared the manuscript. X. W. and S. S. participated in the insulin tolerance test and oral glucose tolerance test. The other authors took part in measuring biochemical indices or participated in the immunofluorescent and histochemical staining. Z. S. also supplied academic and technical support. All of the authors took part in collecting tissue samples at the end of the experiment.

## ETHICAL APPROVAL

All of the protocols were performed in accordance with the "3R" principles and guidelines for laboratory animals (GB14925-2001 and MOST 2006a) established by the People's Republic of China and were

approved by the Experimental Animal Welfare Ethics Committee of the Institute of Materia Medica (Chinese Academy of Medical Sciences and Peking Union Medical College) under No. 00000814.

## ORCID

Zhufang Shen  <https://orcid.org/0000-0003-0690-015X>

## REFERENCES

- Awika, J. M., Rose, D. J., & Simsek, S. (2018). Complementary effects of cereal and pulse polyphenols and dietary fiber on chronic inflammation and gut health. *Food & Function*, 9, 1389–1409. <https://doi.org/10.1039/C7FO02011B>
- Barboza, J. N., da Silva Maia Bezerra Filho, C., Silva, R. O., Medeiros, J. V. R., & de Sousa, D. P. (2018). An overview on the anti-inflammatory potential and antioxidant profile of Eugenol. *Oxidative Medicine and Cellular Longevity*, 2018, 3957262–3957271. <https://doi.org/10.1155/2018/3957262>
- Bodinham, C. L., Smith, L., Wright, J., Frost, G. S., & Robertson, M. D. (2012). Dietary fibre improves first-phase insulin secretion in overweight individuals. *PLoS One*, 7, 40834–40838. <https://doi.org/10.1371/journal.pone.0040834>
- Boland, B. B., Rhodes, C. J., & Grimsby, J. S. (2017). The dynamic plasticity of insulin production in  $\beta$ -cells. *Molecular Metabolism*, 6, 958–973. <https://doi.org/10.1016/j.molmet.2017.04.010>
- Candela, M., Biagi, E., Soverini, M., Consolandi, C., Quercia, S., Severgnini, M., ... Brigidì, P. (2016). Modulation of gut microbiota dysbioses in type 2 diabetic patients by macrobiotic Ma-Pi 2 diet. *British Journal of Nutrition*, 116, 80–93. <https://doi.org/10.1017/S0007114516001045>
- Carroll, I. M., Ringel-Kulka, T., Siddle, J. P., & Ringel, Y. (2012). Alterations in composition and diversity of the intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroenterology and Motility*, 24(6), 521–e248. <https://doi.org/10.1111/j.1365-2982.2012.01891.x>
- Chassaing, B., Koren, O., Goodrich, J. K., Poole, A. C., Srinivasan, S., Ley, R. E., & Gewirtz, A. T. (2015). Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature*, 519, 92–96. <https://doi.org/10.1038/nature14232>
- Christensen, M. H. E., Fadnes, D. J., Røst, T. H., Pedersen, E. R., Andersen, J. R., Våge, V., ... Mellgren, G. (2018). Inflammatory markers, the tryptophan-kynurenine pathway, and vitamin B status after bariatric surgery. *PLoS One*, 13, e0192169–e0192183. <https://doi.org/10.1371/journal.pone.0192169>
- Collado, M. C., Isolauri, E., Laitinen, K., & Salminen, S. (2008). Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *American Journal of Clinical Nutrition*, 88, 894–899. <https://doi.org/10.1093/ajcn/88.4.894>
- Cox, A. J., West, N. P., & Cripps, A. W. (2015). Obesity, inflammation, and the gut microbiota. *Lancet Diabetes Endocrinol*, 3, 207–215. [https://doi.org/10.1016/S2213-8587\(14\)70134-2](https://doi.org/10.1016/S2213-8587(14)70134-2)
- Davison, K. M., & Temple, N. J. (2018). Cereal fiber, fruit fiber, and type 2 diabetes: Explaining the paradox. *Journal of Diabetes and Its Complications*, 32, 240–245. <https://doi.org/10.1016/j.jdiacomp.2017.11.002>
- Desai, M. S., Seekatz, A. M., Koropatkin, N. M., Kamada, N., Hickey, C. A., Wolter, M., ... Martens, E. C. (2016). A Dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell*, 167, 1339–1353.e21. <https://doi.org/10.1016/j.cell.2016.10.043>
- Fei, N., & Zhao, L. (2013). An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. *ISME Journal*, 7, 880–884. <https://doi.org/10.1038/ismej.2012.153>
- Fiorucci, S., & Distrutti, E. (2019). Chenodeoxycholic acid: An update on its therapeutic applications. *Handbook of Experimental Pharmacology*, 256, 265–282.
- Fujii, H., Iwase, M., Ohkuma, T., Ogata-Kaizu, S., Ide, H., Kikuchi, Y., ... Kitazono, T. (2013). Impact of dietary fiber intake on glycemic control, cardiovascular risk factors and chronic kidney disease in Japanese patients with type 2 diabetes mellitus: The Fukuoka Diabetes Registry. *Nutrition Journal*, 12, 159–166. <https://doi.org/10.1186/1475-2891-12-159>
- Hashemi, Z., Fohse, J., Im, H. S., Chan, C. B., & Willing, B. P. (2017). Dietary pea fiber supplementation improves glycemia and induces changes in the composition of gut microbiota, serum short chain fatty acid profile and expression of mucins in glucose intolerant rats. *Nutrients*, 9, 1236–1249. <https://doi.org/10.3390/nu9111236>
- Holscher, H. D. (2017). Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes*, 8, 172–184. <https://doi.org/10.1080/19490976.2017.1290756>
- Houghton, D., Hardy, T., Stewart, C., Errington, L., Day, C. P., Trenell, M. I., & Avery, L. (2018). Systematic review assessing the effectiveness of dietary intervention on gut microbiota in adults with type 2 diabetes. *Diabetologia*, 61, 1700–1711. <https://doi.org/10.1007/s00125-018-4632-0>
- Howlett, J. F., Betteridge, V. A., Champ, M., Craig, S. A., Meheust, A., & Jones, J. M. (2010). The definition of dietary fiber – discussions at the Ninth Vahouny Fiber Symposium: Building scientific agreement. *Food & Nutrition Research*, 54, 5750–5754. <https://doi.org/10.3402/fnr.v54i0.5750>
- Huang, Y. L., Chassard, C., Hausmann, M., von Itzstein, M., & Hennet, T. (2015). Sialic acid catabolism drives intestinal inflammation and microbial dysbiosis in mice. *Nature Communications*, 6, 8141–8151. <https://doi.org/10.1038/ncomms9141>
- Huang, Y. S., Huang, W. C., Li, C. W., & Chuang, L. T. (2011). Eicosadienoic acid differentially modulates production of pro-inflammatory modulators in murine macrophages. *Molecular and Cellular Biochemistry*, 358, 85–94. <https://doi.org/10.1007/s11010-011-0924-0>
- Jefferson, A., & Adolphus, K. (2019). The effects of intact cereal grain fibers, including wheat bran on the gut microbiota composition of healthy adults: A systematic review. *Frontiers in Nutrition*, 6, 33–53. <https://doi.org/10.3389/fnut.2019.00033>
- Kim, Y. S., & Ho, S. B. (2010). Intestinal goblet cells and mucins in health and disease: Recent insights and progress. *Current Gastroenterology Reports*, 12(5), 319–330. <https://doi.org/10.1007/s11894-010-0131-2>
- Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell*, 165, 1332–1345. <https://doi.org/10.1016/j.cell.2016.05.041>
- Koliada, A., Syzenko, G., Moseiko, V., Budovska, L., Puchkov, K., Perederiy, V., ... Vaiserman, A. (2017). Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiology*, 17, 120–125. <https://doi.org/10.1186/s12866-017-1027-1>
- Li, C., Wang, X., Lei, L., Liu, M., Li, R., Sun, S., ... Shen, Z. (2019). Berberine combined with stachyose induces better glycometabolism than berberine alone through modulating gut microbiota and fecal metabolomics in diabetic mice. *Phytotherapy Research*, 34(5), 1166–1174. <https://doi.org/10.1002/ptr.6588>. Epub ahead of print
- Li, C., Yang, M., Hou, G., Liu, S., Huan, Y., Yu, D., ... Shen, Z. (2017). A human glucagon-like peptide-1-albumin recombinant protein with prolonged hypoglycemic effect provides efficient and beneficial control of glucose metabolism in diabetic mice. *Biological & Pharmaceutical Bulletin*, 40, 1399–1408. <https://doi.org/10.1248/bpb.b17-00169>

- Lontchi-Yimagou, E., Sobngwi, E., Matsha, T. E., & Kengne, A. P. (2013). Diabetes mellitus and inflammation. *Current Diabetes Reports*, 13, 435–444. <https://doi.org/10.1007/s11892-013-0375-y>
- Marchesi, J. R., Adams, D. H., Fava, F., Hermes, G. D., Hirschfield, G. M., Hold, G., ... Hart, A. (2016). The gut microbiota and host health: A new clinical frontier. *Gut*, 65, 330–339. <https://doi.org/10.1136/gutjnl-2015-309990>
- Mejías-Luque, R., Lindén, S. K., Garrido, M., Tye, H., Najdovska, M., Jenkins, B. J., ... de Bolós, C. (2010). Inflammation modulates the expression of the intestinal mucins MUC2 and MUC4 in gastric tumors. *Oncogene*, 29, 1753–1762. <https://doi.org/10.1038/onc.2009.467>
- Morgan, X. C., Tickle, T. L., Sokol, H., Gevers, D., Devaney, K. L., Ward, D. V., ... Huttenhower, C. (2012). Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biology*, 13, R79–R96. <https://doi.org/10.1186/gb-2012-13-9-r79>
- Most, J., Goossens, G. H., Reijnders, D., Canfora, E. E., Penders, J., & Blaak, E. E. (2017). Gut microbiota composition strongly correlates to peripheral insulin sensitivity in obese men but not in women. *Benef Microbes*, 8, 557–562. <https://doi.org/10.3920/BM2016.0189>
- Normann, E., Fahlén, A., Engstrand, L., & Lilja, H. E. (2013). Intestinal microbial profiles in extremely preterm infants with and without necrotizing enterocolitis. *Acta Paediatrica*, 102, 129–136. <https://doi.org/10.1111/apa.12059>
- Peng, J., Huan, Y., Jiang, Q., Sun, S., Jia, C., & Shen, Z. (2014). Effects and potential mechanisms of pioglitazone on lipid metabolism in obese diabetic KKAy mice. *PPAR Research*, 2014, 538183–538196. <https://doi.org/10.1155/2014/538183>
- Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., ... Wang, J. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*, 490, 55–60. <https://doi.org/10.1038/nature11450>
- Russell, W. R., Baka, A., Björck, I., Delzenne, N., Gao, D., Griffiths, H. R., ... Weickert, M. O. (2016). Impact of diet composition on blood glucose regulation. *Critical Reviews in Food Science and Nutrition*, 56, 541–590. <https://doi.org/10.1080/10408398.2013.792772>
- Shin, N. R., Whon, T. W., & Bae, J. W. (2015). Proteobacteria: Microbial signature of dysbiosis in gut microbiota. *Trends in Biotechnology*, 33, 496–503. <https://doi.org/10.1016/j.tibtech.2015.06.011>
- Sommer, F., & Bäckhed, F. (2013). The gut microbiota—masters of host development and physiology. *Nature Reviews Microbiology*, 11, 227–238.
- Suez, J., Korem, T., Zeevi, D., Zilberman-Schapira, G., Thaiss, C. A., Maza, O., ... Elinav, E. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature*, 514, 181–186. <https://doi.org/10.1038/nature13793>
- Tawiah, A., Cornick, S., Moreau, F., Gorman, H., Kumar, M., Tiwari, S., & Chadee, K. (2018). High MUC2 mucin expression and misfolding induce cellular stress, reactive oxygen production, and apoptosis in goblet cells. *American Journal of Pathology*, 188, 1354–1373. <https://doi.org/10.1016/j.ajpath.2018.02.007>
- Vieira, A. T., Galvão, I., Macia, L. M., SernagliaÉ, M., Vinolo, M. A., Garcia, C. C., ... Teixeira, M. M. (2017). Dietary fiber and the short-chain fatty acid acetate promote resolution of neutrophilic inflammation in a model of gout in mice. *Journal of Leukocyte Biology*, 101, 275–284. <https://doi.org/10.1189/jlb.3A1015-453RRR>
- Wang, T., Cai, G., Qiu, Y., Fei, N., Zhang, M., Pang, X., ... Zhao, L. (2012). Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. *ISME Journal*, 6, 320–329. <https://doi.org/10.1038/ismej.2011.109>
- Weickert, M. O., & Pfeiffer, A. F. H. (2018). Impact of dietary fiber consumption on insulin resistance and the prevention of type 2 diabetes. *Journal of Nutrition*, 148, 7–12. <https://doi.org/10.1093/jn/nxx008>
- Wolever, T. M., Campbell, J. E., Geleva, D., & Anderson, G. H. (2004). High-fiber cereal reduces postprandial insulin responses in hyperinsulinemic but not normoinsulinemic subjects. *Diabetes Care*, 27, 1281–1285. <https://doi.org/10.2337/diacare.27.6.1281>
- Zabłocka-Stowińska, K., Dzielska, E., Gryszkin, I., & Grajeta, H. (2014). Dietary supplementation during diabetes therapy and the potential risk of interactions. *Advances in Clinical and Experimental Medicine*, 23, 939–946. <https://doi.org/10.17219/acem/37348>
- Zhang, Z., Shi, L., Pang, W., Liu, W., Li, J., Wang, H., & Shi, G. (2016). Dietary fiber intake regulates intestinal microflora and inhibits ovalbumin-induced allergic airway inflammation in a mouse model. *PLoS One*, 11, e0147778–e047793. <https://doi.org/10.1371/journal.pone.0147778>

## SUPPORTING INFORMATION

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

**How to cite this article:** Li C, Wang X, Sun S, et al. Effects of a ready-to-eat cereal formula powder on glucose metabolism, inflammation, and gut microbiota in diabetic db/db mice. *Food Sci Nutr*. 2020;8:4523–4533. <https://doi.org/10.1002/fsn3.1761>