Diosmetin ameliorates glucose metabolism in KK-Ay diabetic mice through regulation of Corynebacterium glutamicum via IRS/PI3K/AKT signaling pathway

Xiaobao Gong[1], Li Xiong[2], Caihong Bi[3], and Baoshun Zhang[1]

[1]Southwest University
[2]The First Affiliated Hospital of Chongqing Medical University
[3]Service Center for Technology Extension of Linyi Fruit and Tea

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Abstract

Background and Purpose: Diosmetin (Dios), a flavonoid compound with multiple pharmacological activities. However, fewer studies have reported its effects on diabetes. Here, we address the effect of Dios on glucose metabolism and gut microbiota in KK-Ay diabetic mice. Experimental Approach: Wild type C57BL/6J mice or diabetic KK-Ay mice were treated with vehicle or Dios for one month. The liver RNA-Seq was used to reveal the key signaling pathway interfered with Dios. The liver 16S rRNA gene sequencing was used to reveal the effects of Dios on gut microbiota. The experiment of C. glu transplantation was used to reveal the relationship between Dios and C. glu on glucose metabolism. Key Results: Dios treatment significantly decreased blood glucose and increased serum insulin concentrations. Transcriptome sequencing analysis found that the underlining mechanism of diosmetin on T2DM by regulating signal pathways of glucose metabolism, which was proved by up-regulating IRS/PI3K/AKT signaling pathway to promote glycogen synthesis and GLUT4 translocation. Besides, Dios treatment reshaped the unbalanced gut microbiota by suppressing the ratio of Firmicutes/Bacteroidetes and markedly increasing the richness of C. glu. Moreover, Treatment with C. glu and Dios together can markedly ameliorate glucose metabolism by up-regulating IRS/PI3K/AKT signaling pathway to promoting glycogen synthesis and GLUT4 translocation. Conclusions and Implications: Dios treatment remarkably ameliorated glucose metabolism in KK-Ay diabetic mice by the regulation of C. glu on IRS/PI3K/AKT signaling pathway and reshaped the unbalanced gut microbiota. Our study provided evidence for the application of Dios to the treatment of T2DM.

Introduction

Type 2 diabetic mellitus (T2DM), an endocrine and metabolic syndrome with glucose metabolism disorder, has become the third high-risk chronic disease after cardiovascular diseases and tumors (Al-Rubeaan, 2015). Statistical data from the International Diabetes Federation show that 9.3% of adults aged 20-79 years, a staggering 463 million people, are living with diabetes, and the number will jump to a staggering 700 million by 2045 if the situation is uncontrolled (Saito et al., 2019). Moreover, more than half (56%) of people with diabetes remain undiagnosed and are at a higher risk of developing harmful and costly complications, which seriously reduce the quality of people's life (Kattan et al., 2018). Thus, effective strategies to prevent and control T2DM are of utmost importance, which is a major challenge for the medical community.

The pancreas is no longer able to make insulin or the body cannot make good use of the insulin it produces is the main cause of T2DM (Bergeron et al., 2018). In the state of insufficient insulin, the resulting excess of hepatic glucose production contributes to hyperglycemia and also profoundly inhibits lipolysis in adipocytes (Xia et al., 2019). A recent study further suggests that the gut microbiota acts as an "exteriorized organ", which plays an important impact on the onset and development of diabetes or obesity (Wei et al., 2020). For

example, an increased Firmicutes/Bacteroidetes ratio is a commonly used indicator of obese and diabetic (ob/ob) mice (Fugmann et al., 2015). Other studies show that gut microbiota and microbial metabolic activities are known to affect lipid and glucose metabolism and chronic low-grade inflammation in the metabolic syndrome (Jayachandran et al., 2017). There is increasing evidence that the change of those factors plays a vital role in the evolution of insulin resistance. In addition, many treatment drugs for T2DM patients, including metformin (Wu et al., 2017) and gliclazide (van Bommel et al., 2020) have been reported to influence the gut microbiota to ameliorate T2DM. Moreover, a series of treatments, including prebiotics and antibiotics, have been evaluated for the regulation of obesity and T2DM and its related metabolic disorders. For example, antibiotic treatment alters the structure of gut microbiota, relieves metabolic endotoxemia, and improves glucose tolerance in the ob/ob diabetic mice (Lange et al., 2016). Besides, a fermentable dietary fiber (oligofructose) was reported to promote the growth of beneficial bacteria and improve intestinal barrier functions and decrease hepatic inflammation in diabetic mice (Zheng et al., 2018). Those studies indicated that gut microbiota may have important effects on T2DM and its associated metabolic disorders.

Diosmetin (3',5,7-trihydroxy-4'-methoxyflavone, Dios), an aglycone of the natural flavonoid abundantly present in legumes, olive leaves, and citrus plants, possesses anticancer, antimicrobial, antioxidant, and anti-inflammatory properties (Yang et al., 2017). Recently, several reports have shown the anti-diabetic activity of Dios. For example, Jiang et al. (Jiang et al., 2018) found that Dios exhibited the neuroprotective effect in streptozotocin (STZ)-induced diabetic nephropathy (DN) mice, via modulating the Akt/NF-ϰB/iNOS signaling pathway. Moreover, based on its strong anti-oxidant property, Dios attenuates the effects of diabetes in STZ-induced diabetic rats. Until now, there was no study confirming the role of Dios in alleviating abnormal glucose metabolism and gut microbiota on T2DM. Hence, we sought to determine whether Dios could improve the disturbance in glucose metabolism and gut microbiota in KK-Ay diabetic mice. Our results implied that Dios could ameliorate glucose metabolism in KK-Ay diabetic mice by the regulation of *C. glu* on IRS/PI3K/AKT signaling pathway and reshaped the unbalanced gut microbiota, which demonstrated that Dios might act as a potential anti-diabetic agent to relieve T2DM and its related metabolic disorders.

Material and methods

Chemicals and reagents

DIOS (purity 98% by HPLC) is provided by the Chemistry Institute of Pharmaceutical Resource of Southwest University (China). The mouse insulin ELISA kit was purchased from Shanghai enzyme-linked Biotechnology Co., Ltd (ml001827-1). The primary antibodies used for western blot were: IRS (CST#2383, 1:1000), p-IRS (Ser307, CST#2381, 1:1000), PI3K (CST#4257, 1:1000), PI3Kα (CST#4255, 1:1000), AKT (CST#3063, 1:1000), p-AKT (Ser473, CST#4060, 1:1000), GSK-3β (CST#9315, 1:1000), p-GSK-3β (Ser9, CST#5558, 1:1000), GS (CST#3886, 1:1000), AS160 (CST#2670, 1:1000),p-AS160 (Thr642, CST#8881, 1:1000), Glut4 (CST#2213, 1:1000), β-actin (Proteintech 20536-1-AP, 1:5000) and secondary antibody (cat. #SA00001-2, 1:10000) were purchased from Proteintech (Wuhan, China), and total protein extraction kits are from Sangon Biotech Co. Ltd (Shanghai, China).

Animals and Experimental Design

Eight-week-old male C57BL/6J mice and KK-Ay diabetic mice (C57BL/6J background) purchased from Beijing Huafukang Biotechnology Co., Ltd. The animals were maintained at standard environmental conditions temperature 25 °C, humidity 50%, and free access to sterile food and water. After one week of acclimation, the mice were randomly divided into 5 groups with 6 mice in each group. C57BL/6J mice were fed with a standard diet and KK-Ay mice were fed with a high fat diet (HFD) for two weeks, and the blood glucose was measured and the mice with blood glucose[?]11 mmol/L were selected as the diabetic model. The diabetic mice were randomly divided into four groups: (1) Model group, treated with the vehicle; (2) L-Dios group, treated with 20 mg/kg.d Dios; (3) H-Dios group, treated with 60 mg/kg.d Dios; (4) Metformin (Met) group, treated with 60mg/kg.d Met. For the experiment of *Corynebacterium glutamicum(C. glu)*transplantation, the diabetic mice were also randomly divided into four groups: (1) Model group, treated with the vehicle; (2) Dios group, treated with 60 mg/kg.d Dios; (3)*C. glu* group, treated with $5x10^9$CFU/100ul/10g*C. glu*

; (4) Dios+*C. glu* group, treated with 60mg/kg.d Dios and 5x10^{9}CFU/100ul/10g *C. glu* for four weeks. During the treatment period, body weight and blood glucose levels were monitored weekly. On the last day of the experiment, fresh mouse stool samples were directly collected in the ultra-clean workbench and immediately stored at -80degC for subsequent analysis. Tissues including liver and skeletal muscle were carefully harvested and kept in liquid nitrogen and then stored at -80degC until analysis.

Ethics statement

All animal experiments in this study are carried out in accordance with the Guide for the Care and Use of Laboratory Animals, which was formulated by the National Institutes of Health and approved by the Office of Experimental Animal Management Committee of Chongqing, China.

Measurement of serumbiochemical indicators and serum insulin

The serum biochemical indicators (TC, TG, LDL-C, HDL-C) were measured using a BK-800 Fully Automatic Biochemical Analyzer (BIOBASE, China) according to the manufacturer's protocol. The serum samples were collected for serum insulin concentrations detection using a commercial ELISA kit based on the manufacturer's instructions.

Morphometry analysis

Liver and skeletal muscle tissues were fixed in 10% formaldehyde, embedded in paraffin, and cut into 5 μm serial sections for staining with H&E and Oil Red O. Histopathological changes were observed using a fluorescence microscope system (TE2000, Nikon Japan) and representative images were presented.

Transcriptomic analysis

Total RNA was extracted from liver tissue using TRIZOL reagent and purified using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA) to eliminate ribosomal RNA, and then stored at -80°C prior for further analysis by NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations. Sample sets were constructed with six individual samples from C57, KK-Ay and KK-Ay+Dios group (three samples per group). RNA-sequence analysis was conducted by RNA-sequence analysis conducted by Beijing Novogene Genomics Technology Co. Ltd. (Beijing, China). The Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the clusterProfiler R package, in which gene length bias was corrected. GO terms with corrected P value less than 0.05 were considered significantly enriched by differential expressed genes. ClusterProfiler R package was used to test the statistical enrichment of differential expression genes in KEGG pathways.

16S rRNA gene sequencing

Total DNA was extracted from the stool samples using the fecal DNA isolation Kit (DP328, TIANamp Beijing, China) based on the manufacturer's instructions, and its concentration and purity were assessed on 1% agarose gels. The 16S bacterial rRNA genes were amplified with the 515F-806R primers specific for the V4 hypervariable regions (5'-GTGCCAGCMGCCGCGGTAA-3' and 5'-GGACTACHVGGGTWTCTA

AT-3'). All PCR reactions were performed in a 30 μL reaction mix containing 15 μl of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), PCR products were quantified and equal amounts were loaded into a 2% agarose gel for electrophoresis. The target bands were excised and analyzed by paired-end sequencing on the Illumina MiSeq platform in accordance with the manufacturer's instruction, provided by Beijing Novogene Genomics Technology Co. Ltd. (Beijing, China).

Western blot analysis

A total of 200 mg of liver and skeletal muscles were lysed with 1 ml RIPA buffer and followed by centrifugation at 13,000 r/min for 10 min. The supernatants were collected and protein concentrations were measured using 660 nm Protein Assay Reagent kit (ThermoFish scientific, China). A 3 μg/μL of protein extract was supplied with lysis buffer and denatured by boiling at 95 °C for 10 min. The denatured proteins were resolved by 12% and 10% SDS-PAGE and transferred to nitrocellulose membrane. The membranes were blocked with

5% skim milk at room temperature for 2 h, incubated with primary antibodies overnight at 4 °C, and then incubated with horseradish peroxidase-conjugated secondary antibodies for 2 h. The protein bands were visualized and quantitated using the Image Jet software and with β-actin as an internal standard.

Statistical analysis

Results were expressed as mean±S.D. The statistical significance of the differences between groups was determined by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test analysis using the GraphPad Prism6 software. Post hoc tests were conducted only if F was significant, and there was no variance in homogeneity. $p<0.05$ was considered statistically significant.

Results

Dios treatment reduced HFD-induced body weight andblood glucose gain in KK-Ay diabetic mice.

To investigate the effect of Dios on body weight and blood glucose, we conducted the KK-Ay mice that were chronically administrated with Dios for 4 weeks. As shown in Fig. 1A, the administration of Dios significantly reduced the body weight compared to the model group at the end of the feeding period. Meanwhile, after 4 weeks of Dios and Met administration, both Dios and Met group exhibited significantly decreased blood glucose (Fig. 1B). In addition, we found that weight gain (Fig. 1C), adipose index (Fig. 1D, 1F) and liver index (Fig. 1E, 1F) of Dios and Met administration were significantly lower than that of KK-Ay diabetic mice. Those results demonstrated that Dios reduces body weight and glucose in KK-Ay diabetic mice.

Diosameliorated lipid accumulation in KK-Ay diabetic mice.

As shown in Fig. 2A-2E, the serum levels of TC, TG, LDL-C were increased and the level of HDL-C and serum insulin decreased in KK-Ay diabetic mice compared with NC group. Notably, Dios treatment significantly decreased the serum levels of TC, TG, LDL-C and increased the serum level of HDL-C and insulin. To explore the effect of Dios on preventing hepatic steatosis, liver and skeletal muscle histopathological changes by using H&E and oil-red staining (Fig. 2F) were performed. H&E staining of liver sections shows widespread vacuolation in KK-Ay diabetic mice but not in Dios and Met treated group. Moreover, fewer lipid droplets in liver and skeletal muscles were observed in the mice of Dios treatment as compared to the KK-Ay diabetic mice.

Dios reversed sustained transcriptional changes in KK-Ay diabetic mice.

To study the mechanism of Dios on KK-Ay diabetic mice, the liver samples were collected for transcription analysis. We calculated FPKM for all samples and performed PCA analysis (Fig. 3A) and correlation analysis (Fig. 3B), which indicates that our samples have good repeatability. To identify gene sets with a statistically significant difference in hepatocytes, we screened the differentially expressed genes (DEGs) between Control vs Model and Model vs Dios by the following criteria: $|\log FC| > 1$, FDR < 0.05 and p value < 0.05. We found 4182 DEGs between model and control (Fig.3C), of those DEGs, 1967 genes were up-regulated and 2215 genes were down-regulated. Besides, a total of 173 DEGs between Model and Dios were revealed. Among them, 101 genes were up-regulated and 72 genes were down-regulated respectively. We used hierarchical cluster analysis (Fig. 3D-3E) to compare the DEGs in three groups, which shows that the similarity of expression patterns of three biological replicates. To identify the biological features of DEGs, we performed significant GO analysis by the clusterProfiler R package, and the biological processes analysis revealed that the DEGs between Model and Dios group (Fig 3F) were associated with glucose metabolism including fructose metabolic process, carbohydrate metabolic process, carboxylic acid biosynthetic process. Among significant GO terms of down-regulated genes (Fig 3G), it was obvious that most GO terms were associated with organic anion transport, carboxylic acid transport, organic acid transport and lipid transport. To further explore the potential mechanism of DEGs between Model and Dios group, KEGG pathway analysis was performed using the clusterProfiler R package. The results of KEGG analysis revealed that DEGs were mainly associated with glucose metabolism including glycolysis/gluconeogenesis, biosynthesis of amino acids, fructose and mannose metabolism (Fig 3H). Interestingly, we found that pathways involved in glucose metabolism were the most

enriched up-regulated pathways (Fig 3I), which is consistent with the reactome functional enrichment analysis (Fig 3J). Those results revealed that the potential mechanism of Dios ameliorated T2DM may be through regulating glucose metabolism.

Dios treatment ameliorated HFD-induced glucose metabolism disorder inKK-Ay diabetic mice.

To clarify the potential mechanism of Dios in KK-Ay diabetic mice, the glucose metabolism and insulin resistance-related signaling proteins in liver and skeletal muscle were observed by western blot analysis. Our data showed that a continuous HFD diet can lead to severe insulin resistance in the liver (Fig. 4A-4C) and skeletal muscle (Fig. 4D-4F). However, treatment with Dios and Met could significantly ameliorate insulin resistance by activating the IRS/PI3K/AKT signaling pathway. Once AKT is activated, it participates in glucose metabolism related pathways caused by insulin. We found that Dios promoted glycogen synthesis in liver and skeletal muscle by phosphorylating GSK-3β and activating GS. Besides, the level of phosphorylation AS160 and Glut4 were significantly up-regulated in skeletal muscle by Dios treatment, which suggests that Dios promote glucose transport.

Dios treatment reverses HFD-induced gut dysbiosis in KK-Ay diabetic mice.

To further explore the effects of Dios on gut microbiota composition, the 16S bacterial rRNA genes composing the V4 hypervariable regions in the faeces of KK-Ay mice were performed. After removing low-quality sequences, a total of 72292 tags were generated. The remaining clean tags were clustered into OTUs (Operational Taxonomic Units) based on 97% similarity. UniFrac distance-based principal coordinate analysis (PCoA) revealed a relative clustering of gut microbiota within each group (Fig 5A). Remarkably, the microbes in Dios and Met groups were more closely clustered relative to ND groups, which is an indication that Dios treatment induced similar microbial composition changes. Unweighted Pair-group Method with Arithmetic Mean (UPGMA) indicates a statistically significant separation between Model group and H-Dios, Met groups (Fig 5B). Notably, *Firmicutes* and *Bacteroidetes* are the most abundant microbiota at the phylum level. Compared with the CK group, the content of the phylum *Firmicutes* in the Model group significantly increased and the*Bacteroidetes* abundance significantly increased. However, a remarkable reversal of this pattern was observed following Dios and Met treatment (Fig 5C-5F). An increased*Firmicutes/Bacteroidetes* ratio is a commonly used indicator of diabetic mice as reported in a previous study, as shown in Fig 5D, the increased ratio of the phylum *Firmicutes* /*Bacteroidetes*were observed in the diabetic model group. On the contrary, Dios and Met treatment remarkably suppressed the ratio of the phylum *Firmicutes/Bacteroidetes* . At the same time, we interestingly found that the ratio of*Actinobacteria* phylum was significantly higher in the H-Dios group than in the other groups. Next, we further analyzed the relative abundances of the gut microbiota at the species level, as illustrated in Fig 5G-5K, there were several bacteria decreased in the diabetic Model group, which were markedly increased by Dios treatment, including*Aerococcus_viridans* (70 fold), *C._glu* (155 fold), Combined, these data suggested that Dios treatment may effectively reverse gut microbiota dysbiosis in diabetic mice.

C. glu **and Dios treatmentreducedHFD-induced body weight and blood glucose gain in KK-Ay diabetic mice.**

Recent studies have shown that the ability of the gut microbiota to modulate obesity can be transferred to other animals. To explore whether the gut microbiota of Dios-treated animals may improve the condition of KK-Ay diabetic mice, we selected the highest abundance bacterium*(C.glu*) for further study. After treatment with Dios and*C.glu* for 4 weeks, the related indexes were measured. As shown in Fig. 6A-6B, it is noteworthy that the body weight and blood glucose of Dios, *C.glu* and Dios+*C. glu* groups were significantly reduced compared to the Model group at the end of the feeding period, similar to the results shown above (Fig 1A and 1B). In addition, our results further showed that the weight gain (Fig. 6C), liver index (Fig. 6D) and adipose index (Fig. 6E) of Dios,*C.glu* and Dios+*C. glu* groups were significantly lower than that of KK-Ay diabetic mice. On the other hand, Expression of TC(Fig 6F), TG (Fig 6G), LDL-C (Fig 6H) was also increased and HDL-C (Fig 6I) and insulin (Fig 6J) decreased in KK-Ay diabetic mice compared with NC group at the end of transplantation. Surprisingly, Dios, *C.glu* and Dios+*C. glu* treatment

significantly decreased the serum levels of TC, TG, LDL-C and increased the serum level of HDL-C and insulin. Furthermore, the liver histopathology also produced significant changes in less vacuolation and lipid droplets when treatment with Dios,*C.glu* and Dios+*C. glu.* compared with KK-Ay diabetic mice (Fig 6K).

C. glu and Dios treatment ameliorated HFD-induced glucose metabolism disorder in KK-Ay diabetic mice.

Treatment with Dios and*C.glu* , glucose metabolism and insulin resistance-related signaling proteins in liver and skeletal muscle were observed by western blot analysis. Our data showed that a continuous HFD diet can lead to severe insulin resistance in liver (Fig. 7A-7C) and skeletal muscle (Fig. 7D-7F), however, treatment with Dios, *C.glu* and Dios+*C. glu* . could significantly ameliorate insulin resistance by activating the IRS/PI3K/AKT signaling pathway. Once AKT is activated, it participates in glucose metabolism related pathways caused by insulin. We found that Dios promoted glycogen synthesis in liver and skeletal muscle by phosphorylating GSK-3β and activating GS. furthermore, the level of phosphorylation AS160 and Glut4 were significantly up-regulated in skeletal muscle by Dios, *C.glu* and Dios+*C. glu* . treatment.

Discussion

Previous studies have shown that gut microbiota and its metabolic products are vital for many metabolic diseases, including obesity (Turnbaugh et al., 2006), insulin resistance (Cani et al., 2007), metabolic syndrome (Vijay-Kumar et al., 2010) and liver steatosis (Spencer et al., 2011). Increasing data indicate that diabetic metabolic disorders are closely related to changes in gut microbiota composition, gut microbiota affects host metabolism by altering gluconeogenesis, glycogenolysis, lipogenesis, inflammation and hormone action (Federico et al., 2017, (Muñoz-Garach et al., 2016). Hence, reverse gut dysbiosis may represent a novel strategy to treat or prevent diabetes. In this study, we demonstrated that Dios treatment remarkably ameliorated glucose metabolism in KK-Ay diabetic mice by the regulation of *C. glu* on IRS/PI3K/AKT signaling pathway and reshaped the unbalanced gut microbiota.

Glycogen is supposed to be the principal storage form of glucose and its metabolisms are primarily in the liver and skeletal muscle. The concerted regulation of the rate of glycogen synthesis and the rate of glycogenolysis is one of the principal methods in the maintenance of glycogen homeostasis. Defects in these two processes can be major contributors to hyperglycemia and insulin resistance (Petersen et al., 2017). Thus, glycogen homeostasis plays an important role in the development of T2DM. Insulin is the main hormone proteins produced by the β cells of pancreatic islets and regulates many important biological functions, which control the metabolism and storage of the three major nutrients (Niswender, 2011). Insulin mainly stimulates lipogenesis, glycogen and protein synthesis and inhibits lipolysis and protein breakdown, to stimulate cell growth and differentiation, and promote the storage of substrates in fat, liver and muscle (Edgerton et al., 2006). When insulin resistance or insulin deficiency occurs, it will cause a serious imbalance of these processes and metabolic disorders, which will lead to the destruction of body homeostasis (Tzeng et al., 2012). The IR and related IRS, the PI3K heterodimer, and AKT are three well-defined and essential mediators of the insulin signaling pathway. Insulin binds to the insulin receptor tyrosine kinase and activates the receptor and then phosphorylates the insulin receptor substrate (IRS), phosphorylated IRS then activates PI3K, and subsequently phosphorylates Akt, leading to Akt activation. When AKT is activated, it participates in the metabolic pathways of glucose transport and glycogen synthesis (Beale, 2013). In our study, through transcription analysis, we predicted that the potential mechanism of Dios ameliorated T2DM may be through regulating glucose metabolism and insulin resistance. As would be predicted, we found that Dios could predominantly up-regulating IRS/PI3K/AKT signaling pathway to promoting glycogen synthesis and GLUT4 translocation to regulate the balance of glucose level.

In recent years, several studies have shown that gut microbiota dysbiosis has been suggested as a prominent feature of T2DM (Ahmad et al., 2019, (Sabatino et al., 2017). In diabetic mice, an obvious shift between*Firmicutes* and *Bacteroidetes* when compared with non-diabetic mice, which indicates that these major phyla may play a role in T2DM, as reported previously (Qin et al., 2012). In our study, we found that Dios effectively reversed gut microbiota dysbiosis when compared to untreated diabetic mice. Moreover, Dios

treatment restored the *Firmicutes/Bacteroidetes* ratio close to that observed in non-diabetic mice. Intriguingly, the ratio of*Actinobacteria* phylum was also significantly higher in the H-Dios group than in the other groups. The*Actinobacteria*phylum represents one of the largest groups of bacteria. It is well known that the*Actinomycetes* , a potentially beneficial bacteria, are a diverse phylum of Gram-positive bacteria found in the human intestinal tract and soil (Bhatti et al., 2017). Thus, we want to further explore the impact of Dios on the relative abundance of the gut microbiota at the species level. In our study, the *C._glu* is markedly increased by Dios (60 mg/kg) treatment compared with KK-Ay diabetic mice. Numerous studies have indicated that *C. glu* is a Gram-positive soil bacteria belonging to the order*Corynebacteriales* within the *Actinobacteria* (Becker et al., 2018). Meanwhile, *C. glu*is an important industrial metabolite producer, and has been widely used for the industrial production of various amino acids, vitamins, and nucleotides (Lee et al., 2016). Previous studies suggested that *C. glu* plays a crucial role in the process of glycogen synthesis. At the same time, by encoding the glg C gene of C. glu, a key enzyme in glycogen synthesis, and studying its correlation with ADP-glucose pyrophosphorylase, it was found that the cells of *C. glu* grown in a medium containing glucose, sucrose or fructose as glycolytic substrates showed rapid glycogen accumulation in the early exponential growth phase (Seibold et al., 2007). Therefore, we hypothesized that*C. glu* may be a potential beneficial bacteria in the development of T2DM. To test this hypothesis, KK-Ay diabetic mice were treated with*C. glu* , Dios or *C. glu* +Dios for 4 weeks. We found that the addition of *C. glu* alone can remarkably reduce HFD-induced body weight and blood glucose gain and significantly ameliorated lipid accumulation compared with KK-Ay diabetic mice group. However, when treatment with *C. glu* and Dios together, the body weight and blood glucose gain down-regulated more significantly. Besides, we found that treatment with*C. glu* and Dios together can markedly ameliorate glucose metabolism by up-regulating IRS/PI3K/AKT signaling pathway to promoting glycogen synthesis and GLUT4 translocation compared with KK-Ay mice.

In summary, in the present study, we demonstrated for the first time that Dios could ameliorate glucose metabolism in KK-Ay diabetic mice by the regulation of *C. glu* on IRS/PI3K/AKT signaling pathway and reshaped the unbalanced gut microbiota. Collectively, our results suggested that Dios may function as potential anti-diabetic agent to effectively play a positive role in the management of T2DM patients.

Author contributions

B.Z. designed the research and C.B. provided suggestions to research. X.G. and L.X. performed most of the experiments, L.X. analyzed the data and C.B. contributed analytic tools. X.G. wrote the primary manuscript, B.S. revised the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

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Figure Legends

Figure 1. Dios treatment reduced HFD-induced body weight and blood glucose gain in KK-Ay diabetic mice. (A,B) Change of body weight and blood glucose in different weeks of the experimental period in KK-Ay diabetic mice. (C) Increased body weight in different groups. (D) Adipose index in different groups. (E) Liver index in different groups. (F) Representative pictures of retroperitoneal fats and livers from different groups. These data represent the mean ± SD (n = 6). *p < 0.05 vs Control group; #p < 0.05vs Model group.

Figure 2. Dios ameliorated lipid accumulation in KK-Ay diabetic mice. the serum levels of (A) TC, (B) TG, (C) LDL-C, (D) HDL-C and (E) Insulin of different groups. (F) Representative images of hematoxylin-eosin and oil-red-O staining (Original magnifications were 200×) of liver and skeletal muscle sections of different groups. These data represent the mean ± SD (n = 6). *p < 0.05 vs Control group; #p < 0.05vs Model group.

Figure 3. Dios reversed sustained transcriptional changes in KK-Ay diabetic mice. (A) PCA analysis of different groups. (B) Heat map of correlation analysis. (C) Identification of DEGs in different groups. The green represents all regulated genes. The blue represents upregulated genes. The grey represents upregulated genes. (D-E) Heat map analysis was employed to the discrimination of expression profile of DEGs

across the samples. Red and green areas separately represent highly and lowly expressed genes in different groups. (F) GO analysis of DEGs between Model and Dios group. (G) GO analysis of down-regulated genes between Model and Dios group. (H) KEGG pathway analysis of DEGs between Model and Dios group. (I) KEGG pathway analysis of up-regulated genes between Model and Dios group. (J) Reactome functional enrichment analysis. n = 3 individuals/group.

Figure 4. Dios treatment ameliorated HFD-induced glucose metabolism disorder in KK-Ay diabetic mice. (A-C) The glucose metabolism and insulin resistance-related signaling proteins in liver were observed by western blot analysis. (D-F) The glucose metabolism and insulin resistance-related signaling proteins in skeletal muscle were observed by western blot analysis. These data represent the mean +- SD (n = 3). *p < 0.05 vs Control group; #p < 0.05vs Model group.

Figure 5. Dios treatment reverses HFD-induced gut dysbiosis in KK-Ay diabetic mice. (A) PCoA of gut microbiota in mice based on weighted UniFrac. (B) UPGMA of gut microbiota in mice. (C) Changes in intestinal bacteria community at the phylum level from different groups. (D) The ratio of the phylum *Firmicutes* to *Bacteroidetes* . (E) The abundance of the phylum level of *Firmicutes.* (F) The abundance of the phylum level of *Bacteroidetes* . (G) Changes in intestinal bacteria community at the species level from different groups. Relative abundance of significant differences at the species level: (H) *Lactobacillus_gasseri;* (I)*Aerococcus_viridans;* (J) *Corynebacterium_glutamicum.* (K)*Bacteroides_acidifaciens* . These data represent the mean +- SD (n = 3). *p < 0.05 vs CK group; #p < 0.05vs M group.

Figure 6. *C. glu* and Dios treatment reduced HFD-induced body weight and blood glucose gain in KK-Ay diabetic mice. (A,B) Change of body weight and blood glucose in different weeks of the experimental period in KK-Ay diabetic mice. (C) Increased body weight in different groups. (D) Liver index in different groups. (E) Adipose index in different groups. The serum levels of (F) TC, (G) TG, (H) LDL-C, (I) HDL-C and (J) Insulin of different groups. (K) Representative images of hematoxylin-eosin and oil-red-O staining (Original magnifications were 200x) of liver sections of different groups. These data represent the mean +- SD (n = 6). *p < 0.05 vs Control group; #p < 0.05 vs Model group.

Figure 7. *C. glu* and Dios treatment ameliorated HFD-induced glucose metabolism disorder in KK-Ay diabetic mice. (A-C) The glucose metabolism and insulin resistance-related signaling proteins in liver were observed by western blot analysis. (D-F) The glucose metabolism and insulin resistance-related signaling proteins in skeletal muscle were observed by western blot analysis. These data represent the mean +- SD (n = 3). *p < 0.05 vs Control group; #p < 0.05 vs Model group.

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